Unravelling the ecological and evolutionary mysteries of the rough fescue grasses *Festuca hallii* and *F. campestris*

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Abstract

The rough fescue grasslands of western North America are diverse, unique ecosystems that provide essential ecological and agricultural services, yet have been significantly fragmented by land conversion, urbanization, and energy infrastructure. Only around 5% of their historic 137,000km^2 extent remains unmodified, and remaining prairie fragments face ongoing threat from unsustainable grazing practices, invasive crop species, and disruption of their natural disturbance regimes. Conserving and restoring rough fescue grasslands remains challenging due to the sensitivity of their defining species, the rough fescue grasses (*Festuca hallii* and *F. campestris*), and a limited understanding of how rough fescue ecology and life history affects their fitness and persistence in disturbed environments.

This dissertation addresses three components of rough fescue autecology that have direct applications for conservation and restoration; Chapter 2 investigates the role of dormancy and cold exposure on rough fescue seed germination using a comparative germination assay between *F. hallii* and *F. campestris.* It finds that the temperature and duration of seed storage has significant impacts on rough fescue germination, and can greatly enhance or stifle germination in seeds of the same age and provenance. Additionally, the two grass species had drastically different germination responses, suggesting that species-specific life history/physiology differences should be taken into account when using wild-collected seed for restoration. Chapter 3 examines the prevalence of *Epichloë* fungal endophytes in wild rough fescue populations across a mosaic of disturbance conditions at Glenbow Ranch Provincial Park near Cochrane, Alberta. Here, I found high (µ= >80%) levels of endophyte infection across all sites, and confirmed, for the first time, the presence of an *Epichloë* symbiosis in *F. hallii.* My final chapter explores the taxonomic and genetic relationship between *F. hallii* and *F. campestris* through a province-wide genomic survey of both allopatric and sympatric rough fescue populations. I discovered the occurrence of extensive and ongoing hybridization between the two species in their ranges of overlap, and additionally, found evidence of *F. campestris* introgression into allopatric populations of *F. hallii.* Collectively, these findings provide actionable ecological insights into the early life stages, mutualistic interactions, and genetic structure of rough fescue grasses, offering a foundation for more targeted and effective restoration practices in this imperilled grassland ecosystem.

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Chapter 1: General Introduction

This vast prairie is in summer a sea of waving grasses.
— J. E. Weaver, *North American Prairie* (1944)

1.1 The ecology of the rough fescue grasslands

The temperate grasslands of central North America, also known as the *prairies* or the *Great Plains*, are among the most anthropogenically altered, yet least protected biomes in the world (Hoekstra et al. 2005; Wilsey 2021). Once covering more than 2 million km^2 of Canada and the United States, only around 38% of the Great Plains remain intact (Downey et al. 2013; Comer et al. 2018), with the remainder converted primarily to agricultural land for growing crops or grazing cattle (Anderson 2006b). Despite growing recognition of the significant contributions grasslands make to global biodiveristy, carbon sequestration, and other essential ecological services (Bengtsson et al. 2019; Lyons et al. 2023), only around 3% of the remaining temperate grasslands in North America fall within protected areas (Comer et al. 2018). Additionally, restoration work carried out in prairie biomes has been hampered by the fragmentary nature of remaining grassland sites (Floate et al. 2017; Török et al. 2021; Elsinger et al. 2023), the historic use of unsuitable invasive species in seed mixes (Samson and Knopf 1994; Woosaree and Otfinowski 2018; Elsinger et al. 2023), and the slow-growing nature of most prairie communities, necessitating restoration methods that are often labour and cost-intensive (Downey et al. 2013; Chazdon et al. 2024).

One region of the Great Plains that has been particularly imperilled is Canada's rough fescue prairies, which now occupy only 5–13% of their former extent across the prairie provinces (Downey et al. 2013; Comer et al. 2018). In addition to the reasons mentioned above, restoration of the rough fescue grasslands remains particularly elusive due to the fact that their dominant species—the rough fescue grasses—remain notoriously unpredictable and poorly understood (Anderson 2006a; Woosaree 2012; Palit et al. 2015). Because these grasses play a central role in the structure and function of rough fescue prairies, it's therefore important to first understand how their ecological idiosyncrasies and life histories affect their persistence in a restoration context (Woosaree 2012; Palit et al. 2015).

The rough fescues (*Festuca hallii* and *Festuca campestris*) are long-lived perennial bunchgrasses that provide the vegetative foundation of the ecosystems in which they occur (Tirmenstein 2000; Moisey et al. 2006). These foundational species, with their unique morphology, growth habits, and life history characteristics, in turn shape and sustain many of the ecological processes and species interactions of their respective communities (Weaver 1944; Bork et al. 2012). True to the name "bunchgrasses", rough fescue grows in dense tufts ("crowns") of tightly packed leaves (Desserud 2006; Anderson 2006a), which serve to protect the main growth points (basal meristems) at or below ground level (Fig. 1.1; Tirmenstein 2000).

F. campestris is the larger of the two, forming dense crowns 20–50cm across and up to 1.4m tall (Tirmenstein 2000; Anderson 2006a), while *F. hallii* tends to grow in smaller tufts, connected extensively by creeping underground stems (rhizomes; Fig. 1.2; Pavlick and Looman 1984; Aiken and Darbyshire 1990).

This capacity for extensive vegetative growth makes rough fescue grasses both highly resistant to fire, and capable of living for a very long time, with mature plants estimated to be decades, if not hundreds of years old (Qiu et al. 2009; de Witte and Stöcklin 2010; Tannas 2014). The "rough" moniker refers to the grasses' stiff, wiry leaves (Bork et al. 2012), which have a distinct sandpaper-like surface texture capable of cutting skin (a fact I can personally attest to). This roughness is due to the leaves' high silica content, which, coupled with their narrow inrolled shape, grants them significant tolerance to water loss and drought (Fig. 1.4; Blackman and Bailey 1971; Weerstra and Holcroft Weerstra 1998; Rizwan et al. 2015).

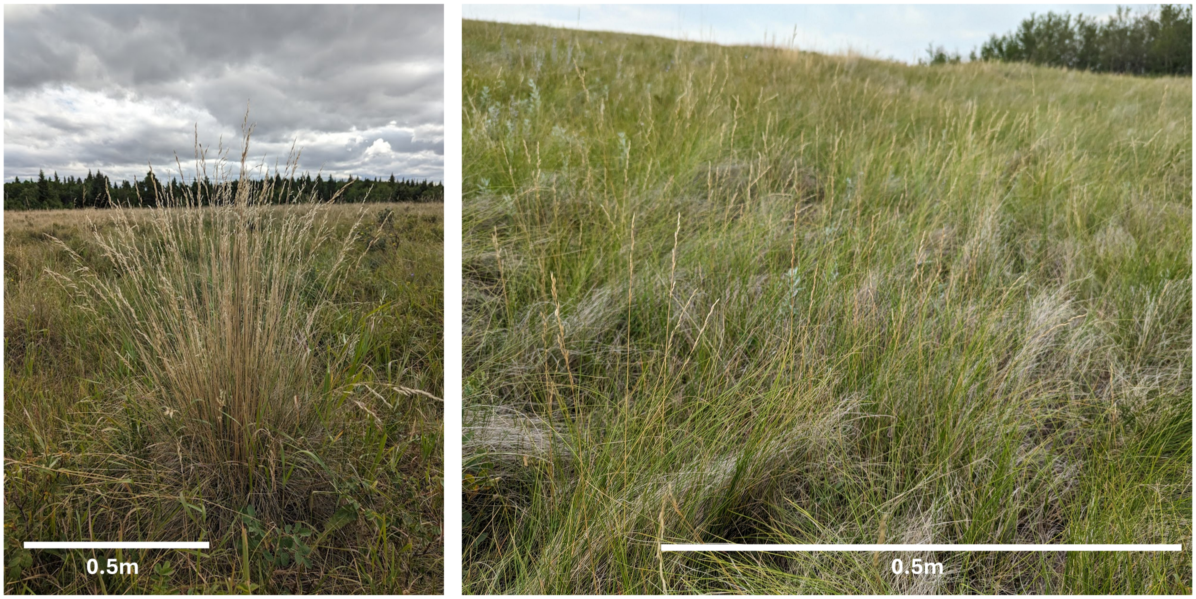
This tolerance is further boosted by an extensive web of dense, underground roots (Stout et al. 1981; Desserud 2006) that help to capture and retain soil moisture and nutrients, in turn contributing to the creation of rich Black Chernozem soil, high in organic matter and nutrients (Dormaar and Willms 1993; Bailey et al. 2010; Labaz et al. 2024). In addition to contributing to hospitable belowground conditions for their fellow prairie plants, rough fescue grasses aid in regulating aboveground conditions by producing copious quantities of persistent litter (i.e., dead leaf material that remains attached to the plant; Tirmenstein 2000). This leaf litter forms dense blankets of "prairie wool" around the grass crowns (Desserud 2016), helping to trap and retain moisture, reduce surface evaporation, and regulate soil temperatures for the grasses and their fellow prairie plants (Fig. 1.5; Deutsch et al. 2010; Bork et al. 2012; Chen et al. 2024).

Collectively, these traits give rise to ecosystems that support exceptional biological diversity, including many rare and endangered plants (Samson and Knopf 1994; Floate et al. 2017), native pollinators (Worthy et al. 2024), breeding birds, and mammals (Otfinowski et al. 2017).

1.2 The value of the rough fescue prairie

Rough fescues grasslands also provide valuable ecosystem services for human communities, in addition to the plants and animals of the prairies. The rich black chernozem soils associated with rough fescue are a major underground carbon sink, capturing and storing an estimated 0.7 to 1.5 tonnes of carbon/hectare annually, compared to managed cropland, which sequesters only 0.031–0.063 tonnes $\mathrm{ha}^{-1}\ \mathrm{yr}^{-1}$ (Alcock 2017; Labaz et al. 2024). In addition, the mosaic of deep roots and thick litter work in tandem to filter pollutants from runoff, capture and recycle nutrients, improve water retention and prevent soil erosion (Tirmenstein 2000; Bailey et al. 2010; Bork et al. 2012). Another ecosystem service that has long been recognized is rough fescue's value as highly productive, highly nutritious forage for grazing herbivores, namely cattle (Willms and Fraser 1992; Palit et al. 2017). In addition to being highly palatable, it also has the unique ability to "cure on the stem", meaning

Figure 1.1: Tightly-packed basal sheaths give rough fescue grasses their distinctive "bunched" appearance, and protect their vegetative buds close to the ground.



Festuca campestris **Festuca hallii**

Figure 1.2: *Festuca campestris* (left) typically grows in densely tufted “crowns” up to 50cm across and 1.4m tall. *F. hallii* (left) shows a more “mat-forming” growth, with smaller grass tufts joined by creeping underground stems (rhizomes).

it retains its high nutritional content even after going dormant (Desserud 2006; Moisey et al. 2006). Historically, this made rough fescue a valuable source of fall and winter forage for plains bison (*Bison bison bison*), whose grazing would in turn clear away excess litter and promote more vigorous regrowth in the spring (Bork et al. 2012; Otfinowski et al. 2017). Since the advent of the ranching industry in North America, this has become perhaps the most valued ecosystem service provided by rough fescue grasslands, and a primary driver for interest in their management and conservation (Willms et al. 1985; Willms et al. 1986; Dormaar and Willms 1990; Bork et al. 2012; Krzic et al. 2014; Pyle et al. 2018). Taken together, the climatic, ecological, and economic services provisioned by native prairies like these are valued at an estimated CAD\$3,300–6,000 ha^{-1} yr^{-1} (Dupras et al. 2016; Liu et al. 2022). Unfortunately, however, the very qualities that make rough fescue grasslands productive, distinct, and ecologically valuable are also the qualities that have made them highly susceptible to exploitation and decline (Woosaree 2012; Otfinowski et al. 2017).

1.3 Past and current threats to rough fescue grasslands

Fescue grasslands ought to be robust to ecological and anthropogenic disturbance, considering that fire and grazing were historically key to maintaining their diversity and resilience (Tirmenstein 2000; Bork et al. 2012). Dormant season grazing by bison helped maintain a shifting mosaic of diverse prairie patches (Anderson 2006b). Periodic fires, both natural and those curated by Indigenous Nations like the Blackfoot Confederacy, cleared away woody overgrowth, replenished soil nutrients,

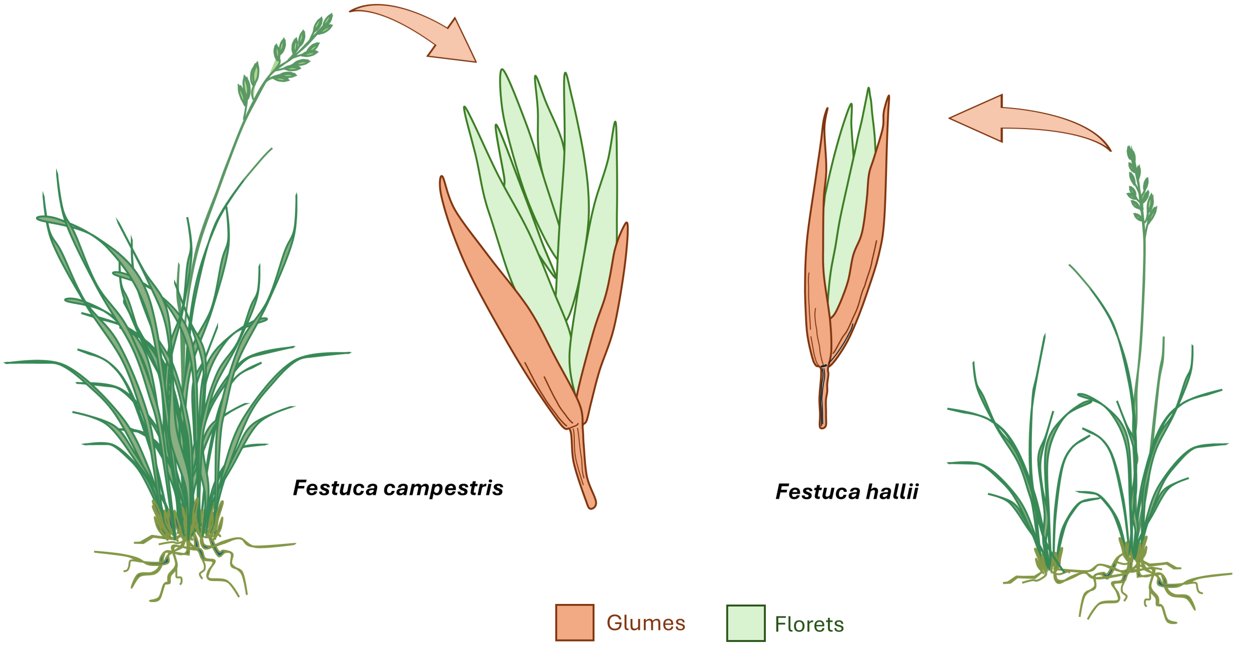


Figure 1.3: Visual comparison of *F. campestris* and *F. hallii*'s growth form and flower cluster (spikelet) differences. *F. campestris* have 3–7 flowers (florets) per spikelet, and bracts (glumes) of unequal length, which are shorter than the rest of the flower cluster. *F. hallii* have 2–3 florests per spikelet, with glumes of approximately equal length, that almost fully enclose the flowers within. Colours are for illustrative purposes only.

Figure 1.4: Three leaves from the same rough fescue plant, showcasing their ability to roll tightly during dry conditions to reduce moisture loss. Inset shows a cross section of the rolled leaf. The thick walls and u-shaped cross section of the leaves give them a stiff, wiry feel and confer significant drought tolerance.

Figure 1.5: Mature rough fescue prairie, persisting amid the maintenance of an adjacent railway track. The mats of dead leaf litter at the base of each plant (light grey patches) form a blanket of “prairie wool” that serves to insulate the surrounding soil and reduce moisture loss.

and encouraged new germination from the prairie seedbank (Bailey et al. 2010; Pylypec 2017; Yao et al. 2017; SpearChief-Morris 2025). Mature fescue plants are therefore highly adapted to both kinds of disturbance; capable of regrowing even after heavy winter grazing or intense fire (Moisey et al. 2006; Desserud 2016). However, the arrival of European settlers to western Canada in the late 19th and early 20th centuries marked a dramatic disruption to the systems that enabled this resilience (Samson et al. 2004; Floate et al. 2017). Native sod was quickly broken and replaced with more "productive" non-native forage crops to take advantage of the rich, dark soils associated with fescue communities, while the remaining unconverted prairie was often subject to intensive year-round cattle grazing (Hanson and Vorhies 1938; Dormaar and Willms 1990; Samson and Knopf 1994). In combination, the legacy effects of unregulated grazing, disruption of natural disturbance regimes, and ongoing threat of aggressive non-native crop species have reduced the once-dominant fescue grasslands to few scattered fragments (Otfinowski et al. 2017; Palit et al. 2017), which present significant conservation and restoration challenges for those interested in protecting them (Desserud 2016; Woosaree and Otfinowski 2018).

Ecologists and restoration practitioners face considerable difficulty in reestablishing disturbed prairie sites (Desserud 2016; Woosaree and Otfinowski 2018), and some of this difficulty is due to rough fescue's life history and other ecological characteristics. As perennial climax species that primarily reproduce vegetatively, rough fescue are slow growing, produce seed sporadically, and have low/unpredictable germination rates in the field (Desserud 2006; Elsinger et al. 2022). They are also poor competitors against invasive forage grasses (Desserud 2016; Otfinowski et al. 2017), and are intolerant to grazing during the growing season (Moisey et al. 2006; Bork et al. 2012). Most restoration efforts that relied on traditional broadcast seeding have failed (Desserud et al. 2010; Desserud and Naeth 2013; Lancaster et al. 2015), while more effective techniques like planting plugs or transplanting native sod are both labour and cost-intensive (Downey et al. 2013; Chazdon et al. 2024). Additionally, over 80% of remaining rough fescue prairie in Canada is held in private ownership, and is used primarily for either cattle grazing, or energy infrastructure such as oil and gas pipelines or wellsites (Bailey et al. 2010). What limited restoration occurs on these lands is therefore primarily intended to maximize forage output or reclaim "equivalent land capability" (ESRD 2015; Pyle et al. 2018), rather than restore ecological integrity (Samson and Knopf 1994). The result of this is that most rough fescue restoration research is hampered by a reliance on inefficient "one-size-fits-all" treatment methods (Desserud 2006; Woosaree 2012; Palit et al. 2017), and several prominent research gaps remain unanswered by the bodies most interested in effectively conserving native grasslands (Grassland Restoration Forum 2009; Lancaster et al. 2015).

1.4 Purpose and scope of the current research

I aimed to address some of the prominent knowledge gaps that have made rough fescue so difficult to restore, through an autecological perspective. That is, I have prioritized an ecological understanding of rough fescue grasses as individual species, and how their life histories and relationships with their environment affect their fitness and persistence (Palit et al. 2015; Palit et al. 2017).

In taking this approach, I hope to generate knowledge that will provide not only a better understanding of rough fescue grasses, but also a means of developing more targeted and effective approaches to their restoration.

The first chapter of my thesis addresses rough fescue seed dormancy and stratification (i.e., cold storage) requirements. Restoration practitioners often have to rely on fescue seed that has been collected in years previous, stored in unknown conditions, and then distributed as needed for projects (Desserud 2006; Woosaree 2012; Palit et al. 2017). Little to no research has been conducted on how such storage treatments might affect the subsequent germination of rough fescue seedlings, and so I aimed to address these potential effects with a comparative germination assay of *F. hallii* and *F. campestris*. I found that, not only can rough fescue germination be enhanced or suppressed based on the storage conditions of their seeds, but that the two species had drastically different responses to identical storage and germination conditions. *F. hallii* germination was greatly improved (from 69% to 94%) by long periods of cold (-20°C) storage and moisture priming treatment, while *F. campestris* exhibited low overall germination (13%) that was only improved to 33% by long periods of storage around 4°C. This work highlights the importance of considering seed storage and handling as an influential and species-specific part of the restoration pipeline, rather than a simple logistical hurdle.

Chapter 2 of my thesis examines the relationship between rough fescue grasses and their symbiotic fungal endophytes. Endophytes can strongly influence plant fitness and persistence, especially in disturbed or environmentally stressful environments (Saikkonen et al. 2016; Lee et al. 2021). To understand potential associations between fescue endophtyes and levels of grassland disturbance/health, I conducted a landscape-scale survey of remnant rough fescue grassland populations at Glenbow Ranch Provincial Park in Alberta, Canada. I did not identify any associations between endophyte prevalence and the site metrics I captured (functional diversity, litter cover, % cover of increaser, invader, and rough fescue species), however I did observe high levels (>80%) of endophyte infection across all sites, and documented the first known association between *Epichloë* endophytes and *F. hallii*. These results expand our understanding of the extent of *Epichloë* symbioses in rough fescue grasses, building on the work of Rudgers et al. (2016), and opening the door for further exploration of the exact nature of this relationship.

My final data chapter takes a closer look at the taxonomic and genetic relationship between *Festuca hallii* and *Festuca campestris*. The two species have a long and contentious taxonomic history, which has never been fully explored in a genetic context (Pavlick and Looman 1984; Harms 1985; Anderson 2006a). Because the identification of taxonomically distinct populations is important to conservation and species at risk policy (COSEWIC 2023), and because hybridization can both increase (Urfusová et al. 2021; Faske et al. 2024) and decrease (Sinaga et al. 2024; Andreev et al. 2025) available genetic diversity, I wanted to explore the possibility that the two species were hybridizing in areas of range overlap. To do so, I conducted a genomic study of rough fescue populations across Alberta, to map their relationships in areas of allopatry and sympatry. Through this, I found that not only are *F. hallii* and *F. campestris* hybridizing where their ranges overlap (98% of sympatric samples were hybrids), but that there is actually fairly extensive introgression of *F. campestris* ancestry into populations of *F. hallii* previously thought to be allopatric (40% of allopatric samples were hybrids). These results shed light on the two species' debated taxonomic history and the existence of confusing morphologically intermediate specimens (Johnston and Cosby 1966; Harms 1985), while also raising questions about the true extent of their ranges in Alberta.

Taken together, these different aspects of my dissertation represent insight into new, specific, and actionable ecological knowledge about two important species that have long eluded proper restoration success. Helping us understand more about what influences their crucial early life stages, how they might be interacting with mutualists in their environments, and how the two species relate to one another across their ranges in the province.

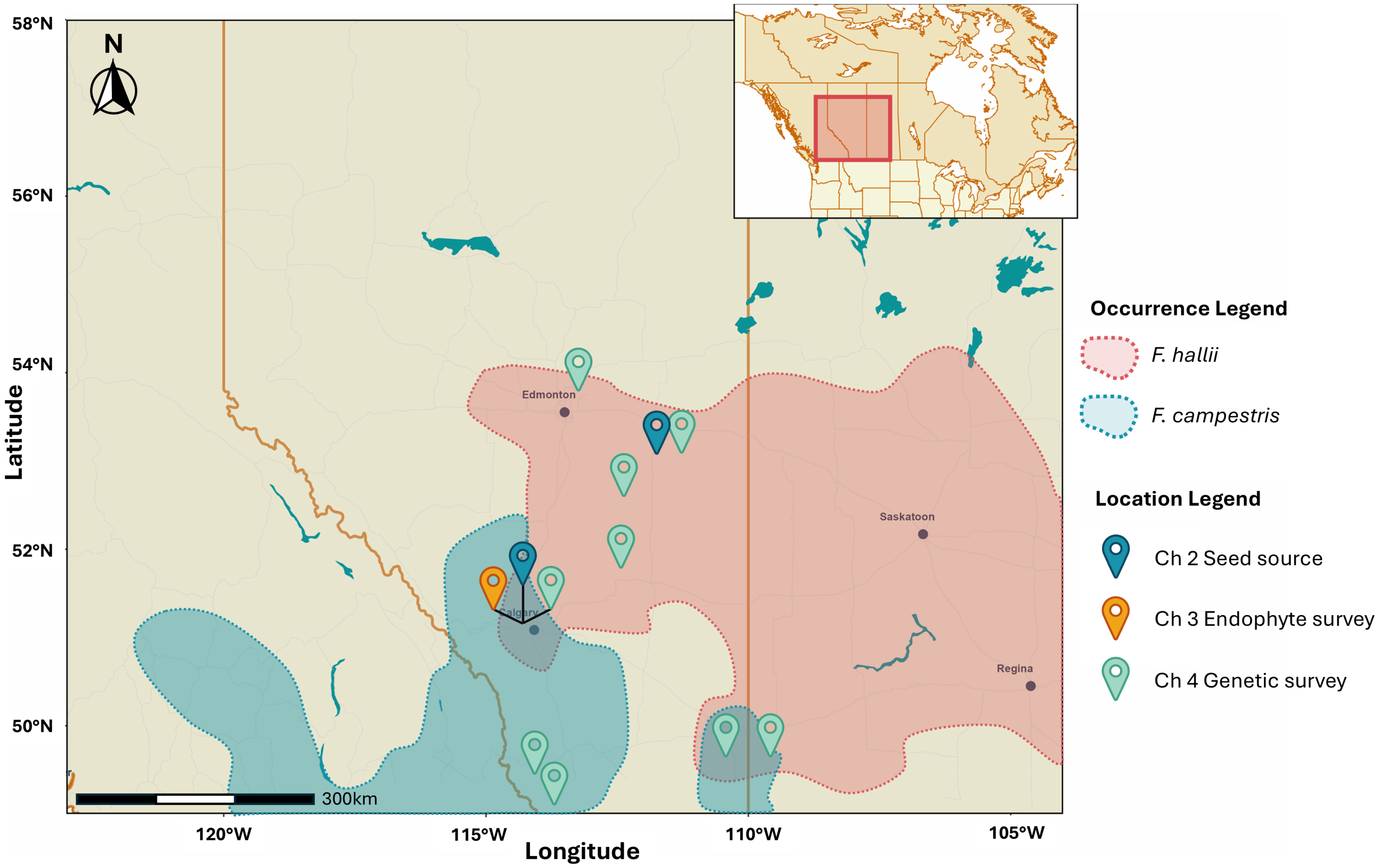


Figure 1.6: Map denoting the locations of all sites surveyed or sourced for my three data chapters. Blue pins show the locations from which seeds were sourced for Chapter 2's comparative germination experiment. The orange pin shows Glenbow Ranch Provincial Park, where Chapter 3's endophyte surveys were carried out. Green pins show sampling locations for my genetic survey of allopatric and sympatric fescue populations in Chapter 4. Shaded regions denote the approximate ranges for both parental species, adapted from Pavlick and Looman (1984) and iNaturalist occurrence records

References

Aiken, S. G. and S. J. Darbyshire (1990). *Fescue grasses of Canada.* Publication 1844/E. Ottawa, Canada: Agriculture Canada. ISBN: 0-660-13483-7.

Alcock, J. (2017). *Wetland and Grassland Retention and Restoration as an Effective Carbon Management Strategy in Alberta.* Tech. rep. Alberta, Canada: Viresco Solutions.

Anderson, G. (2006a). Festuca hallii *(Vasey) Piper (Hall's fescue): A Technical Conservation Assessmen.* Technical Conservation Assessment. Fort Collins, Colorado: Colorado Natural Heritage Program, Colorado State University.

Anderson, R. C. (2006b). Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers1. The Journal of the Torrey Botanical Society 133.4, pp. 626–647. DOI: `10.3159/1095-5674(2006)133[626:EAOOTC]2.0.CO;2`.

Andreev, V., J. Puzey, E. Davies, C. Olson-Manning, S. Kreutzmann, and M. Fishbein (2025). History and Dynamics of an Extensive Plant Hybrid Zone on the Great Plains of North America. Molecular Ecology 34.18, e70048. DOI: `10.1111/mec.70048`.

Bailey, A. W., D. McCartney, and M. Schellenberg (2010). *Management of Canadian prairie rangeland / [by] Arthur W. Bailey, Duane McCartney, Michael P. Schellenberg. : A52-178/2010E - Government of Canada Publications - Canada.ca.* Monograph A52-178/2010E. Ottawa, Canada: Agriculture and Agri-Food Canada.

Bengtsson, J., J. M. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. J. O'Farrell, H. G. Smith, and R. Lindborg (2019). Grasslands—more important for ecosystem services than you might think. Ecosphere 10.2, e02582. DOI: `10.1002/ecs2.2582`.

Blackman, E. and C. B. Bailey (1971). Dissolution of silica from dried grass in nylon bags placed in the rumen of a cow. Canadian Journal of Animal Science 51.2, pp. 327–332. DOI: `10.4141/cjas71-045`.

Bork, E., W. Willms, S. Tannas, and M. Alexander (2012). Seasonal Patterns of Forage Availability in the Fescue Grasslands Under Contrasting Grazing Histories. Rangeland Ecology & Management 65.1, pp. 47–55. DOI: `10.2111/REM-D-11-00087.1`.

Chazdon, R. L., D. A. Falk, L. F. Banin, M. Wagner, S. J. Wilson, R. C. Grabowski, and K. N. Suding (2024). The intervention continuum in restoration ecology: rethinking the active–passive dichotomy. Restoration Ecology 32.8, e13535. DOI: `10.1111/rec.13535`.

Chen, W., L. Jiang, R. Jia, B. Tang, H. Jiang, Y. Wang, X. Lu, J. Su, and Y. Bai (2024). Plant litter loss exacerbates drought influences on grasslands. New Phytologist 241.1, pp. 142–153. DOI: `10.1111/nph.19374`.

Comer, P. J., J. C. Hak, K. Kindscher, E. Muldavin, and J. Singhurst (2018). Continent-Scale Landscape Conservation Design for Temperate Grasslands of the Great Plains and Chihuahuan Desert. Natural Areas Journal 38.2, pp. 196–211. DOI: `10.3375/043.038.0209`.

COSEWIC (2023). *COSEWIC Guidelines for Recognizing Designatable Units.* URL: `https://cosewic.ca/index.php/en/reports/preparing-status-reports/guidelines-recognizing-designatable-units.html`.

de Witte, L. C. and J. Stöcklin (2010). Longevity of clonal plants: why it matters and how to measure it. Annals of Botany 106.6, pp. 859–870. DOI: `10.1093/aob/mcq191`.

Desserud, P. (2006). *Restoration of Rough Fescue (*Festuca campestris*) Grassland on Pipelines in Southwestern Alberta*. Tech. rep. T/121. Lethbridge, Alberta: Rangeland Management Branch, Public Lands Division, Alberta Sustainable Resource Development, p. 77.

Desserud, . and M. A. Naeth (2013). Natural Recovery of Rough Fescue (*Festuca hallii* (Vasey) Piper) Grassland After Disturbance by Pipeline Construction in Central Alberta, Canada. Natural Areas Journal 33.1, pp. 91–98. DOI: `10.3375/043.033.0111`.

Desserud, P. (2016). *Industrial activity in the central Parkland and northern fescue native grasslands: strategies for minimizing surface disturbance*. Tech. rep. Edmonton, Alberta: Alberta Environment and Parks.

Desserud, P., C. C. Gates, B. Adams, and R. D. Revel (2010). Restoration of foothills rough fescue grassland following pipeline disturbance in southwestern Alberta. Journal of Environmental Management 91.12, pp. 2763–2770. DOI: `10.1016/j.jenvman.2010.08.006`.

Deutsch, E. S., E. W. Bork, and W. D. Willms (2010). Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. Agriculture, Ecosystems & Environment 135.1, pp. 1–9. DOI: `10.1016/j.agee.2009.08.002`.

Dormaar, J. F. and W. D. Willms (1990). Sustainable production from the Rough Fescue Prairie. Journal of Soil and Water Conservation 45.1, pp. 137–140. DOI: `10.1080/00224561.1990.12456450`.

Dormaar, J. F. and W. D. Willms (1993). Decomposition of Blue Grama and Rough Fescue Roots in Prairie Soils. Journal of Range Management 46.3, pp. 207–213. DOI: `10.2307/4002608`. JSTOR: `4002608`.

Downey, B. A., P. F. Jones, F. Blouin, J. D. Richman, and B. L. Downey (2013). Restoring Mixed Grass Prairie in Southeastern Alberta, Canada. Rangelands 35.3, pp. 16–20. DOI: `10.2111/RANGELANDS-D-12-00082.1`.

Dupras, J., J. He, C. L'Ecuyer-Sauvageau, J. Auclair, and T. Podeer (2016). *Natural capital: the economic value of the National Capital Commission's green network*. Tech. rep. Ottawa, Ontario: National Capital Commission.

Elsinger, M. E., A. Dhar, and M. A. Naeth (2022). Recovery of plains rough fescue grasslands on reclaimed well sites. Journal for Nature Conservation 66, p. 126122. DOI: `10.1016/j.jnc.2021.126122`.

Elsinger, M. E., A. Dhar, and M. A. Naeth (2023). Plains rough fescue grassland restoration using natural regeneration after pipeline disturbances. Restoration Ecology 31.3, e13703. DOI: `10.1111/rec.13703`.

ESRD (2015). *2010 Reclamation Criteria for Wellsites and Associated Facilities for Native Grasslands (July 2013 Update)*. Tech. rep. Edmonton, Alberta: Environment and Sustainable Development, p. 92.

Faske, T. M., A. C. Agneray, J. P. Jahner, C. Osuna-Mascaró, L. M. Sheta, B. A. Richardson, E. A. Leger, and T. L. Parchman (2024). Environment predicts the maintenance of reproductive isolation in a mosaic hybrid zone of rubber rabbitbrush. Evolution 78.2, pp. 300–314. DOI: `10.1093/evolut/qpad207`.

Floate, K. D., J. D. Shorthouse, D. J. Giberson, and H. A. Cárcamo (2017). Arthropods of Canadian grasslands: a retrospective of a 40-year project of the Biological Survey of Canada. The Canadian Entomologist 149.6, pp. 702–717. DOI: `10.4039/tce.2017.46`.

Grassland Restoration Forum (2009). *Prospectus for a shared approach to research: Conserving and Restoring Rough Fescue Grasslands.* Tech. rep. Pincher Creek, AB: Grassland Restoration Forum. URL: `https://grasslandrestorationforum.ca/wp-content/uploads/2021/03/FRF-Prospectus-July-2009-S.pdf`.

Hanson, H. C. and C. T. Vorhies (1938). Need for Research on Grasslands. The Scientific Monthly 46.3, pp. 230–241. JSTOR: `16318`.

Harms, V. L. (1985). A Reconsideration of the Nomenclature and Taxonomy of the Festuca Altaica Complex (poaceae) in North America. Madroño 32.1, pp. 1–10. JSTOR: `41424511`.

Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts (2005). Confronting a biome crisis: global disparities of habitat loss and protection. Ecology Letters 8.1, pp. 23–29. DOI: `10.1111/j.1461-0248.2004.00686.x`.

Johnston, A. and H. E. Cosby (1966). Rhizomatous form of *Festuca scabrella.* Canadian Journal of Plant Science 46.2, pp. 211–212. DOI: `10.4141/cjps66-034`.

Krzic, M., S. F. Lamagna, R. F. Newman, G. Bradfield, and B. M. Wallace (2014). Long-term grazing effects on rough fescue grassland soils in southern British Columbia. Canadian Journal of Soil Science 94.3, pp. 337–345. DOI: `10.4141/cjss2013-019`.

Labaz, B., J. B. Kowalska, C. Kabala, M. Kobierski, J. Waroszewski, M. Dudek, K. Szopka, and D. Gruszka (2024). Distribution and Pools of Soil Organic Carbon in Chernozemic Soils Impacted by Intensive Farming and Erosion in the Loess Plateau in South-East Poland. Agronomy 14.11, p. 2544. DOI: `10.3390/agronomy14112544`.

Lancaster, J., B. Adams, R. Adams, and P. Desserud (2015). *Long-term Revegetation Success of Industry Reclamation Techniques for Native Grassland: Foothills Fescue, Foothills Parkland and Montane Natural Subregions; Phase 1 - Literature Review and Case Studies.* Tech. rep. Land and Forest Policy Branch, Alberta Environment and Sustainable Resource Development.

Lee, K., A. Missaoui, K. Mahmud, H. Presley, and M. Lonnee (2021). Interaction between Grasses and Epichloë Endophytes and Its Significance to Biotic and Abiotic Stress Tolerance and the Rhizosphere. Microorganisms 9.11, p. 2186. DOI: `10.3390/microorganisms9112186`.

Liu, H., L. Hou, N. Kang, Z. Nan, and J. Huang (2022). The economic value of grassland ecosystem services: A global meta-analysis. Grassland Research 1.1, pp. 63–74. DOI: `10.1002/glr2.12012`.

Lyons, K. G., P. Török, J.-M. Hermann, K. Kiehl, A. Kirmer, J. Kollmann, G. E. Overbeck, S. Tischew, E. B. Allen, J. D. Bakker, C. Brigham, E. Buisson, K. Crawford, P. Dunwiddie, J. Firn, D. Grobert, K. Hickman, S. L. Stradic, and V. M. Temperton (2023). Challenges and opportunities for grassland restoration: A global perspective of best practices in the era of climate change. Global Ecology and Conservation 46, e02612. DOI: `10.1016/j.gecco.2023.e02612`.

Moisey, D. M., W. D. Willms, and E. W. Bork (2006). Effect of Standing Litter on Rough Fescue Utilization by Cattle. Rangeland Ecology & Management 59.2, pp. 197–203. DOI: `10.2111/05-039R1.1`.

Otfinowski, R., H. G. Pinchbeck, and P. A. Sinkins (2017). Using Cattle Grazing to Restore a Rough Fescue Prairie Invaded by Kentucky Bluegrass. Rangeland Ecology & Management 70.3, pp. 301–306. DOI: `10.1016/j.rama.2016.10.008`.

Palit, R., Y. Bai, J. Romo, B. Coulman, and R. St Pierre (2015). Variations in vernalization requirements among ecotypes of *Festuca hallii*. Grass and Forage Science 70.2, pp. 353–364. DOI: `10.1111/gfs.12101`.

Palit, R., Y. Bai, J. Romo, B. Coulman, and R. Warren (2017). Seed Production in Festuca Hallii is Regulated by Adaptation to Long-Term Temperature and Precipitation Patterns. Rangeland ecology & management 70.2, pp. 238–243.

Pavlick, L. E. and J. Looman (1984). Taxonomy and nomenclature of rough fescues, *Festuca altaica* , *F . campestris* (*F . scabrella* var. *major*), and *F . hallii* , in Canada and the adjacent part of United States. Canadian Journal of Botany 62.8, pp. 1739–1749. DOI: `10.1139/b84-235`.

Pyle, L., L. M. Hall, and E. W. Bork (2018). Linking management practices with range health in northern temperate pastures. Canadian Journal of Plant Science 98.3, pp. 657–671. DOI: `10.1139/cjps-2017-0223`.

Pylypec, B. (2017). Trends in Bird Densities at a Remnant Fescue Grassland in Saskatchewan. The Canadian Field-Naturalist 131.2, pp. 170–178. DOI: `10.22621/cfn.v131i2.1904`.

Qiu, J., Y.-B. Fu, Y. Bai, and J. F. Wilmshurst (2009). Genetic variation in remnant *Festuca hallii* populations is weakly differentiated, but geographically associated across the Canadian Prairie. Plant Species Biology 24.3, pp. 156–168. DOI: `10.1111/j.1442-1984.2009.00251.x`.

Rizwan, M., S. Ali, M. Ibrahim, M. Farid, M. Adrees, S. A. Bharwana, M. Zia-ur-Rehman, M. F. Qayyum, and F. Abbas (2015). Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. Environmental Science and Pollution Research 22.20, pp. 15416–15431. DOI: `10.1007/s11356-015-5305-x`.

Saikkonen, K., C. A. Young, M. Helander, and C. L. Schardl (2016). Endophytic Epichloë species and their grass hosts: from evolution to applications. Plant Molecular Biology 90, pp. 665–675. DOI: `10.1007/s11103-015-0399-6`.

Samson, F. and F. Knopf (1994). Prairie conservation in North America. BioScience 44.6, pp. 418–421. DOI: `10.2307/1312365`.

Samson, F. B., F. L. Knopf, and W. R. Ostlie (2004). Great Plains ecosystems: past, present, and future. Wildlife Society Bulletin 32.1, pp. 6–15. DOI: `10.2193/0091-7648(2004)32[6:GPEPPA]2.0.CO;2`.

Sinaga, P., E. Klichowska, A. Nowak, and M. Nobis (2024). Hybridization and introgression events in cooccurring populations of closely related grasses (Poaceae: Stipa) in high mountain steppes of Central Asia. PLOS ONE 19.2, e0298760. DOI: `10.1371/journal.pone.0298760`.

SpearChief-Morris, J. (2025). Kainai Nation: the first Indigenous fire guardians program in Canada — The Narwhal. The Narwhal.

Stout, D. G., A. McLean, and D. A. Quinton (1981). Growth and Phenological Development of Rough Fescue in Interior British Columbia. Journal of Range Management 34.6, pp. 455–459. DOI: `10.2307/3898097`. JSTOR: `3898097`.

Tannas, S. (2014). *The Successful Restoration of Fescue Grasslands in Southwestern Alberta.* Regina, SK.

Tirmenstein, D. (2000). *Festuca altaica, F. campestris, F. hallii.* URL: `https://www.fs.usda.gov/database/feis/plants/graminoid/fesspp/all.html`.

Török, P., L. A. Brudvig, J. Kollmann, J. N. Price, and B. Tóthmérész (2021). The present and future of grassland restoration. Restoration Ecology 29.S1, e13378. DOI: `10.1111/rec.13378`.

Urfusová, R., V. Mahelka, F. Krahulec, and T. Urfus (2021). Evidence of widespread hybridization among couch grasses (Elymus, Poaceae). Journal of Systematics and Evolution 59.1, pp. 113–124. DOI: `10.1111/jse.12563`.

Weaver, J. E. (1944). North American Prairie. The American Scholar 13.4, pp. 391–407.

Weerstra, B. G. and A. C. Holcroft Weerstra (1998). *Preliminary Classification of Plains Rough Fescue (*Festuca hallii*) Community Types within the Central Parkland Subregion of Alberta.* Tech. rep. Cochrane, Alberta: Biota Consultants.

Willms, W. D., S. Smoliak, and A. W. Bailey (1986). Herbage Production following Litter Removal on Alberta Native Grasslands. Journal of Range Management 39.6, pp. 536–540. DOI: `10.2307/3898766`. JSTOR: `3898766`.

Willms, W. D., S. Smoliak, and J. F. Dormaar (1985). Effects of Stocking Rate on a Rough Fescue Grassland Vegetation. Journal of Range Management 38.3. DOI: `10.2307/3898970`.

Willms, W. D. and J. Fraser (1992). Growth characteristics of rough fescue (*Festuca scabrella* var. campestris) after three years of repeated harvesting at scheduled frequencies and heights. Canadian Journal of Botany 70.11, pp. 2125–2129. DOI: `10.1139/b92-264`.

Wilsey, B. (2021). Restoration in the face of changing climate: importance of persistence, priority effects, and species diversity. Restoration Ecology 29.S1, e13132. DOI: `10.1111/rec.13132`.

Woosaree, J. (2012). *Strategies for Evaluating the Effectiveness of Reclamation Successes on Native Landscapes.* Tech. rep. Project #911651. Edmonton, Alberta: Petroleum Technology Alliance Canada (PTAC).

Woosaree, J. and R. Otfinowski (2018). Importance of species diversity in the revegetation of Alberta's northern fescue prairies. Biodiversity and Conservation 27.3, pp. 665–680. DOI: `10.1007/s10531-017-1456-z`.

Worthy, S. H., J. H. Acorn, and C. M. Frost (2024). Biodiversity measures of a grassland plant-pollinator community are resilient to the introduction of honey bees (Apis mellifera). PLOS ONE 19.10, e0309939. DOI: `10.1371/journal.pone.0309939`.

Yao, L., M. A. Naeth, and F. P. O. Mollard (2017). Ecological role of pyrolysis by-products in seed germination of grass species. Ecological Engineering 108, pp. 78–82. DOI: `10.1016/j.ecoleng.2017.08.018`.

Chapter 2: Seed storage conditions shape germination outcomes: Differing responses in two closely related rough fescue grasses

> *[It has been] reported that, while the possibilities of indigenous species for use in depleted rangelands have been suggested frequently, the general experience has been that few plants, regardless of species, establish from broadcast seedings.*
> — A. Johnston, *Some factors affecting Germination, Emergence, and Early growth of Three Range Grasses* (1961)

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Chapter Summary

Seed dormancy is a protective adaptation meant to reduce the risk of germination in unsuitable or unpredictable conditions. However, dormancy can complicate or undermine restoration efforts if care is not taken to understand how dormancy-influencing seed storage conditions can affect germination outcomes. Additionally, assuming that closely related species will respond similarly to the same storage and germination conditions may obscure important life history differences, potentially leading to ineffective or biased restoration strategies. I examined how seed storage conditions influence germination outcomes in two foundational grassland species (*Festuca hallii* (Vasey) Piper and *Festuca campestris* Rydb.) by subjecting seeds of both species to different combinations of storage duration, temperature, and moisture, before conducting a germination assay under identical controlled conditions. I found that the two species had drastically different responses. *F. campestris* exhibited low overall germination (13%), that was not meaningfully influenced by any of the storage treatment combinations. *F. hallii*, meanwhile, had a mean germination of 69%, which increased to 94% simply by soaking the otherwise untreated seeds for 24 hours prior to the germination assay. These findings highlight the importance of considering seed handling and storage as critical steps in the restoration pipeline, with the potential to significantly impact germination and establishment success. They also highlight the need to consider species-specific management strategies, even among closely-related taxa, and for clear communication among seed suppliers, land managers, and restoration practitioners to ensure shared understanding of seed histories and conditions prior to planting.

2.1 Introduction

Germination is the first and most crucial stage in a plant's life cycle, setting the stage for its successful establishment and survival (Larson et al. 2015; Zhang et al. 2021; Bargali and Bargali 2016). A plant seed must first avoid being predated or destroyed, and then encounter a specific combination of physical and physiological conditions (temperature, moisture, light, etc.) in order to successfully germinate (Dalling et al. 2011). If the seed does not overcome these initial barriers and find suitable conditions, it may fail to germinate entirely, leading to the plant's death before its life cycle even begins (Larson et al. 2015; Daws et al. 2007). Even in the case of a successful germination, the location and conditions under which the seed has germinated will then determine the conditions that the plant experiences for the rest of its life, significantly affecting its growth, survival, and reproductive success (Donohue et al. 2010; Vibhuti et al. 2015; Pathak et al. 2025). Given how crucial this stage is for a plant's development, it is therefore important to examine how diverse germination strategies are expressed and maintained in wild populations.

Despite being a universal requirement for successful seed establishment, plants display a wide variety of different germination strategies (Zhang et al. 2021). The timing, required conditions, and proportion of seeds that germinate are influenced by a plant's life-history traits, evolutionary history, and local environmental conditions (Kildisheva et al. 2020; Zhang et al. 2021). However, maternal effects (i.e., environmental conditions experienced by the parent plant before and during seed development) also significantly impact germination behaviour (Donohue et al. 2010; Klupczyńska and Pawłowski 2021; Zhang et al. 2021). Species in regions with seasonal climate shifts typically germinate in response to predictable changes in temperature or moisture (Probert 2000; Dalling et al. 2011). Conversely, species in environments that experience rare but intense disturbances such as fire or flooding may rely on the physical and chemical shifts brought about by such events to trigger germination (Baskin and Baskin 2004; Dalling et al. 2011). Germination timing in turn influences a plant's competitive interactions within its surrounding community; species which germinate quickly and easily may gain a competitive advantage from early access to space and resources, while species with delayed germination are more likely to avoid mortality events (e.g. flooding, frost) and increase the likelihood of encountering favourable conditions (Donohue et al. 2010).

An additional factor influencing germination timing is dormancy. That is, physical, physiological, or other intrinsic mechanisms that must be overcome before a seed can germinate (Knapp 2000; Probert 2000, Table A1). While dormancy is meant to be a protective adaptation which reduces the risk of a seed germinating in inhospitable conditions (Finch-Savage and Leubner-Metzger 2006; Johnson et al. 2023), it adds significant complexity and sensitivity to the germination process. Dormant seeds cannot germinate unless their dormancy mechanisms are overcome, even when environmental conditions are otherwise suitable (Kildisheva et al. 2020; Finch-Savage and Leubner-Metzger 2006). By contrast, non-dormant seeds can usually germinate as soon as their basic temperature and moisture needs are met, without the need for dormancy-breaking processes (Knapp 2000; Baskin and Baskin 2004). Dormant seeds may also possess multiple dormancy mechanisms, each requiring their own specific conditions to overcome (Kildisheva et al. 2020, Table A1). In nature, seeds overcome dormancy in response to environmental cues such as seasonal temperature and precipitation shifts, increasing day length, wildfire, digestion and excretion by animals, and mechanical abrasion in rocky or sandy soils (Finch-Savage and Leubner-Metzger 2006; Dalling et al. 2011; Kleyheeg et al. 2018). Many of these cues can also be replicated artificially, allowing us to manipulate and break seed dormancy more quickly or predictably.

Seed dormancy can be manipulated relatively easily through treatments such as scarification, chemical or growth regulator applications, and cold stratification (e.g. Russell 2011; Yan and Chen 2020; Johnson et al. 2023). However, while these methods are well-established, applying them without an understanding of a plant's specific dormancy mechanisms can be counterproductive. Improper timing or application of dormancy-breaking treatments can inadvertently re-induce dormancy, leading to reduced germinability and seed vigour (Knapp 2000; Shen et al. 2001). Subsequently, mistimed plantings leave seeds vulnerable to pathogens, predation, and competition from other plants, further reducing their chances of successful establishment (Dalling et al. 2011; Kildisheva et al. 2020). Given the substantial variability that can arise from the interplay of local adaptation, germination cues, and dormancy requirements, understanding how these factors manifest in a species of interest is essential; not only for effective conservation and restoration planning, but also to avoid inadvertently undermining these efforts (Baskin et al. 2006; Baskin and Baskin 2020; Kildisheva et al. 2020). In this context, rough fescue grasses provide an ideal model for studying how seed handling, storage, and management practices influence germination, as their dormancy mechanisms remain largely unexplored, despite their ecological importance and the significant interest and effort invested in trying to restore them.

Rough fescue (*Festuca hallii* and *Festuca campestris*) are perennial bunch grasses that play a foundational role in North America's mesic grasslands, performing key ecological functions such as moisture retention, erosion control, and habitat and forage provisioning (Anderson 2006a; Sherritt 2012). Despite their ecological importance, rough fescue restoration has proven challenging, particularly when attempting to restore the species from seed. While germination rates are typically high under laboratory conditions (e.g., *F. hallii*: Romo et al. 1991; Qiu et al. 2010; Yao et al. 2017; Small et al. 2019) (*F. campestris*: Yao et al. 2017), field restoration efforts often see little to no establishment of rough fescue from seed (Desserud et al. 2010; Desserud and Naeth 2013).

Rough fescue has been shown to germinate under a wide range of temperature and light conditions, though moisture availability is a critical limiting factor (Romo et al. 1991; Romo and Eddleman 1995; Mollard and Naeth 2014). What remains unclear is how seed storage and dormancy-related treatments affect their germination. Species that experience similar seasonal conditions commonly possess physiological dormancy mechanisms and require prolonged exposure to properly germinate (Probert 2000; Klupczyńska and Pawłowski 2021; Zhang et al. 2021). Therefore, clarifying how pre-germination seed storage or handling affects rough fescue germination could provide valuable insight into why these species remain so difficult to restore, and help improve future restoration outcomes (Kövendi-Jakó 2017; Kildisheva et al. 2020). Additionally, identifying whether closely related species like *Festuca hallii* and *Festuca campestris* respond differently to the same seed treatments is crucial, as effective restoration relies on understanding the specific requirements of each species (Klupczyńska and Pawłowski 2021; Zhang et al. 2021).

This study focuses on two primary research questions: 1) how do different seed storage conditions (e.g., Temperature, Duration, and Hydration) affect the germination of rough fescue grass seeds? and 2) do *Festuca hallii* and *Festuca campestris* exhibit different germination responses to the same seed storage treatments? I hypothesize that seed storage conditions are a significant determinant of germination success, due to dormancy mechanisms shaped by species-specific environmental adaptations and life histories. Specific predictions regarding germination response to seed storage conditions can be found in Table 2.1. While their absolute germination percentages may differ, I predict that both species will respond similarly to the same treatments, due to their close relatedness and comparable environmental conditions. (Stout et al. 1981; Anderson 2006a; Pavlick and Looman 1984). More generally, I expect this research will help clarify if (and how) seed storage conditions and species life history differences should be accounted for in restoration contexts.

2.2 Methods

2.2.1 Data collection

To test how seed storage conditions affect the germination of *F. hallii* and *F. campestris*, I used a factorial experiment where seeds from each species were exposed to different combinations of cold storage factors, before being germinated under identical conditions (Table 2.1). To ensure that the seeds had not undergone any kind of natural or artificial cold stratification before the experiment began, I used seeds collected directly from wild plants in fall 2023, before the onset of winter conditions. *F. hallii* seeds were collected in mid-October from the Kinsella Research Ranch (Beaver County, AB, 53.0036, -111.5196). *F. campestris* seeds were collected in early November from Glenbow Ranch Provincial Park (Cochrane, AB, 51.1577, -114.3710). I cleaned and sorted seeds by hand before portioning into treatment groups.

Seed storage treatment combinations and germination assay

I created the experimental treatment groups by combining different levels of three storage treatment factors: Duration, Temperature, and Hydration. Descriptions and justification for these treatment levels are provided in Table 2.1. In total, 27 treatment groups were created (3 levels of Duration x 3 levels of Temperature x 3 levels of Hydration; Table 2.2). In addition, five control groups were kept at room temperature for the five month storage period to serve as comparisons for the treatment groups; an unsoaked baseline, a post-soaked baseline, and three groups presoaked at 1, 3, and 5 months (Table 2.3). Following storage, seeds from each treatment and control group were plated under standardized germination conditions.

Approximately 50 seeds per species were portioned out for each treatment/control group, and

Table 2.1: Fescue seed storage treatment factors, descriptions, and predictions for effect on germination

Treatment Factor	**Factor Description**	**Factor Level**	**Level Description**	**Prediction**
Duration	Length of time the seeds were kept in cold storage	1 month	1 month of cold storage prior to germination assay	One month is an insufficient amount of time to overcome dormancy, and may even push seeds further into dormancy, reducing germination (Knapp 2000; Yan and Chen 2020)
		3 months	3 months of cold storage prior to germination assay	Represents a shortened winter scenario as may result from climate change; will reduce germination if seeds require five months of cold to break dormancy (Probert 2000; Klupczyńska and Pawłowski 2021)
		5 months	5 months of cold storage prior to germination assay	Represents typical winter duration in the seeds' native range; expected to improve germination compared to untreated seeds (DeMaere et al. 2012; Zhang et al. 2021)
Temperature	Temperature at which the seeds were stored during treatment	Fridge	Storage in a laboratory refrigerator at 3–4°C	Warmer than typical winter temperatures in the species' native range; likely insufficient to break dormancy, resulting in reduced germination (Johnston and MacDonald 1967; Molnar 2022)
		Mild Freeze	Storage in a freezer maintained at -6 to -8°C	Represents typical winter temperatures in the native range; expected to effectively break dormancy and improve germination compared to untreated seeds
		Deep Freeze	Storage in a -20°C freezer	Colder than natural winter conditions; predicted to damage seeds and reduce germination (Boyd and Lemos 2013; Baskin and Baskin 2020)
Hydration	Hydration treatment applied to the seeds before or after the cold storage period	No soak	Seeds were kept dry before and during storage	Control condition; should match expected baseline germination response.
		Pre-soak	Seeds soaked in dH_2O for 24h, then air dried prior to being placed in storage	Hydration will prime seeds for germination, but subsequent drying and cold storage may induce deeper dormancy or damage seed embryo. Expected to reduce germination (Grilz et al. 1994; Shen et al. 2001)
		Post-soak	Seeds soaked in dH_2O for 24h after storage, just prior to germination assay	Will prime seeds for germination and overcome effects of moisture limitation; expected to increase germination (Romo et al. 1991; Madsen et al. 2018)

Table 2.2: The 27 treatment groups, representing every combination of duration, temperature, and hydration. Each treatment group was replicated 3 times, with 18 seeds per replicate, for each of the two species.

Duration	**Temperature**	**Hydration**	**Treatment ID Code**
1 month	Fridge	No Soak	1M-FR-NS
		Pre-Soak	1M-FR-PR
		Post-Soak	1M-FR-PO
	Mild Freeze	No Soak	1M-MF-NS
		Pre-Soak	1M-MF-PR
		Post-Soak	1M-MF-PO
	Deep Freeze	No Soak	1M-DF-NS
		Pre-Soak	1M-DF-PR
		Post-Soak	1M-DF-PO
3 months	Fridge	No Soak	3M-FR-NS
		Pre-Soak	3M-FR-PR
		Post-Soak	3M-FR-PO
	Mild Freeze	No Soak	3M-MF-NS
		Pre-Soak	3M-MF-PR
		Post-Soak	3M-MF-PO
	Deep Freeze	No Soak	3M-DF-NS
		Pre-Soak	3M-DF-PR
		Post-Soak	3M-DF-PO
5 months	Fridge	No Soak	5M-FR-NS
		Pre-Soak	5M-FR-PR
		Post-Soak	5M-FR-PO
	Mild Freeze	No Soak	5M-MF-NS
		Pre-Soak	5M-MF-PR
		Post-Soak	5M-MF-PO
	Deep Freeze	No Soak	5M-DF-NS
		Pre-Soak	5M-DF-PR
		Post-Soak	5M-DF-PO

Table 2.3: The five control groups used in my germination assay. Each control was replicated 3 times, with 18 seeds per replicate, for each of the two species.

Control Group	**Description**	**Prediction**	**Treatment Combo ID**
Untreated baseline	Seeds kept at room temperature for entire storage period with no hydration treatment	Expected to match germination levels seen with similarly untreated seeds (Fig. A1)	C-RT-NS
Post-soaked	Seeds kept at room temperature for entire storage period, but soaked in dH_2O for 24h prior to germination assay	Expected to improve germination compared to the unsoaked baseline (Madsen et al. 2018; Small et al. 2019)	C-RT-PO
1, 3, and 5-month Pre-soaked	Seeds kept at room temperature for the entire storage period, soaked in dH_2O for 24h at 1, 3, or 5 months prior to the germination assay, then dried and returned to room temperature	Compares the effect of soaking and drying on germination, regardless of cold storage duration	1M-RT-PR, 3M-RT-PR, 5M-RT-PR

seeds were stored in glassine envelopes for the duration of their respective storage treatments. To ensure that all seeds were removed from storage at the same time and could be assessed under identical germination conditions, I staggered the start of the cold storage treatments according to their duration. Seeds in the 5-month treatment groups were placed into storage first, followed by the 3-month and 1-month groups at their respective intervals.

I plated 3 replicates of 18 seeds (per species) for each of the 32 treatment groups. Seeds were spaced evenly onto two layers of moistened brown paper towel within a lidded petri dish, then each dish was placed within one of two Sanyo versatile environmental test chambers (MLR-351, Sanyo Scientific, USA). The chambers maintained a constant temperature of 18°C, and relative humidity of 80% (Romo et al. 1991; Small et al. 2019). I rotated shelves within and between the chambers three times during the course of the observation period to mitigate potential effects of shelf position on the germination conditions. The number of seeds and replicates per treatment were chosen to align with similar multi-factor germination experiments (e.g. Small et al. 2019; Russell 2011; Yao et al. 2017), while accounting for the number of seeds and amount of space available to me.

Because light exposure can inhibit the germination of rough fescue seeds (Romo and Eddleman 1995; Mollard and Naeth 2014), the chambers were set to a 23hr dark cycle, with 1 hour of light to allow for inspection of germinating seeds. I added 1-2mL of distilled water to the dishes with a pipette, as needed, to keep the paper towel and seeds moist.

I monitored the seeds every 2-3 days for signs of germination. Because my focus was on the seeds' ability to overcome dormancy and initiate germination, I counted a seed as germinated if there was any sign that the radicle or coleoptile had emerged from the seed coat (Palazzo and Brar

1997; Meyer and Narem 2019), rather than waiting for the seedling to reach a certain length (Romo et al. 1991; Vivanco et al. 2021). Germinated seeds were recorded, and then removed from the Petri dish after counting (Qiu et al. 2010; Yao et al. 2017).

I ended the observation period after 41 days, having observed no germination in >90% of the dishes for 5 consecutive days (Russell 2011; Cheng et al. 2022). Once the germination assay had concluded, I checked all ungerminated seeds to determine their viability (i.e., whether they were capable of germinating at all). Moldy seeds were considered nonviable (Dalling et al. 2011; Yao et al. 2017), as were empty seeds, and seeds that were soft/rotten when pressed or dissected (Qiu et al. 2010; Yao et al. 2017; Vivanco et al. 2021). Non-viable seeds were excluded from the total seed counts used in my data analysis.

2.2.2 Statistical analysis

Characterizing germination responses with Time-to-Event curves

To assess differences in germination between the two species of fescue and among the different storage treatment combinations, I fit several log-logistic time-to-event models to the germination assay data (Ritz et al. 2015). These models are particularly useful for analyzing germination data because they account for interval censoring (i.e., uncertainty regarding the exact time germination occurred), are flexible enough to accommodate the sigmoidal shape of most germination curves, and generate parameters that have useful biological interpretations, such as the maximum proportion of germinated seeds and median germination time (Onofri et al. 2022).

I observed a drastic and significant difference in germination response between *F. hallii* and *F. campestris* (Likelihood Ratio Test: LR = 664.43, df = 3, p = 1.32×10^{-126}; Fig. S2), leading me to analyze each species separately. For both species, I fit models using the number of germinations per observation day as a function of each combination of Duration, Temperature, and Hydration.

I encountered difficulties fitting the *F. campestris* data due to four groups exhibiting zero germination (1M-DF-PO, 3M-MF-PR, 3M-DF-PO, and 5M-FR-PR), which prevented the model from converging properly. To address this, I removed those four groups from my data set and allowed the model to fit separate curve functions for the remaining treatment groups (Onofri et al. 2022). This reduced the functionality and informativeness of the *F. campestris* model, but still allowed me to analyze its overall germination trends.

To compare germination responses across the different treatment groups, I extracted curve-defining parameters from each model. For *F. hallii*, I applied cluster-robust sandwich estimation to account for the non-independent nature of the data, as the seeds were grouped within petri dishes (Onofri et al. 2022). This method adjusts the curve parameters, standard errors, and p-values by measuring the variability among clusters (i.e. seeds within a petri dish), rather than treating each observation as independent (Zeileis et al. 2020). This accounts for correlations among seeds in the same dish, and provides more accurate standard error and p-values. Robust estimation was not applicable to the *F. campestris* model due to the limitations described above (i.e., extremely low germination rates), and so I extracted standard parameter estimates instead.

Germination success as a function of storage treatment

I analyzed the likelihood of germination in regards to seed storage treatment using logistic regression. For each species, I modelled the number of germinated seeds (successes) vs ungerminated seeds (failures) against a categorical "Treatment combination" variable, which was created by combining each treatment group's Duration, Temperature, and Hydration levels into a single factor (Tables 2.3 and 2.2). The untreated control group C-RT-NS (5 months at room temperature with no soaking treatment) was set as the model's reference condition for comparison. This approach allowed me to account for the unbalanced nature of the experimental design; while the treatment groups followed a fully factorial structure (3 levels of Duration x 3 levels of Temperature x 3 levels of Hydration), the inclusion of the 5 control groups created additional groups outside of the factorial structure. This imbalance caused challenges when fitting the logistic regression model, as the model requires all factorial combinations to be present in the data. Combining these into a single factor re-balances the groups and avoids issues with trying to fit a fully-factorial model.

I conducted all statistical analyses using `R` (v4.3.1; R Core Team 2023). I used the the `drcte` package (v1.0.30; Onofri 2023) for the time-to-event models, and used `emmeans` (v1.10.4; Lenth 2024) and `broom` (v1.0.6; Robinson et al. 2024) for the logistic regressions.

2.3 Results

2.3.1 Post-germination assay observations

Germination rates between *F. hallii* and *F. campestris* were significantly different, both overall, and across individual storage treatment combinations (Figs. 2.1 & A2). *F. hallii* had a mean germination of 69% (SE ± 2%) across all treatments, with a minimum of 28% germination and a maximum of 100%. *F. campestris* had a mean germination of 13% (SE ± 2%) across all treatments, with a minimum of 0% germination and a maximum of 60% (Table A2).

Mold was a significant factor impacting the *F. campestris* seeds; mold affected 30% (SE ± 2%) of the seeds per dish on average, with some dishes having 80% of seeds infected with mold (Table A2). *Festuca hallii* seeds fared better, with an average of 8% (SE ± 1%) of seeds affected by mold, to a maximum of 44%.

After accounting for moldy and empty seeds at the end of the experiment, I determined that an average of 72% (SE ± 1%) of *F. hallii* seeds per dish were viable (i.e., alive and able to germinate), while only 43% (SE ± 2%) of *F. campestris* seeds per dish were viable.

2.3.2 Germination trends in response to seed storage treatment

Festuca hallii

F. hallii germination was significantly affected by the combination of duration, temperature, and soaking treatments (observed LR value = 211.5,df = 93, p = 3.23×10^{-11}). The untreated baseline group (seeds stored for 5 months at room temperature with no soaking treatment) had an estimated maximum germination of 58%, though this estimate was not statistically distinguishable from zero (SE = 0.90, t = 0.64, p = 0.52). Germination was highest in seeds which were also kept at room temperature for 5 months, but soaked for 24 hours prior to germination. This group saw an estimated 94% germination rate (SE = 0.05, t = 18.72, p = 3.52×10^{-70}). Seeds stored in the deep freeze for one month with no soaking treatment had the lowest germination rate at 39% (SE = 0.09, t = 4.4259, p = 1.03×10^{-11}). Overall, most treatment groups for *F. hallii* had germination rates estimated at >70% (Fig. A3).

Festuca campestris

F. campestris germination was highest in seeds stored in the fridge for 5 months and either soaked prior to germination (33%; SE = 0.12, t = 2.74, p = 0.006), or not soaked at all (32%; SE = 0.11, t = 2.96, p = 0.003), with the remaining statistically significant germination estimates falling between 13 and 26% (Table A4). However, most of the treatment groups had germination estimates that were so low as to be statistically insignificant. This included the untreated baseline group (5 months at room temperature with no soaking treatment; 4.5% germination; SE = 0.04, t = 1.0238, p = 0.31).

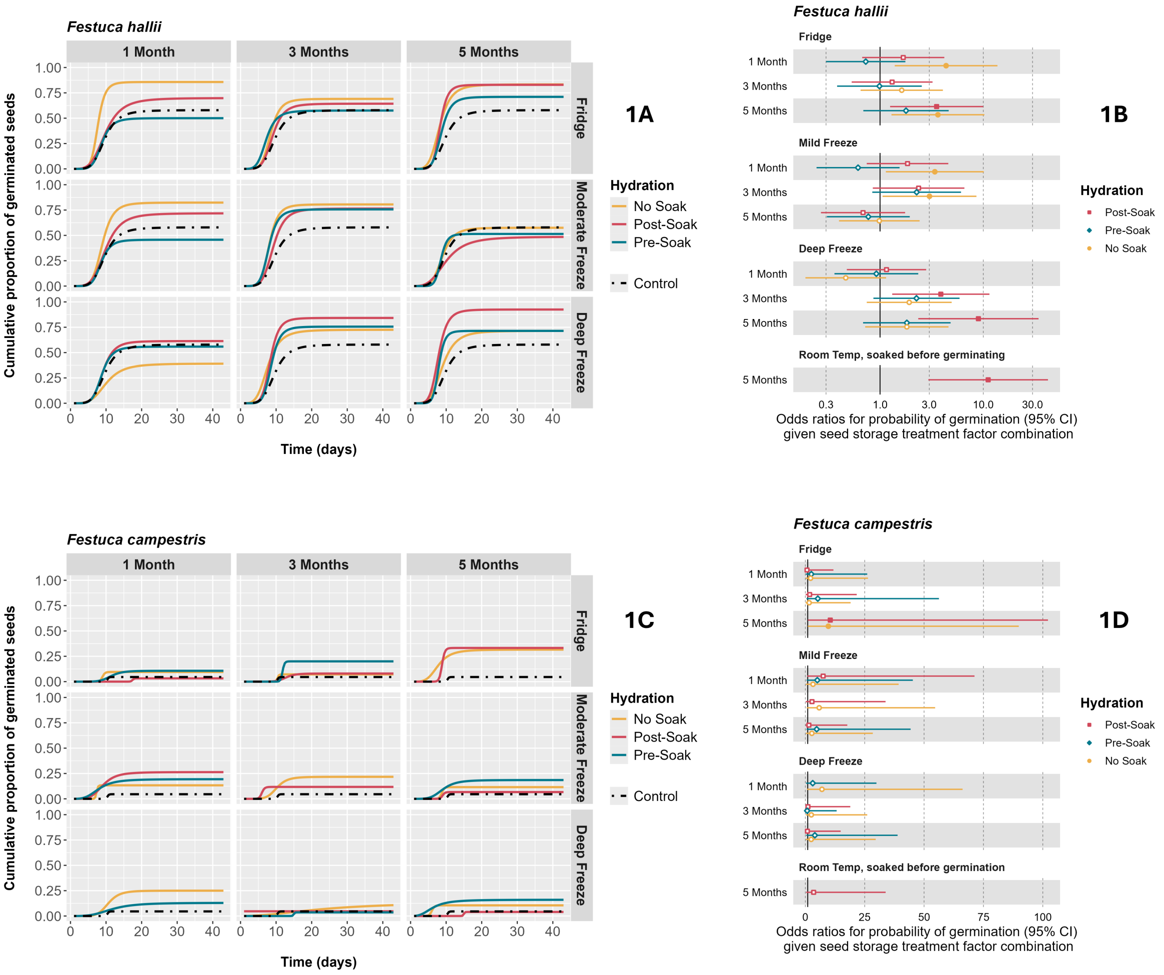

Figure 2.1: Time-to-Event curves (1A and 1C) showing germination response of *F. hallii* and *F. campestris* seeds to different combinations of cold storage treatment, and Forest plots (1B and 1D) showing likelihood of germination (Odds Ratio) for different treatment combinations. The control group (dotted line in TtE curves; Odds Ratio = 1 in forest plots) corresponds to seeds which were kept at room temperature for 5 months, with no hydration treatment.

2.3.3 Effects of storage treatment on likelihood of germination

Festuca hallii

F. hallii seeds were 11 times more likely to eventually germinate than the untreated baseline if simply soaked for 24 hours prior to germination, with no cold storage treatment (SE = 0.68, z = 3.54, p = 3.94×10^{-4}). Additionally, seeds stored in the deep freeze for 5 months were 9 times more likely to germinate if soaked before germination (SE = 0.68, z = 3.21, p = 1.35×10^{-3}). At milder temperatures (i.e., Fridge and Mild Freezer treatments), germination seemed to be most improved (3-4 times) by not soaking the seeds at all (e.g. 1M and 5M FR, 1M and 3M MF; Panel 1B, Fig. 2.1), however this was not consistent across durations. Finally, seeds that were pre-soaked (i.e., prior to cold storage) tended to have a reduced likelihood of germination compared to the baseline control, though none of these estimates were statistically significant (Table A5; Panel 1B, Fig. 2.1).

Festuca campestris

For *F. campestris*, the only treatment combination that significantly changed the likelihood of germination compared to the untreated control was 5-months of storage in the fridge. Both the No Soak and Post-Soak treatment significantly improved the likelihood of germination when compared to untreated seeds (see table, OR of no soak was 9.7, p-value 0.046, OR of Post soak was 10.5, p-value 0.043). However, confidence intervals for these estimates were extremely high, so these results should be interpreted with caution (Panel 1D; Fig. 2.1).

2.4 Discussion

Although *F. hallii* and *F. campestris* are closely related species, my results show that the two can have significantly different germination responses to identical storage and germination conditions. *F. hallii* exhibited moderate germination overall, with most groups germinating between 70-90%, which is commonly reported for this species (Romo et al. 1991; Small et al. 2019; Yao et al. 2017). By contrast, *F. campestris* exhibited extremely low germination rates across most treatments. While germination rates of ~80% have been documented under specific conditions (e.g. Johnston 1961; Yao et al. 2017), data on typical germination rates for this species remain scarce. Comparative germination studies have also observed *F. campestris* to have lower germination rates than *F. hallii* under identical conditions (Fig. A1), and *F. campestris* is noted to have unreliable germination when using wild-harvested seed (Desserud 2006).

One reason for *F. campestris'* low germination rate was the relatively high proportion of non-viable seeds present in my samples (Baskin et al. 2006). Many seeds were visibly overtaken by mold, and/or soft and rotten when checked at the end of the germination assay (Table A2). Because viable, mature seeds typically possess physical and physiological defenses against fungal infection (Dalling et al. 2011; Kremer et al. 1984), it is likely that the moldy seeds were underdeveloped, rather than the result of general contamination. Anecdotally, I observed many moldy seeds surrounded by unaffected seeds, suggesting an issue inherent to the moldy seeds themselves, rather than plate-level contamination.

F. campestris' low germination rate may reflect a different life history strategy when compared to *F. hallii*. The high number of non-viable seeds present in my samples suggest that *F. campestris* seeds may require an extended period of "after-ripening" in order to be physiologically capable of germinating (Probert 2000; Baskin and Baskin 2020). Here, after-ripening refers to a period of time after seed dispersal that some species require before their seeds are fully developed and capable of germinating. This process minimizes germination in unfavourable conditions and is common among high-elevation species with short, unpredictable growing seasons, such as *F. campestris* (Romo 1996; Shen et al. 2001). Such species are typically adapted to stagger their germination across multiple years, in order to maximize the chance of encountering a favourable growing season (Zhang et al. 2021; Daws et al. 2007). The low and unpredictable germination rates observed in *F. campestris* may reflect this "bet-hedging" strategy (Pausas et al. 2022), in contrast to *F. hallii*, which is native to lower elevations and more open habitats (Pavlick and Looman 1984; Aiken and Darbyshire 1990). Species in such environments typically exhibit rapid, high levels of germination in order to capitalize on the narrow window of optimal germination conditions at the beginning of the growing season (Zhang et al. 2021; Daws et al. 2007).

Effect of storage conditions on germination of rough fescue

In addition to having significantly different germination rates, the two species responded very differently to seed storage conditions. Unfortunately, the overall low germination of *F. campestris* limited my ability to fully explore the effects of the different treatment combinations, as only two treatments resulted in a significant improvement over baseline conditions. It is possible that this extended period of cool storage helped to initiate or accelerate the "after-ripening" process that I hypothesize *F. campestris* seeds require to germinate (Baskin and Baskin 2020). Otherwise, all other treatment groups exhibited high variability and insufficient germination to yield reliable estimates. As such, I focus on the germination response of *F. hallii* to explore the differences among storage treatments.

There is significant variability in *F. hallii* germination response across treatment combinations. While some treatments significantly improved the likelihood of germination, I did not find any consistent trends for either main or interacting effects of Temperature, Duration, and Hydration. It is likely that the high variation in germination is due to natural variability in the seeds' environmental tolerances and dormancy triggers (Shen et al. 2001; Qiu et al. 2010; Daws et al. 2007). Rough fescue can initiate growth at temperatures as low as 2°C (Johnston and MacDonald 1967), which suggests that the fridge, and potentially even mild freezer conditions may not have been sufficiently cold enough to effectively induce and break dormancy for all seeds (Baskin et al. 2006). The interacting effects of insufficiently low temperatures and/or insufficiently long storage times may have exacerbated dormancy differences among the seeds, enhancing germination in some groups while inducing deeper dormancy in others (Milberg and Andersson 1998; Knapp 2000; Probert 2000). Future studies could focus on isolating the interaction between storage temperature and duration using a gradient of more finely-spaced intervals (e.g. storage durations of 2, 4, 6...20 weeks, storage temperature at -30°C, -25°C, -20°C...5°C). A larger sample size of conditions with smaller incremental increases could allow me to generate a more continuous curve of germination responses (Romo et al. 1991), rather than relying on broader, more discrete categories. This could help identify more precise thresholds or "tipping points", beyond which dormancy is either broken or reinforced. For example, Shen et al. (2001) observed that Switchgrass (*Panicum virgatum*) germination remained below 20% when cold stratified for only 15 days, but jumped sharply to over 80% if stratified for more than 20 days.

Despite the variability across duration and temperature treatments, I did observe dramatic and relatively consistent improvements (up to 11 times higher than baseline) to germination in seeds that were soaked for 24 hours prior to the germination assay (Pre-germination/PG soak). This treatment aligns with the common practice of "priming" seeds for germination by soaking them in water or chemical/hormonal solutions (Madsen et al. 2018; Small et al. 2019). Priming allows the seed to be fully hydrated, which helps to overcome both physical and physiological dormancy barriers and kickstart germination before a seed is planted (Falleri et al. 2004; Finch-Savage and Leubner-Metzger 2006; Daws et al. 2007). It is possible that the control (room temperature) seeds never entered dormancy (Klupczyńska and Pawłowski 2021; Knapp 2000), and that the soaking treatment simply provided them with optimal moisture conditions to maximize their germination (Falleri et al. 2004; Daws et al. 2007). For seeds stored in the deep freeze, the extended cold period may have simulated natural overwintering, after which the soaking replicated increased moisture from spring snowmelt, allowing the seeds to complete their dormancy cycle (Johnston 1961; Stout et al. 1981; Romo 1996). In future studies, additional tests could be implemented to verify whether the ungerminated seeds were truly dormant or actually non-viable. Treating imbibed seeds with gibberellic acid can trigger germination in seeds that are still viable but physiologically dormant (Duncan et al. 2019), while tetrazolium staining helps to identify viable seeds from dead ones (Falleri et al. 2004; Baskin et al. 2006).

Moisture availability has been identified as the most significant limiting factor in rough fescue germination (Romo et al. 1991; Romo 1996; Qiu et al. 2010), and seed moisture content is a primary determinant of germination success and seedling fitness (Tannas 2014; Falleri et al. 2004; Daws et al. 2007). Given how large an improvement I observed from the baseline germination rate simply by soaking the *F. hallii* seeds prior to germination, restoration practitioners should consider incorporating seed priming as an additional step prior to field seeding rough fescue. While cost and logistical constraints may not always make this practical, the potential for significantly improved field establishment is worth exploring in future fescue restoration efforts.

Despite differences in their ecology and life history traits, *F. hallii* and *F. campestris* are not often treated as distinct species when it comes to best practices for conservation and restoration (Anderson 2006a; Anderson 2006b; Tannas 2014). However, my results suggest that their germination responses can vary significantly, meaning that seed storage and handling practices that work for *F. hallii* may not be equally effective for *F. campestris.* For *F. hallii*, soaking seeds for 24h prior to planting is a reasonable and effective approach to improving germination across a range of seed storage conditions. For *F. campestris*, longer periods of cool, dry storage may help accelerate the development of fresher seeds to combat low/unpredictable germination.

2.4.1 Management Implications and Conclusions

This study provides evidence that the temperature, duration, and moisture conditions that seeds experience during storage can drastically alter their germination outcomes. It also provides evidence of significant, species-specific responses to identical seed storage treatment and germination conditions. These findings have implications not only for restoration practitioners, but for seed suppliers and conservation researchers, as they suggest that seed collection and storage should be considered an influential and integral component of the restoration pipeline, rather than simply a logistical issue. They also highlight the importance of accounting for specific environmental adaptation and life history differences when planning and implementing conservation and restoration initiatives. Simple pre-treatment methods, such as cold storage or seed priming can greatly improve germination outcomes, but should not be treated as "one-size-fits-all" solutions, even when working with closely-related species. Further research and integration of biologically informed, species-specific restoration strategies could lead to more targeted and effective restoration outcomes, and a more comprehensive understanding of focal species and biomes for researchers and practitioners alike.

References

Aiken, S. G. and S. J. Darbyshire (1990). *Fescue grasses of Canada.* Publication 1844/E. Ottawa, Canada: Agriculture Canada. ISBN: 0-660-13483-7.

Anderson, G. (2006a). Festuca campestris *Rydberg (rough fescue): A Technical Conservation Assessment.* Tech. rep. Rocky Mountain Region: USDA Forest Service.

Anderson, G. (2006b). Festuca hallii *(Vasey) Piper (Hall's fescue): A Technical Conservation Assessmen.* Technical Conservation Assessment. Fort Collins, Colorado: Colorado Natural Heritage Program, Colorado State University.

Bargali, K. and S. S. Bargali (2016). Germination capacity of seeds of leguminous plants under water deficit conditions: implication for restoration of degraded lands in Kumaun Himalaya. Tropical Ecology 57.3.

Baskin, C. C. and J. M. Baskin (2004). Germinating Seeds of Wildflowers, an Ecological Perspective. HortTechnology. DOI: `10.21273/HORTTECH.14.4.0467`.

Baskin, C. C. and J. M. Baskin (2020). Breaking Seed Dormancy during Dry Storage: A Useful Tool or Major Problem for Successful Restoration via Direct Seeding? Plants 9.5, p. 636. DOI: `10.3390/plants9050636`.

Baskin, C. C., K. Thompson, and J. M. Baskin (2006). Mistakes in germination ecology and how to avoid them. Seed Science Research 16.3, pp. 165–168. DOI: `10.1079/SSR2006247`.

Boyd, C. S. and J. A. Lemos (2013). Freezing Stress Influences Emergence of Germinated Perennial Grass Seeds. Rangeland Ecology & Management 66.2, pp. 136–142. DOI: `10.2111/REM-D-12-00135.1`.

Cheng, J., H. Huang, W. Liu, Y. Zhou, W. Han, X. Wang, and Y. Zhang (2022). Unraveling the Effects of Cold Stratification and Temperature on the Seed Germination of Invasive Spartina alterniflora Across Latitude. Frontiers in Plant Science 13, p. 911804. DOI: `10.3389/fpls.2022.911804`.

Dalling, J. W., A. S. Davis, B. J. Schutte, and A. Elizabeth Arnold (2011). Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. Journal of Ecology 99.1, pp. 89–95. DOI: `10.1111/j.1365-2745.2010.01739.x`.

Daws, M. I., S. Bolton, D. F. R. P. Burslem, N. C. Garwood, and C. E. Mullins (2007). Loss of desiccation tolerance during germination in neo-tropical pioneer seeds: implications for seed mortality and germination characteristics. Seed Science Research 17.4, pp. 273–281. DOI: `10.1017/S0960258507837755`.

DeMaere, C., M. Alexander, and M. Willoughby (2012). *Range Plant Communities and Range Health Assessment Guidelines for the Foothills Parkland Subregion of Alberta.* Publication No. T/274. Accessed: YYYY-MM-DD. Pincher Creek, Alberta, Canada: Alberta Environment and Sustainable Resource Development, Lands Division, p. 136. URL: `https://open.alberta.ca/dataset/1b1b2f60-1a43-4ed9-b1a7-8e4b52b7cb30/resource/44b0544b-8935-4a07-8c14-bb962bb65d75/download/srd-range-health-foothills-parkland-2012.pdf`.

Desserud, P. (2006). *Restoration of Rough Fescue (*Festuca campestris*) Grassland on Pipelines in Southwestern Alberta.* Tech. rep. T/121. Lethbridge, Alberta: Rangeland Management Branch, Public Lands Division, Alberta Sustainable Resource Development, p. 77.

Desserud, P., C. C. Gates, B. Adams, and R. D. Revel (2010). Restoration of foothills rough fescue grassland following pipeline disturbance in southwestern Alberta. Journal of Environmental Management 91.12, pp. 2763–2770. DOI: `10.1016/j.jenvman.2010.08.006`.

Desserud, P. A. and M. A. Naeth (2013). Promising results in central Alberta with rough fescue (*Festuca hallii*) seeding following disturbance. Native Plants Journal 14.1, pp. 25–32. DOI: `10.3368/npj.14.1.25`.

Donohue, K., R. R. de Casas, L. Burghardt, K. Kovach, and C. G. Willis (2010). Germination, Post-germination Adaptation, and Species Ecological Ranges. Annual Review of Ecology, Evolution, and Systematics 41.Volume 41, 2010, pp. 293–319. DOI: `10.1146/annurev-ecolsys-102209-144715`.

Duncan, C., N. Schultz, W. Lewandrowski, M. Good, and S. Cook (2019). *Protocol to determine seed dormancy, imbibition, viability, longevity and germination responses to temperature (version 1).* DOI: `10.17504/protocols.io.6cehate`. URL: `https://www.protocols.io/view/protocol-to-determine-seed-dormancy-imbibition-via-6cehate`.

Falleri, E., C. Muller, and E. Laroppe (2004). Effect of water stress on germination of beechnuts treated before and after storage. Canadian Journal of Forest Research 34.6, pp. 1204–1209. DOI: `10.1139/x04-003`.

Finch-Savage, W. E. and G. Leubner-Metzger (2006). Seed dormancy and the control of germination. New Phytologist 171.3, pp. 501–523. DOI: `10.1111/j.1469-8137.2006.01787.x`.

Grilz, P. L., J. T. Romo, and J. A. Young (1994). Comparative Germination of Smooth Brome and Plains Rough Fescue. The Prairie Naturalist 26.2, pp. 157–170.

Johnson, A. J., B. Geary, A. Hulet, and M. D. Madsen (2023). Breaking Dormancy and Increasing Restoration Success of Native Penstemon Species Using Gibberellic Acid Seed Coatings and U-Shaped Furrows. Plants 12.23, p. 4005. DOI: `10.3390/plants12234005`.

Johnston, A. and M. D. MacDonald (1967). Floral initiation and seed production in festuca scabrella torr. Canadian Journal of Plant Science 47.5, pp. 577–583. DOI: `10.4141/cjps67-100`.

Johnston, A. (1961). Some factors affecting germination, emergence, and early growth of three range grasses. Canadian Journal of Plant Science 41.1, pp. 59–70. DOI: `10.4141/cjps61-009`.

Kildisheva, O. A., K. W. Dixon, F. A. O. Silveira, T. Chapman, A. Di Sacco, A. Mondoni, S. R. Turner, and A. T. Cross (2020). Dormancy and germination: making every seed count in restoration. Restoration Ecology 28.S3, S256–S265. DOI: `10.1111/rec.13140`.

Kleyheeg, E., M. Claessens, and M. B. Soons (2018). Interactions between seed traits and digestive processes determine the germinability of bird-dispersed seeds. PLoS ONE 13.4, e0195026. DOI: `10.1371/journal.pone.0195026`.

Klupczyńska, E. A. and T. A. Pawłowski (2021). Regulation of Seed Dormancy and Germination Mechanisms in a Changing Environment. International Journal of Molecular Sciences 22.3, p. 1357. DOI: `10.3390/ijms22031357`.

Knapp, A. D. (2000). “An Overview of Seed Dormancy in Native Warm-Season Grasses”. In: *Native Warm-Season Grasses: Research Trends and Issues.* John Wiley & Sons, Ltd. Chap. 7, pp. 107–122. ISBN: 978-0-89118-609-0. DOI: `10.2135/cssaspecpub30.c7`.

Kövendi-Jakó, A. (2017). Relationship of germination and establishment for twelve plant species in restored dry grassland. Applied Ecology and Environmental Research 15.4, pp. 227–239. DOI: `10.15666/aeer/1504_227239`.

Kremer, R. J., L. B. Hughes Jr., and R. J. Aldrich (1984). Examination of Microorganisms and Deterioration Resistance Mechanisms Associated with Velvetleaf Seed1. Agronomy Journal 76.5, pp. 745–749. DOI: `10.2134/agronj1984.00021962007600050009x`.

Larson, J. E., R. L. Sheley, S. P. Hardegree, P. S. Doescher, and J. J. James (2015). Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. Journal of Applied Ecology 52.1, pp. 199–209. DOI: `10.1111/1365-2664.12350`.

Lenth, R. (2024). *emmeans: Estimated Marginal Means, aka Least-Squares Means.* R package version 1.10.4. URL: `https://CRAN.R-project.org/package=emmeans`.

Madsen, M. D., L. Svejcar, J. Radke, and A. Hulet (2018). Inducing rapid seed germination of native cool season grasses with solid matrix priming and seed extrusion technology. PLOS ONE 13.10, e0204380. DOI: `10.1371/journal.pone.0204380`.

Meyer, M. H. and D. M. Narem (2019). Prairie Dropseed Germination Highest with Warm, Moist Conditions. HortTechnology 29.6, pp. 830–832. DOI: `10.21273/HORTTECH04407-19`.

Milberg, P. and L. Andersson (1998). Does cold stratification level out differences in seed germinability between populations? Plant Ecology 134.2, pp. 225–234. DOI: `10.1023/A:1009793119466`.

Mollard, F. P. O. and M. A. Naeth (2014). Photoinhibition of germination in grass seed – Implications for prairie revegetation. Journal of Environmental Management 142, pp. 1–9. DOI: `10.1016/j.jenvman.2014.04.007`.

Molnar, P. (2022). Differences between soil and air temperatures: Implications for geological reconstructions of past climate. Geosphere 18.2, pp. 800–824. DOI: `10.1130/GES02448.1`.

Onofri, A. (2023). *drcte: Statistical Approaches for Time-to-Event Data in Agriculture.* R package version 1.0.30. URL: `https://CRAN.R-project.org/package=drcte`.

Onofri, A., M. B. Mesgaran, and C. Ritz (2022). A unified framework for the analysis of germination, emergence, and other time-to-event data in weed science. Weed Science 70.3, pp. 259–271. DOI: `10.1017/wsc.2022.8`.

Palazzo, A. J. and G. S. Brar (1997). *The Effects of Temperature on Germination of Eleven Festuca Cultivars.:* tech. rep. Fort Belvoir, VA: Defense Technical Information Center. DOI: `10.21236/ADA330578`.

Pathak, D. K., K. Bargali, S. S. Bargali, and A. Fartyal (2025). Seed germination and early seedling growth behavior of critically endangered Catamixis baccharoides to variation in soil type. Vegetos 38.3, pp. 1019–1028. DOI: `10.1007/s42535-024-00865-9`.

Pausas, J. G., B. B. Lamont, J. E. Keeley, and W. J. Bond (2022). Bet-hedging and best-bet strategies shape seed dormancy. New Phytologist 236.4, pp. 1232–1236. DOI: `10.1111/nph.18436`.

Pavlick, L. E. and J. Looman (1984). Taxonomy and nomenclature of rough fescues, *Festuca altaica* , *F* . *campestris* (*F* . *scabrella* var. *major*), and *F* . *hallii* , in Canada and the adjacent part of United States. Canadian Journal of Botany 62.8, pp. 1739–1749. DOI: `10.1139/b84-235`.

Probert, R. J. (2000). "The role of temperature in the regulation of seed dormancy and germination." In: *Seeds: the ecology of regeneration in plant communities.* Ed. by M. Fenner. 2nd ed. UK: CABI Publishing, pp. 261–292. ISBN: 978-0-85199-947-0. DOI: `10.1079/9780851994321.0261`.

Qiu, J., Y. Bai, Y.-B. Fu, and J. F. Wilmshurst (2010). Spatial variation in temperature thresholds during seed germination of remnant *Festuca hallii* populations across the Canadian prairie. Environmental and Experimental Botany 67.3, pp. 479–486. DOI: `10.1016/j.envexpbot.2009.09.002`.

R Core Team (2023). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing. Vienna, Austria. URL: `https://www.R-project.org/`.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard (2015). Dose-Response Analysis Using R. PLOS ONE 10.12. Ed. by Y. Xia, e0146021. DOI: `10.1371/journal.pone.0146021`.

Robinson, D., A. Hayes, and S. Couch (2024). *broom: Convert Statistical Objects into Tidy Tibbles.* R package version 1.0.6. URL: `https://CRAN.R-project.org/package=broom`.

Romo, J. T. and L. E. Eddleman (1995). Use of Degree-Days in Multiple-Temperature Experiments. Journal of Range Management 48.5, p. 410. DOI: `10.2307/4002244`. JSTOR: `4002244`.

Romo, J. T., P. L. Grilz, C. J. Bubar, and J. A. Young (1991). Influences of Temperature and Water Stress on Germination of Plains Rough Fescue. Journal of Range Management 44.1, pp. 75–81. DOI: `10.2307/4002644`. JSTOR: `4002644`.

Romo, J. T. (1996). Seed age-germination relationships in Plains Rough Fescue, Festuca altaica subspecies hallii. The Canadian field-naturalist 110.2, pp. 294–297. DOI: `10.5962/p.357454`.

Russell, M. (2011). Dormancy and Germination Pre-Treatments in Willamette Valley Native Plants. Northwest Science 85.2, pp. 389–402. DOI: `10.3955/046.085.0222`.

Shen, Z.-X., D. J. Parrish, D. D. Wolf, and G. E. Welbaum (2001). Stratification in Switchgrass Seeds Is Reversed and Hastened by Drying. Crop Science 41.5, pp. 1546–1551. DOI: `10.2135/cropsci2001.4151546x`.

Sherritt, D. E. (2012). "*Festuca hallii* (Vasey) Piper (Plains rough fescue) and *Festuca campestris* RYDB (Foothills rough fescue) Response to Seed Mix Diversity and Mycorrhizae". PhD thesis. Edmonton: University of Alberta. DOI: `10.7939/R34P68`.

Small, C. C., D. Degenhardt, and T. McDonald (2019). Plant growth regulators for enhancing Alberta native grass and forb seed germination. Ecological Engineering 142, p. 100003. DOI: `10.1016/j.ecoena.2019.100003`.

Stout, D. G., A. McLean, and D. A. Quinton (1981). Growth and Phenological Development of Rough Fescue in Interior British Columbia. Journal of Range Management 34.6, pp. 455–459. DOI: `10.2307/3898097`. JSTOR: `3898097`.

Tannas, S. (2014). *The Successful Restoration of Fescue Grasslands in Southwestern Alberta.* Regina, SK.

Vibhuti, V., C. Shahi, K. Bargali, and S. S. Bargali (2015). Seed germination and seedling growth parameters of rice (Oryza sativa) varieties as affected by salt and water stress. The Indian Journal of Agricultural Sciences 85.1, pp. 102–108. DOI: `10.56093/ijas.v85i1.46046`.

Vivanco, P., J. A. Oliveira, and I. Martín (2021). Optimal germination conditions for monitoring seed viability in wild populations of fescues. Spanish Journal of Agricultural Research 19.3, e0804. DOI: `10.5424/sjar/2021193-18025`.

Yan, A. and Z. Chen (2020). The Control of Seed Dormancy and Germination by Temperature, Light and Nitrate. The Botanical Review 86.1, pp. 39–75. DOI: `10.1007/s12229-020-09220-4`.

Yao, L., M. A. Naeth, and F. P. O. Mollard (2017). Ecological role of pyrolysis by-products in seed germination of grass species. Ecological Engineering 108, pp. 78–82. DOI: `10.1016/j.ecoleng.2017.08.018`.

Zeileis, A., S. Köll, and N. Graham (2020). Various Versatile Variances: An Object-Oriented Implementation of Clustered Covariances in R. Journal of Statistical Software 95, pp. 1–36. DOI: `10.18637/jss.v095.i01`.

Zhang, C., C. G. Willis, K. Donohue, Z. Ma, and G. Du (2021). Effects of environment, life-history and phylogeny on germination strategy of 789 angiosperms species on the eastern Tibetan Plateau. Ecological Indicators 129, p. 107974. DOI: `10.1016/j.ecolind.2021.107974`.

Chapter 3: Patterns of *Epichloë* endophyte infection in rough fescue grasses across gradients of disturbance in Glenbow Ranch Provincial Park, Alberta

> *It has long been known that the grains of the Darnel . . . grass contain a definite fungus-mycelium, always situated in a definite layer of the seed . . . in the growing point of the seedling stem and in the base of the young leaf rudiments.*
>
> — E. M. Freeman, *On the Seed-Fungus* (1904)

Chapter Summary

Fungal endophytes in the genus *Epichloë* are mutualists of cool-season grasses which confer multiple physiological benefits to their plant hosts, including drought tolerance, increased competitive ability, and resistance to grazing by herbivores. Levels of endophyte infection within different populations of grass are variable, but have been shown to correspond to increasing or decreasing levels of disturbance and environmental stress. This responsiveness makes endophyte infection levels useful indicators of site disturbance and health. I conducted a survey of disturbance metrics and endophyte infection prevalence in rough fescue grasses (*Festuca hallii* and *F. campestris*) across 33 sites in Glenbow Ranch Provincial Park (GRPP), Alberta. GRPP is a working ranch with a natural mosaic of disturbed agricultural rangeland and remnant rough fescue grassland, which makes it an ideal location for testing the connection between endophytes and site health. I was unable to draw a direct connection between endophyte prevalence and site disturbance at any of the sites, however, I did find consistently high levels of endophyte infection (mean = 81%) across all sites. Additionally, this work is the first to confirm and report the presence of a fungal endophyte symbiosis in *Festuca hallii*.

3.1 Introduction

Fungal endophytes are symbionts which carry out their entire life cycle within the tissues of their host plant (i.e., systemic), without causing symptoms of disease (i.e., asymptomatic; Saikkonen et al. 1998; Clay and Schardl 2002; Kuldau and Bacon 2008; Rodriguez et al. 2009). While the nature of the host-endophyte relationship can vary depending on environmental conditions and the class of endophyte present (Saikkonen et al. 1998; Clay and Schardl 2002; Rodriguez et al. 2009), these relationships are often mutualistic in nature. The endophyte benefits from nutrients and protection provided by the host plant (Saikkonen et al. 1998; Lee et al. 2021), while the host in turn may benefit from a variety of biotic and abiotic protections provided by the endophyte (Saona et al. 2010; Bastías et al. 2024). Such benefits include defense against insect and mammalian herbivores (Saikkonen et al. 1998; Saona et al. 2010; Hewitt et al. 2021), increased resistance to drought, competition and other abiotic stresses (Bacon 1993; Rodriguez et al. 2009; Afkhami and Strauss 2016), and general improvements to fitness and growth (Schardl et al. 2013; Lee et al. 2021).

While some fungal endophytes can reproduce sexually by forming external spore-producing structures on their host's flowers (Rodriguez et al. 2009; Clay and Schardl 2002), endophytes in the genus *Epichloë* primarily reproduce asexually, through vertical transmission of fungal tissue from the host plant to its seeds (Fig. 3.1; Saunders et al. 2010; Popay et al. 2021; von Cräutlein et al. 2021). This method of vertical transmission promotes mutualistic, or at least, net-neutral relationships between *Epichloë* endophytes and their hosts, as the endophytes become entirely reliant on their hosts for survival and reproduction (Saikkonen et al. 1998; Kuldau and Bacon 2008; Faeth 2009; Saona et al. 2010; Newman et al. 2022; von Cräutlein et al. 2021). While an endophyte may offer beneficial protections at moderate levels of disturbance, it can become a costly burden to the host plant if environmental stress levels are too high, leading to the host rejecting the endophyte (Lee et al. 2021; Rudgers et al. 2012; Bastías and Gundel 2022). Conversely, if disturbance is low and resources are not limited, the host may no longer require the benefits offered by an endophyte. Indeed, host plants have been observed to reduce transmission of their fungal endophytes to the next generation when environmental conditions become less stressful, thereby regulating the prevalence of endophytes in the population according to environmental context (Rodriguez et al. 2009; Saona et al. 2010; Rudgers et al. 2012; Gibert et al. 2015; Rudgers et al. 2016). In this way, variations in fungal endophyte prevalence can serve as a bimodal ecological indicator of environmental stress or disturbance levels experienced by different host populations across a landscape (Granath et al. 2007; Koh and Hik 2007; Colón Carrión et al. 2022).

One system where greater study of fungal endophytes could be particularly valuable, both as ecological indicators and beneficial mutualists, is the rough fescue prairies of western North America. Once covering 255,000km^2 of western North America, these diverse and highly productive grasslands have been reduced to less than 5% of their former range due to combined factors including agricultural development, energy infrastructure, the introduction of invasive, non-native forage grasses, and the suppression of natural fire cycles (Desserud et al. 2010; Otfinowski et al. 2017;

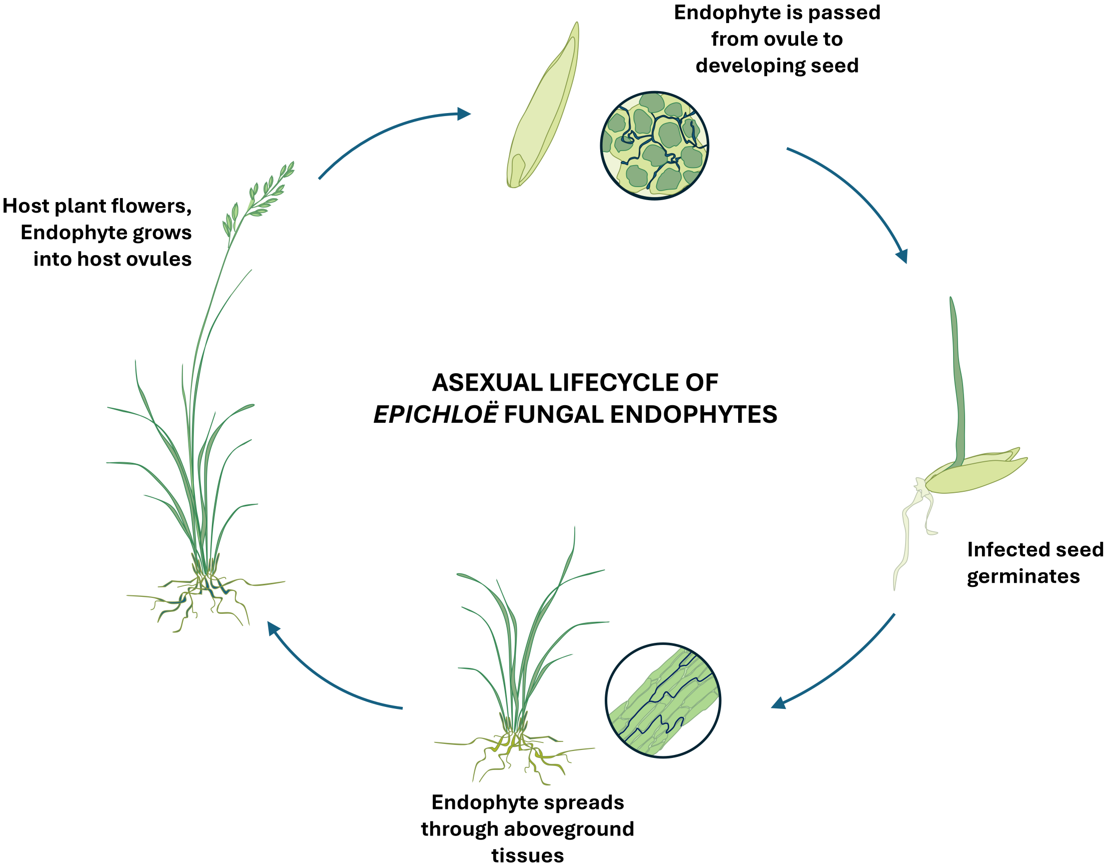

Figure 3.1: The asexual life cycle of *Epichloë* fungal endophytes. Fungal hyphae spread through the intercellular spaces of their host plant's tissues, eventually being transmitted to the next generation via infected seeds. Circular insets show the typical growth pattern of fungal hyphae in leaf and seed tissues. Adapted from Johnson et al. 2013.

Elsinger et al. 2023). True to their name, the characteristic vegetation of the rough fescue prairies are rough fescue grasses; *Festuca hallii* and *Festuca campestris.* These long-lived perennial bunchgrasses are both ecologically and economically valuable, providing critical habitat and high-quality forage for both livestock and wild herbivores (Desserud and Naeth 2013b). Additionally, their deep roots and abundant leaf litter create stable microclimates within the arid prairies by reducing erosion, retaining soil moisture, and buffering surface temperatures (Deutsch et al. 2010; Desserud et al. 2010; Bengtsson et al. 2019). However, rough fescue grasses are also notoriously sensitive to anthropogenic disturbance (Willms and Fraser 1992; Desserud and Naeth 2013a). Particularly, being grazed during the growing season (Willms 1988; Krzic et al. 2014; Otfinowski et al. 2017) and being outcompeted by introduced forage grasses such as smooth brome (*Bromus inermis*) and Kentucky bluegrass (*Poa pratensis;* Tannas et al. 2015; Otfinowski et al. 2017). This sensitivity contributes to their poor recovery and restoration once disturbed (Elsinger et al. 2023; Desserud et al. 2010). Few attempts to actively restore (i.e through seeding) rough fescue grasses have been successful (Desserud and Naeth 2013a; Stover et al. 2018), and as a result, the recommended recovery strategy for these communities is usually to minimize disturbance as much as possible, and rely on natural regeneration to slowly restore the original community (Desserud et al. 2010; Desserud and Naeth 2013a; Elsinger et al. 2023). Thus, investigating ways to better understand and improve the resilience and recovery of rough fescue grasses is critical to informing more effective restoration outcomes.

Epichloë endophytes have been detected in both *F. campestris* and *F. hallii*, however the exact benefits they provide for their host plants remain poorly understood. The most commonly cited endophyte-provisioned benefit in grasses is the production of alkaloids which make the host plant unpalatable and/or toxic to mammalian and insect herbivores, thus offering protection against predation (Finch et al. 2016; Collinson et al. 2024; Schardl et al. 2013; Saona et al. 2010; Saikkonen et al. 1998). *Epichloë* FcaTG-1, the endophyte associated with *F. campestris*, can produce insect-deterring alkaloids, but lacks the genes needed to produce alkaloids that deter mammalian grazers (Schardl et al. 2013; Rudgers et al. 2016). As mammalian grazing is a more significant threat to rough fescue fitness and persistence than insect damage (Johnston and MacDonald 1967; Desserud et al. 2010; Krzic et al. 2014), this suggests that anti-herbivore defense may not be the primary driver of the rough fescue-endophyte symbiosis. The grasses could instead be benefiting from improved resistance to other disturbance-related stressors such as drought, or competitive exclusion (Afkhami and Strauss 2016; Bastías et al. 2024). Indeed, the endophytes could be instead offering a form of indirect herbivore resistance; not against grazing, but against the effects of erosion, soil compaction and moisture loss brought about by the presence of large herbivores (Adams et al. 2016; Rudgers et al. 2016).

I wanted to understand the potential benefits of endophytes to rough fescue, given that they seemingly do not deter grazing mammals. While endophyte-host interactions may be negative, positive, or neutral (Saikkonen et al. 1998; Newman et al. 2022), *Epichloë* endophytes tend towards net-positive relationships with their hosts, since their reproduction is so closely tied to their host's wellbeing (Saunders et al. 2010; Popay et al. 2021; Newman et al. 2022). I therefore hypothesized that fungal endophytes were being maintained in rough fescue populations because they offered some enhanced tolerance to environmental stressors that significantly hamper rough fescue persistence. Namely, drought (Stout et al. 1981; Palit et al. 2012; White et al. 2014) and competition (Grilz et al. 1994; Tannas et al. 2015), both of which can be introduced and exacerbated by prolonged grazing pressure (Willms et al. 1985; Adams et al. 2016). I therefore predicted that endophyte prevalence would be highest at sites with intermediate levels of disturbance related to these stressors. Conversely, sites with very high or very low levels of disturbance would see a lower prevalence of endophytes, as the symbiosis would be less beneficial for the host grass at either extreme.

3.2 Methods

3.2.1 Study area

To investigate whether endophyte infection rates in rough fescue are linked to site-level anthropogenic disturbance, I conducted a field study across 33 sites in Glenbow Ranch Provincial Park (GRPP), Alberta, during July and August 2022. GRPP is a 3,200-acre provincial park located along the Bow River valley between Calgary and Cochrane, AB (51.16702, -114.392653). The park provides an ideal study site due to the presence of several intact rough fescue populations and its historical and ongoing use as a working ranch (High Range Ecological Consultants 2008; Alberta Parks 2025). GRPP's ranching legacy has created a natural gradient of disturbance across the park. While some areas remain relatively undisturbed and support robust native grassland communities, others have been subject to persistent cattle grazing and foot traffic, resulting in reduced diversity, greater incidence of aggressive non-native species, and reduced water-retaining capacity (Tannas 2013; Adams et al. 2016). This variation in disturbance levels, within the same geographical area and climatic conditions, offers a unique opportunity to study the relationship between rough fescue its endophytes across a range of ecological contexts.

3.2.2 Site selection

I selected 33 sites across Glenbow Ranch Provincial Park (Fig. 3.2), using a combination of previously surveyed transects (High Range Ecological Consultants 2008; Tannas 2013) and newly identified locations to capture a range of disturbance levels and fescue abundance. 18 sites were selected based on transects originally surveyed in 2007 and 2013 by Tannas Conservation Services as part of a series of ecological assessments (Tannas 2013; High Range Ecological Consultants 2008). Using these surveys allowed me to identify locations where fescue had been recorded in previous years, and provided baseline information on site health and expected vegetation communities. To complement these sites and capture disturbance conditions not represented in the original Tannas surveys, I selected 15 additional sites where I had identified rough fescue during my initial reconnaissance.

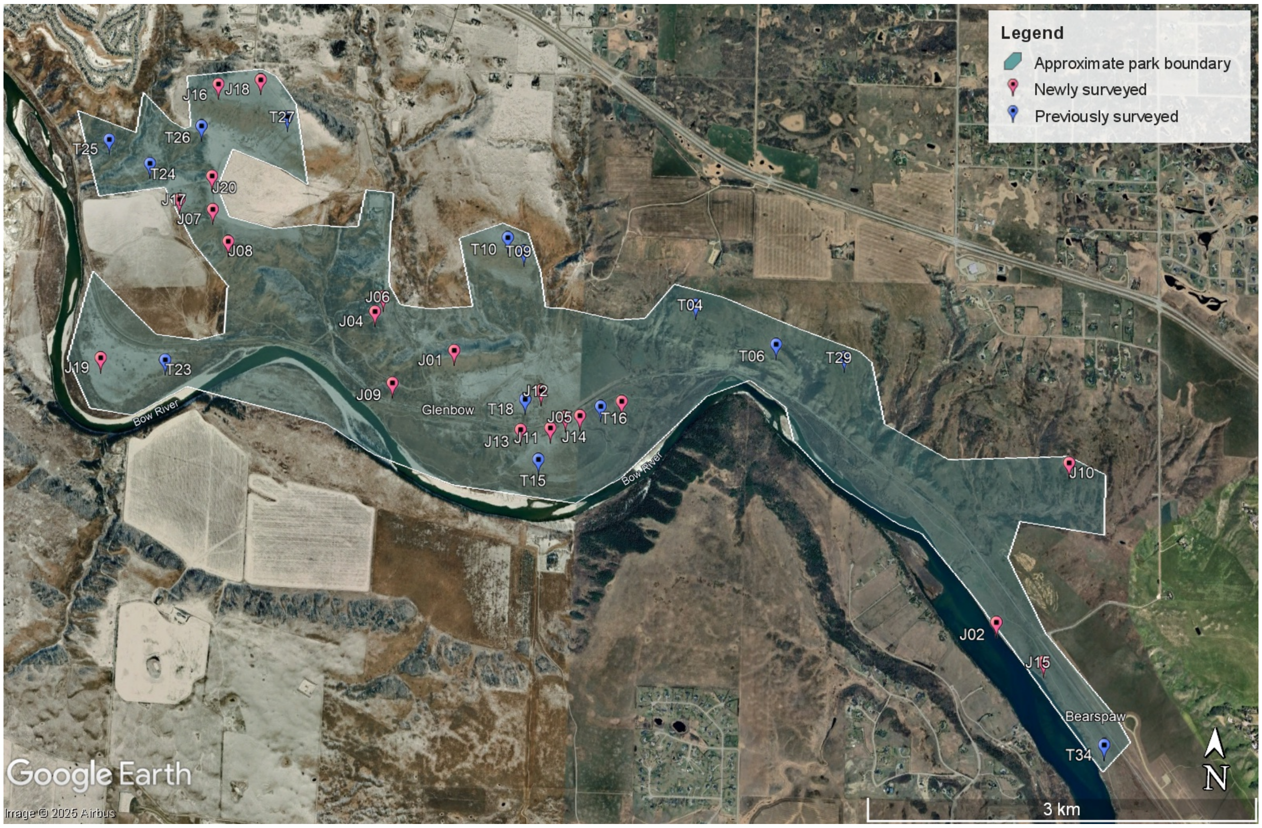

Figure 3.2: Field sites surveyed across Glenbow Ranch Provincial Park in 2022. Blue pins represent sites that had been previously inventoried by Tannas Conservation Services in 2007 and 2013, and were re-surveyed for this study. Pink pins represent sites that were newly surveyed for the purposes of this study.

3.2.3 Data Collection

Rough fescue collection and processing

To assess the level of endophyte infection at each site, I randomly chose 15-20 rough fescue plants per site and collected 3 vegetative tillers from each. To process the samples, I removed the outermost sheath from each grass tiller, and cut the bottom 5cm into ≈0.5cm fragments. For most of the grasses sampled at each site, I placed the fragments in a glassine envelope to dry. I also "fixed" a subset of samples (3-5 individuals per site) in Carnoy's Solution I (1 part glacial acetic acid : 3 parts absolute ethanol), to preserve endophyte structures for later microscopic analysis (Koh et al. 2006).

The Range Health Assessment Tool

My initial plan for assessing disturbance and ecological integrity at each site was to use the Range Health Assessment (RHA) guidelines developed by the Government of Alberta (Adams et al. 2016; Government of Alberta 2016). The RHA is a practical, field-based tool for evaluating rangeland condition in response to different management regimes, including livestock grazing (Pyle et al. 2018). It is based around five key ecological criteria which capture multiple aspects of ecological function and anthropogenic disturbance, and were chosen as reliable indicators of rangeland health (Pyke et al. 2002). Because GRPP is a working ranch with a long history of grazing (Glenbow Ranch Park Foundation 2018), the RHA provided a good framework for assessing anthropogenic disturbance at each site.

However, the RHA presents some limitations that made it less applicable for my specific research purposes. As a rapid assessment tool, it is designed for quick, mostly qualitative assessments of each range health indicator, which receive a numerical score based on field observations. The scores from each section are then summed to generate an overall "Site Health" score from 0 to 100 (Adams et al. 2016). While this is a convenient system for rangeland managers, these scores have limited use in more detailed statistical analysis. Additionally, the primary purpose of the RHA scoring system is to help inform land management decisions by providing a general overview of a site's integrity (Adams et al. 2016; Pyle et al. 2018). It is not intended to identify specific trends or relationships among site characteristics, which was necessary for addressing my specific research questions. Therefore, I adapted the RHA metrics into more quantifiable measurements of the same ecological indicators, which allowed me to retain the practical baseline of the RHA tool, while providing metrics that were more suited for my analyses. Of the five original RHA metrics, I adapted four (Table 3.1). I also added an additional metric of my own (rough fescue cover), which serves as a measure of ecological integrity and endophyte transmissibility (Afkhami and Rudgers 2008; Adams et al. 2016). I describe the data collection methods and calculations for each site metric below.

Table 3.1: The five ecological indicators evaluated in the Rangeland Health Assessment (RHA) and the corresponding quantitative metrics adapted for this study.

RHA Metric	**RHA Criteria**	**Adapted Metric**	**Range Health Relevance**	**Endophyte Relevance**
Integrity and Ecological Status	Assesses successional stage of the site and compares species composition to a reference plant community	% cover of 'increaser' species	Prolonged anthropogenic disturbance shifts the community towards more ruderal species (i.e., 'increasers') (Landsberg et al. 2003; Wroe et al. 2013).	Endophytes increase fitness and persistence in disturbance-prone, competitive environments (i.e., those where 'increasers' may be thriving; Rudgers et al. 2012; Lee et al. 2021)
Plant Community Structure	Assesses the presence/absence of expected vegetation layers (e.g., low shrubs, tall graminoids, medium forbs, etc.)	Gini-Simpson index of plant functional group diversity	Greater structural and functional diversity supports more complete and efficient partitioning of resources, enhancing ecosystem productivity and resource availability (Pyke et al. 2002; Adams et al. 2016)	Endophytes improve uptake and allocation in resource-limited environments, which may be less necessary in functionally diverse communities (Rodriguez et al. 2009; Lee et al. 2021)
Hydrologic Function and Nutrient Cycling	Estimates quantity of plant litter at the site in lbs/acre, as a proxy for moisture retention	Litter quantity in lbs/acre (unchanged)	In grasslands, plant litter reduces evaporation, improves water infiltration, and regulates soil surface temperatures. Anthropogenic disturbance reduces litter levels (Adams et al. 2016).	Litter loss exacerbates drought conditions and increases environmental stress. Endophytes improve drought tolerance via several mechanisms (Bacon 1993; Hewitt et al. 2021; Lee et al. 2021)
Prohibited Noxious and Noxious Weeds	Assesses the density and distribution of prohibited noxious and noxious weeds (i.e., invasive, non-native species) at a site	% cover of invasive species	Invasive plants are both indicators and sources of anthropogenic disturbance, capable of displacing native species and reducing site functioning/diversity (Pyke et al. 2002; Adams et al. 2016)	Endophytes can improve persistence and competitive ability of host plant, offering resistance against invasive species (Rudgers et al. 2012; Afkhami and Strauss 2016)
Site Stability	Assesses levels of accelerated erosion and human-caused bare ground at a site	NA	Quantifying site stability requires large-scale, longer term monitoring of landscape-level erosion dynamics (Adams et al. 2016). I omitted this metric due to time and logistical restraints	NA
NA	NA	**Rough Fescue % Cover**	Rough fescue cover decreases with increasing anthropogenic disturbance (Willms et al. 1985; Krzic et al. 2014)	Endophyte transmission between generations is imperfect. Higher density of host plants means a greater chance of endophytes persisting in the population (Afkhami and Rudgers 2008; Rudgers et al. 2009)

Vegetation surveys and site characteristics

To assess plant community composition and functional diversity, I established a 100m x 100m survey area at each site (Fig. 3.3). I first recorded all identifiable vascular plant species within the survey area to get a general overview of site composition and vegetation patterns (Adams et al. 2016). I then sampled three quadrats along the center of the survey area by tossing a 1x1m frame at the 0, 50, and 100m marks (Reilly and MacKay 2013) and taking detailed percent cover estimates where each quadrat landed (Tannas 2013). At sites with significant vegetation heterogeneity (i.e. plants clustered in patches rather than distributed evenly across the site) I visually estimated percent cover at the site-level rather than by quadrat. I also estimated tree and shrub cover at the site level. In both cases, sampling via quadrat can result in biased and unrepresentative cover estimates (Adams et al. 2016). Because invasive plants can be both indicators and sources of site-level disturbance in grassland communities, I estimated percent cover for these species at the site level as well (Adams et al. 2016).

To estimate the amount of plant litter (i.e. dead but persisting plant material) across each site, I hand-raked the litter within a 0.25m x 0.25m section of the three quadrats along the transect, and compared the collected litter to visual standards to estimate mass in lbs/acre (Adams et al. 2016).

3.2.4 Organizing and calculating site metrics

Species lists and classifications

To calculate the site health metrics I would use as predictors of endophyte prevalence, I first assembled a master list of each species I recorded across my 33 survey sites. For each species, I sourced and compiled relevant trait data, including common and binomial names, growth habit, native/invasive status and grazing response (Table B3), using data from the Canadensys Database of Vascular Plants of Canada (VASCAN), resources from Agriculture Alberta (Wroe et al. 2013), and regional field guides (Tannas 2003; Kershaw and Allen 2020). I then integrated the species trait data with the species composition and percent cover data from each site survey, allowing me to calculate relevant site-level metrics, as detailed below.

Two species were specially classified after all vegetation data was compiled. Kentucky bluegrass (*Poa pratensis*) and smooth brome (*Bromus inermis*) are two non-native grasses that have been widely introduced across North American grasslands as forage crops (Grilz et al. 1994; Otfinowski et al. 2017), and were commonly found at my sites. While these grasses are not officially classified as invasive or noxious species in Alberta, they are known to have profoundly disruptive effects on rough fescue grasslands (Grilz et al. 1994; Sinkins and Otfinowski 2012; Tannas et al. 2015). For this reason, I classified both species as invasive for the purposes of my analyses.

Functional diversity and the Gini-Simpson Index

For each site, I calculated a Gini-Simpson Index of plant functional groups within the community. I defined a plant's functional group as the combination of its size and growth habit (e.g., tall grass,

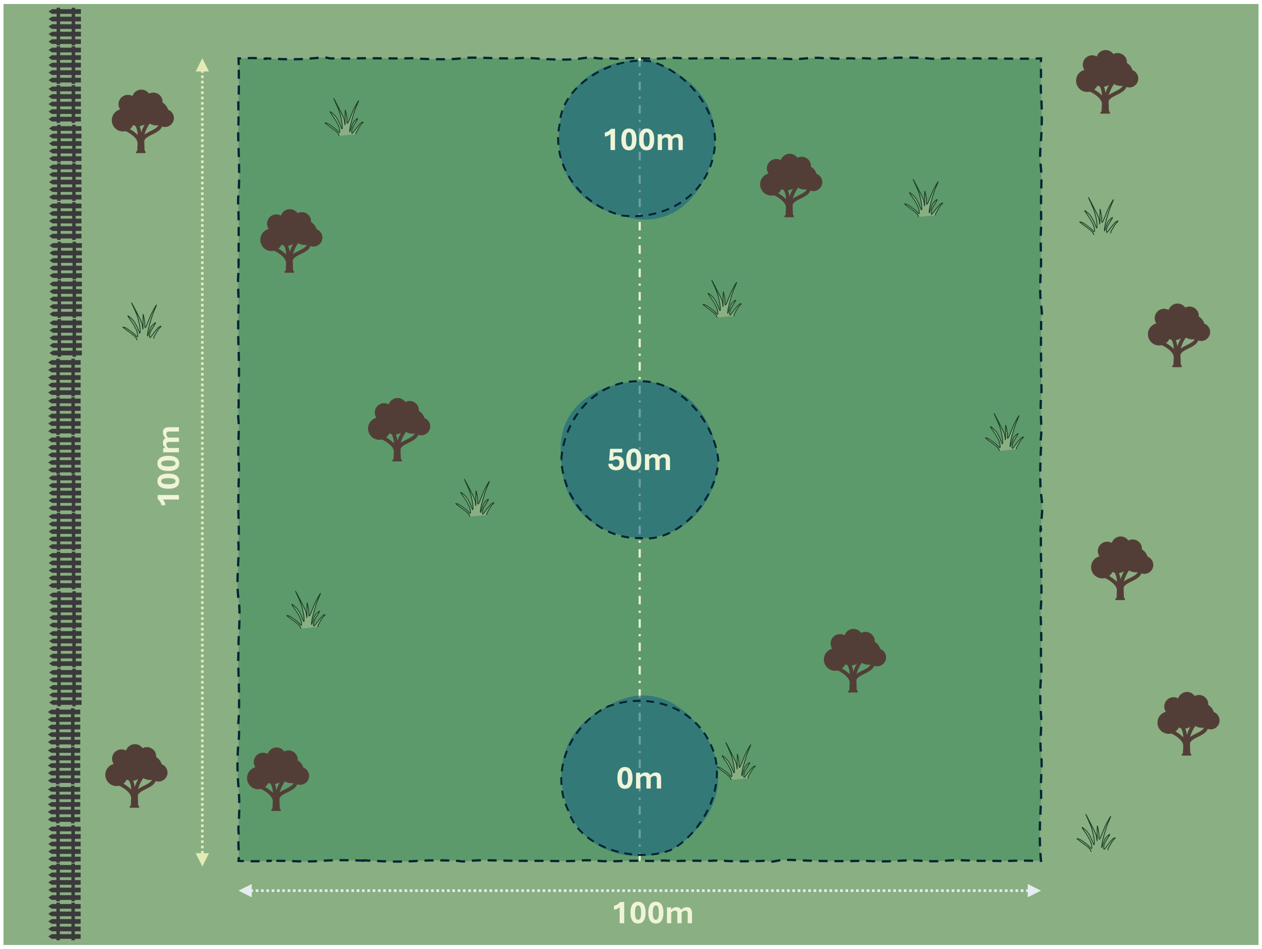

Figure 3.3: Overview of the vegetation survey layout used at each site. I recorded all identifiable plants within a 100x100m area (shaded square), and took detailed percent cover estimates at three 1x1m quadrats placed randomly within the beginning, middle, and end zones of each survey area (shaded circles).

ground cover forb, etc.), which corresponds to the grassland structural layer classifications outlined in (Adams et al. 2016).

The **Simpson Index** (D) is a measure of diversity and evenness, which is calculated as:

$$D = \sum p_i^2$$

Where p_i is the proportion of total plant cover made up by group i.

The **Gini-Simpson Index** is the complement of the Simpson Index (i.e., $1 - D$). This inversion makes the index easier to interpret; higher values indicate greater diversity and evenness compared to lower values (Rad et al. 2009).

Litter cover

To calculate litter cover for each site, I simply averaged the litter mass estimates from the three quadrats sampled along each survey area.

Proportional Percent Cover

I calculated proportional percent cover for rough fescue, 'increaser' species (i.e., species that are native, but opportunistically increase at a site under increased disturbance), and invasive species by dividing the percent cover of each species by the total plant cover at that site. This approach standardizes percent cover estimates for sites that may have greater or less than 100% total cover due to the presence of bare ground or overlapping vegetation layers (Adams et al. 2016).

Endophyte screening

I screened all sampled tillers for the presence of endophytes using Agrinostics Phytoscreen field tiller endophyte detection kits (Agrinostic Ltd. Co., Watkinsville, GA SKU: ENDO797-3) in accordance with the protocol outlined in Koh et al. (2006). I placed small cross-sections of each grass tiller onto a nitrocellulose membrane that was soaked overnight in a buffer solution to extract endophyte-specific proteins and bind them to the membrane. I then dried the membrane and treated it with a series of antibody reagents that bind to the endophyte proteins and can be stained pink with chromagen solution. After staining, I examined the membranes; if a pink spot had developed where a grass tiller was in contact with the membrane, I scored that grass as endophyte-positive (E+). If no colour developed, I scored the sample as endophyte-negative (E-) (Fig. 3.4).

While endophyte infection is sometimes graded according to the "intensity" of the infection level as it varies from plant to plant (e.g., number of infected tillers/plant, density of fungal hyphae within tillers; Koh et al. 2006; Koh and Hik 2007; Rudgers et al. 2016), I recorded infection as a simple measure of presence/absence within each plant, due to time and logistical constraints (Bazely et al. 2007; Granath et al. 2007).

While I intended to collect and screen an equivalent number of *F. campestris* and *F. hallii* tillers,

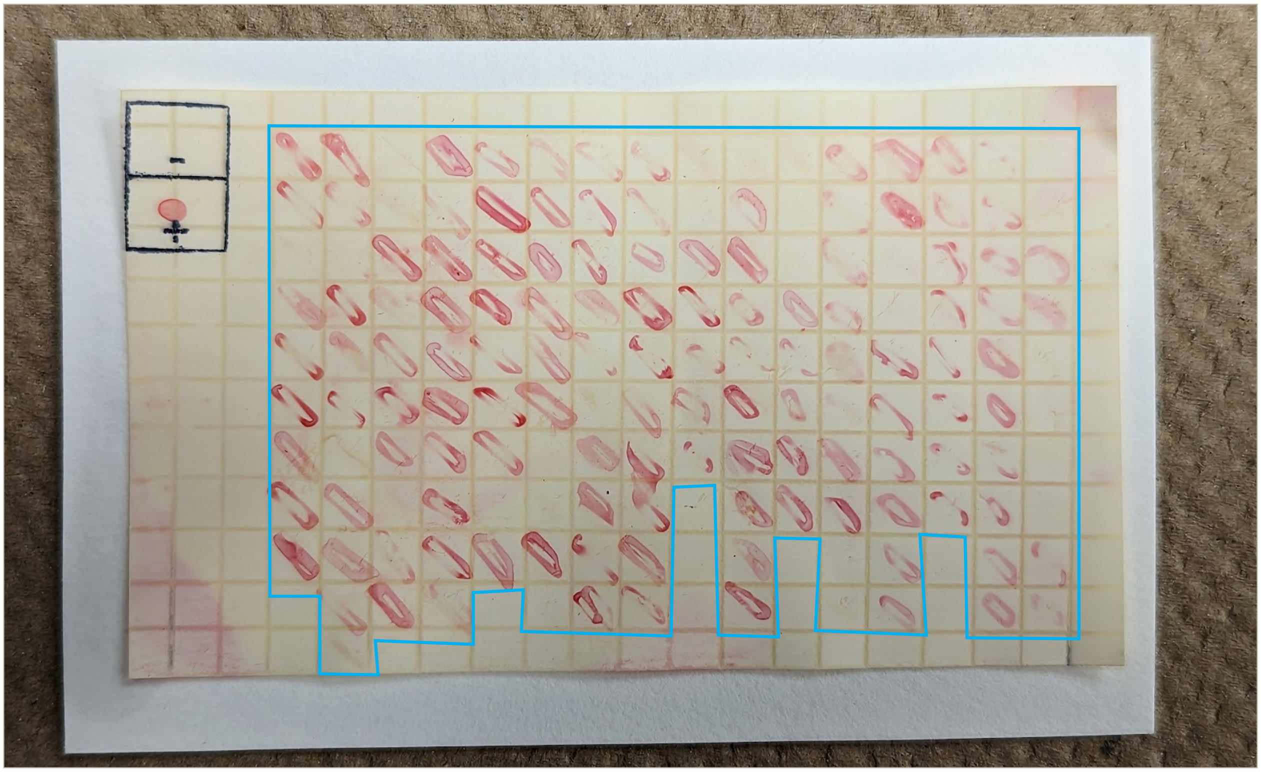

Figure 3.4: One of the endophyte screening cards. All cells which contained a fescue sample are outlined in blue, and the dark pink stains indicate that the tiller in that cell was positive for endophytes. A confirmed positive and negative control are shown in the top left corner of the card for comparison.

my sampling was limited by two factors. The first was that *F. hallii* is less common at GRPP, and even after consulting prior vegetation surveys to locate areas where *F. hallii* had previously been identified, I was often unable to relocate these populations due to shifts in the community composition. Second, I frequently encountered morphologically ambiguous fescue populations that I was unable to confidently identify, even after consulting a provincial fescue taxonomist (Lysandra Pyle, pers. comm.). As a result, my final collection consisted of 126 *F. campestris* specimens, 43 *F. hallii*, and 32 ambiguous and unidentified rough fescue samples.

3.2.5 Statistical Analysis

Predicting endophyte prevalence in relation to site metrics via logistic regression

To determine if disturbance-related site characteristics influenced the level of endophyte infection within each rough fescue population, I used binary logistic regression, which is useful for estimating the probability of binary outcomes (i.e., infected vs. not infected) as a function of multiple predictors (i.e., my five site metrics; Harris 2021). I modelled the proportion of endophyte-infected grasses at each site (no. infected/total no. sampled per site) against each of the five site metrics as predictor variables (Table 3.1). Before proceeding, I checked the model for outlier values and multicolinearity (i.e., correlation between two or more of my predictor variables) which may have skewed my results (Kassambara 2018; Harris 2021). There were no problematic outliers or multicolinearities detected, so I retained all five site metrics in my model. Because the initial logistic regression did not identify any significant predictors, I applied LASSO regression (Least Absolute Shrinkage and Selection Operator) as a regularization technique to identify the most important site metrics. LASSO regression systematically shrinks a model's predictor variable coefficients to identify which predictors are the most informative, thereby reducing complexity and overfitting (Ranstam and Cook 2018).

Due to the unequal samples sizes of *F. campestris* and *F. hallii*, and the presence of ambiguous/unidentified specimens among my samples, I combined all fescue endophyte data together, rather than analyzing the species separately. Because there was no significant difference between the endophyte infection levels of the two species ($\chi^2 = 0.54143$, df = 1, p-value = 0.4618), I proceeded under the assumption that the difference in sample sizes would not skew the outcomes of the model.

Creating a unified "disturbance score" with PCA

To see if the five site characteristics could function as a unified and predictive "disturbance score", I conducted a Principal Component Analysis (PCA) using the `prcomp` function in `R` (v4.3.1; R Core Team 2024). Prior to analysis, I used the `scale` function to standardize my site metrics (i.e., mean = 0, SD = 1), and ensure they shared the same scale of measurement. I also examined scatterplot matrices of the five variables to verify that their relationships were approximately linear (Jolliffe and Cadima 2016). I felt confident that my data met the assumptions needed for an insightful PCA, so I proceeded with the analysis. The PCA reduced each site's functional diversity, litter cover, fescue cover, increaser cover, and invasive cover to a series of principal components (PCs) that best explained the variation in site characteristics (Jolliffe and Cadima 2016). I assessed the relative importance of each principal component using a scree plot (Auer and and Gervini 2008), which showed that the first two components (PC1 and PC2) captured the majority of variance in site characteristics. I plotted PC1 and PC2 to visualize the relationships among the five site health metrics (Fig. 3.11), and also used these PCs as predictive variables in a second logistic regression to determine if either of them influenced the prevalence of endophytes at each site.

I conducted all statistical analyses using `R` (v4.3.1; R Core Team, 2023). Logistic and LASSO regressions were performed using the `glmnet` package (v4.1.8, Friedman et al. 2023). I used the `broom` and `ggplot2` packages (v1.0.7, Robinson et al. 2023; v3.5.1, Wickham et al. 2016) for tidying the model outputs and generating visualizations.

3.3 Results

The average endophtye infection rate for *F. campestris* was 83%, while the average for *F. hallii* was 77%. The infection rate for the unknown ambiguous samples was 76% (Fig. 3.5). Although the infection rate for *F. campestris* was higher than that of *F. hallii*, this was not a significant difference ($\chi^2 = 0.54143$, df = 1, p-value = 0.4618).

The average endophyte infection rate per site across all species was 81% ±0.03, minimum was 33%, max was 100% (Fig. 3.6). Over 90% of sites had an infection rate greater than 50%, and over 75% of the sites had an infection rate over 75%. In fact, almost 40% of sites had an infection rate above 90% (Fig. 3.7)

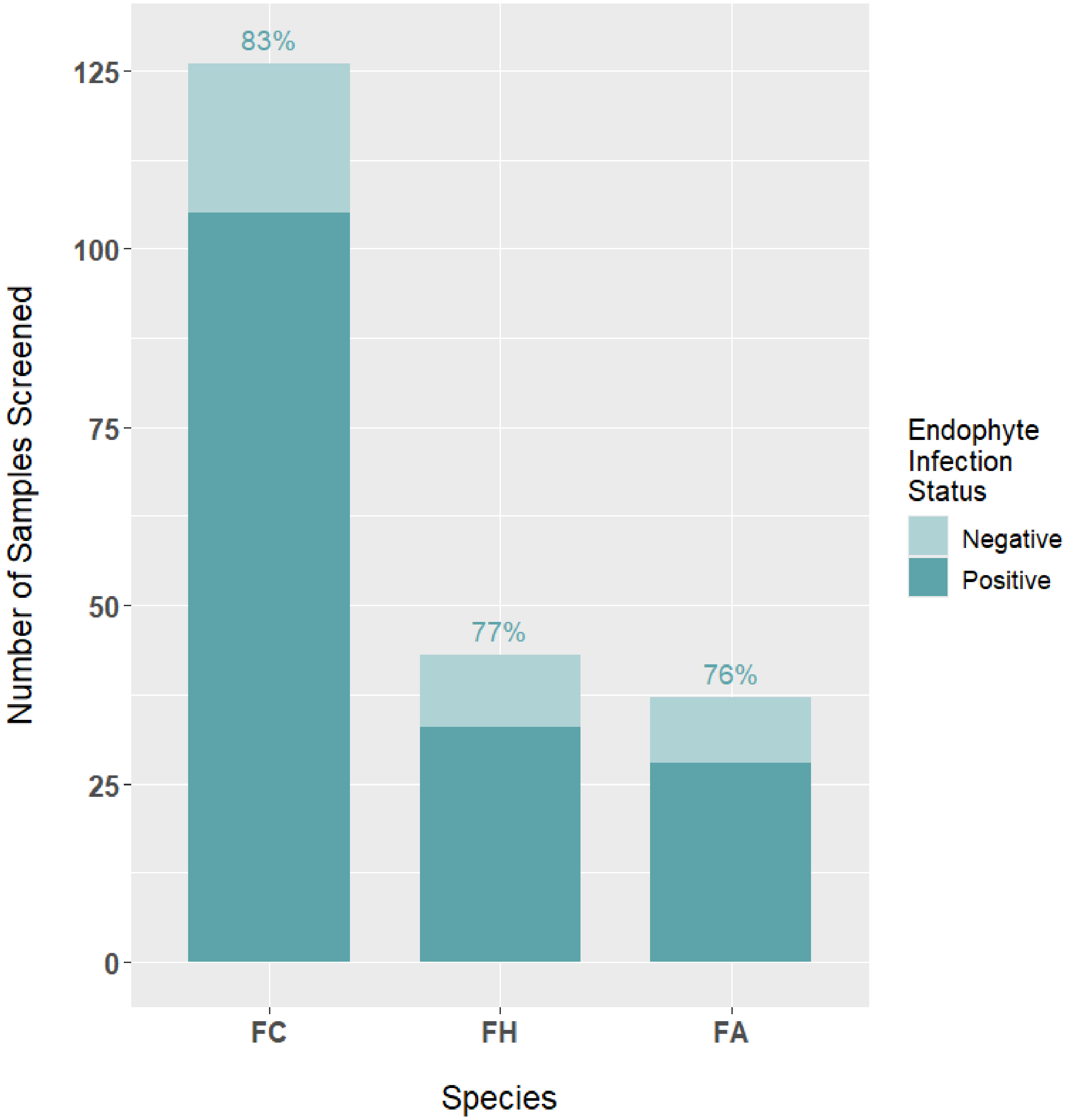

Figure 3.5: Endophyte infection levels and total sample sizes for *Festuca campestris* (FC), *F. hallii* (FH), and the unidentified, ambiguous fescue specimens (FA)

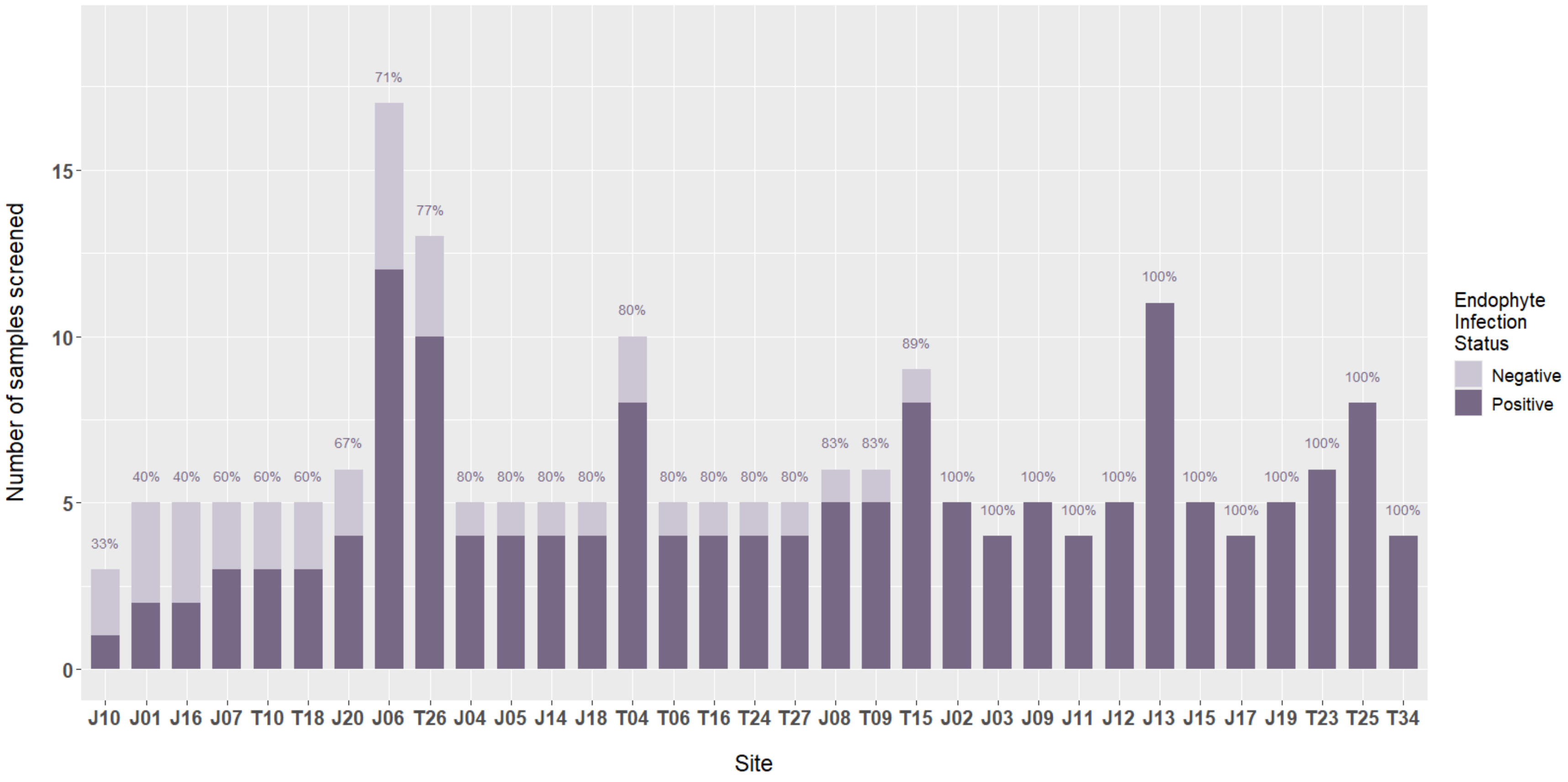

Figure 3.6: The proportion of rough fescue samples that tested positive for endophyte infection at each of 33 sites sampled across Glenbow Ranch Provincial Park

I found no evidence that any of the five disturbance metrics influenced the prevalence of endophytes at my sites. This was the case whether I assessed them individually (Figs. 3.8 and 3.9), or as a combined “site health” variable using the PCA components (Fig. 3.10). Even when the environmental factors were evaluated in a LASSO regression framework, no effect was large enough to be retained in the model.

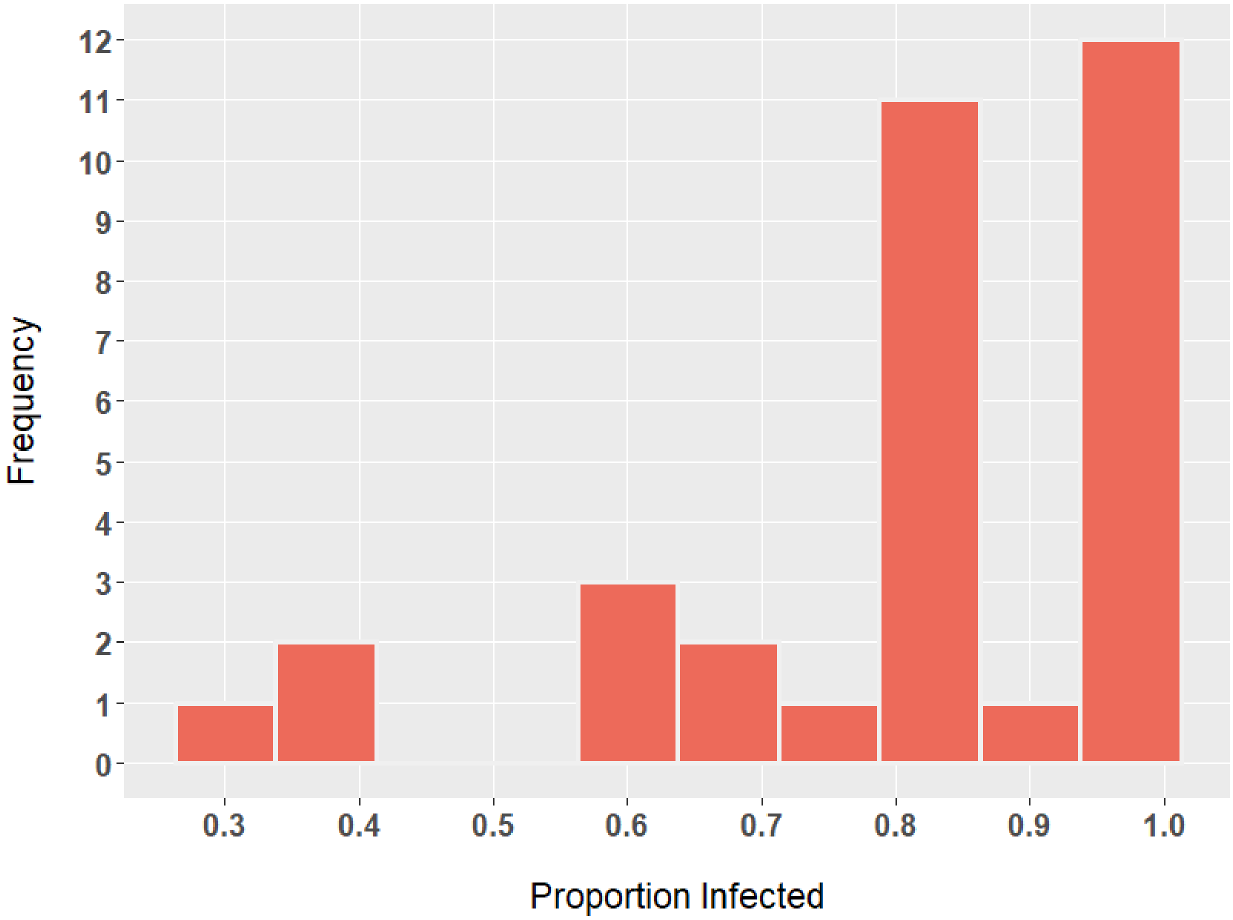

Figure 3.7: The distribution of endophyte infection levels across 33 sites at GRPP shows that the majority of sites had infection levels exceeding 80%

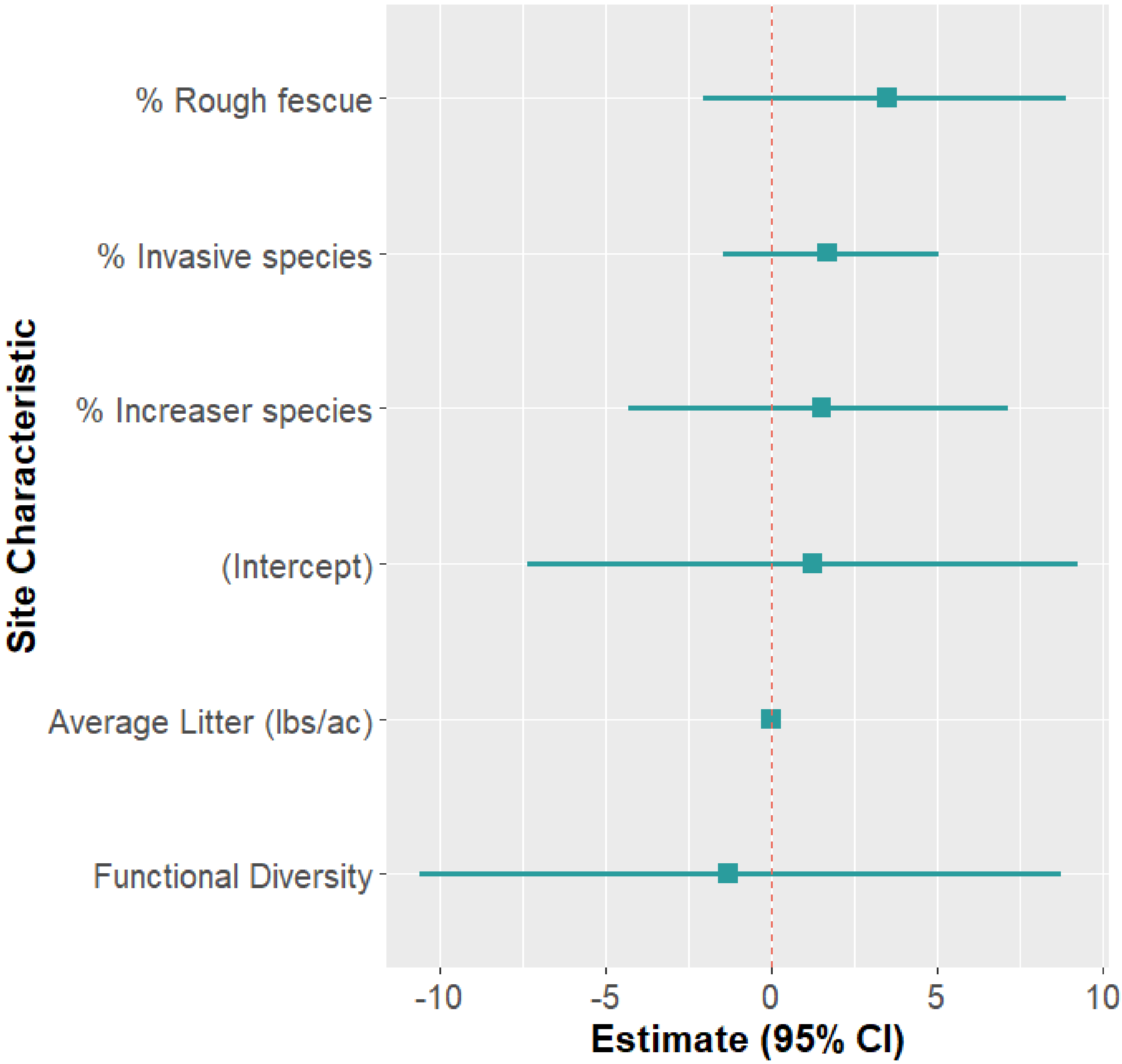

Figure 3.8: None of the five site characteristics measured across 33 sites at Glenbow Ranch PP were significantly associated with endophyte prevalence in rough fescue.

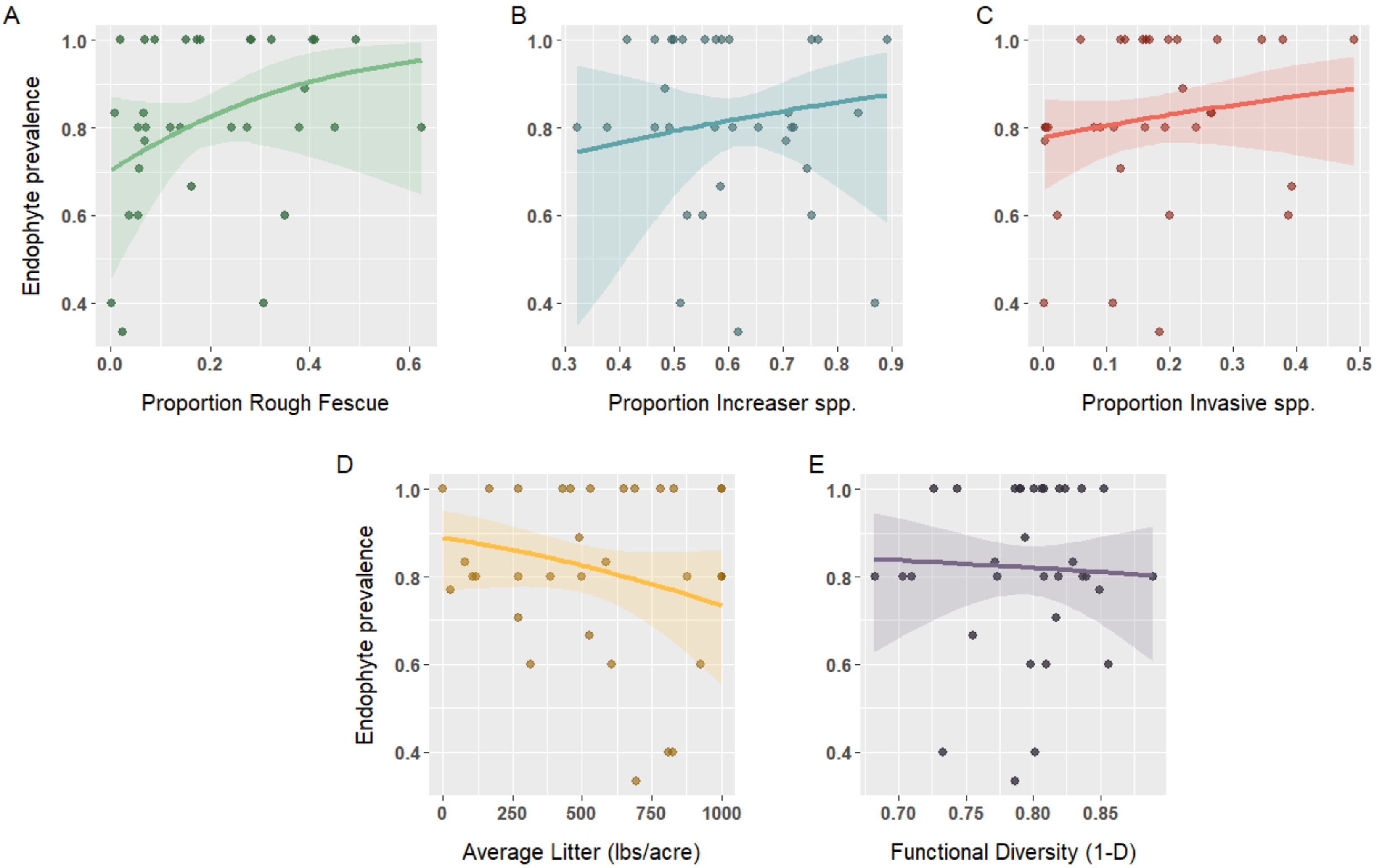

Figure 3.9: The relationship between each of the five site characteristics and the prevalence of fungal endophytes across all sites. Each line represents the predicted proportion of endophyte infection + 95% CI, each point represents an individual site.

There were two primary “site health” gradients among my sites which could be described as composite variables. The first gradient was positively associated with the proportion of increaser species at a site, and negatively associated with the proportion of rough fescue (PC1; loadings = 0.590 and -0.618 respectively), which explained 42% of the variation in the data. The second gradient was positively associated with both plant litter and the proportion of invasive species (loadings = 0.526 and 0.279, respectively), and negatively associated with a site’s functional diversity (loading = -0.627) (Fig. 3.11). Loadings for all components can be found in Table B2.

3.4 Discussion

The primary takeaway from this study is the confirmation that *F. hallii* hosts endophytic fungi, which is the first reported instance of such a mutualism in this species. This is not particularly surprising, as fungal endophytes are nearly ubiquitous across plant taxa (Rodriguez et al. 2009), however confirmation of this symbiosis is still valuable for improving our understanding of this species and its autecology. There is some debate regarding whether immunoblot assays are specific enough to to confirm the identity of these endophytes as *Epichloë*, specifically (Koh et al. 2006; Jensen et al. 2011). However, given the close relationship between *F. hallii* and *F. campestris* (which does host an *Epichloë* endophyte; Rudgers et al. 2016), it is likely that *F. hallii’s* endophyte falls within this genus as well (Schardl et al. 2013; Lee et al. 2021). Confirmation through microscopic and genetic analysis would be useful for future studies, but regardless, this finding opens the door for future research into the ecological influences of fungal endophytes in *F. hallii*.

As for my initial goal of linking fungal endophyte prevalence to site-level indicators of disturbance and rangeland health; I did not find a significant relationship among any of these factors, but my findings do reveal some interesting patterns that are worth further future exploration. Though the identity of *F. hallii’s* endophyte remains unknown for the time being, infection rates were similar to that of *F. campestris*, with no significant difference between the two (Fig. 3.5). Since this study is the first to report the presence of fungal endophytes in *F. hallii*, there is no additional information about its frequency of infection in other populations. However, the average infection rate of 83% that I observed in *F. campestris* is consistent with the ~75% infection rate reported by Rudgers et al. (2016) during their survey of wild *F. campestris* populations in western Montana.

Also notable is how consistently high the rates of endophyte infection were across all sites. 12 of the 33 sites (36%) had an infection level of 100%, and the majority of sites (72%) had infection rates at or above 80% (Figs. 3.6 and 3.7). These levels are at the upper end of infection rates reported for wild populations of fescue, which can range anywhere from 0–20% (Wäli et al. 2007; Semmartin et al. 2015) to 90-100% (Zabalgogeazcoa et al. 1999; Saona et al. 2010; Semmartin et al. 2015; Pereira et al. 2019; Vikuk et al. 2019).

The consistently high levels of endophyte infection observed across sites likely reflected the limited range of disturbance conditions present in my dataset; i.e., the fact that the most of the sites had moderate levels of disturbance, rather than being ‘very healthy’ or ‘very unhealthy’.

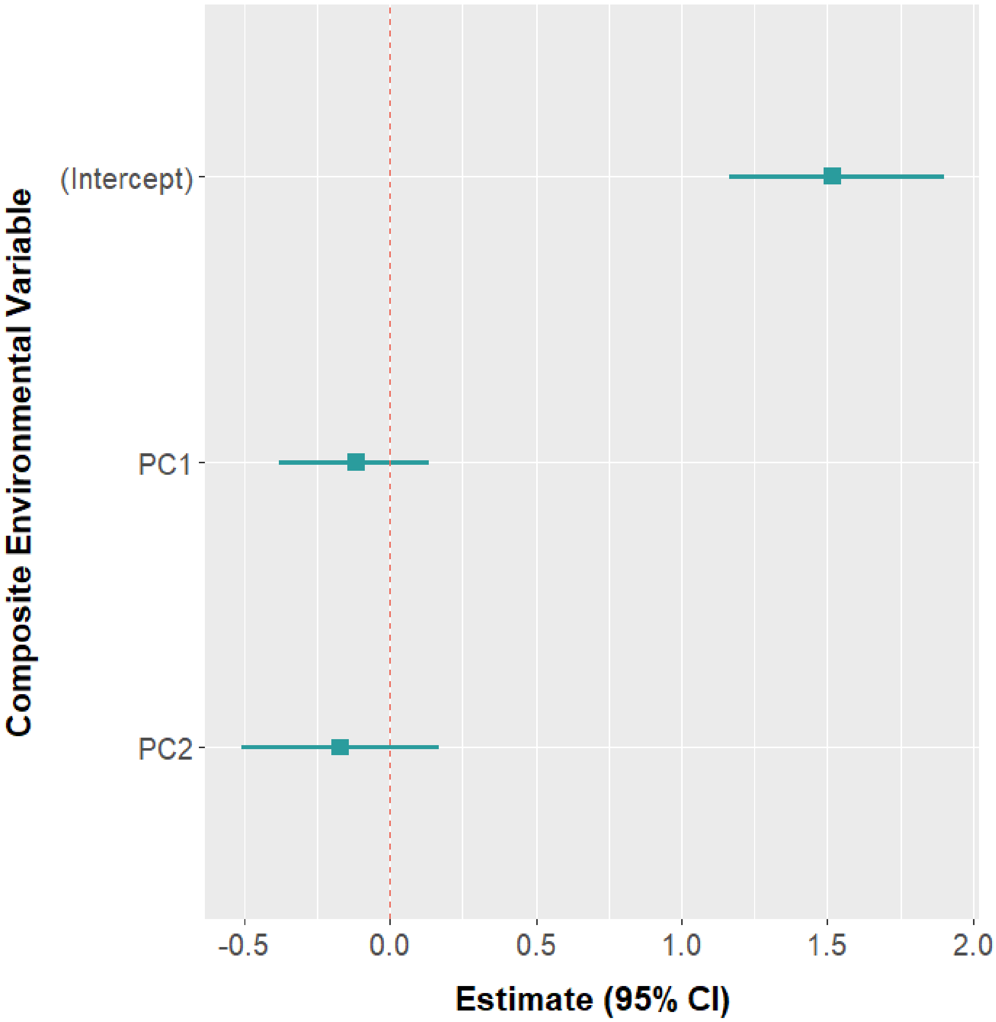

Figure 3.10: Neither of the two most explanatory principal components from a principle component analysis of the five environmental parameters meaningfully influenced the proportion of endophyte infection at my sites when included in a logistic regression

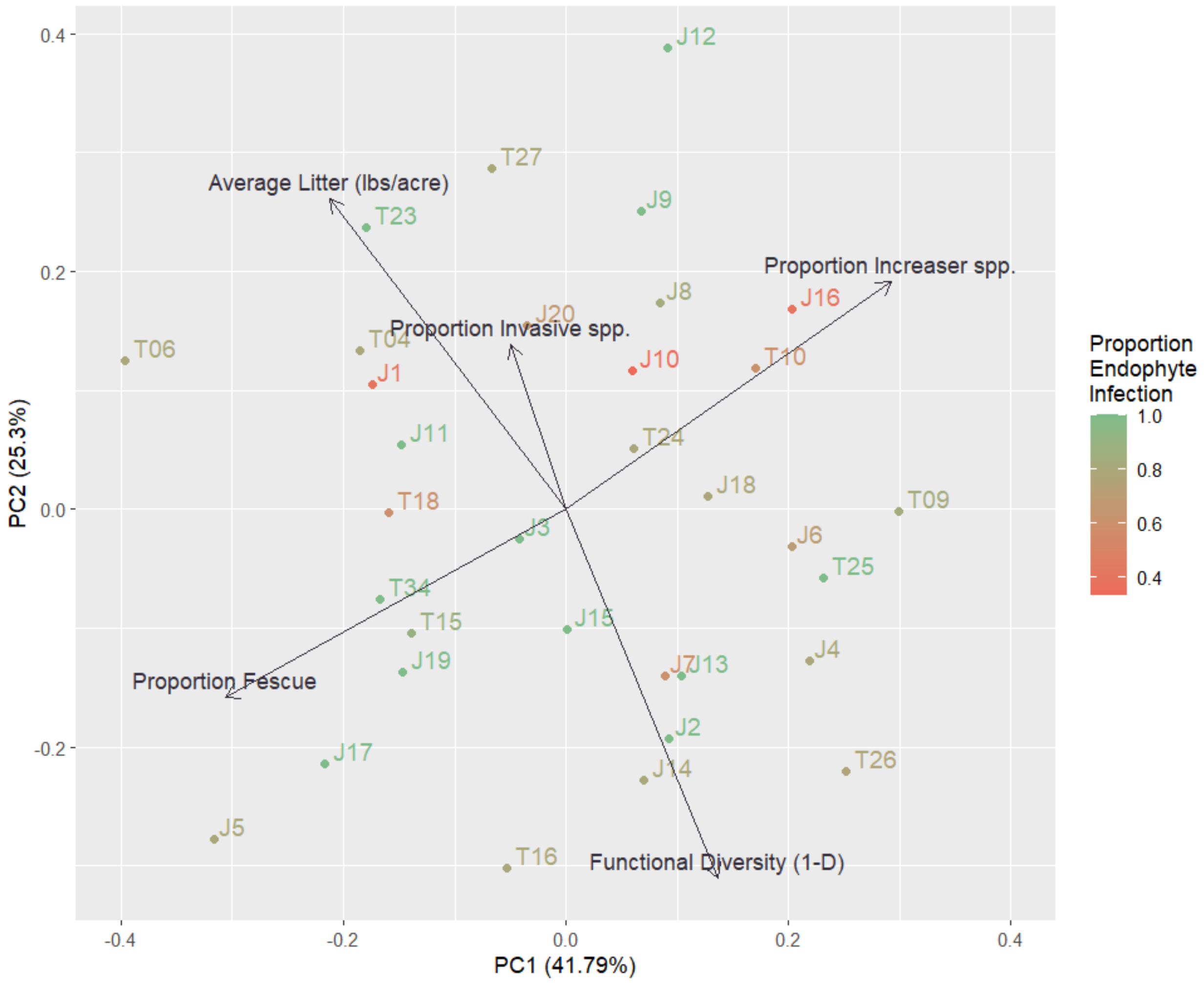

Figure 3.11: PCA biplot showing the relationships among my five site health characteristics. Each point is a specific site, while colours denote the proportion of plants at that site with endophytes.

Because rough fescue disappears quickly from sites that are too disturbed (Willms et al. 1985; Krzic et al. 2014), there were very few truly "high disturbance" sites with enough fescue for me to sample and screen for endophytes. Conversely, Glenbow Ranch's long history of ranching, industry, and human settlement (Glenbow Ranch Park Foundation 2018) means that few (if any) sites in the park could be classified as "low disturbance" (Tannas 2013). While I did try to capture sites with the broadest possible range of disturbance conditions, this meant that most of the sites sampled had been subjected to intermediate levels of disturbance.

The PCA revealed some notable relationships among site health metrics (Fig. 3.11). PC1 defined a clear gradient between the proportion of rough fescue found at a site vs. the proportion of increaser species found at a site. Increaser species are, typically, early-successional species which are opportunistic in nature and tolerate a broad range of stressful environmental conditions. As a result, they tend to increase in abundance in response to anthropogenic disturbance (Landsberg et al. 2003; Wroe et al. 2013). Conversely, rough fescue grasses are late-successional species which are highly sensitive to grazing-related disturbance, and will decrease in response (Bork et al. 2012; Otfinowski et al. 2017). Therefore, one can interpret PC1 as representing the plant community shifting from later to earlier seral stages in response to increasing disturbance (Willms et al. 1985; Adams et al. 2016).

PC2 showed a positive association between plant litter and invasive species cover, and a negative association with functional diversity. High functional diversity and high litter cover are both generally associated with higher range health (Pyke et al. 2002; Adams et al. 2016), so it seems counterintuitive for them to be on opposing ends of the PC2 axis. However, this can be explained in two ways: 1) One of the defining characteristics of rough fescue grasses is the production of large quantities of persistent leaf litter. This litter serves to retain soil moisture and regulate soil temperatures (Desserud et al. 2010; Chen et al. 2024), but if left completely ungrazed, litter cover can become so dense as to smother and exclude other plant species (Looman 1969; Deutsch et al. 2010). Therefore, a site with a high volume of litter may have plenty of rough fescue, but a low diversity of other species (e.g. Site T06; Figs. 3.11 and B1). 2) Alternatively, forage grasses such as Kentucky bluegrass and smooth brome are also known to produce large quantities of dense leaf litter (Otfinowski et al. 2017), and grow to the exclusion of other native grassland species. In the same way, a site with a high proportion of these aggressive species could also have a high volume of litter and low functional diversity (e.g. Site J12; Figs. 3.11 and B1). This may also explain why "Proportion Invasive spp." loads in the same direction as litter cover along PC2; since Kentucky bluegrass and smooth brome were classified as invasive species for my analysis, they would contribute to both the litter cover and invasive species cover loadings.

While the low variation in disturbance levels among my sampled sites was likely the main factor affecting my results, there are additional ecological/methodological factors that may have contributed to my lack of significant findings. One is sample size; following the "10 Events per Variable" rule of thumb (Peduzzi et al. 1995; Austin and Steyerberg 2017), I would have ideally surveyed 50-60 sites (i.e., 10 sites per each of the 5 site metrics) to ensure sufficient statistical power for my logistic regression, rather than just 33. While I tried to account for the limited number of sample sites by using LASSO regression, the fact that no variables were retained suggests that any real relationships between the metrics I measured would be very subtle, even with enough sites. Another possibility is that I simply did not measure the environmental metrics that directly predict endophyte prevalence. While the metrics chosen are important indicators of site health (Pyke et al. 2002; Adams et al. 2016), they serve more as proxy indicators, rather than direct measurements of disturbance. I did not directly measure metrics such as grazing pressure or soil moisture; both of which have been shown to have more direct relationships with endophyte prevalence (Koh et al. 2006; Saona et al. 2010; Rudgers et al. 2016). It is also possible some unmeasured background variable (e.g., altitude, climate; Bazely et al. 2007; Semmartin et al. 2015) was confounding and masking any significant patterns. For example, Saona et al. (2010) observed that, while grazing and nutrient levels significantly affected endophyte prevalence when soil moisture was low, neither factor showed an effect when soil moisture was adequate.

3.4.1 Conclusions and future directions

This study set out to assess whether the prevalence of fungal endophytes in rough fescue populations is associated with particular indicators of environmental stress and anthropogenic disturbance. Although I did not identify any significant relationships, I did observe consistently high levels of endophyte infection in both *F. hallii* and *F. campestris.* My assumption was that, because *Epichloë* symbioses are usually tightly regulated by the host grass (Saona et al. 2010; Gibert et al. 2015; Lee et al. 2021; Newman et al. 2022), I would see some indication of endophyte infection levels responding to some measurable indicator of disturbance at my sites (Casler and Waldron 2023). Herbivory pressure and soil moisture have already been linked to endophyte prevalence in wild rough fescue populations (Rudgers et al. 2016), however my research was unable to find any such relationships. Further studies, that directly compare the fitness and persistence of infected vs. uninfected grasses in disturbed environments (e.g., Kannadan and Rudgers 2008; Faeth 2009; Chen et al. 2017) would be necessary to draw any conclusions about the role these endophytes might play.

References

Adams, B., G. Ehlert, C. Stone, M. Alexander, D. Lawrence, M. Willoughby, D. Moisey, C. Hincz, A. Burkinshaw, J. Richman, C. DeMaere, T. Kupsch, T. France, T. Broadbent, L. Blonski, A. Miller, and K. France (2016). *Rangeland Health Assessment for Grassland, Forest and Tame Pasture.*

Afkhami, M. E. and J. A. Rudgers (2008). Symbiosis Lost: Imperfect Vertical Transmission of Fungal Endophytes in Grasses. The American Naturalist 172.3, pp. 405–416. DOI: `10.1086/589893`.

Afkhami, M. E. and S. Y. Strauss (2016). Native fungal endophytes suppress an exotic dominant and increase plant diversity over small and large spatial scales. Ecology 97.5, pp. 1159–1169. DOI: `10.1890/15-1166.1`.

Alberta Parks (2025). *History - Glenbow Ranch Provincial Park — Alberta Parks.* URL: `https://www.albertaparks.ca/parks/kananaskis/glenbow-ranch-pp/information-facilities/history`.

Auer, P. and D. and Gervini (2008). Choosing Principal Components: A New Graphical Method Based on Bayesian Model Selection. Communications in Statistics - Simulation and Computation 37.5, pp. 962–977. DOI: `10.1080/03610910701855005`.

Austin, P. C. and E. W. Steyerberg (2017). Events per variable (EPV) and the relative performance of different strategies for estimating the out-of-sample validity of logistic regression models. Statistical Methods in Medical Research 26.2, pp. 796–808. DOI: `10.1177/0962280214558972`.

Bacon, C. W. (1993). Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. Agriculture, Ecosystems & Environment. Acremonium/Grass Interactions 44.1, pp. 123–141. DOI: `10.1016/0167-8809(93)90042-N`.

Bastías, D. A. and P. E. Gundel (2022). Plant stress responses compromise mutualisms with Epichloë endophytes. Journal of Experimental Botany 74.1, pp. 19–23. DOI: `10.1093/jxb/erac428`.

Bastías, D. A., S. Prakash, Y. Morozova, W. J. Mace, J. Sprosen, W. Simpson, and R. D. Johnson (2024). *Epichloë* endophytes can alleviate water deficit effects on perennial ryegrass through host morpho-physiological modulation. Environmental and Experimental Botany 226, p. 105927. DOI: `10.1016/j.envexpbot.2024.105927`.

Bazely, D. R., J. P. Ball, M. Vicari, A. J. Tanentzap, M. Bérenger, T. Rakocevic, and S. Koh (2007). Broad-scale geographic patterns in the distribution of vertically-transmitted, asexual endophytes in four naturally-occurring grasses in Sweden. Ecography 30.3, pp. 367–374. DOI: `10.1111/j.0906-7590.2007.04985.x`.

Bengtsson, J., J. M. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. J. O'Farrell, H. G. Smith, and R. Lindborg (2019). Grasslands—more important for ecosystem services than you might think. Ecosphere 10.2, e02582. DOI: `10.1002/ecs2.2582`.

Bork, E., W. Willms, S. Tannas, and M. Alexander (2012). Seasonal Patterns of Forage Availability in the Fescue Grasslands Under Contrasting Grazing Histories. Rangeland Ecology & Management 65.1, pp. 47–55. DOI: `10.2111/REM-D-11-00087.1`.

Casler, M. D. and B. L. Waldron (2023). Endophytic Fungal Infection of Meadow Fescue in the Driftless Area of the Upper Mississippi River Valley: Impacts on Agronomic Fitness. Grasses 2.4, pp. 263–275. DOI: `10.3390/grasses2040019`.

Chen, W., L. Jiang, R. Jia, B. Tang, H. Jiang, Y. Wang, X. Lu, J. Su, and Y. Bai (2024). Plant litter loss exacerbates drought influences on grasslands. New Phytologist 241.1, pp. 142–153. DOI: `10.1111/nph.19374`.

Chen, W., H. Liu, Wurihan, Y. Gao, S. D. Card, and A. Ren (2017). The advantages of endophyte-infected over uninfected tall fescue in the growth and pathogen resistance are counteracted by elevated CO2. Scientific Reports 7.1, p. 6952. DOI: `10.1038/s41598-017-07183-y`.

Clay, K. and C. Schardl (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. The American Naturalist 160 Suppl 4, S99–S127. DOI: `10.1086/342161`.

Collinson, N. P., K. Giri, J. Kaur, G. Spangenberg, M. Malipatil, R. C. Mann, and I. Valenzuela (2024). Evaluating the Effects of Epichloë Fungal Endophytes of Perennial Ryegrass on the Feeding Behaviour and Life History of Rhopalosiphum padi. Insects 15.10, p. 744. DOI: `10.3390/insects15100744`.

Colón Carrión, N., C. L. Troche, and A. E. Arnold (2022). Communities of endophytic fungi in a Puerto Rican rainforest vary along a gradient of disturbance due to Hurricane Maria. Ecology and Evolution 12.12, e9618. DOI: `10.1002/ece3.9618`.

Desserud, . and M. A. Naeth (2013a). Natural Recovery of Rough Fescue (*Festuca hallii* (Vasey) Piper) Grassland After Disturbance by Pipeline Construction in Central Alberta, Canada. Natural Areas Journal 33.1, pp. 91–98. DOI: `10.3375/043.033.0111`.

Desserud, P., C. C. Gates, B. Adams, and R. D. Revel (2010). Restoration of foothills rough fescue grassland following pipeline disturbance in southwestern Alberta. Journal of Environmental Management 91.12, pp. 2763–2770. DOI: `10.1016/j.jenvman.2010.08.006`.

Desserud, P. A. and M. A. Naeth (2013b). Promising results in central Alberta with rough fescue (*Festuca hallii*) seeding following disturbance. Native Plants Journal 14.1, pp. 25–32. DOI: `10.3368/npj.14.1.25`.

Deutsch, E. S., E. W. Bork, and W. D. Willms (2010). Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. Agriculture, Ecosystems & Environment 135.1, pp. 1–9. DOI: `10.1016/j.agee.2009.08.002`.

Elsinger, M. E., A. Dhar, and M. A. Naeth (2023). Plains rough fescue grassland restoration using natural regeneration after pipeline disturbances. Restoration Ecology 31.3, e13703. DOI: `10.1111/rec.13703`.

Faeth, S. H. (2009). Asexual fungal symbionts alter reproductive allocation and herbivory over time in their native perennial grass hosts. The American Naturalist 173.5, pp. 554–565. DOI: `10.1086/597376`.

Finch, S. C., C. G. L. Pennell, J. W. F. Kerby, and V. M. Cave (2016). Mice find endophyte-infected seed of tall fescue unpalatable – implications for the aviation industry. Grass and Forage Science 71.4, pp. 659–666. DOI: `10.1111/gfs.12203`.

Gibert, A., D. Magda, and L. Hazard (2015). Interplay between Endophyte Prevalence, Effects and Transmission: Insights from a Natural Grass Population. PLOS ONE 10.10. Ed. by G. Berg, e0139919. DOI: `10.1371/journal.pone.0139919`.

Glenbow Ranch Park Foundation (2018). *Glenbow Ranch Provincial Park.* URL: `https://grpf.ca/wp-content/uploads/2020/07/2018_Glenbow_Ranch_Web_English.pdf`.

Government of Alberta (2016). *Rangeland health assessment for grassland, forest and tame pasture.* URL: `https://open.alberta.ca/publications/9781460127858/resource/2cadf8f4-ef65-4e48-812e-10f8daf027a8`.

Granath, G., M. Vicari, D. R. Bazely, J. P. Ball, A. Puentes, and T. Rakocevic (2007). Variation in the abundance of fungal endophytes in fescue grasses along altitudinal and grazing gradients. Ecography 30.3, pp. 422–430. DOI: `10.1111/j.0906-7590.2007.05027.x`.

Grilz, P. L., J. T. Romo, and J. A. Young (1994). Comparative Germination of Smooth Brome and Plains Rough Fescue. The Prairie Naturalist 26.2, pp. 157–170.

Harris, J. K. (2021). Primer on binary logistic regression. Family Medicine and Community Health 9.Suppl 1. DOI: `10.1136/fmch-2021-001290`.

Hewitt, K. G., A. J. Popay, R. W. Hofmann, and J. R. Caradus (2021). *Epichloë* - a lifeline for temperate grasses under combined drought and insect pressure. Grass Research 1.1, pp. 1–12. DOI: `10.48130/GR-2021-0007`.

High Range Ecological Consultants (2008). *Glenbow Ranch Provincial Park 2007 vegetation and range resource inventory and development of range management recommendations.* Tech. rep. Alberta Tourism, Parks and Recreation.

Jensen, J. B., V. T. González, D. U. Guevara, T. V. Bhuvaneswari, P. R. Wäli, M. V. Tejesvi, A. M. Pirttilä, D. Bazely, M. Vicari, and K. A. Bråthen (2011). Kit for detection of fungal endophytes of grasses yields inconsistent results. Methods in Ecology and Evolution 2.2, pp. 197–201. DOI: `10.1111/j.2041-210X.2010.00066.x`.

Johnson, L. J., A. C. M. de Bonth, L. R. Briggs, J. R. Caradus, S. C. Finch, D. J. Fleetwood, L. R. Fletcher, D. E. Hume, R. D. Johnson, A. J. Popay, B. A. Tapper, W. R. Simpson, C. R. Voisey, and S. D. Card (2013). The exploitation of epichloae endophytes for agricultural benefit. Fungal Diversity 60.1, pp. 171–188. DOI: `10.1007/s13225-013-0239-4`.

Johnston, A. and M. D. MacDonald (1967). Floral initiation and seed production in festuca scabrella torr. Canadian Journal of Plant Science 47.5, pp. 577–583. DOI: `10.4141/cjps67-100`.

Jolliffe, I. T. and J. Cadima (2016). Principal component analysis: a review and recent developments. Philosophical transactions. Series A, Mathematical, physical, and engineering sciences 374.2065, p. 20150202. DOI: `10.1098/rsta.2015.0202`.

Kannadan, S. and J. A. Rudgers (2008). Endophyte symbiosis benefits a rare grass under low water availability. Functional Ecology 22.4, pp. 706–713. DOI: `10.1111/j.1365-2435.2008.01395.x`.

Kassambara, A. (2018). *Logistic Regression Assumptions and Diagnostics in R.* Accessed: 2025-10-08. STHDA. URL: `https://www.sthda.com/english/articles/36-classification-methods-essentials/148-logistic-regression-assumptions-and-diagnostics-in-r/`.

Kershaw, L. and L. Allen (2020). *Vascular Flora of Alberta: An Illustrated Guide*. Vol. 1. Alberta, Canada: Linda Kershaw. ISBN: 1-77724-410-2.

Koh, S., M. Vicari, J. P. Ball, T. Rakocevic, S. Zaheer, D. S. Hik, and D. R. Bazely (2006). Rapid detection of fungal endophytes in grasses for large-scale studies. Functional Ecology 20.4, pp. 736–742. DOI: `10.1111/j.1365-2435.2006.01150.x`.

Koh, S. and D. S. Hik (2007). Herbivory Mediates Grass–Endophyte Relationships. Ecology 88.11, pp. 2752–2757. DOI: `10.1890/06-1958.1`.

Krzic, M., S. F. Lamagna, R. F. Newman, G. Bradfield, and B. M. Wallace (2014). Long-term grazing effects on rough fescue grassland soils in southern British Columbia. Canadian Journal of Soil Science 94.3, pp. 337–345. DOI: `10.4141/cjss2013-019`.

Kuldau, G. and C. Bacon (2008). Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. Biological Control. Special Issue: Endophytes 46.1, pp. 57–71. DOI: `10.1016/j.biocontrol.2008.01.023`.

Landsberg, J., C. D. James, S. R. Morton, W. J. Müller, and J. Stol (2003). Abundance and composition of plant species along grazing gradients in Australian rangelands. Journal of Applied Ecology 40.6, pp. 1008–1024. DOI: `10.1111/j.1365-2664.2003.00862.x`.

Lee, K., A. Missaoui, K. Mahmud, H. Presley, and M. Lonnee (2021). Interaction between Grasses and Epichloë Endophytes and Its Significance to Biotic and Abiotic Stress Tolerance and the Rhizosphere. Microorganisms 9.11, p. 2186. DOI: `10.3390/microorganisms9112186`.

Looman, J. (1969). The Fescue grasslands of Western Canada. Vegetatio 19.1, pp. 128–145. DOI: `10.1007/BF00259007`.

Newman, J. A., S. Gillis, and H. A. Hager (2022). Costs, Benefits, Parasites and Mutualists: The Use and Abuse of the Mutualism–Parasitism Continuum Concept for “Epichloë” Fungi. Philosophy, Theory, and Practice in Biology 14.0. DOI: `10.3998/ptpbio.2103`.

Otfinowski, R., H. G. Pinchbeck, and P. A. Sinkins (2017). Using Cattle Grazing to Restore a Rough Fescue Prairie Invaded by Kentucky Bluegrass. Rangeland Ecology & Management 70.3, pp. 301–306. DOI: `10.1016/j.rama.2016.10.008`.

Palit, R., J. F. Cahill, and E. G. Lamb (2012). Irrigation but not N fertilization enhances seedhead density in plains rough fescue (*Festuca hallii*). Grass and Forage Science 68.1, pp. 120–124. DOI: `10.1111/j.1365-2494.2012.00878.x`.

Peduzzi, P., J. Concato, A. R. Feinstein, and T. R. Holford (1995). Importance of events per independent variable in proportional hazards regression analysis II. Accuracy and precision of regression estimates. Journal of Clinical Epidemiology 48.12, pp. 1503–1510. DOI: `10.1016/0895-4356(95)00048-8`.

Pereira, E., B. R. Vázquez de Aldana, L. San Emeterio, and I. Zabalgogeazcoa (2019). A Survey of Culturable Fungal Endophytes From Festuca rubra subsp. pruinosa, a Grass From Marine Cliffs, Reveals a Core Microbiome. Frontiers in Microbiology 9, p. 3321. DOI: `10.3389/fmicb.2018.03321`.

Popay, A. J., N. R. Cox, A. J. Popay, and N. R. Cox (2021). Costs and benefits of hosting *Epichloë* endophytes: a comparison of three haplotypes in *Lolium perenne* with contrasting effects on two herbivores. Grass Research 1.1, pp. 1–10. DOI: `10.48130/GR-2021-0008`.

Pyke, D. A., J. E. Herrick, P. Shaver, and M. Pellant (2002). Rangeland Health Attributes and Indicators for Qualitative Assessment. Journal of Range Management 55.6, pp. 584–597. DOI: `10.2307/4004002`. JSTOR: `4004002`.

Pyle, L., L. M. Hall, and E. W. Bork (2018). Linking management practices with range health in northern temperate pastures. Canadian Journal of Plant Science 98.3, pp. 657–671. DOI: `10.1139/cjps-2017-0223`.

R Core Team (2024). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. URL: `https://www.R-project.org/`.

Rad, J. E., M. Manthey, and A. Mataji (2009). Comparison of plant species diversity with different plant communities in deciduous forests. International Journal of Environmental Science & Technology 6.3, pp. 389–394. DOI: `10.1007/BF03326077`.

Ranstam, J. and J. A. Cook (2018). LASSO regression. British Journal of Surgery 105.10, p. 1348. DOI: `10.1002/bjs.10895`.

Reilly, D. and B. MacKay (2013). "Annotating ecology: looking to biological fieldwork for mobile spatial annotation workflows". In: *Proceedings of the 15th international conference on Human-computer interaction with mobile devices and services*. MobileHCI '13. New York, NY, USA: Association for Computing Machinery, pp. 35–44. ISBN: 978-1-4503-2273-7. DOI: `10.1145/2493190.2493245`.

Rodriguez, R. J., J. F. White Jr, A. E. Arnold, and R. S. Redman (2009). Fungal endophytes: diversity and functional roles. New Phytologist 182.2, pp. 314–330. DOI: `10.1111/j.1469-8137.2009.02773.x`.

Rudgers, J. A., M. E. Afkhami, M. A. Rúa, A. J. Davitt, S. Hammer, and V. M. Huguet (2009). A fungus among us: broad patterns of endophyte distribution in the grasses. Ecology 90.6, pp. 1531–1539. DOI: `10.1890/08-0116.1`.

Rudgers, J. A., R. A. Fletcher, E. Olivas, C. A. Young, N. D. Charlton, D. E. Pearson, and J. L. Maron (2016). Long-term ungulate exclusion reduces fungal symbiont prevalence in native grasslands. Oecologia 181.4, pp. 1151–1161. DOI: `10.1007/s00442-016-3620-7`.

Rudgers, J. A., T. E. X. Miller, S. M. Ziegler, and K. D. Craven (2012). There are many ways to be a mutualist: Endophytic fungus reduces plant survival but increases population growth. Ecology 93.3, pp. 565–574. JSTOR: `23143944`.

Saikkonen, K., S. H. Faeth, M. Helander, and T. J. Sullivan (1998). FUNGAL ENDOPHYTES: A Continuum of Interactions with Host Plants. Annual Review of Ecology and Systematics 29.1, pp. 319–343. DOI: `10.1146/annurev.ecolsys.29.1.319`.

Saona, N. M., B. R. Albrectsen, L. Ericson, and D. R. Bazely (2010). Environmental stresses mediate endophyte–grass interactions in a boreal archipelago. Journal of Ecology 98.2, pp. 470–479. DOI: `10.1111/j.1365-2745.2009.01613.x`.

Saunders, M., A. E. Glenn, and L. M. Kohn (2010). Exploring the evolutionary ecology of fungal endophytes in agricultural systems: using functional traits to reveal mechanisms in community processes. Evolutionary Applications 3.5-6, pp. 525–537. DOI: `10.1111/j.1752-4571.2010.00141.x`.

Schardl, C. L., S. Florea, J. Pan, P. Nagabhyru, S. Bec, and P. J. Calie (2013). The epichloae: alkaloid diversity and roles in symbiosis with grasses. Current opinion in plant biology 16.4, 10.1016/j.pbi.2013.06.012. DOI: `10.1016/j.pbi.2013.06.012`.

Semmartin, M., M. Omacini, P. E. Gundel, and I. M. Hernández-Agramonte (2015). Broad-scale variation of fungal-endophyte incidence in temperate grasses. Journal of Ecology 103.1, pp. 184–190. DOI: `10.1111/1365-2745.12343`.

Sinkins, P. A. and R. Otfinowski (2012). Invasion or retreat? The fate of exotic invaders on the northern prairies, 40 years after cattle grazing. Plant Ecology 213.8, pp. 1251–1262. DOI: `10.1007/s11258-012-0083-8`.

Stout, D. G., A. McLean, and D. A. Quinton (1981). Growth and Phenological Development of Rough Fescue in Interior British Columbia. Journal of Range Management 34.6, pp. 455–459. DOI: `10.2307/3898097`. JSTOR: `3898097`.

Stover, H. J., M. A. Naeth, and S. R. Wilkinson (2018). Transplanting Following Non-Native Plant Control in Rocky Mountain Foothills Fescue Grassland Restoration. Ecological Restoration 36.1, pp. 19–27.

Tannas, K. (2003). *Common Plants of the Western Rangelands - Volume 1: Grasses and Grass-like Species*. Vol. 1. Edmonton, Alberta: Olds College. Alberta Agriculture, Food and Rural Development.

Tannas, S. (2013). *Glenbow Ranch Provincial Park 2013 Vegetation and Range Resource Inventory.* Tech. rep. Cremona, AB: Tannas Conservation Services.

Tannas, S., D. B. Hewins, and E. W. Bork (2015). Isolating the role of soil resources, defoliation, and interspecific competition on early establishment of the late successional bunchgrass *Festuca campestris*. Restoration Ecology 23.4, pp. 366–374. DOI: `10.1111/rec.12207`.

Vikuk, V., C. A. Young, S. T. Lee, P. Nagabhyru, M. Krischke, M. J. Mueller, and J. Krauss (2019). Infection Rates and Alkaloid Patterns of Different Grass Species with Systemic Epichloë Endophytes. Applied and Environmental Microbiology 85.17, e00465–19. DOI: `10.1128/AEM.00465-19`.

von Cräutlein, M., M. Helander, H. Korpelainen, P. H. Leinonen, B. R. Vázquez de Aldana, C. A. Young, I. Zabalgogeazcoa, and K. Saikkonen (2021). Genetic Diversity of the Symbiotic Fungus Epichloë festucae in Naturally Occurring Host Grass Populations. Frontiers in Microbiology 12. DOI: `10.3389/fmicb.2021.756991`.

Wäli, P. R., J. U. Ahlholm, M. Helander, and K. Saikkonen (2007). Occurrence and Genetic Structure of the Systemic Grass Endophyte Epichloë festucae in Fine Fescue Populations. Microbial Ecology 53.1, pp. 20–29. DOI: `10.1007/s00248-006-9076-2`.

White, S. R., J. F. Cahill Jr., and E. W. Bork (2014). Implications of Precipitation, Warming, and Clipping for Grazing Resources in Canadian Prairies. Agronomy Journal 106.1, pp. 33–42. DOI: `10.2134/agronj2013.0085`.

Willms, W. D. (1988). Response of rough fescue (*Festuca scabrella*) to light, water, temperature, and litter removal, under controlled conditions. Canadian Journal of Botany 66.3, pp. 429–434. DOI: `10.1139/b88-068`.

Willms, W. D., S. Smoliak, and J. F. Dormaar (1985). Effects of Stocking Rate on a Rough Fescue Grassland Vegetation. Journal of Range Management 38.3. DOI: `10.2307/3898970`.

Willms, W. D. and J. Fraser (1992). Growth characteristics of rough fescue (*Festuca scabrella* var. campestris) after three years of repeated harvesting at scheduled frequencies and heights. Canadian Journal of Botany 70.11, pp. 2125–2129. DOI: `10.1139/b92-264`.

Wroe, R., S. Smoliak, and G. Wheeler (2013). *Alberta range plants and their classification.*

Zabalgogeazcoa, I., B. R. Vazquez de Aldana, B. Garcia Criado, and A. Gacia Ciudad (1999). The infection of Festuca rubra by the fungal endophyte Epichloe festucae in Mediterranean permanent grasslands. Grass and forage science 54.1, pp. 91–95.

Chapter 4: Patterns of hybridization and introgression between *F. campestris* and *F. hallii* across Alberta, Canada

> *Although I agree that [the] North American [rough fescue] taxa are at least broadly distinguishable . . . personal experience in both field and herbarium strongly suggests to me that these are rather less discrete.*
>
> — V. L. Harms, *A reconsideration of the nomenclature and taxonomy of the* Festuca altaica *complex in North America* (1985)

Chapter Summary

Hybridization during secondary contact between sympatric populations is a common phenomenon in plants, and can greatly complicate the identification and delineation of co-occurring species. This is particularly true among grasses (Poaceae), which hybridize readily, and often. Only with the advent of more advanced molecular techniques have we begun to discover the true diversity and complexity of the grass family, which, until recently, relied primarily on morphological identification for species delineation, often resulting in obscured or incomplete understandings of species relationships. One such case is the rough fescue grasses (*F. campestris* and *F. hallii*) of western Canada, whose taxonomic relationship has been contended for decades, due to the presence of morphologically ambiguous individuals in areas of range overlap. Hybridization between the two has been theorized, but never confirmed. Using Reduced Representation genome Sequencing (RRS) data from 26 allopatric and sympatric rough fescue populations across Alberta and Saskatchewan, I found that there is extensive and ongoing hybridization between the two species where their ranges overlap. Moreover, I found evidence of *F. campestris* introgression into populations of *F. hallii* previously considered to be allopatric, suggesting historic or long-distance gene flow between the two. These results serve as a cautionary tale against making assumptions about species boundaries in areas of historical overlap. They may also carry practical implications for conservation and restoration, as the occurrence of "hidden hybrids" in allopatric populations may necessitate increased caution when sourcing or transplanting seeds across different regions.

4.1 Introduction

Properly delineating biological species and their ranges is an important and yet elusive endeavour (Barton and Hewitt 1985; Christie et al. 2022)—not only for taxonomists and systematists, but also for the practical purposes of management, conservation, and restoration (Baiakhmetov et al. 2021; Faske et al. 2024). While there are many conceptual clashes that can complicate the definition of a "species", one real-world complication is the ever-shifting nature of different species' ranges and levels of contact. Once-contiguous populations may become geographically or ecologically separated from each other (Barton and Hewitt 1985; Rundle and Nosil 2005; Cozzolino et al. 2022), prompting genetic divergence via selection pressures or natural genetic drift (Hoskin et al. 2005; Lindtke et al. 2014). Without regular gene flow, the populations will continue to diverge, eventually leading to the emergence of distinct species (Rundle and Nosil 2005; Cozzolino et al. 2022; Benítez-Benítez et al. 2025). However, the process of speciation can be interrupted or muddied if the barriers to gene flow are prematurely weakened or removed, resulting in secondary contact between the previously isolated populations (Li et al. 2016; Tomasello et al. 2020). And, when once-contiguous populations are reunited, there are a few possible ecological outcomes (Anderson and Matute 2025).

One outcome of secondary contact is competitive exclusion (i.e. local extinction) of one or both of the species (Germain et al. 2021; Anderson and Matute 2025). Because recently diverged populations often largely share the same general niche (Vallin et al. 2012), their reunion often results in niche overlap and increased competition for space and resources (Pfennig and Pfennig 2010; Beans 2014; Cozzolino et al. 2022). Depending on the nature of this competition, one species may exclude the other by either better competing for the same ecological resources (Konuma and Chiba 2007; Beans 2014; Germain et al. 2021), or by interfering with their reproduction (Kyogoku 2020; Mitchell et al. 2022; Anderson and Matute 2025). Therefore, if both species are to persist after reestablishing contact, some level of adaptation and divergence is expected to reduce the intensity of this competition (Stuart and Losos 2013; Beans 2014; Anderson and Matute 2025).

One means of reducing sympatric competition and promoting increased coexistence is via character displacement (Konuma and Chiba 2007; Germain et al. 2021; Anderson and Matute 2025). That is, the divergence of morphological, physiological, behavioural or developmental traits between species in direct competition with each other (Pfennig and Pfennig 2010; Beans 2014). This divergence may be ecological in nature, including divergence in morphology (Hoskin et al. 2005; Pfennig and Pfennig 2010), growth habit (Beans 2014), or habitat usage (Stuart and Losos 2013) to reduce competition for the same space or resources. Character displacement may also be reproductive in nature (Hoskin et al. 2005; Cozzolino et al. 2022). For example, divergence in the timing of flowering/pollen release in grasses (Petit et al. 1997; Silvertown et al. 2005), shifting call characteristics in frogs (Hoskin et al. 2005), or switching to self-reproduction rather than outcrossing in insect-pollinated plants (Randle et al. 2018). Displacement of some characters may also affect both resource and reproductive overlap (Beans 2014). For example, development of differing floral structures to alleviate competition for the same pollinator can also reduce the chance of cross-pollination between two diverged species (Randle et al. 2018; Cozzolino et al. 2022). Because this differentiation is driven by competition within zones of sympatry but not allopatry (Beans 2014), the result is that sympatric populations end up exhibiting more exaggerated phenotypic differences than allopatric ones. A clear example of this exaggerated differentiation is seen in Stroud et al.'s (2024) study of anoles in south Florida. The two species, which normally occupy the same tree perches in their respective ranges, have shifted both their behaviour and morphology to avoid competition. One species has adapted to more ground-dwelling behaviour, while the other has become more arboreal, and both species have developed more exaggerated limb differences to suit these divergent lifestyles (Stroud et al. 2024). Rapid phenotypic displacements such as these can in turn promote rapid reproductive exclusivity and isolation between populations, further enforcing their divergence (Beans 2014; Andreev et al. 2025). However, if reproductive barriers remain somewhat permeable and neither species displaces the other, secondary contact can also result in hybridization between the overlapping populations (Germain et al. 2021; Anderson and Matute 2025).

The consequences of hybridization between diverged species are varied. While the exchange of genes offers the opportunity to increase diversity and adaptation potential between populations (Baiakhmetov et al. 2021; Sinaga et al. 2024; Faske et al. 2024), it also risks wasting resources and genetic material if hybrids are non-viable or sterile (Petit et al. 1997; Allendorf et al. 2001; Sinaga et al. 2024). Additionally, hybrids may be poorly suited for either parent's niche (Pfennig and Pfennig 2010; Sinaga et al. 2024), reducing overall fitness and adaptive potential of the resulting population (Anderson and Matute 2025; Andreev et al. 2025). As a result, many populations have a variety of prezygotic (e.g., differing behaviour, morphology, phenology) and postzygotic (e.g., hybrid inviability/sterility) mechanisms in place to discourage hybridization and select against hybrids (Petit et al. 1997; Ramsey et al. 2003; Faske et al. 2024). This is not universally true across taxa, however. In fact, in many plant groups, hybridization is not only common, but is in fact a major driver of diversity and speciation (López-Caamal and Tovar-Sánchez 2014; Urfusová et al. 2021; Sinaga et al. 2024).

Stable hybridization among sympatric populations is particularly prevalent in grasses (Baiakhmetov et al. 2021; Sinaga et al. 2024), in part due to their use of wind, rather than insects, as a pollination vector. Grasses typically produce dense clusters of flowers, adapted to effectively pull in and capture as much airborne pollen as possible (Stanley 1999; Friedman and Barrett 2009). They also produce large quantities of pollen that can travel great distances (Qiu et al. 2007; Friedman and Barrett 2009), which increases the likelihood of cross-pollination between different populations and species (Pereira et al. 2023; Stull et al. 2023). Conversely, insect-pollinated plants typically rely on specialized pollinators and/or complex floral architecture to reduce the incidence of heterospecific pollen transfer (Cozzolino et al. 2022; Pereira et al. 2023). Grass hybrids are also generally quite fit (Lindtke et al. 2014; Sinaga et al. 2024), and because many grasses are able to self-fertilize or propagate vegetatively (Stanley 1999), hybrids and parents can persist alongside each other for extended periods (Petit et al. 1997; Sinaga et al. 2024). This in turn increases the likelihood of future hybridization events if gene flow remains unrestricted (Brown et al. 2024; Faske et al. 2024).

Indeed, several foundational grass lineages originated from past hybridization events (Baiakhmetov et al. 2021; Tkach et al. 2020) and hybridization continues to be a major driver of grass diversity and speciation today (Winterfeld et al. 2014; Urfusová et al. 2021; Sinaga et al. 2024). Stable, multi-species hybridization complexes are not uncommon (e.g., Baiakhmetov et al. 2021; Urfusová et al. 2021; Sinaga et al. 2024), and pose significant challenges for identification and classification of species. Indeed, because grass taxonomy historically relied on morphological characteristics to differentiate between species, many hybrids have gone undetected until recently (Tkach et al. 2020; Baiakhmetov et al. 2021), due to the mosaic of intermediate or transgressive morphological traits that hybrids may present (Allendorf et al. 2001; López-Caamal and Tovar-Sánchez 2014). Given the prevalence of grass hybridization in wild populations, it is therefore worth revisiting taxa with disputed classifications to determine if hybridization could be underlying their taxonomic uncertainty (Baiakhmetov et al. 2021).

I posit that the rough fescue grasses *F. campestris* and *F. hallii* are one such case where hybridization has contributed to significant taxonomic uncertainty. These grasses are foundational species in the mesic grasslands of western north America (Pavlick and Looman 1984), where they contribute valuable ecological services in their respective habitats (Anderson 2006a; Anderson 2006b; Desserud and Naeth 2013). The two species are generally separated by geography and habitat, with *F. campestris* occupying the higher elevation foothills/montane regions of southern B.C. and Alberta, and *F. hallii* occurring throughout the lower parkland and grasslands of eastern Alberta, into Saskatchewan (Fig. 4.3; Aiken and Darbyshire 1990; Pavlick and Looman 1984). However, there are regions of range overlap where both species co-occur (Fig. 4.3); likely the result of secondary contact after the advance and retreat of glaciers during the Pleistocene (Qiu et al. 2009; Tomasello et al. 2020; Faske et al. 2024). The two species, along with a third in the rough fescue complex (*F. altaica*) have a contentious taxonomic history; once classified as a single species, *Festuca scabrella*, they have alternatively been described as different forms of the same species (Johnston and Cosby 1966; Aiken and Darbyshire 1990), as subspecies (Harms 1985), and most recently, as three distinct species (Fig. 4.1; Pavlick and Looman 1984). Much of this taxonomic confusion stems from their morphology; while allopatric populations can be reliably differentiated by several morphological characters (Table 4.1), individuals in sympatric populations often possess intermediate or ambiguous traits that make them impossible to classify as one species or the other (Johnston and Cosby 1966; Harms 1985; L. Pyle, E. Bork, and W. Willms, pers. comm.). The existence of these intermediate specimens is strongly suggestive of historic or ongoing hybridization between the two rough fescue species (Winterfeld et al. 2014), which could account for the long-standing uncertainty about their taxonomic classification (Tkach et al. 2020).

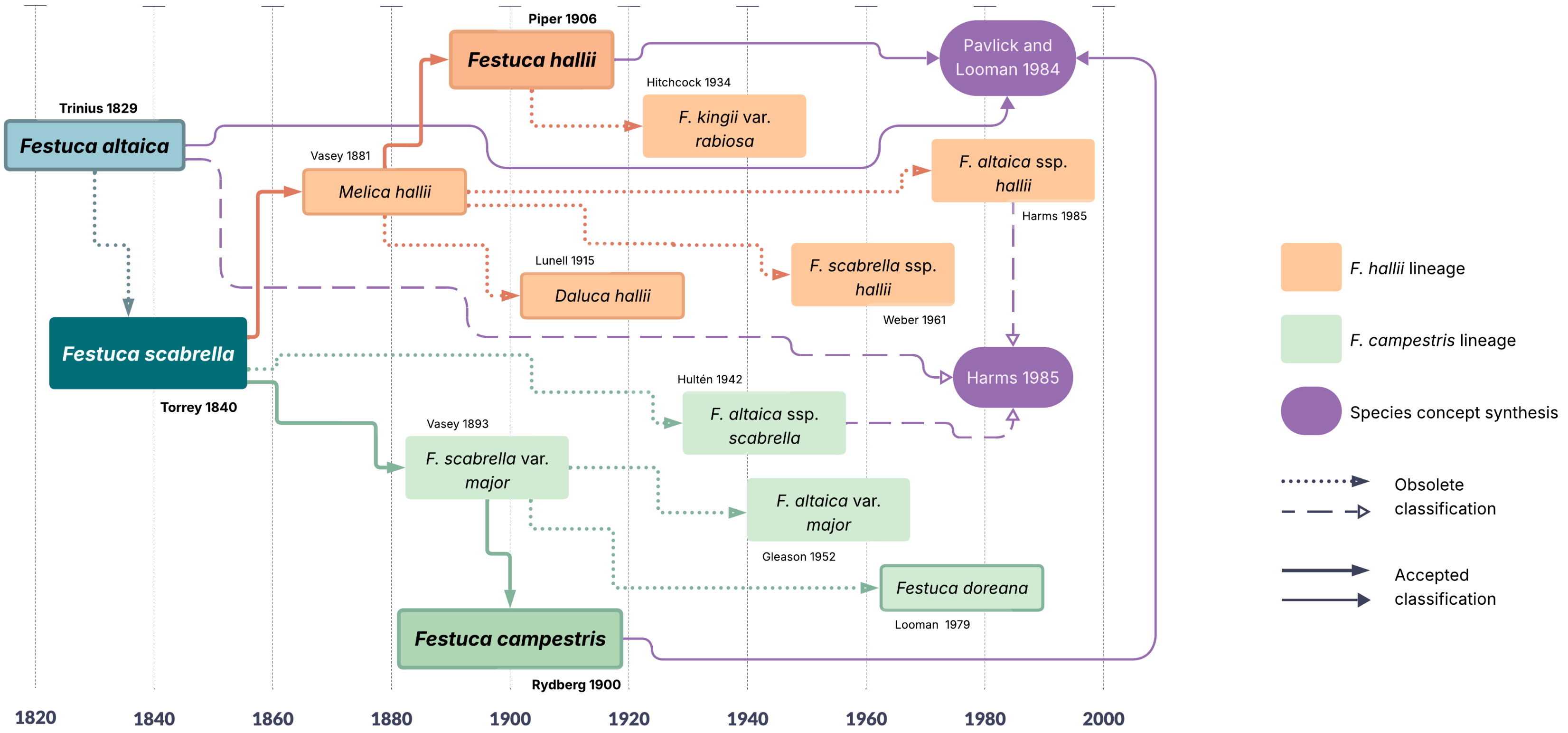

Figure 4.1: A timeline of the convoluted taxonomic history of *Festuca hallii* (orange) and *F. campestris* (green). Along with the previously described *F. altaica* (blue), both species were initially lumped together under the name *F. scabrella* in 1840. Over the following decades, they were variously described as separate species, subspecies, and varieties, but *F. scabrella* remained the most widely used name until the mid-1980s, when two competing species concepts emerged. Harms (1985) proposed classifying all rough fescues as subspecies of *F. altaica*, while Pavlick and Looman (1984) argued they should be recognized as separate species based on chromosomal differences. This latter interpretation is the one used today. Dotted/dashed lines represent proposed but obsolete classifications, while solid lines trace the accepted taxonomy. Purple lines and nodes link the species names used in Harms' (1985) and Pavlick and Looman's (1984) competing species concepts. Small text beside each node indicates the author and year of the proposed taxonomic name. Adapted from Pavlick and Looman 1984, Aiken and Darbyshire 1990, Anderson 2006a, and Anderson 2006b.

Table 4.1: Morphological characteristics commonly used for distinguishing *F. hallii* and *F. campestris* in the field. Adapted from Pavlick and Looman 1984 and Tannas 2013.

	Festuca campestris	***Festuca hallii***
Growth		
Growth form	Densely tufted in large tussocks	Smaller tufts forming mats
Rhizomes	Occasionally present	Frequently present
Culm height (cm)	40–90	25–65
Inflorescence		
No. florets/spikelet	3–7	2–3
Glume ratio	Glumes unequal in length	Glumes nearly equal in length
Glume length	Glumes shorter than florets	Glumes as long as florets

Working under the assumption that these species have not sufficiently diverged to become reproductively isolated, I hypothesized that the two species are actively hybridizing in regions of sympatry, and that the morphologically intermediate specimens found in these regions represent hybrids. I predicted that individuals found in zones of range overlap would show intermediate admixture values between the two parent species, while those in zones of allopatry would show pure parentage.

4.2 Methods

4.2.1 Sampling and Study sites

To determine whether there is ongoing hybridization between *Festuca hallii* and *F. campestris*, I collected samples for genetic sequencing and analysis from nine sites across Alberta and Saskatchewan in July 2023. To choose sampling sites, I first identified four broad zones of interest based on historic distribution information (Pavlick and Looman 1984), occurrence records from iNaturalist (iNaturalist community, 2023), and consultation with provincial taxonomists (Lysandra Pyle, pers. comm.).

Allopatric zones "FH" and "FC" covered areas where *F. hallii* and *F. campestris* are found exclusively; i.e., areas where I could be reasonably confident that I was sampling "pure" parental species (Fig. 4.3; Pavlick and Looman 1984; Shastry et al. 2021). Sympatric zones "GR" and "CH" were areas of overlap between the two species, where ambiguous/intermediate specimens had previously been reported. Zone "GR" encompassed Glenbow Ranch, a provincial park northwest of Calgary that contains a mosaic of remnant *F. hallii* and *F. campestris* populations. Zone "CH" covered Cypress Hills, an inter-provincial park between Alberta and Saskatchewan that is contiguous with *F. hallii's* broader range and hosts an isolated population of *F. campestris* (Fig. 4.3). Within each of the four zones, I chose multiple sampling sites with known rough fescue populations to maximize coverage and potentially identify the geographic extent of hybridization between the two

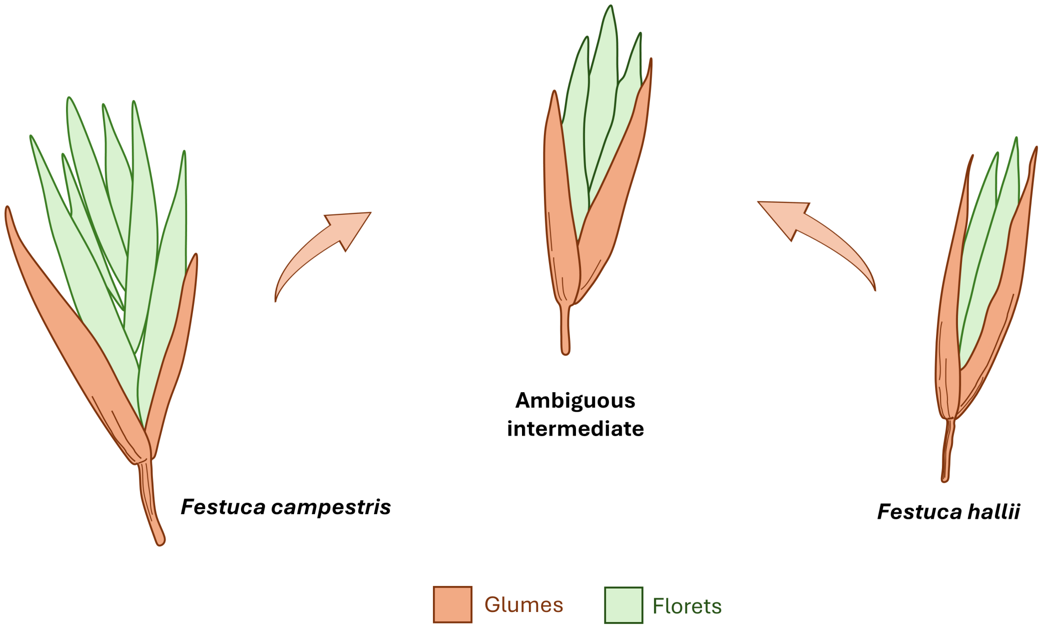

Figure 4.2: Visual comparison of *F. campestris* and *F. hallii*'s growth form and flower cluster (spikelet) differences, vs. an ambiguous intermediate specimen. *F. campestris* have 3–7 flowers (florets) per spikelet, and bracts (glumes) of unequal length, which are shorter than the rest of the flower cluster. *F. hallii* have 2–3 florets per spikelet, with glumes of approximately equal length, that almost fully enclose the flowers within. Ambiguous specimens may possess a mosaic of intermediate traits, e.g., as pictured here: 3 florets, and glumes that are moderately equal in length, but neither enclose nor fully sit below the flower cluster. Colours are for illustrative purposes only.

Table 4.2: Summary of all nine sites where I collected rough fescue samples for sequencing. “Pops.” indicates the number of populations sampled at each site, latitude and longitude provide the general coordinates for each location. Site names are also abbreviated into short codes for ease of reference throughout the manuscript.

Site Name	**Site Code**	**Pops.**	**Latitude**	**Longitude**
Allopatric Zone - *F. campestris*				
Beauvais Lake Provincial Park	BEAU	1	49.408401	-114.110103
Waterton Lakes National Park	WAT	4	49.090663	-113.938166
Allopatric Zone - *F. hallii*				
Donalda Badlands	DON	1	52.584178	-112.553093
Gibbons Prairie-Badlands	GIB	3	53.837549	-113.328884
Kinsella Research Ranch	KIN	3	53.084552	-111.560937
Rumsey Natural Area	RUM	3	51.798050	-112.634729
Sympatric Zone - Cypress Hills				
Cypress Hills Interprovincial Park (Alberta)	CHAB	3	49.623482	-110.345506
Cypress Hills Interprovincial Park (Sask.)	CHSK	3	49.665028	-109.656170
Sympatric Zone - Glenbow Ranch				
Glenbow Ranch Provincial Park	GLEN	5	51.160621	-114.376392

species (Baiakhmetov et al. 2021). In allopatric zones, I simply sampled the presumed parental populations as broadly as possible within the constraints of time and permit access. In sympatric zones, I tried to locate and collect representative samples of both *F. campestris* and *F.hallii*, as well as their putative hybrids. To do this, I sampled multiple individuals that phenotypically resembled either parental species, and/or had been identified as either parental species by previous species inventories (Tannas 2013, C. Lockerbie, pers. comm.). I also collected multiple samples with ambiguous and intermediate morphologies that could not be assigned to either parent species, under the assumption that these specimens were hybrid individuals (Andreev et al. 2025; Sinaga et al. 2024). In total, I sampled 26 different fescue populations across 9 different sites within the 4 zones (Fig. 4.3, Table 4.2).

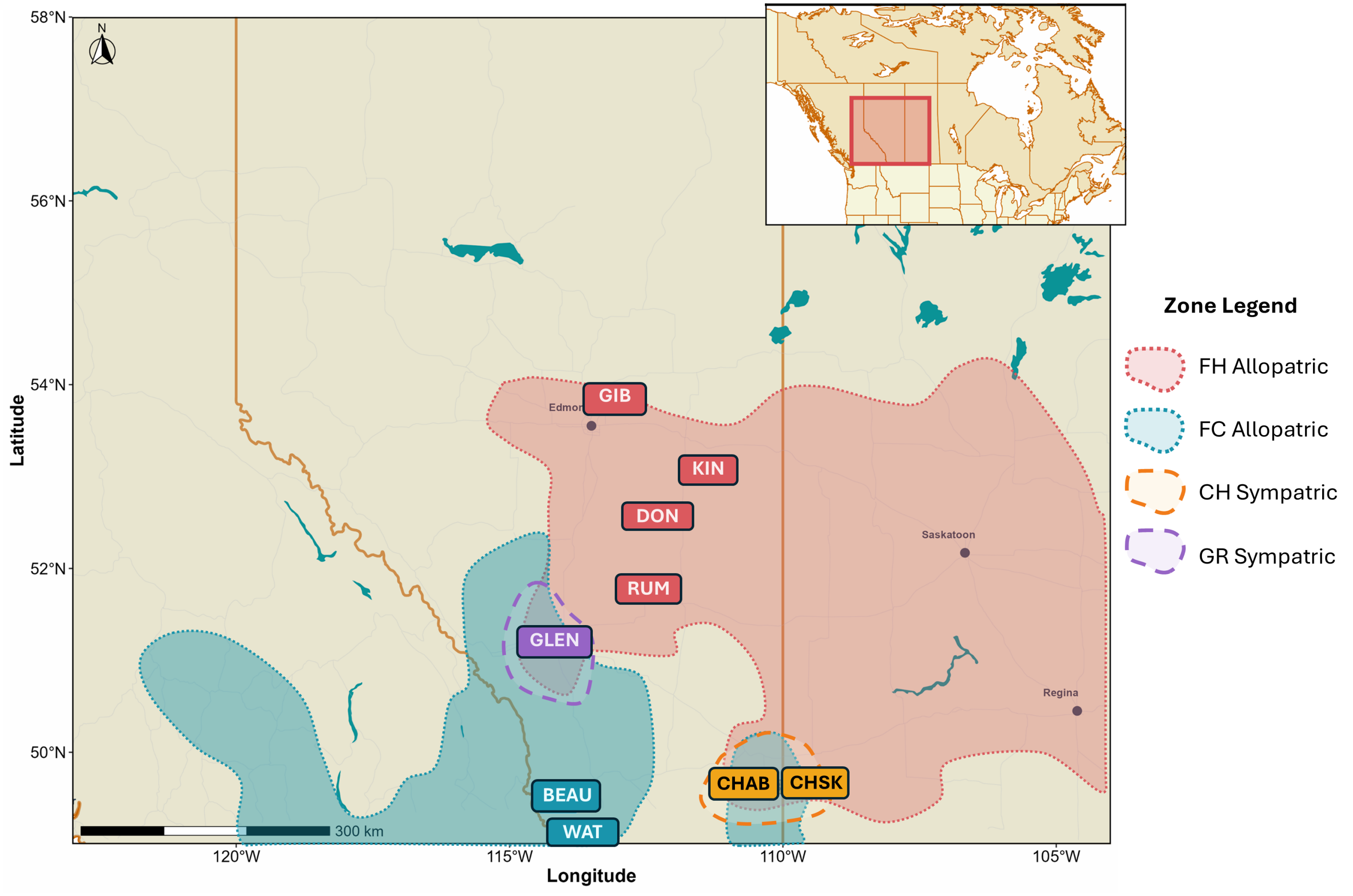

Figure 4.3: I collected rough fescue samples for sequencing from nine sites across Alberta and Saskatchewan in 2023, targeting both allopatric and sympatric zones of occurrence for *F. campestris* and *F. hallii*. Shaded regions denote the approximate ranges for both parental species, adapted from Pavlick and Looman (1984) and iNaturalist occurrence records.

Sample collection and processing

Within each population, I collected material from 3–5 individual grasses for DNA extraction and sequencing. To do so, I pulled 2–3 tillers from the base of the plant, which I wrapped in damp paper towel, placed in a plastic zip-top bag, and stored in a cooler during transport (Čertner et al. 2022). The bags were transferred to a refrigerator and stored for a maximum of 48 hours before being further processed, wherein I peeled away the outer leaves of each tiller to expose the young, fresh tissue of the pseudostem (Center for Plant Conservation 2019), and clipped the bottom of the tiller into short pieces directly into an Eppendorf tube. All tubes were then frozen and stored at -20°C until extraction. After returning from the field, I freeze-dried all samples to further preserve the tissues, and to make tissue disruption/DNA extraction easier and more effective (Karakas et al. 2023). In total, I collected 112 fescue samples across all sampling sites.

DNA extraction and genomic library preparation

In order to identify potential hybrids among my rough fescue samples, I used a reduced representation sequencing (RRS) pipeline to generate genome-wide SNP data for each fescue sample (Peterson et al. 2014; Faske et al. 2024; Meuser et al. 2025). I first extracted genomic DNA from all 112 samples using DNeasy Plant Kits (Qiagen, Germany), according to the manufacturer's instructions ("Mini" plant kit protocol). To first disrupt the plant tissue, I placed $\approx 20\,\mathrm{mg}$ of freeze-dried material from each sample into bead beating tubes preloaded with 3mm stainless steel beads and milled the samples for 3 minutes each using a Fisherbrand™ Bead Mill 4 Mini Homogenizer (Thermo Fisher Scientific, Massachusetts, USA). I then proceeded with the Qiagen DNA extraction protocol as written.

To reduce the size of the sample genomes and prepare them for sequencing, I followed the RRS protocols outlined in Parchman et al. (2012). I double-digested the extracted DNA with EcoRI and MseI restriction enzymes, then ligated adaptors containing custom inline barcodes for each individual to the digested fragments. I then amplified the fragments and annealed Illumina primers to the ends using PCR, which allows the fragments to be recognized and sequenced. I pooled all of the annealed samples together, then size-selected for fragments between 190-290bp using a Sage Science Pippin Prep (Sage Science, Massachusetts, USA), to standardize the fragment lengths and reduce the size of the sequencing template. After confirming that the target fragment sizes had been isolated with automated electrophoresis (Agilent 4200 TapeStation System, Santa Clara, CA), I sent the samples to The Centre for Applied Genomics (TCAG) in Toronto, Canada for sequencing. Sequencing was performed on an Illumina NovaSeq X platform using 2 × 150 bp chemistry, and only the forward read was retained for downstream analysis.

Processing and filtering sequence data

After receiving the sequenced data back from TCAG, I prepared the reads for analysis with the following protocol, based on Meuser et al. (2025). I first demultiplexed the raw FASTQ files using `sabre` (github.com/najoshi/sabre), to sort the jumbled sequence reads and match them to the correct individual (`-m` set to 2 to allow up to 2 barcode mismatches). I then quality checked the parsed sequences using `FASTQC` (Andrews 2010, version 0.11.9) to assess overall data quality before aligning the fragments to a reference genome. I used the Kyuss v2.0 *Lolium perenne* genome assembly (GCF_019359855.2_Kyuss_2.0; Chen et al. 2024) as a reference genome for alignment. Although *L. perenne* is somewhat distantly related to *F. campestris* and *F. hallii* (Catalán et al. 2004), it can and does hybridize with the *Festuca* genus, suggesting reasonable genetic compatibility (Aiken and Darbyshire 1990). Additionally, confamilial species can provide reliable reference genomes for alignment and SNP discovery (Galla et al. 2019), so I felt confident using the Kyuss assembly in this context (Prasad et al. 2022). I indexed the reference genome and aligned my reads using the Burrows-Wheeler Alignment tool (`BWA`; v0.7.19) with the `BWA-MEM` algorithm (Li and Durbin 2009; Li 2013).

After quality checking and aligning the raw reads, I then sorted and processed them using `SAMtools` (Li et al. 2009; Danecek et al. 2021, version 1.15). I called variants using `bcftools mpileup` (Danecek et al. 2021, version 1.19), and converted the resulting BCF file into a VCF file using `bcftools call`, which compiled the called variants into a format that could be filtered and used for downstream analysis. The initial variant call yielded over 1.5 million loci, which I filtered using `VCFtools` (Danecek et al. 2011, version 0.1.16). I set `--max-missing` to 0.8 to retain only the variants present in at least 80% of the samples. I also filtered for a minimum quality score of 20 (`--minQ 20`) and a minor allele count of at least 3 (`--mac 3`), leaving me with 24,932 high-quality variants. I then filtered out individuals with greater than 70% missing data, which retained 97 of the original 112 samples. Finally, I removed rare variants by filtering by minor allele frequency (`--maf 0.05`), removed multi-allelic sites (`--max-alleles 2, --min-alleles 2`), and removed indels (`--remove-indels`), leaving a final dataset of 23,462 variants across 97 fescue samples. Filtering thresholds were informed by similar RRS studies (Omire et al. 2022; Meuser et al. 2025; Pyne et al. 2025) and adjusted as needed to ensure only high-quality samples were retained.

As a final quality control step, I checked that none of my samples were duplicates or clones of one another. I used `PLINK` (v2.00a5.8; Chang et al. 2015) to calculate pairwise identity-by-descent (IBD) as a measure of genetic similarity between individuals. I also used `PLINK` to calculate average call rate, average Minor Allele Frequency (MAF), and Minor Allele Count (MAC) for all of the SNPs retained after filtering. There was no indication of any duplicates (i.e., no $\widehat{\pi}$ values were close to 1; Ellingson and Fardo 2016), so I proceeded with my analysis using all 97 samples.

Identifying hybridization with `ENTROPY`

To assess the number of distinct species within my samples, and whether hybridization was occurring between them, I used `ENTROPY` (v2.0; Shastry et al. 2021) to assess population clustering (k), admixture proportions (q) and inter-source ancestry (Q) for each fescue sample. Admixture proportion (q) represents the fraction of an individual's genome that comes from a given source population. For example, $q = 1$ for Species A would denote a genome entirely derived from Species

A, while $q = 0$ would indicate no ancestry from that species. Meanwhile, q values between 0 and 1 indicate some degree of admixture (i.e., hybridization) between the source populations (Gompert et al. 2014; Shastry et al. 2021). In contrast, inter-source ancestry (Q) looks at patterns of allele inheritance across the genome to determine how recently hybridization occurred (i.e., distinguishing F1 hybrids from later generations or backcrosses; Gompert et al. 2014; Shastry et al. 2021)

To estimate these values, I first converted my filtered VCF file to an MPGL format compatible with `ENTROPY`. This step also generated initial genotype likelihoods and admixture estimates (q), which were used to initialize the Markov Chain Monte Carlo (MCMC) algorithm used by `ENTROPY`. (Gompert et al. 2014). While my assumption was that the fescue samples would sort into just two population clusters (i.e., two different species; $k = 2$), I tested k values 2–9 to make sure I wasn't overlooking other potential clustering patterns in the data. For each value of k, I ran three independent MCMC chains, each consisting of 60,000 iterations, with a burn-in of 20,000 and a thinning rate of 20 (Faske et al. 2024). I assessed how well each value of k fit the data using extracted DIC (Deviance Information Criterion) values, and checked model convergence using the $\hat{\mathrm{R}}$ values and Effective Sample Size (ESS; Gelman and Rubin 1992; Shastry et al. 2021). $k = 2$ had both the best fit and best convergence values (Table 4.3) so I proceeded with my analysis using $k = 2$ as the best-supported population structure. When estimating inter-source ancestry (Q) for each sample, I re-ran `ENTROPY` using the $k = 2$ q values obtained from the first run to initialize the MCMC chains (Shastry et al. 2021).

To classify samples as pure parental species or as hybrids, I identified all individuals where the 95% confidence intervals for admixture (q) estimates overlapped with 0 (for *F. campestris*) or 1 (for *F. hallii*). All other individuals with intermediate values were classified as hybrids (McFarlane and Pemberton 2019; McFarlane et al. 2020).

Population structure and differences

To determine whether the four sympatric/allopatric zones differed significantly in their admixture composition, I compared the distribution of admixture (q) values across zones using pairwise Kolmogorov-Smirnov (KS) tests (Mandeville et al. 2015). I also explored population structure using Principal Component Analysis (PCA), which is an effective way to reduce the variation from many thousands of genetic markers to visualize population differences across geographic space (Price et al. 2006; Jolliffe and Cadima 2016). To do this, I used the genotype probability matrix produced by `ENTROPY` for $k = 2$, and performed a PCA using the `prcomp` function in `R` (v4.4.1; R Core Team 2024). I used the first two principal components (PCs) as plotting axes, and distinguished samples by site (colour) and zone (symbol) to see if and how the nine sample populations would cluster (Faske et al. 2024).

4.3 Results

After quality control and filtering, 97 individuals remained in my dataset; 15 from Allopatric FC, 42 from Allopatric FH, 24 from Sympatric GR, and 16 from Sympatric CH. 23,462 of the original

Table 4.3: Convergence diagnostics and Deviance Information Criterion (DIC) values for `ENTROPY` runs testing population clusters of $k = 2$–9. Effective Sample Size (ESS) indicates the number of independent samples obtained by the MCMC (higher values = more reliable parameter estimates). The Gelman–Rubin diagnostic ($\hat{R}$) assesses the convergence of the MCMC chains, with values near 1 indicating good convergence. DIC is a measure of model fit, with lower values indicate a better-fitting model.

k	**Effective Sample Size (ESS)**			**Convergence Diagnostic ($\hat{R}$)**			**DIC**
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	
2	70.91	2538.60	1461.63	1	1.02	1.00	6.48E+06
3	7.70	2118.01	469.98	1	1.44	1.02	6.85E+06
4	10.34	2131.87	371.32	1	1.18	1.02	1.54E+07
5	1.46	2397.69	260.62	1	18.73	2.81	1.56E+07
6	3.78	2672.21	336.75	1	1.52	1.03	2.79E+07
7	1.27	2105.07	250.96	1	37.59	3.54	2.66E+07
8	1.41	2279.60	249.91	1	15.29	1.89	1.08E+08
9	1.53	2659.13	260.00	1	17.78	1.81	7.09E+07

Table 4.4: Results of Kolmogorov-Smirnov (K-S) tests comparing the distributions of admixture (q) values among my four allopatric and sympatric zones. D indicates the level of divergence between two populations, with $D = 1$ indicating that the populations do not overlap at all.

Zone 1	**Zone 2**	**D**	**p-value**
Sympatric 1 (GR)	Sympatric 2 (CH)	0.65	1.86E-03
	FH allopatric	0.93	2.47E-14
	FC allopatric	0.93	1.86E-08
Sympatric 2 (CH)	FH allopatric	0.98	2.55E-12
	FC allopatric	1.00	3.99E-08
FH allopatric	FC allopatric	1.00	5.44E-13

1,538,878 SNPs were retained, with an average call rate of 97.42%, average MAF of 0.2172, and average MAC of 41.14.

The genetic variation among my samples was best explained by two distinct population clusters ($k = 2$; Table 4.3), supporting the classification of *F. campestris* and *F. hallii* as distinct species, as asserted by Pavlick and Looman (1984). And, as predicted, the analysis also revealed the occurrence of substantial admixing (i.e., hybridization) occurring between the two species, both in regions of sympatry, and in areas thought to be allopatric (Figs. 4.5 and 4.6)

My genotypic species assignments were consistent, but not completely correlated, with my original phenotypic assignments. I had originally assigned 15 individuals as *F. campestris*, 42 as *F. hallii*, and 40 as hybrids based on phenotypic characteristics in the field. However, after reclassifying species with q CI-overlapping, there were 14 "pure" *F. campestris* (mean $q = 0.999$, SD =

Table 4.5: The number and proportion of samples classified as *F. campestris*, *F. hallii*, or hybrid in each of the four sampling zones. Classifications are based on 95% confidence interval overlap with admixture (q) values of 0 (*F. campestris*) or 1 (*F. hallii*), with intermediate specimens being classified as hybrids.

Zone	***F. campestris***		***F. hallii***		**Hybrids**	
	n	**proportion**	**n**	**proportion**	**n**	**proportion**
FC allopatric	14	0.93	0	0	1	0.07
FH allopatric	0	0	25	0.59	17	0.41
Sympatric 1 (GR)	0	0	1	0.04	23	0.96
Sympatric 1 (CH)	0	0	0	0	16	1

0.001), 26 "pure" *F. hallii* (mean $q = 0.999$, SD = 0.001), and 57 hybrids with admixture values ranging between the two (mean $q = 0.443$, SD = 0.273; Fig. 4.5). 17 samples that I originally classified as *F. hallii* in the FH Allopatric zone turned out to be hybrids, with q scores indicating 4–18% *F. campestris* ancestry. Inter-source ancestry values (Q) for these allopatric hybrids seem to indicate that they are late generation backcrosses with *F. hallii* (Fig. 4.8). In contrast, only one *F. campestris* sample from the FC Allopatric zone was reclassified as a hybrid, with a q score of 89%. Finally, only one sample that was phenotypically classified as a hybrid was reclassified as a pure parent species (Fig. 4.4).

All individuals found in the two sympatric zones were classified as hybrids, except for one *F. hallii* identified at Glenbow Ranch (Fig. 4.6). However, I suspect this individual may be the result of a sample mix-up with the "hybrid" individual found at the allopatric site "GIB", considering that both their admixture values and PCA positions are almost perfectly swapped (Figs. 4.6 and 4.9). Both sympatric zones contained first generation (F1) hybrids (i.e., direct offspring of the two parental species; $Q \approx 1$), in addition to possible first-generation backcrosses ($0.75 < Q < 1$). Additionally, sympatric zone GR appears to have several later-generation backcrosses with *F. campestris* ($0.25 < Q < 0.50$).

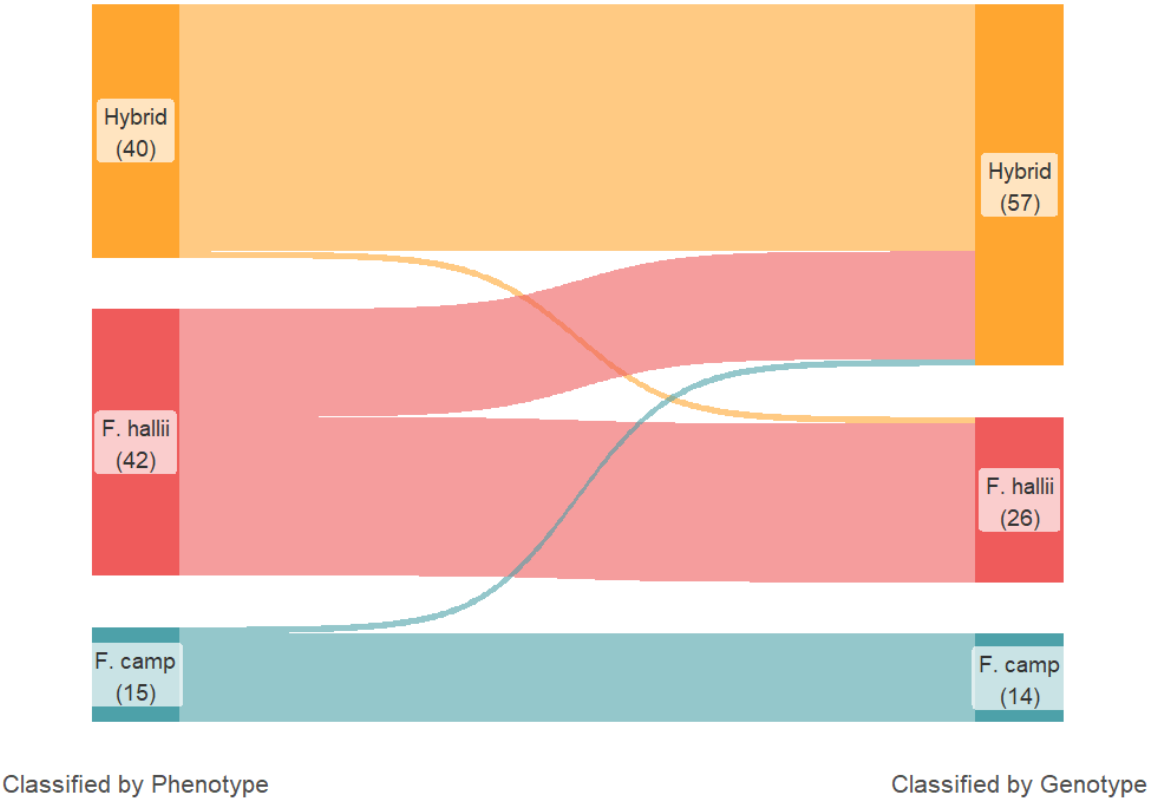

Figure 4.4: Sankey diagram showing rough fescue species reassignments before and after genetic analysis. On the left is a breakdown of how the 97 individuals were originally classified based on morphological characteristics (i.e., phenotype). On the right are the new classifications, based on the ancestry data (q scores) obtained from our genotypic analysis.

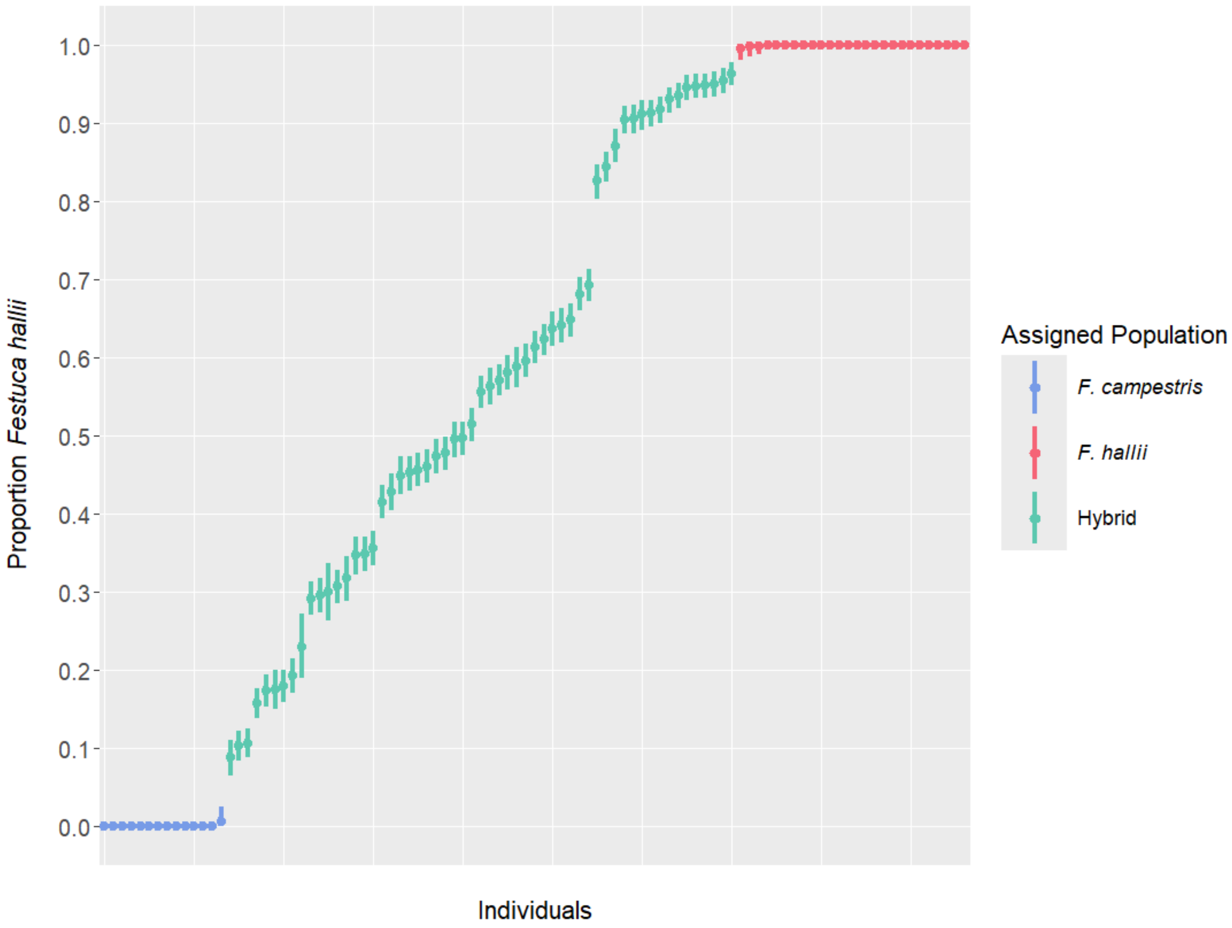

Figure 4.5: Admixture proportions q and 95% confidence intervals for individual grasses across all sample sites using 23,462 SNPs. Individuals were assessed as parental species if their CIs overlapped 0 (*F. campestris*, blue) or 1 (*F. hallii*, red). Otherwise, they were classified as hybrids (green).

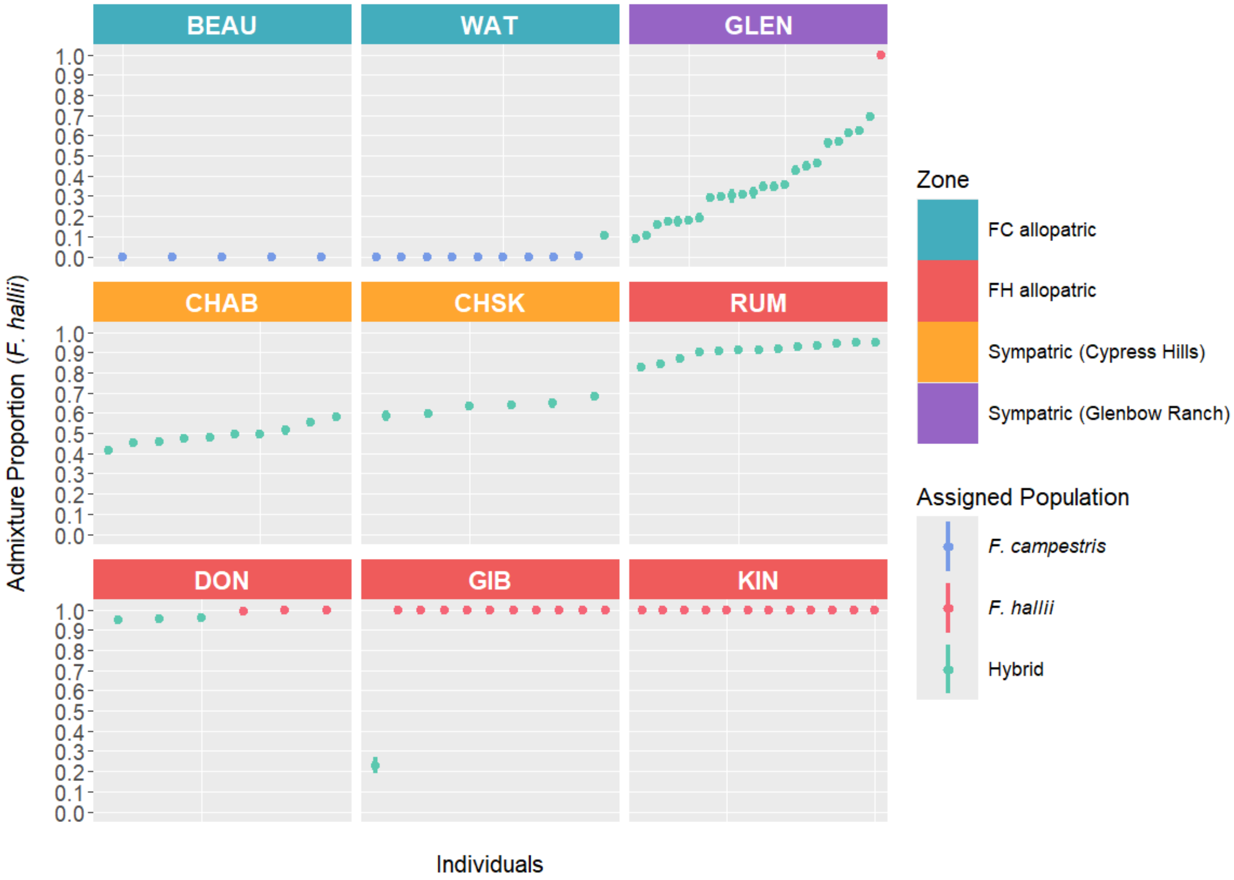

Figure 4.6: Admixture proportions (q) and 95% confidence intervals for 97 grasses sampled across 9 sites show that hybrid individuals (green) were present in both sympatric and allopatric zones.

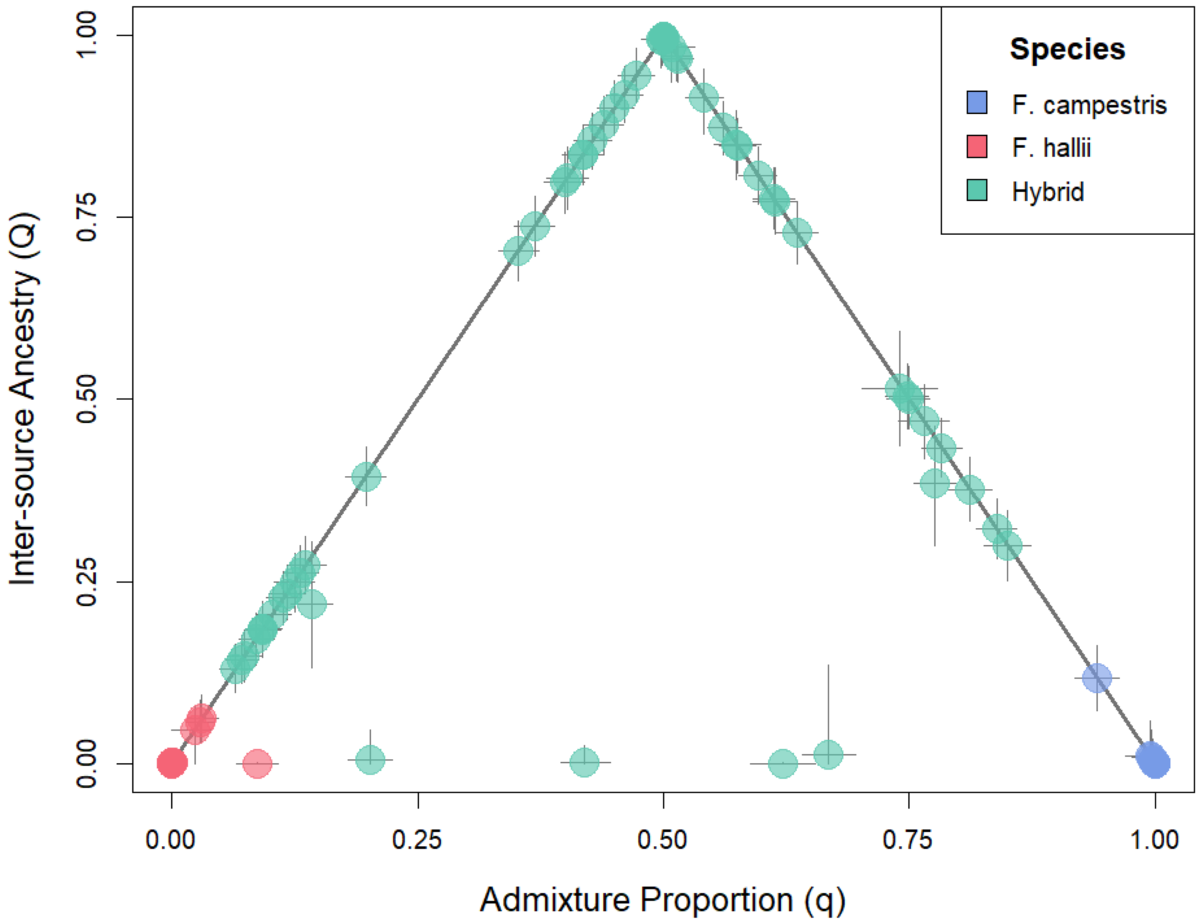

Figure 4.7: Triangle plot of all fescue samples across all zones surveyed. The x-axis shows admixture proportion (q); i.e., the proportion of ancestry from each parental species. The y-axis shows inter-source ancestry (Q); i.e., the recency of hybridization, with values near 1 corresponding to first-generation hybrids

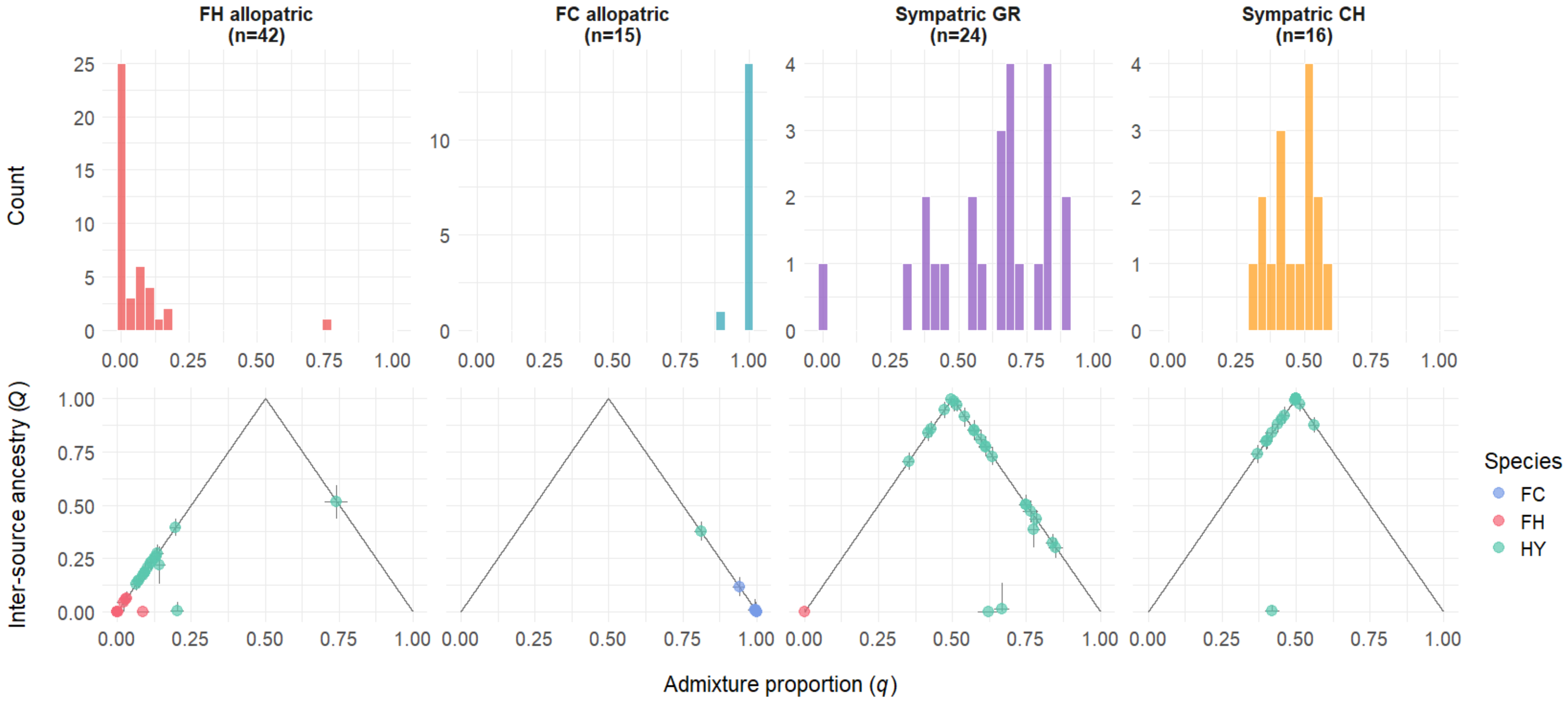

Figure 4.8: Admixture histograms and corresponding triangle plots for each of the four sampling zones. Histograms show the distribution of q values (i.e., the proportion of ancestry from each parental species) for individuals within each zone. In triangle plots, the x-axis shows q, while the y-axis shows inter-source ancestry, Q (i.e., the recency of hybridization), with values near 1 corresponding to first generation hybrids.

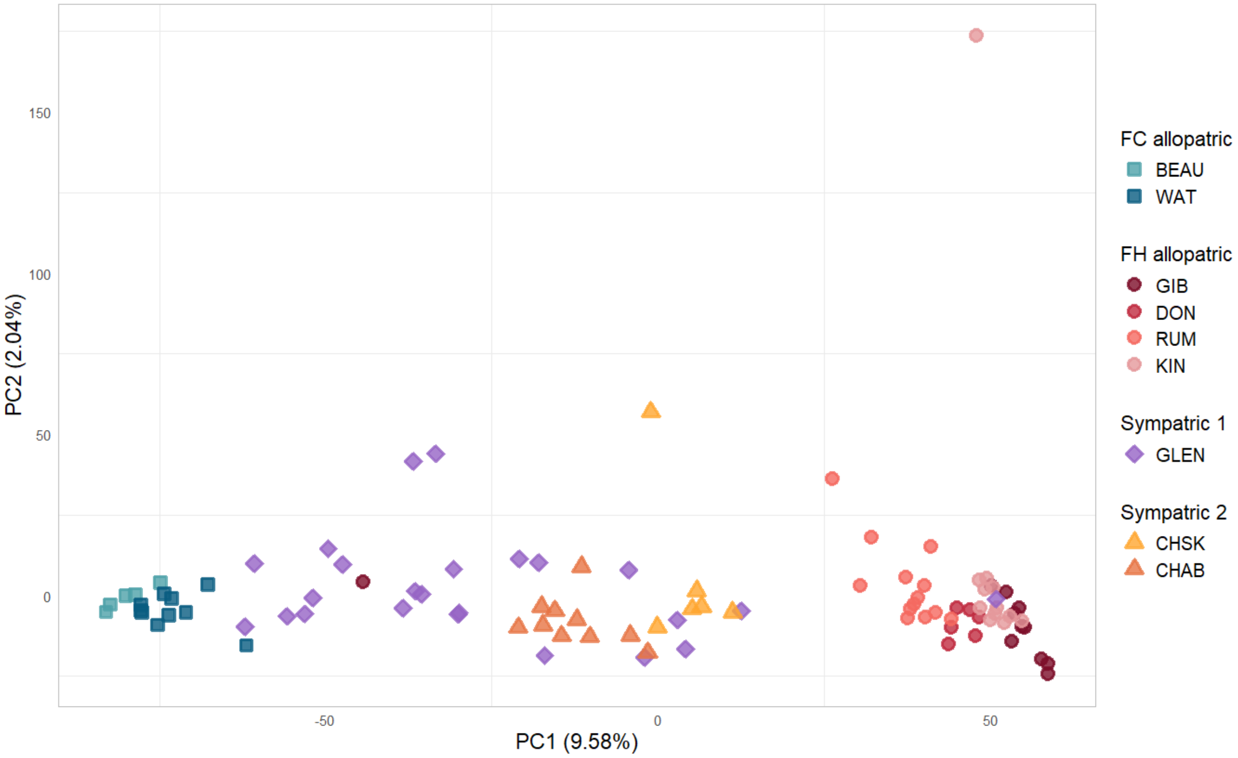

Figure 4.9: Principal Component Analysis of rough fescue genotype likelihood estimates across all sample sites. Genotype likelihoods estimated by `ENTROPY`, $k = 2$ ancestry model.

Populations from the two allopatric zones are clearly very genetically distinct from one another, clustering distinctly at opposite ends of our first PCA axis (PC1 = 9.58% of variation). Meanwhile, individuals from sympatric populations had intermediate genetic composition. Individuals from sympatric zone CH are evenly clustered between the two parental zones, while those from sympatric zone GR are skewed towards and slightly overlapping the *F. campestris* allopatric zone (Fig. 4.9).

4.4 Discussion

I found evidence of extensive hybridization between *F. hallii* and *F. campestris* in two distinct and geographically isolated zones of sympatry (Figs. 4.5 and 4.6). The presence of F1 hybrids in both sympatric zones suggests that hybridization between the two species is recent and ongoing, while the presence of several generations of backcrossed individuals suggests that at least some of these F1 hybrids are fit enough to reproduce with the parental species (Fig. 4.8; Shastry et al. 2021; Abbott 2017).

Hybrids were present not only in the sympatric populations, but in the so-called allopatric populations as well (Fig. 4.6). This is somewhat surprising, as the two species are not reported as co-occurring in the areas I surveyed (Pavlick and Looman 1984, Lysandra Pyle, pers. comm.). The presence of admixed individuals in our allopatric populations could therefore suggest historic contact zones between the two species that have since been disrupted (Nieto Feliner et al. 2023).

Alternatively, this could be the result of long distance cross-pollination between the two species; wind-borne pollen can travel great distances (Robledo-Arnuncio 2011), and gene flow has been observed across disjunct rough fescue populations many kilometres apart from each other (Qiu et al. 2007; Qiu et al. 2009). This could also explain why the two allopatric zones have differing proportions of admixed individuals (Table 4.5). Alberta's prevailing winds run west from the mountains to east over the prairie and parkland regions (Huffman et al. 2021), which would facilitate pollen dispersal from the range of *F. campestris* into the range of *F. hallii* (Mengli et al. 2008). By contrast, the forested landscape and higher elevation of the FC allopatric sites would likely restrict much east-to-west gene flow (Pavlick and Looman 1984; Polato et al. 2017). Also notable is that the highest proportion of hybrids in an allopatric zone was found at site "RUM", which is closest to the GR sympatric zone (Fig. 4.3), while the second-highest was at site "DON" (second-closest to the contact zone; Fig 4.6). This pattern is mirrored in my PCA (Fig. 4.9), which shows a continuous distribution of genotypes between the western *F. campestris* allopatric zone and the two sympatric zones. Conversely, all of the allopatric *F. hallii* populations form a distinct cluster, with no overlap into the sympatric populations. The clustering does not seem to be a matter of geographical distance between populations, as both sympatric zones are as close or closer to the *F. hallii* allopatric region as they are to that of *F. campestris* (Fig. 4.3). However, this pattern could also be related to post-glacial expansion of rough fescue from western refugia (Qiu et al. 2009). To fully explore this seeming west-to-east introgression, future work could sample a more contiguous gradient of rough fescue populations between the west and east, to see if there truly is asymmetric gene flow from one direction to the other (Andreev et al. 2025).

Despite the overall prevalence of hybrids in both sympatric zones, the two did differ significantly in their underlying population structure (Table 4.4; histograms in Fig. 4.8). At Cypress Hills I found first generation hybrids and what appear to be early generation backcrosses, with a narrow concentration of admixture (q) values and high intersource ancestry (Q; Fig. 4.8). This suggests a high number of F1 individuals in this population. Glenbow Ranch on the other hand showed a wider range of mixed ancestry, with several individuals that appear to be late generation backcrosses with *F. campestris*. This divergence in hybrid zone structure is not particularly surprising; Glenbow Ranch PP is located in the west of Alberta, close to existing contiguous populations of *F. campestris*, which would provide greater opportunity for backcrossing. Cypress Hills meanwhile, is more geographically disjunct from populations of either parental species, being a high-elevation plateau surrounded by low-lying prairie (Kulig 1996; Widenmaier and Strong 2010). Additionally, differences in the structure of replicate hybrid zones may be due simply to differences in local environmental conditions (Faske et al. 2024), or the genetic makeup of the initial F1 individuals that kick off hybridization (McFarlane et al. 2024).

While the structural differences between the two sympatric zones are not unusual, the near-absence of parental species within my collected samples is unexpected (Germain et al. 2021; Anderson and Matute 2025). After all, the F1 hybrids found in the sympatric zones could only have come about via relatively recent reproduction between *F. hallii* and *F. campestris*. This could be the result of sampling bias, though this seems unlikely, as I intentionally sought out populations of the parental species in both sympatric zones. It could also indicate that these hybrid populations have been "orphaned" from their parental populations as a result of the intense development and fragmentation the fescue grasslands have been subject to over the past century (Trottier 1992; Holcroft Weerstra 2003; Nieto Feliner et al. 2023). Because rough fescue grasses are long-lived and capable of extensive vegetative reproduction (i.e., cloning; Qiu et al. 2009; de Witte and Stöcklin 2010; Stojanova et al. 2019), it is possible that these hybrid populations have been sustaining themselves for quite some time without significant input from parental populations (Li et al. 2016; Sinaga et al. 2024; Brown et al. 2024).

This kind of hybrid zone—where parental species are rare but hybrids and backcrosses dominate—creates a "unimodal" distribution of admixture ancestry, (Abbott 2017; Nieto Feliner et al. 2023). Unimodal hybrid zones have been documented in other plant systems (e.g., Marques et al. 2014; Li et al. 2016; Andreev et al. 2025), and typically indicate weak reproductive barriers between the parental species (Nieto Feliner et al. 2023; Brown et al. 2024). By contrast, a "bimodal" hybrid zone will contain mostly parental species, with a small number of F1 hybrids (Abbott 2017; Pickup et al. 2019), and a "trimodal" hybrid zone will have roughly equal proportions of parental species and F1s, but few backcrosses (Lindtke et al. 2014; Pickup et al. 2019). Such systems can arise when reproductive barriers between parent species are stronger (Pickup et al. 2019; Brown et al. 2024), and/or there is postzygotic selection against hybrids (Lindtke et al. 2014; Abbott 2017).

The fact that there were many F1s found in my hybrid zones but seemingly no F2s could suggest that the F1s were well adapted to the environmental conditions of the sympatric zones, but that there was some measure of selection acting against subsequent F2 generations (Lindtke et al. 2014; Abbott 2017). I did not measure or take into account selection against hybrids in my data, but it would be worth exploring in future studies, to see if the prevalence of rough fescue hybrids is connected to any particular habitat or environmental characteristics. Especially in transitional habitats between parental ranges, hybrids may have an advantage over their parents by being well-suited to intermediate environmental conditions (Nieto Feliner et al. 2023; Faske et al. 2024). Glenbow Ranch PP in particular contains many transitional ecotones between the western montane habitat of *F. campestris* and the eastern parkland/grassland habitat of *F. hallii* (Tannas 2013), and it would be interesting to see how hybridization between these two species changes across environmental gradients. Future studies should incorporate the collection of climatic data (Andreev et al. 2025), plant "neighbourhood" demographics (Benítez-Benítez et al. 2025), pollen/seed dispersal distances (Qiu et al. 2007), and hybrid fitness/fertility metrics (Abbott 2017) across multiple zones of species overlap to construct a more comprehensive understanding of what factors are promoting the creation and persistence of rough fescue hybrids (McFarlane and Mandeville 2023), while accounting for the large effect that stochasticity has on differences among hybrid zones (McFarlane et al. 2024).

An additional caveat that could explain this asymmetric distribution of hybrid demographics is the difference in ploidy between the two parental species. One of the strongest arguments in favour of classifying *F. hallii* and *F. campestris* as separate species was cytotype (i.e., chromosome count), with the former determined to be tetraploid ($4x = 28$), and the latter being octoploid ($8x = 56$; Pavlick and Looman 1984; Aiken and Gardiner 1991). Differences in ploidy were once assumed to prevent reproduction between species of different cytotypes (Marques et al. 2014; Pezzi et al. 2024; Kolář et al. 2017; Brown et al. 2024). However, we now know that mixed ploidy hybridization is not only possible, but fairly common (Marques et al. 2014; Brown et al. 2024), especially among plants. Within grasses especially, cross-ploidy hybridization is a source of significant diversity and the originator of entire taxonomic lineages (Otto 2007; Urfusová et al. 2021; Brown et al. 2024).

I did not take ploidy differences into account in my analysis because I lacked confirmed chromosome counts for my collected samples, and while the rough fescue cytotypes provided in the literature were useful reference points, they may not be reliable estimates for all populations. In fact, it's possible for a single species to have multiple different ploidy levels, even within the same geographic area (Petit et al. 1997; Kolář et al. 2017; Brown et al. 2024). Therefore, making assumptions about the ploidy of any of my specimens could have been inaccurate (Otto 2007; Kolář et al. 2017; Brown et al. 2024), especially when considering the hybrids, and their potential 5, 6, or $7x$ cytotypes (Brown et al. 2024). Additionally, `ENTROPY` is currently limited to analyzing hexaploids ($6x$) or lower (Shastry et al. 2021), and can produce biased population structure estimates if the exact cytotypes of the input samples are unknown (Gompert and Mock 2017). In hybrid identification studies such as mine, polyploids can effectively be analyzed as diploids (Shastry et al. 2021), because their genomes generally pair and segregate reliably during meiosis, resulting in diploid-like patterns of inheritance (Svačina et al. 2020; Brown et al. 2024). Additionally, `ENTROPY` takes into account allele depth and genotype likelihoods when estimating population structure, which results in robust estimates, even for mixed ploidy datasets (Shastry et al. 2021). If there was an effect of ploidy on ancestry, I would have expected to see discordance between the population structures estimated when looking at admixture values (q; Fig. 4.5) vs. genotype likelihoods (Fig. 4.9). Instead, both show a distinct clustering of two parental populations, with hybrids in a continuous gradient between them, suggesting the two methods have converged on the same underlying population structure (Shastry et al. 2021). It is possible that some of the variation seen along PC2 (y-axis of Fig. 4.9) of the PCA could come from ploidy differences among my samples (Svačina et al. 2020; Shastry et al. 2021), however it does not interfere with the primary variation seen among the four zones along PC1. Therefore, while I am aware of the potential complications of polyploidy and ploidy differences in hybrid systems, I do not think these differences affect my overall conclusions.

Beyond being robust to differences in ploidy, `ENTROPY`'s integration of allele frequencies and genotype likelihoods into its population estimates also helps to reduce the chance of confusing hybridization with Incomplete Lineage Sorting (ILS). ILS occurs when genetic variation present in a common ancestor is passed down to both descendent species, making individuals appear admixed, without actually hybridizing (Schumer et al. 2014; Meyer et al. 2017; Wang et al. 2018). However, that is unlikely to be the case here. If ILS were driving the differentiation seen in these fescue populations, I would expect to see shared alleles spread not just throughout the sympatric populations, but the allopatric as well (Joly et al. 2009). Given that I used over 23,000 variants in my analysis, it is unlikely that ILS alone could produce admixture patterns localized only in the two species' range of overlap (Meyer et al. 2017; Morales-Briones et al. 2018). This, coupled with `ENTROPY'S` use of multiple genetic parameters for its population structure estimates (Shastry et al. 2021) gives me confidence that the patterns seen in my data reflect true hybridization, and not incomplete lineage sorting.

Overall, the discovery of rough fescue hybrids in these sympatric zones is not particularly surprising, given their close relatedness, the propensity of grasses to hybridize, and this region's history as a secondary contact zone (Tomasello et al. 2020; Baiakhmetov et al. 2021; Sinaga et al. 2024). Nevertheless, the confirmation that these two species are actively and easily introgressing does help to shed some light on their contentious taxonomic history. In addition to giving us further insight into these species, this discovery may also carry practical implications when it comes to future restoration and conservation efforts. Until we know more about the fertility of these hybrids and to what environments they may best be suited, care should be taken when sourcing seeds to reduce potential contamination of admixed populations into allopatric regions (Mengli et al. 2008). Additionally, it would be worthwhile exploring the full geographical extent of the species hybridization, so that we know which species we're actually dealing with in what part of the province. Overall, restoration of a hybridizing species needs special consideration due to the potential for extinction-via-hybridization (Rhymer and Simberloff 1996; Anderson and Matute 2025) and thus, these findings should be considered in future management plans.

References

Abbott, R. J. (2017). Plant speciation across environmental gradients and the occurrence and nature of hybrid zones. Journal of Systematics and Evolution 55.4, pp. 238–258. DOI: `10.1111/jse.12267`.

Aiken, S. G. and S. J. Darbyshire (1990). *Fescue grasses of Canada.* Publication 1844/E. Ottawa, Canada: Agriculture Canada. ISBN: 0-660-13483-7.

Aiken, S. G. and S. E. Gardiner (1991). SDS–PAGE of seed proteins in Festuca (Poaceae): taxonomic implications. Canadian Journal of Botany 69.7, pp. 1425–1432. DOI: `10.1139/b91-185`.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg (2001). The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution 16.11, pp. 613–622. DOI: `10.1016/S0169-5347(01)02290-X`.

Anderson, G. (2006a). Festuca campestris *Rydberg (rough fescue): A Technical Conservation Assessment.* Tech. rep. Rocky Mountain Region: USDA Forest Service.

Anderson, G. (2006b). Festuca hallii *(Vasey) Piper (Hall's fescue): A Technical Conservation Assessmen.* Technical Conservation Assessment. Fort Collins, Colorado: Colorado Natural Heritage Program, Colorado State University.

Anderson, S. A. S. and D. R. Matute (2025). Secondary Sympatry as a Sorting Process. Ecology Letters 28.4, e70108. DOI: `10.1111/ele.70108`.

Andreev, V., J. Puzey, E. Davies, C. Olson-Manning, S. Kreutzmann, and M. Fishbein (2025). History and Dynamics of an Extensive Plant Hybrid Zone on the Great Plains of North America. Molecular Ecology 34.18, e70048. DOI: `10.1111/mec.70048`.

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data.* Accessed: 2025-09-12. URL: `http://www.bioinformatics.babraham.ac.uk/projects/fastqc`.

Baiakhmetov, E., D. Ryzhakova, P. D. Gudkova, and M. Nobis (2021). Evidence for extensive hybridisation and past introgression events in feather grasses using genome-wide SNP genotyping. BMC Plant Biology 21.1, p. 505. DOI: `10.1186/s12870-021-03287-w`.

Barton, N. H. and G. M. Hewitt (1985). Analysis of Hybrid Zones. Annual Review of Ecology, Evolution, and Systematics 16.Volume 16, pp. 113–148. DOI: `10.1146/annurev.es.16.110185.000553`.

Beans, C. M. (2014). The case for character displacement in plants. Ecology and Evolution 4.6, pp. 852–865. DOI: `10.1002/ece3.978`.

Benítez-Benítez, C., P. Jiménez-Mejías, J. A. Calleja-Alarcón, M. Leo, M. Sanz-Arnal, F. Lara, R. Garilleti, S. Martín-Bravo, M. Escudero, M. Fernández-Mazuecos, J. Calatayud, and N. G. Medina (2025). Plant Neighbourhood as Predictor of Allopatric Speciation in Sedges. Molecular Ecology 34.6, e17683. DOI: `10.1111/mec.17683`.

Brown, M. R., R. J. Abbott, and A. D. Twyford (2024). The emerging importance of cross-ploidy hybridisation and introgression. Molecular Ecology 33.8, e17315. DOI: `10.1111/mec.17315`.

Catalán, P., P. Torrecilla, J. Á. L. Rodríguez, and R. G. Olmstead (2004). Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L–F sequences. Molecular Phylogenetics and Evolution 31.2, pp. 517–541. DOI: `10.1016/j.ympev.2003.08.025`.

Center for Plant Conservation (2019). “Guidelines for Tissue Collection and Storage Related to Genetic Studies of Rare Plants”. In: *CPC Best Plant Conservation Practices to Support Species Survival in the Wild.* Escondido, CA: Center for Plant Conservation.

Čertner, M., M. Lučanová, E. Sliwinska, F. Kolář, and J. Loureiro (2022). Plant material selection, collection, preservation, and storage for nuclear DNA content estimation. Cytometry Part A 101.9, pp. 737–748. DOI: `10.1002/cyto.a.24482`.

Chang, C. C., C. C. Chow, L. C. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience 4, p. 7. DOI: `10.1186/s13742-015-0047-8`.

Chen, W., L. Jiang, R. Jia, B. Tang, H. Jiang, Y. Wang, X. Lu, J. Su, and Y. Bai (2024). Plant litter loss exacerbates drought influences on grasslands. New Phytologist 241.1, pp. 142–153. DOI: `10.1111/nph.19374`.

Christie, K., L. S. Fraser, and D. B. Lowry (2022). The strength of reproductive isolating barriers in seed plants: Insights from studies quantifying premating and postmating reproductive barriers over the past 15 years. Evolution 76.10, pp. 2228–2243. DOI: `10.1111/evo.14565`.

Cozzolino, S., G. Scopece, P. Cortis, M. R. Barone Lumaga, D. Cafasso, and M. Ayasse (2022). Reproductive character displacement allows two sexually deceptive orchids to coexist and attract the same specific pollinator. Evolutionary Ecology 36.2, pp. 217–232. DOI: `10.1007/s10682-021-10149-2`.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, and 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools. Bioinformatics (Oxford, England) 27.15, pp. 2156–2158. DOI: `10.1093/bioinformatics/btr330`.

Danecek, P., J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, A. Whitwham, T. Keane, S. A. McCarthy, R. M. Davies, and H. Li (2021). Twelve years of SAMtools and BCFtools. GigaScience 10.2, giab008. DOI: `10.1093/gigascience/giab008`.

de Witte, L. C. and J. Stöcklin (2010). Longevity of clonal plants: why it matters and how to measure it. Annals of Botany 106.6, pp. 859–870. DOI: `10.1093/aob/mcq191`.

Desserud, . and M. A. Naeth (2013). Promising results in central Alberta with rough fescue (*Festuca hallii*) seeding following disturbance. Native Plants Journal 14.1, pp. 25–32.

Ellingson, S. R. and D. W. Fardo (2016). Automated quality control for genome wide association studies. F1000Research 5, p. 1889. DOI: `10.12688/f1000research.9271.1`.

Faske, T. M., A. C. Agneray, J. P. Jahner, C. Osuna-Mascaró, L. M. Sheta, B. A. Richardson, E. A. Leger, and T. L. Parchman (2024). Environment predicts the maintenance of reproductive isolation in a mosaic hybrid zone of rubber rabbitbrush. Evolution 78.2, pp. 300–314. DOI: `10.1093/evolut/qpad207`.

Friedman, J. and S. C. H. Barrett (2009). Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. Annals of Botany 103.9, pp. 1515–1527. DOI: `10.1093/aob/mcp035`.

Galla, S. J., N. J. Forsdick, L. Brown, M. P. Hoeppner, M. Knapp, R. F. Maloney, R. Moraga, A. W. Santure, and T. E. Steeves (2019). Reference Genomes from Distantly Related Species Can Be Used for Discovery of Single Nucleotide Polymorphisms to Inform Conservation Management. Genes 10.1, p. 9. DOI: `10.3390/genes10010009`.

Gelman, A. and D. B. Rubin (1992). Inference from Iterative Simulation Using Multiple Sequences. Statistical Science 7.4, pp. 457–472. DOI: `10.1214/ss/1177011136`.

Germain, R. M., S. P. Hart, M. M. Turcotte, S. P. Otto, J. Sakarchi, J. Rolland, T. Usui, A. L. Angert, D. Schluter, R. D. Bassar, M. T. Waters, F. Henao-Diaz, and A. M. Siepielski (2021). On the Origin of Coexisting Species. Trends in Ecology & Evolution 36.4, pp. 284–293. DOI: `10.1016/j.tree.2020.11.006`.

Gompert, Z., L. K. Lucas, C. A. Buerkle, M. L. Forister, J. A. Fordyce, and C. C. Nice (2014). Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Molecular Ecology 23.18, pp. 4555–4573. DOI: `10.1111/mec.12811`.

Gompert, Z. and K. E. Mock (2017). Detection of individual ploidy levels with genotyping-by-sequencing (GBS) analysis. Molecular Ecology Resources 17.6, pp. 1156–1167. DOI: `10.1111/1755-0998.12657`.

Harms, V. L. (1985). A Reconsideration of the Nomenclature and Taxonomy of the Festuca Altaica Complex (poaceae) in North America. Madroño 32.1, pp. 1–10. JSTOR: `41424511`.

Holcroft Weerstra, . (2003). *Plains Rough Fescue (*Festuca hallii*) Grassland Mapping - Central Parkland Natural Subregion of Alberta.* Tech. rep. Cochrane, Alberta: Biota Consultants.

Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz (2005). Reinforcement drives rapid allopatric speciation. Nature 437.7063, pp. 1353–1356. DOI: `10.1038/nature04004`.

Huffman, A., R. J. Martinuzzi, and D. Sezer (2021). An Exploratory Analysis of Wind Patterns of Alberta, Canada. Environmental Modeling & Assessment 26.5, pp. 737–761. DOI: `10.1007/s10666-021-09783-5`.

Johnston, A. and H. E. Cosby (1966). Rhizomatous form of *Festuca scabrella.* Canadian Journal of Plant Science 46.2, pp. 211–212. DOI: `10.4141/cjps66-034`.

Jolliffe, I. T. and J. Cadima (2016). Principal component analysis: a review and recent developments. Philosophical transactions. Series A, Mathematical, physical, and engineering sciences 374.2065, p. 20150202. DOI: `10.1098/rsta.2015.0202`.

Joly, S., P. A. McLenachan, and P. J. Lockhart (2009). A Statistical Approach for Distinguishing Hybridization and Incomplete Lineage Sorting. The American Naturalist. DOI: `10.1086/600082`.

Karakas, E., P. Ferrante, R. Schafleitner, G. Giuliano, A. R. Fernie, and S. Alseekh (2023). Plant Sample Collection and Shipment for Multi-omic Analyses and Phytosanitary Evaluation. Current Protocols 3.12, e952. DOI: `10.1002/cpz1.952`.

Kolář, F., M. Čertner, J. Suda, P. Schönswetter, and B. C. Husband (2017). Mixed-Ploidy Species: Progress and Opportunities in Polyploid Research. Trends in Plant Science 22.12, pp. 1041–1055. DOI: `10.1016/j.tplants.2017.09.011`.

Konuma, J. and S. Chiba (2007). Ecological character displacement caused by reproductive interference. Journal of Theoretical Biology 247.2, pp. 354–364. DOI: `10.1016/j.jtbi.2007.03.013`.

Kulig, J. J. (1996). The glaciation of the Cypress Hills of Alberta and Saskatchewan and its regional implications. Quaternary International 32, pp. 53–77. DOI: `10.1016/1040-6182(95)00059-3`.

Kyogoku, D. (2020). When does reproductive interference occur? Predictions and data. Population Ecology 62.2, pp. 196–206. DOI: `10.1002/1438-390X.12041`.

Li, H. (2013). *Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.* DOI: `10.48550/arXiv.1303.3997`. arXiv: `1303.3997 [q-bio]`.

Li, H. and R. Durbin (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics (Oxford, England) 25.14, pp. 1754–1760. DOI: `10.1093/bioinformatics/btp324`.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, and 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics (Oxford, England) 25.16, pp. 2078–2079. DOI: `10.1093/bioinformatics/btp352`.

Li, Y., F. Tada, T. Yamashiro, and M. Maki (2016). Long-term persisting hybrid swarm and geographic difference in hybridization pattern: genetic consequences of secondary contact between two Vincetoxicum species (Apocynaceae–Asclepiadoideae). BMC Evolutionary Biology 16.1, p. 20. DOI: `10.1186/s12862-016-0587-2`.

Lindtke, D., Z. Gompert, C. Lexer, and C. A. Buerkle (2014). Unexpected ancestry of *Populus* seedlings from a hybrid zone implies a large role for postzygotic selection in the maintenance of species. Molecular Ecology 23.17, pp. 4316–4330. DOI: `10.1111/mec.12759`.

López-Caamal, A. and E. Tovar-Sánchez (2014). Genetic, morphological, and chemical patterns of plant hybridization. Revista Chilena de Historia Natural 87.1, p. 16. DOI: `10.1186/s40693-014-0016-0`.

Mandeville, E. G., T. L. Parchman, D. B. McDonald, and C. A. Buerkle (2015). Highly variable reproductive isolation among pairs of Catostomus species. Molecular ecology 24.8, pp. 1856–1872. DOI: `10.1111/mec.13118`.

Marques, I., D. Draper, L. Riofrío, and C. Naranjo (2014). Multiple hybridization events, polyploidy and low postmating isolation entangle the evolution of neotropical species of Epidendrum (Orchidaceae). BMC Evolutionary Biology 14.1, p. 20. DOI: `10.1186/1471-2148-14-20`.

McFarlane, S. E., D. C. Hunter, H. V. Senn, S. L. Smith, R. Holland, J. Huisman, and J. M. Pemberton (2020). Increased genetic marker density reveals high levels of admixture between red deer and introduced Japanese sika in Kintyre, Scotland. Evolutionary Applications 13.2, pp. 432–441. DOI: `10.1111/eva.12880`.

McFarlane, S. E., J. P. Jahner, D. Lindtke, C. A. Buerkle, and E. G. Mandeville (2024). Selection leads to remarkable variability in the outcomes of hybridisation across replicate hybrid zones. Molecular Ecology 33.11, e17359. DOI: `10.1111/mec.17359`.

McFarlane, S. E. and E. G. Mandeville (2023). Diverse data sources and new statistical models offer prospects for improving the predictability of anthropogenic hybridization. Global Change Biology 29.4, pp. 923–925. DOI: `10.1111/gcb.16527`.

McFarlane, S. E. and J. M. Pemberton (2019). Detecting the True Extent of Introgression during Anthropogenic Hybridization. Trends in Ecology & Evolution 34.4, pp. 315–326. DOI: `10.1016/j.tree.2018.12.013`.

Mengli, Z., H. Bing, and W. D. Willms (2008). Detection of genetic diversity in rough fescue (*Festuca campestris* Rydb) populations of southern Alberta and British Columbia, Canada, using RAPD markers. Canadian Journal of Plant Science 88.2, pp. 307–312. DOI: `10.4141/CJPS07071`.

Meuser, A. V., A. R. Pitura, S. E. McFarlane, and E. G. Mandeville (2025). *Extensive multi-species hybridization between Leuciscidae minnow species.* DOI: `10.1101/2025.07.14.664671`.

Meyer, B. S., M. Matschiner, and W. Salzburger (2017). Disentangling Incomplete Lineage Sorting and Introgression to Refine Species-Tree Estimates for Lake Tanganyika Cichlid Fishes. Systematic Biology 66.4, pp. 531–550. DOI: `10.1093/sysbio/syw069`.

Mitchell, C., S. Leigh, L. Alphey, W. Haerty, and T. Chapman (2022). Reproductive interference and Satyrisation: mechanisms, outcomes and potential use for insect control. Journal of Pest Science 95.3, pp. 1023–1036. DOI: `10.1007/s10340-022-01476-6`.

Morales-Briones, D. F., A. Liston, and D. C. Tank (2018). Phylogenomic analyses reveal a deep history of hybridization and polyploidy in the Neotropical genus Lachemilla (Rosaceae). New Phytologist 218.4, pp. 1668–1684. DOI: `10.1111/nph.15099`.

Nieto Feliner, G., D. Criado Ruiz, I. Álvarez, and I. Villa-Machío (2023). The puzzle of plant hybridisation: a high propensity to hybridise but few hybrid zones reported. Heredity 131.5-6, pp. 307–315. DOI: `10.1038/s41437-023-00654-1`.

Omire, A., J. Neondo, N. L. M. Budambula, L. Wangai, S. Ogada, and C. Mweu (2022). Genetic Diversity and Population Structure of Doum Palm (Hyphaene compressa) Using Genotyping by Sequencing. Frontiers in Genetics 13, p. 762202. DOI: `10.3389/fgene.2022.762202`.

Otto, S. P. (2007). The Evolutionary Consequences of Polyploidy. Cell 131.3, pp. 452–462. DOI: `10.1016/j.cell.2007.10.022`.

Pavlick, L. E. and J. Looman (1984). Taxonomy and nomenclature of rough fescues, *Festuca altaica* , *F* . *campestris* (*F* . *scabrella* var. *major*), and *F* . *hallii* , in Canada and the adjacent part of United States. Canadian Journal of Botany 62.8, pp. 1739–1749. DOI: `10.1139/b84-235`.

Pereira, L., P.-A. Christin, and L. T. Dunning (2023). The mechanisms underpinning lateral gene transfer between grasses. PLANTS, PEOPLE, PLANET 5.5, pp. 672–682. DOI: `10.1002/ppp3.10347`.

Peterson, G. W., Y. Dong, C. Horbach, and Y.-B. Fu (2014). Genotyping-By-Sequencing for Plant Genetic Diversity Analysis: A Lab Guide for SNP Genotyping. Diversity 6.4, pp. 665–680. DOI: `10.3390/d6040665`.

Petit, C., P. Lesbros, X. Ge, and J. D. Thompson (1997). Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid Arrhenatherum elatius (Poaceae). Heredity 79.1, pp. 31–40. DOI: `10.1038/hdy.1997.120`.

Pezzi, P. H., L. C. Wheeler, L. B. Freitas, and S. D. Smith (2024). Incomplete lineage sorting and hybridization underlie tree discordance in *Petunia* and related genera (Petunieae, Solanaceae). Molecular Phylogenetics and Evolution 198, p. 108136. DOI: `10.1016/j.ympev.2024.108136`.

Pfennig, D. W. and K. S. Pfennig (2010). Character displacement and the origins of diversity. The American Naturalist 176.Suppl 1, S26–S44. DOI: `10.1086/657056`.

Pickup, M., Y. Brandvain, C. Fraïsse, S. Yakimowski, N. H. Barton, T. Dixit, C. Lexer, E. Cereghetti, and D. L. Field (2019). Mating system variation in hybrid zones: facilitation, barriers and asymmetries to gene flow. New Phytologist 224.3, pp. 1035–1047. DOI: `10.1111/nph.16180`.

Polato, N. R., M. M. Gray, B. A. Gill, C. G. Becker, K. L. Casner, A. S. Flecker, B. C. Kondratieff, A. C. Encalada, N. L. Poff, W. C. Funk, and K. R. Zamudio (2017). Genetic diversity and gene flow decline with elevation in montane mayflies. Heredity 119.2, pp. 107–116. DOI: `10.1038/hdy.2017.23`.

Prasad, A., E. D. Lorenzen, and M. V. Westbury (2022). Evaluating the role of reference-genome phylogenetic distance on evolutionary inference. Molecular Ecology Resources 22.1, pp. 45–55. DOI: `10.1111/1755-0998.13457`.

Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick, and D. Reich (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38.8, pp. 904–909. DOI: `10.1038/ng1847`.

Pyne, C. B., J. N. Campbell, S. E. McFarlane, and E. G. Mandeville (2025). A novel sex-associated genomic region in Catostomus fish species. Journal of Heredity, esaf007. DOI: `10.1093/jhered/esaf007`.

Qiu, J., Y.-B. Fu, Y. Bai, and J. F. Wilmshurst (2007). Patterns of amplified restriction fragment polymorphism in natural populations and corresponding seed collections of plains rough fescue (*Festuca hallii*). Canadian Journal of Botany 85.5, pp. 484–492. DOI: `10.1139/B07-037`.

Qiu, J., Y.-B. Fu, Y. Bai, and J. F. Wilmshurst (2009). Genetic variation in remnant *Festuca hallii* populations is weakly differentiated, but geographically associated across the Canadian Prairie. Plant Species Biology 24.3, pp. 156–168. DOI: `10.1111/j.1442-1984.2009.00251.x`.

R Core Team (2024). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing. Vienna, Austria. URL: `https://www.R-project.org/`.

Ramsey, J., H. D. Bradshaw, and D. W. Schemske (2003). Components of reproductive isolation between the monkeyflowers Mimulus lewisii and M. cardinalis (Phrymaceae). Evolution; International Journal of Organic Evolution 57.7, pp. 1520–1534. DOI: `10.1111/j.0014-3820.2003.tb00360.x`.

Randle, A. M., R. B. Spigler, and S. Kalisz (2018). Shifts to earlier selfing in sympatry may reduce costs of pollinator sharing. Evolution 72.8, pp. 1587–1599. DOI: `10.1111/evo.13522`.

Rhymer, J. M. and D. Simberloff (1996). Extinction by Hybridization and Introgression. Annual Review of Ecology and Systematics 27, pp. 83–109. JSTOR: `2097230`.

Robledo-Arnuncio, J. J. (2011). Wind pollination over mesoscale distances: an investigation with Scots pine. New Phytologist 190.1, pp. 222–233. DOI: `10.1111/j.1469-8137.2010.03588.x`.

Rundle, H. D. and P. Nosil (2005). Ecological speciation. Ecology Letters 8.3, pp. 336–352. DOI: `10.1111/j.1461-0248.2004.00715.x`.

Schumer, M., G. G. Rosenthal, and P. Andolfatto (2014). How common is homoploid hybrid speciation? Evolution 68.6, pp. 1553–1560. DOI: `10.1111/evo.12399`.

Shastry, V., P. E. Adams, D. Lindtke, E. G. Mandeville, T. L. Parchman, Z. Gompert, and C. A. Buerkle (2021). Model-based genotype and ancestry estimation for potential hybrids with mixed-ploidy. Molecular Ecology Resources 21.5, pp. 1434–1451. DOI: `10.1111/1755-0998.13330`.

Silvertown, J., C. Servaes, P. Biss, and D. Macleod (2005). Reinforcement of reproductive isolation between adjacent populations in the Park Grass Experiment. Heredity 95.3, pp. 198–205. DOI: `10.1038/sj.hdy.6800710`.

Sinaga, P., E. Klichowska, A. Nowak, and M. Nobis (2024). Hybridization and introgression events in cooccurring populations of closely related grasses (Poaceae: Stipa) in high mountain steppes of Central Asia. PLOS ONE 19.2, e0298760. DOI: `10.1371/journal.pone.0298760`.

Stanley, K. E. (1999). Evolutionary Trends in the Grasses (Poaceae): A Review. The Michigan Botanist 38.1. HDL: `2027/spo.0497763.0038.101`.

Stojanova, B., V. Koláříková, M. Šurinová, J. Klápště, V. Hadincová, and Z. Münzbergová (2019). Evolutionary potential of a widespread clonal grass under changing climate. Journal of Evolutionary Biology 32.10, pp. 1057–1068. DOI: `10.1111/jeb.13507`.

Stroud, J. T., S. T. Giery, R. J. P. Heathcote, S. Tiatragul, M. L. Yuan, K. J. Feeley, and J. B. Losos (2024). Observing character displacement from process to pattern in a novel vertebrate community. Nature Communications 15.1, p. 9862. DOI: `10.1038/s41467-024-54302-1`.

Stuart, Y. E. and J. B. Losos (2013). Ecological character displacement: glass half full or half empty? Trends in Ecology & Evolution 28.7, pp. 402–408. DOI: `10.1016/j.tree.2013.02.014`.

Stull, G. W., K. K. Pham, P. S. Soltis, and D. E. Soltis (2023). Deep reticulation: the long legacy of hybridization in vascular plant evolution. The Plant Journal 114.4, pp. 743–766. DOI: `10.1111/tpj.16142`.

Svačina, R., P. Sourdille, D. Kopecký, and J. Bartoš (2020). Chromosome Pairing in Polyploid Grasses. Frontiers in Plant Science 11. DOI: `10.3389/fpls.2020.01056`.

Tannas, S. (2013). *Glenbow Ranch Provincial Park 2013 Vegetation and Range Resource Inventory.* Tech. rep. Cremona, AB: Tannas Conservation Services.

Tkach, N., J. Schneider, E. Döring, A. Wölk, A. Hochbach, J. Nissen, G. Winterfeld, S. Meyer, J. Gabriel, M. H. Hoffmann, and M. Röser (2020). Phylogenetic lineages and the role of hybridization as driving force of evolution in grass supertribe Poodae. TAXON 69.2, pp. 234–277. DOI: `10.1002/tax.12204`.

Tomasello, S., K. Karbstein, L. Hodač, C. Paetzold, and E. Hörandl (2020). Phylogenomics unravels Quaternary vicariance and allopatric speciation patterns in temperate-montane plant species: A case study on the Ranunculus auricomus species complex. Molecular Ecology 29.11, pp. 2031–2049. DOI: `10.1111/mec.15458`.

Trottier, G. C. (1992). *A Landowner's Guide: Conservation of Canadian Prairie Grasslands*. Tech. rep. CW66-120/1992E-PDF. Edmonton, Alberta: Environment Canada, Canadian Wildlife Service, p. 92.

Urfusová, R., V. Mahelka, F. Krahulec, and T. Urfus (2021). Evidence of widespread hybridization among couch grasses (Elymus, Poaceae). Journal of Systematics and Evolution 59.1, pp. 113–124. DOI: `10.1111/jse.12563`.

Vallin, N., A. M. Rice, H. Arntsen, K. Kulma, and A. Qvarnström (2012). Combined effects of interspecific competition and hybridization impede local coexistence of Ficedula flycatchers. Evolutionary Ecology 26.4, pp. 927–942. DOI: `10.1007/s10682-011-9536-0`.

Wang, K., J. A. Lenstra, L. Liu, Q. Hu, T. Ma, Q. Qiu, and J. Liu (2018). Incomplete lineage sorting rather than hybridization explains the inconsistent phylogeny of the wisent. Communications Biology 1.1, p. 169. DOI: `10.1038/s42003-018-0176-6`.

Widenmaier, K. J. and W. L. Strong (2010). Tree and forest encroachment into fescue grasslands on the Cypress Hills plateau, southeast Alberta, Canada. Forest Ecology and Management 259.10, pp. 1870–1879. DOI: `10.1016/j.foreco.2010.01.049`.

Winterfeld, G., J. Schneider, K. Perner, and M. Röser (2014). Polyploidy and hybridization as main factors of speciation: complex reticulate evolution within the grass genus Helictochloa. Cytogenetic and Genome Research 142.3, pp. 204–225. DOI: `10.1159/000361002`.

Chapter 5: General Conclusions and Recommendations

> *It appears therefore that a large proportion of the agricultural wealth ... of our region [has] its origin in the activity of just one kind of higher plant,* Festuca scabrella
>
> — Moss and Campbell, *The Fescue Grassland of Alberta* (1947)

5.1 Conclusions

I began this thesis with the recognition that rough fescue grasslands are difficult to restore, in large part because we have a limited understanding of their foundational species, *Festuca hallii* and *F. campestris*. Through the work presented here, I have addressed several fundamental knowledge gaps that clarify our understanding of these two species, their relationships with their environment, and their relationships with each other. Together, these insights will hopefully provide not just a greater appreciation of these valuable species, but also a stronger foundation for informing future restoration work.

We now know that rough fescue seeds are highly sensitive to the storage conditions they are exposed to, and that their germination can be greatly enhanced through relatively simple moisture priming treatments. We have also observed high levels of symbiosis between rough fescue grasses and *Epichloë* endophytes, though the exact nature of this relationship remains unknown. And perhaps most remarkably, we now know that these two species, with their long-debated taxonomic history, are in fact extensively hybridizing with each other in regions of range overlap.

The discovery that *F hallii* and *F campestris* hybridize in regions of sympatry is particularly significant, because it also has potential consequences for the findings of my previous chapters. For example, I now know that, rather than comparing the germination of *F. hallii* vs. *F. campestris*, I likely was actually comparing the performance of *F. hallii* (sourced from a confirmed allopatric population) against seeds of mixed ancestry, since my "*F. campestris*" seeds were sourced from a known hybridization zone (Glenbow Ranch Provincial Park). This was unintentional and unavoidable, since I had not yet realized the two species were hybridizing at GRPP. The seeds were also collected from plants that were morphologically consistent with *F. campestris*, therefore I had no reason at the time to doubt their identity. Could this be the reason for that group's abysmal germination performance? It's certainly possible, though confirming this would necessitate a repeat of the germination assay, using seeds sourced from confirmed allopatric populations, and ideally subjected to some preliminary genotyping. The germination performance of hybrid seeds in their own right should also be considered. Clearly, *F. hallii* and *F. campestris* are capable of producing viable seeds, and the resulting F1 hybrids are themselves capable of reproducing, at least with their parental species. However, the actual rates of rough fescue hybrid seed germination are unknown.

Previous studies of other grass systems have found that hybrid seeds can actually exhibit increased germination, emergence, and competitive performance compared to their parents (Lipińska 2005; Stanisavljevic et al. 2020; Mostow et al. 2024). Additionally, this advantage may extend to seed dormancy requirements, with grass hybrids needing less time in storage to "after ripen" and reach maximum germination potential (Voigt et al. 1996; Stanisavljevic et al. 2020). However, a potential tradeoff is that hybrid seeds may deteriorate more quickly than parents if stored for extended periods of time (Stanisavljevic et al. 2020). A germination assay comparing the performance of hybrid seeds against pure *F. hallii* and *F. campestris* would be a valuable undertaking, even without the inclusion of seed storage condition variables. However, testing the impacts of extended storage on hybrid vs parental species would be particularly useful, considering how reliant rough fescue restoration projects are on wild-harvested and stored seed reserves.

Comparing germination performance between endophyte-infected (E+) and uninfected (E-) grasses would also be a valuable undertaking, considering the mixed effects that endophyte symbiosis can have on seeds. Endophytes may improve (e.g., Wang et al. 2020; Chen et al. 2021), inhibit (e.g., Gundel et al. 2006), or have no impact (e.g., Mack and Rudgers 2008) on grass germination, depending on the species tested and the germination conditions (e.g., temperature, moisture, salinity) experienced by the seeds. Additionally, endophytes within seeds that are collected and placed in storage tend to decay faster than in seeds buried in soil, resulting in a loss of endophyte symbiosis between generations (Mack and Rudgers 2008; Gundel et al. 2010). The temperature and moisture conditions the seeds are kept under may also accelerate or slow this process (Wiewióra and Żurek 2025). If indeed there is some cost/benefit relationship between rough fescues and their endophytes, then seed collection and storage could be impacting not just their germination, but also the prevalence of their symbiosis.

Regarding the costs and benefits of endophyte symbiosis, one priority for future research should be a direct, experimental comparison of E+ vs. E- rough fescue growth and persistence across a controlled gradient of disturbance. Endophyte infection has been shown to significantly improve plant growth rate and biomass (Mack and Rudgers 2008; Kuldau and Bacon 2008; Wang et al. 2020), increase abiotic stress tolerance and drought tolerance (Rodriguez et al. 2009; Lee et al. 2021; Hewitt et al. 2021), and increase competitive ability (Saikkonen et al. 1998; Rudgers et al. 2010; Lee et al. 2021), and so it is conceivable that they could also be offering some benefit in rough fescue. Previous work has shown that rough fescue endophytes are responsive to changes in soil moisture and grazing pressure (Rudgers et al. 2016), which are key determinants of rough fescue germination, persistence, and growth. Therefore, comparisons of E+ and E- plants against these two environmental factors should be a priority.

In addition to their potential ecological roles, the endophytes associated with *F. hallii* and *F. campestris* warrant further investigation due to the fact that their host grasses can hybridize. Since I collected all of my endophyte samples at Glenbow Ranch Provincial Park, where parental species are rare, it's likely that I was measuring endophyte levels in primarily hybrid grass samples. This raises interesting questions about the identity of these endophytes; since *Epichloë* endophytes are vertically transmitted through seed (Saikkonen et al. 2016), hybrid grasses inherit endophytes only from their mother plant (Gagic et al. 2018; Boller and Kopecký 2020). However, the evolution of these endophytes is also tightly co-regulated by that of their host grasses (Saikkonen et al. 2016), and many grass systems also host endophytes that are themselves hybrid strains (Saari and Faeth 2012; Cagnano et al. 2019). Whether Glenbow Ranch PP represents an ancient or relatively recent hybrid zone, there are interesting implications for the endophytes residing there (Boller and Kopecký 2020). Future work should identify and map the endophytes within allopatric populations of *F. hallii*, as done with *F. campestris* by Rudgers et al. (2016). Then, a more informed mapping of the endophytes in hybrid zones like Glenbow Ranch and Cypress Hills could be undertaken.

Taken together, the intersections between the different components of my thesis raise not only theoretical ecological questions, but also ones that have direct, practical restoration applications. For example, if endophyte infection does have an impact on rough fescue germination rates, seedling performance, or overall fitness/persistence, it may be prudent to introduce some measure of endophyte screening to assess overall infection levels in collected seeds (Aldrich-Markham et al. 2007; Wiewióra and Żurek 2025). Similarly, if hybrid grass seeds have poor germination performance, or reduced longevity when placed in storage, it may necessitate greater care and genotyping when collecting seeds from regions of potential admixture (Mengli et al. 2008; Nieto Feliner et al. 2023).

Even if seeds from hybrid individuals have similar or equivalent performance to their parents, care should still be taken to better map the extent of admixing between the two species. Collection and transplant of seeds from one region to another could inadvertently be causing or accelerating admixing between the two species, further blurring their boundaries (Faske et al. 2024; Andreev et al. 2025). This is especially relevant given that "taxonomic validity" is a foundational prerequisite for consideration under Canada's Species at Risk Act (SARA; COSEWIC 2015). Discrete and evolutionarily significant "designatable units" are a fundamental concept at the heart of identifying populations for protection and conservation initiatives, and hybridization/gene flow within sympatric populations can affect if/how populations are considered eligible (COSEWIC 2023). "Plains" rough fescue (i.e., *Festuca hallii*) is in fact currently a high priority candidate species for classification by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; COSEWIC 2025). If hybrids between *F. campestris* and *F. hallii* either pose a threat to the integrity of either species, or represent a new and unique population in their own right, this could open the door to stricter and better conservation protections in the future.

5.2 Practical recommendations for rough fescue restoration

The various components of my thesis have, in some ways, raised as many questions as they have answered. Nevertheless, I believe there are several key insights that can be translated to actionable steps for practitioners working to restore rough fescue grasslands (Fig. 5.1).

1. **Identify the age and provenance of your seeds.** Understanding where your seeds came

from, how long they have been stored, and under what conditions should be a top priority for restoration practitioners. Knowing seed source location can help avoid unintentional introgression between *F. campestris* and *F. hallii* populations, while knowing how seeds have been stored between collection and distribution can help inform expectations about seed dormancy or expected germination. Until the extent of introgression between the two species is more thoroughly mapped in the province, it is recommended to only source seeds from well within established allopatric zones. Distances of ≈ 200km from sympatric populations (Figs. 4.3 and 4.6) appear to be far enough to ensure pure parentage.

2. Maximize germination potential by assessing and assisting dormancy. If seed storage history is uncertain, or incomplete dormancy is suspected, a small-scale germination or dormancy test should be conducted to quickly assess baseline expectations (Baskin et al. 2006). If seeds are dormant, it may be possible to accelerate their return to full germination potential through a regimented cycle of alternating temperatures (i.e., a "Move-along" experiment; Baskin and Baskin 2003). Alternatively, planting seeds in fall may allow them to naturally experience a full dormancy cycle and improve germination in the spring (Desserud et al. 2010; Fleenor 2011), however this appears to have mixed effects for rough fescue (Lancaster et al. 2015).

3. Pre-treat seeds to enhance hydration and germination. As seen in Chapter 2, a simple seed priming treatment (i.e., soaking seeds in water for 24 hours) can greatly enhance rough fescue germination (see also Small et al. 2019). Since this treatment is inexpensive and low-risk, practitioners should consider implementing seed priming prior to seeding whenever practical.

4. Maintain high soil moisture during germination and establishment. Since moisture is the most significant limiting factor to rough fescue germination and establishment (Romo et al. 1991; Qiu et al. 2010; Tannas 2014), care should be taken to maximize moisture retention at a site after seeds are sown. One method for doing this is through the application of native straw mulch (Desserud and Naeth 2013; Mollard et al. 2016), which reduces moisture loss and mimics the stabilizing effects of rough fescue leaf litter.

Though not explored during my research, an additional restoration strategy that I believe would complement the above recommendations is the use of seed pellets (i.e., "seed balls" or "seed bombs"). As the name suggests, this involves "pelletizing" seeds with a clay or soil binder to form aggregates that can be distributed across a site similarly to traditional broadcast seeding (Gornish et al. 2019; Teichroew and Rew 2025). The pellet coating serves to reduce moisture loss, stabilize temperatures, and protect the encased seeds while they germinate, and is an effective means of improving germination and establishment of seeds in moisture-limited environments (Berto et al. 2024). Though this technique is perhaps more time and labour-intensive than seed priming or mulching, I believe it would be worthwhile to investigate its effectiveness for improving establishment over traditionally ineffective broadcast seeding (Lancaster et al. 2015).

Collectively, this dissertation highlights the value of targeted autecological research in addressing practical questions about species persistence and restoration. By integrating insights about rough

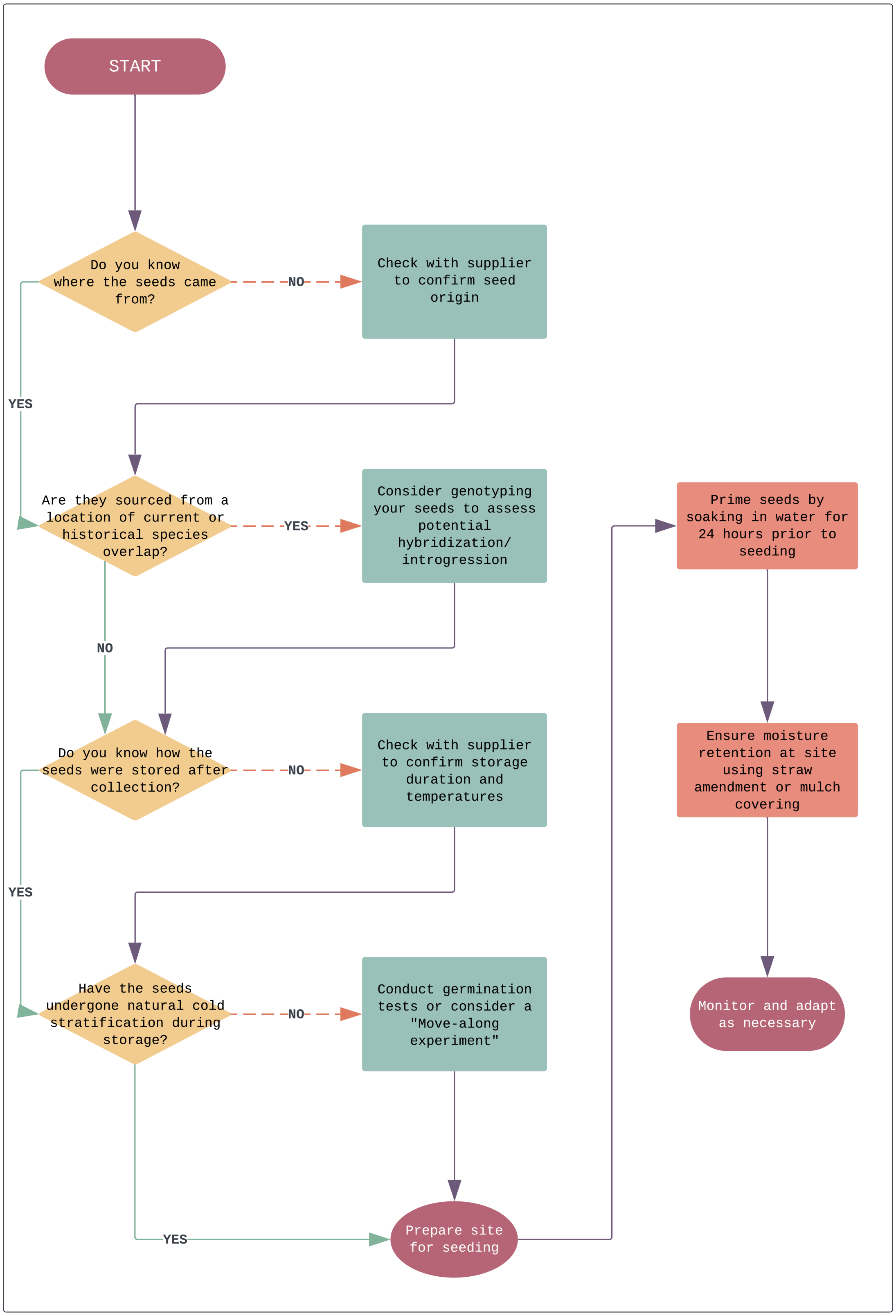

Figure 5.1: A “Fescue Rescue Flowchart”, summarizing my suggestions for improving rough fescue germination and establishment.

fescue seed ecology, species interactions, and population genetics, it provides a deeper understanding of these foundational species and their relationship with their environment, and charts a path for future research and actionable conservation steps.

References

Aldrich-Markham, S., G. Pirelli, and A. M. Craig (2007). *Endophyte Toxins in Grass Seed Fields and Straw.* Tech. rep. EM 8598-E. Corvallis, Oregon: Oregon State University Extension Service.

Andreev, V., J. Puzey, E. Davies, C. Olson-Manning, S. Kreutzmann, and M. Fishbein (2025). History and Dynamics of an Extensive Plant Hybrid Zone on the Great Plains of North America. Molecular Ecology 34.18, e70048. DOI: `10.1111/mec.70048`.

Baskin, C. C. and J. M. Baskin (2003). When Breaking Seed Dormancy is a Problem. Native Plants Journal 4.1. DOI: `10.3368/npj.4.1.17`.

Baskin, C. C., K. Thompson, and J. M. Baskin (2006). Mistakes in germination ecology and how to avoid them. Seed Science Research 16.3, pp. 165–168. DOI: `10.1079/SSR2006247`.

Berto, B., A. L. Ritchie, and T. E. Erickson (2024). The effects of seed enhancements on plant establishment in native grasses: A meta-analysis. Applied Vegetation Science 27.2, e12774. DOI: `10.1111/avsc.12774`.

Boller, B. and D. Kopecký (2020). Triploid forage grass hybrids Festuca apennina × F. pratensis display extraordinary heterosis for yield characteristics. Euphytica 216.9, p. 143. DOI: `10.1007/s10681-020-02680-0`.

Cagnano, G., N. Roulund, C. S. Jensen, F. P. Forte, T. Asp, and A. Leuchtmann (2019). Large Scale Screening of Epichloë Endophytes Infecting Schedonorus pratensis and Other Forage Grasses Reveals a Relation Between Microsatellite-Based Haplotypes and Loline Alkaloid Levels. Frontiers in Plant Science 10.

Chen, Y., K. Su, C. Li, and J. F. White (2021). Interactive Effects of Epichloë Endophyte, Dormancy-Breaking Treatments and Geographic Origin on Seed Germination of Achnatherum inebrians. Microorganisms 9.11, p. 2183. DOI: `10.3390/microorganisms9112183`.

COSEWIC (2015). *Determining eligibility of wildlife species for status assessment.* Tech. rep. Gatineau, QC: Committee on the Status of Endangered Wildlife in Canada - Environment and Climate Change Canada.

COSEWIC (2023). *COSEWIC Guidelines for Recognizing Designatable Units.* URL: `https://cosewic.ca/index.php/en/reports/preparing-status-reports/guidelines-recognizing-designatable-units.html`.

COSEWIC (2025). *COSEWIC Candidate wildlife species.* URL: `https://cosewic.ca/index.php/en/reports/candidate-wildlife-species.html`.

Desserud, P., C. C. Gates, B. Adams, and R. D. Revel (2010). Restoration of foothills rough fescue grassland following pipeline disturbance in southwestern Alberta. Journal of Environmental Management 91.12, pp. 2763–2770. DOI: `10.1016/j.jenvman.2010.08.006`.

Desserud, P. and M. A. Naeth (2013). Establishment of a native bunch grass and an invasive perennial on disturbed land using straw-amended soil. Journal of Environmental Management 114, pp. 540–547. DOI: `10.1016/j.jenvman.2012.11.001`.

Faske, T. M., A. C. Agneray, J. P. Jahner, C. Osuna-Mascaró, L. M. Sheta, B. A. Richardson, E. A. Leger, and T. L. Parchman (2024). Environment predicts the maintenance of reproductive isolation in a mosaic hybrid zone of rubber rabbitbrush. Evolution 78.2, pp. 300–314. DOI: `10.1093/evolut/qpad207`.

Fleenor, R. (2011). *Plant Guide for mountain rough fescue (*Festuca campestris*).* Tech. rep. Spokane, WA: USDA-Natural Resources Conservation Service.

Gagic, M., M. J. Faville, W. Zhang, N. T. Forester, M. P. Rolston, R. D. Johnson, S. Ganesh, J. P. Koolaard, H. S. Easton, D. Hudson, L. J. Johnson, C. D. Moon, and C. R. Voisey (2018). Seed Transmission of Epichloë Endophytes in Lolium perenne Is Heavily Influenced by Host Genetics. Frontiers in Plant Science 9. DOI: `10.3389/fpls.2018.01580`.

Gornish, E., H. Arnold, and J. Fehmi (2019). Review of seed pelletizing strategies for arid land restoration. Restoration Ecology 27.6, pp. 1206–1211. DOI: `10.1111/rec.13045`.

Gundel, P. E., P. H. Maseda, M. M. Vila-Aiub, C. M. Ghersa, and R. Benech-Arnold (2006). Effects of Neotyphodium fungi on Lolium multiflorum seed germination in relation to water availability. Annals of Botany 97.4, pp. 571–577. DOI: `10.1093/aob/mcl004`.

Gundel, P., M. Martínez-Ghersa, W. Batista, and C. Ghersa (2010). Dynamics of Neotyphodium endophyte infection in ageing seed pools: incidence of differential viability loss of endophyte, infected seed and non-infected seed. Annals of Applied Biology 156.2, pp. 199–209. DOI: `10.1111/j.1744-7348.2009.00379.x`.

Hewitt, K. G., A. J. Popay, R. W. Hofmann, and J. R. Caradus (2021). *Epichloë* - a lifeline for temperate grasses under combined drought and insect pressure. Grass Research 1.1, pp. 1–12. DOI: `10.48130/GR-2021-0007`.

Kuldau, G. and C. Bacon (2008). Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. Biological Control. Special Issue: Endophytes 46.1, pp. 57–71. DOI: `10.1016/j.biocontrol.2008.01.023`.

Lancaster, J., B. Adams, R. Adams, and P. Desserud (2015). *Long-term Revegetation Success of Industry Reclamation Techniques for Native Grassland: Foothills Fescue, Foothills Parkland and Montane Natural Subregions; Phase 1 - Literature Review and Case Studies.* Tech. rep. Land and Forest Policy Branch, Alberta Environment and Sustainable Resource Development.

Lee, K., A. Missaoui, K. Mahmud, H. Presley, and M. Lonnee (2021). Interaction between Grasses and Epichloë Endophytes and Its Significance to Biotic and Abiotic Stress Tolerance and the Rhizosphere. Microorganisms 9.11, p. 2186. DOI: `10.3390/microorganisms9112186`.

Lipińska, H. (2005). Evaluation of Festulolium brauni, Lolium perenne and Festuca pratensis development in pure sowing and their mixtures. Agronomy Science 60, pp. 163–174.

Mack, K. M. L. and J. A. Rudgers (2008). Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117.2, pp. 310–320. DOI: `10.1111/j.2007.0030-1299.15973.x`.

Mengli, Z., H. Bing, and W. D. Willms (2008). Detection of genetic diversity in rough fescue (*Festuca campestris* Rydb) populations of southern Alberta and British Columbia, Canada, using RAPD markers. Canadian Journal of Plant Science 88.2, pp. 307–312. DOI: `10.4141/CJPS07071`.

Mollard, F. P. O., M. A. Naeth, and A. Cohen-Fernandez (2016). Mulch amendment facilitates early revegetation development on an abandoned field In northern mixed grass prairies of North America. Ecological Engineering 97, pp. 284–291. DOI: `10.1016/j.ecoleng.2016.10.004`.

Mostow, R. S., F. S. Barreto, and S. D. Hacker (2024). A hybrid beachgrass (Ammophila arenaria × A. breviligulata) is more productive and outcompetes its non-native parent species. Oecologia 205.1, pp. 81–94. DOI: `10.1007/s00442-024-05548-5`.

Nieto Feliner, G., D. Criado Ruiz, I. Álvarez, and I. Villa-Machío (2023). The puzzle of plant hybridisation: a high propensity to hybridise but few hybrid zones reported. Heredity 131.5-6, pp. 307–315. DOI: `10.1038/s41437-023-00654-1`.

Qiu, J., Y. Bai, Y.-B. Fu, and J. F. Wilmshurst (2010). Spatial variation in temperature thresholds during seed germination of remnant *Festuca hallii* populations across the Canadian prairie. Environmental and Experimental Botany 67.3, pp. 479–486. DOI: `10.1016/j.envexpbot.2009.09.002`.

Rodriguez, R. J., J. F. White Jr, A. E. Arnold, and R. S. Redman (2009). Fungal endophytes: diversity and functional roles. New Phytologist 182.2, pp. 314–330. DOI: `10.1111/j.1469-8137.2009.02773.x`.

Romo, J. T., P. L. Grilz, C. J. Bubar, and J. A. Young (1991). Influences of Temperature and Water Stress on Germination of Plains Rough Fescue. Journal of Range Management 44.1, pp. 75–81. DOI: `10.2307/4002644`. JSTOR: `4002644`.

Rudgers, J. A., S. Fischer, and K. Clay (2010). Managing plant symbiosis: fungal endophyte genotype alters plant community composition. Journal of Applied Ecology 47.2, pp. 468–477. DOI: `10.1111/j.1365-2664.2010.01788.x`.

Rudgers, J. A., R. A. Fletcher, E. Olivas, C. A. Young, N. D. Charlton, D. E. Pearson, and J. L. Maron (2016). Long-term ungulate exclusion reduces fungal symbiont prevalence in native grasslands. Oecologia 181.4, pp. 1151–1161. DOI: `10.1007/s00442-016-3620-7`.

Saari, S. and S. H. Faeth (2012). Hybridization of *Neotyphodium* endophytes enhances competitive ability of the host grass. New Phytologist 195.1, pp. 231–236. DOI: `10.1111/j.1469-8137.2012.04140.x`.

Saikkonen, K., S. H. Faeth, M. Helander, and T. J. Sullivan (1998). FUNGAL ENDOPHYTES: A Continuum of Interactions with Host Plants. Annual Review of Ecology and Systematics 29.1, pp. 319–343. DOI: `10.1146/annurev.ecolsys.29.1.319`.

Saikkonen, K., C. A. Young, M. Helander, and C. L. Schardl (2016). Endophytic Epichloë species and their grass hosts: from evolution to applications. Plant Molecular Biology 90, pp. 665–675. DOI: `10.1007/s11103-015-0399-6`.

Small, C. C., D. Degenhardt, and T. McDonald (2019). Plant growth regulators for enhancing Alberta native grass and forb seed germination. Ecological Engineering 142, p. 100003. DOI: `10.1016/j.ecoena.2019.100003`.

Stanisavljevic, R., D. Poštić, R. Štrbanović, M. Tabaković, S. Jovanović, J. Milenković, D. okić, and D. Terzić (2020). Effect of seed storage on seed germination and seedling quality of *Festulolium* in comparison with related forage grasses. Tropical Grasslands-Forrajes Tropicales 8.2, pp. 125–132. DOI: `10.17138/tgft(8)125-132`.

Tannas, S. (2014). *The Successful Restoration of Fescue Grasslands in Southwestern Alberta.* Regina, SK.

Teichroew, E. B. and L. J. Rew (2025). Testing the effects of seed pellet composition to aid in semiarid restoration seeding. Restoration Ecology 33.6, e14349. DOI: `10.1111/rec.14349`.

Voigt, P. W., C. R. Tischler, and M. M. Poverene (1996). Seed Dormancy and Its Alleviation in Lovegrass Hybrids. Crop Science 36.6, cropsci1996.0011183X003600060047x. DOI: `10.2135/cropsci1996.0011183X003600060047x`.

Wang, Z., C. Li, and J. White (2020). Effects of Epichloë endophyte infection on growth, physiological properties and seed germination of wild barley under saline conditions. Journal of Agronomy and Crop Science 206.1, pp. 43–51. DOI: `10.1111/jac.12366`.

Wiewióra, B. and G. Żurek (2025). Endophyte Viability in Grass Seeds: Storage Conditions Affecting Survival and Control Methods. Agronomy 15.8, p. 1977. DOI: `10.3390/agronomy15081977`.

Appendix A

Table A1: The basic classes of seed dormancy, adapted from Finch-Savage and Leubner-Metzger 2006 and Kildisheva et al. 2020. Seeds may also possess a combination of these dormancy types, requiring multiple different dormancy-breaking mechanisms.

Dormancy class	**Description**	**Dormancy-breaking mechanism(s)**
Morphological	Plant embryo is underdeveloped at seed dispersal and therefore unable to germinate	Allowing sufficient time for embryo to grow and mature
Physiological	Plant embryo is fully developed, but germination is inhibited by chemical/hormonal mechanisms	Cold or warm stratification, treatment with gibberellins (hormonal growth regulators), exposure to light
Physical	Plant embryo is fully developed, but germination is inhibited by a hard/impermeable seed coat	Chemical or manual scarification/degradation of the seed coat

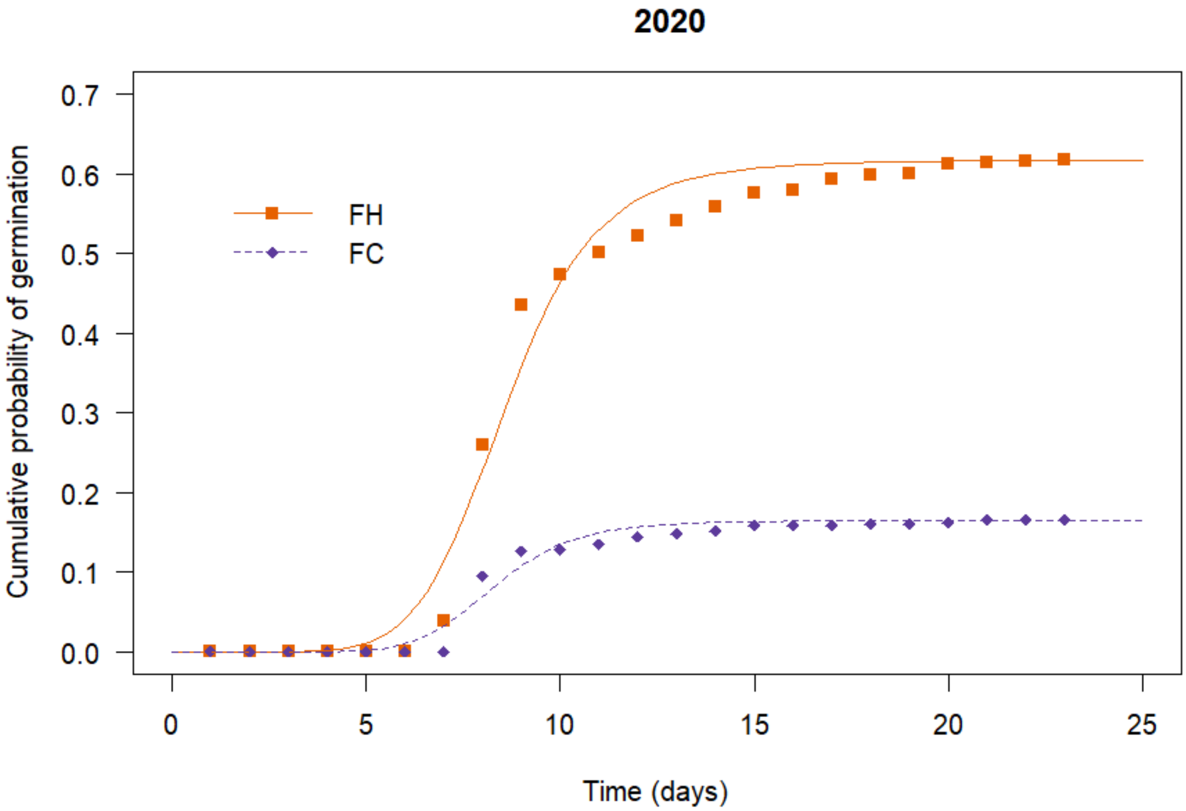

Figure A1: Results from a 2020 germination assay (unpublished data) show significantly higher germination in *F. hallii* compared to *F. campestris* (Likelihood Ratio Test: LR = 271.98, df = 3, p = 1.15×10^{-58}). This experiment used three-year-old, wild-collected seeds provided by Tannas Conservation Services

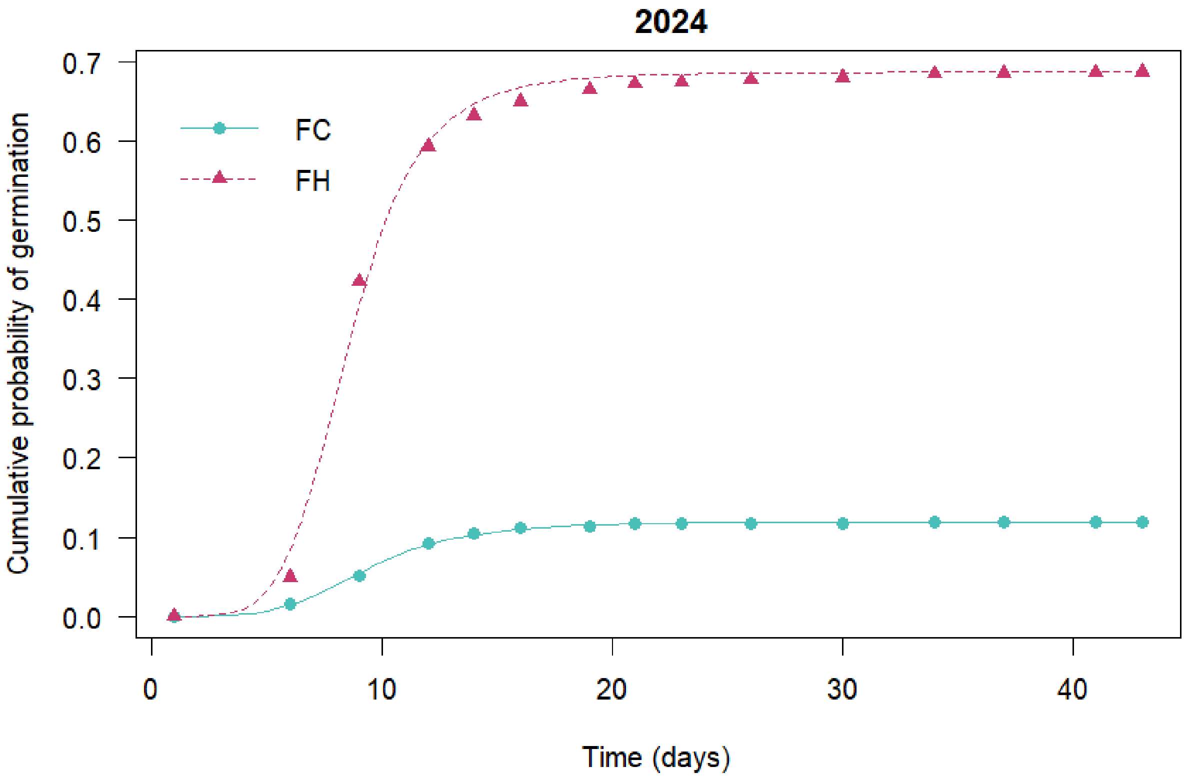

Figure A2: Pooled across all treatments, the 2024 germination experiment showed a similar disparity in germination response to the 2020 experiment, with *F. hallii* having significantly higher germination than *F. campestris* Likelihood Ratio Test: LR = 664.43, df = 3, p = 1.32×10^{-126}

Table A2: Post-assay examination of all ungerminated seeds revealed that *F. campestris* had a higher proportion of nonviable seeds compared to *F. hallii*, with over 50% of seeds being empty or moldy

	Festuca campestris				
	n	Mean	Min	Max	SE
Proportion viable seeds/dish	**96**	**0.43**	**0.00**	**0.78**	**0.02**
Germinated	94	0.13	0.00	0.60	0.02
Ungerminated but viable	96	0.30	0.00	0.65	0.02
Proportion nonviable seeds/dish	**96**	**0.57**	**0.22**	**1.00**	**0.02**
Moldy	96	0.31	0.00	0.83	0.02
Empty	96	0.26	0.00	0.72	0.01
	Festuca hallii				
	n	Mean	Min	Max	SE
Proportion viable seeds/dish	**96**	**0.72**	**0.39**	**1.00**	**0.01**
Germinated	96	0.69	0.28	1.00	0.02
Ungerminated but viable	96	0.03	0.00	0.33	0.01
Proportion nonviable seeds/dish	**96**	**0.28**	**0.00**	**0.61**	**0.01**
Moldy	96	0.08	0.00	0.44	0.01
Empty	96	0.20	0.00	0.39	0.01

Table A3: Estimates of parameter 'd' (i.e. maximum proportion germinated) from Time-to-Event models for each storage treatment combination for *Festuca hallii* seeds. The baseline (control) group is denoted with an asterisk (*).

Group	**Estimate (proportion germinated)**	**SE**	**t-value**	**p-value**
RT:C:PO	0.94	0.050	18.723	**3.52E-70**
DF:5M:PO	0.93	0.044	20.953	**2.66E-85**
FR:1M:NS	0.86	0.067	12.845	**7.88E-36**
DF:3M:PO	0.84	0.092	9.135	**2.15E-19**
FR:5M:NS	0.83	0.102	8.163	**7.07E-16**
FR:5M:PO	0.83	0.131	6.346	**2.95E-10**
MF:1M:NS	0.82	0.142	5.790	**8.65E-09**
MF:3M:NS	0.81	0.197	4.090	**4.55E-05**
MF:3M:PO	0.76	0.056	13.704	**2.86E-40**
MF:3M:PR	0.76	0.250	3.023	**0.003**
DF:3M:PR	0.76	0.102	7.377	**2.72E-13**
RT:5M:PR	0.74	0.157	4.748	**2.26E-06**
DF:3M:NS	0.73	6.287	0.115	0.908
MF:1M:PO	0.72	0.030	23.733	**2.35E-105**
DF:5M:NS	0.71	0.109	6.569	**7.08E-11**
DF:5M:PR	0.71	0.182	3.930	**8.91E-05**
FR:5M:PR	0.71	0.107	6.664	**3.79E-11**
FR:1M:PO	0.70	0.154	4.536	**6.21E-06**
FR:3M:NS	0.69	0.062	11.220	**4.57E-28**
FR:3M:PO	0.64	0.192	3.343	**0.001**
RT:1M:PR	0.62	0.181	3.414	**0.001**
DF:1M:PO	0.61	0.107	5.717	**1.32E-08**
RT:C:NS *	0.58	0.901	0.643	0.520
FR:3M:PR	0.58	0.123	4.698	**2.87E-06**
MF:5M:NS	0.58	0.299	1.924	0.055
DF:1M:PR	0.56	0.135	4.130	**3.84E-05**
MF:5M:PR	0.51	0.036	14.270	**2.61E-43**
FR:1M:PR	0.50	0.111	4.515	**6.86E-06**
MF:5M:PO	0.49	0.803	0.607	0.544
RT:3M:PR	0.49	0.081	5.982	**2.77E-09**
MF:1M:PR	0.46	0.183	2.497	**0.013**
DF:1M:NS	0.39	0.088	4.426	**1.03E-05**

Table A4: Estimates of parameter ‘d’ (i.e. maximum proportion germinated) from Time-to-Event models for each storage treatment combination for *Festuca campestris* seeds. The baseline (control) group is denoted with an asterisk (*).

Group	**Estimate (proportion germinated)**	**SE**	**t-value**	**p-value**
FR:5M:PO	0.333	0.122	2.7386	**0.006**
FR:5M:NS	0.316	0.107	2.9613	**0.003**
MF:1M:PO	0.263	0.101	2.6049	**0.009**
DF:1M:NS	0.250	0.097	2.582	**0.010**
RT:3M:PR	0.226	0.408	0.5547	0.579
MF:3M:NS	0.217	0.086	2.5277	**0.011**
FR:3M:PR	0.199	0.103	1.935	0.053
MF:1M:PR	0.194	0.071	2.7275	**0.006**
MF:5M:PR	0.185	0.075	2.4772	**0.013**
DF:5M:PR	0.160	0.074	2.1817	**0.029**
RT:1M:PR	0.148	0.068	2.1669	**0.030**
RT:C:PO	0.143	0.066	2.1603	**0.031**
DF:3M:NS	0.138	0.105	1.3087	0.191
MF:1M:NS	0.133	0.088	1.5192	0.129
DF:1M:PR	0.130	0.061	2.1411	**0.032**
MF:3M:PO	0.117	0.078	1.5052	0.132
MF:5M:NS	0.115	0.063	1.8416	0.066
FR:1M:PR	0.107	0.058	1.8331	0.067
DF:5M:NS	0.105	0.070	1.4952	0.135
RT:5M:PR	0.105	0.070	1.4952	0.135
FR:1M:NS	0.095	0.064	1.4868	0.137
FR:3M:PO	0.080	0.054	1.4745	0.140
FR:3M:NS	0.071	0.049	1.4678	0.142
MF:5M:PO	0.067	0.045	1.4638	0.143
RT:C:NS *	0.045	0.044	1.0238	0.306
DF:5M:PO	0.040	0.039	1.0209	0.307
DF:3M:PR	0.036	0.035	1.0187	0.308
FR:1M:PO	0.032	0.032	1.017	0.309

Table A5: Likelihood of *F. hallii* germination to be improved by different storage treatment combinations. Logistic regression output, with estimates exponentiated to show odds ratios.

Treatment Group	**Estimate (odds ratio)**	**SE**	**z-value**	**p-value**
(Intercept)	1.38	0.329	0.969	0.332
C.RT.PO	11.15	0.680	3.544	**3.94e-4**
5M.DF.PO	8.97	0.684	3.206	**1.35e-3**
1M.FR.NS	4.36	0.584	2.522	**0.012**
3M.DF.PO	3.88	0.553	2.451	**0.014**
5M.FR.NS	3.64	0.529	2.442	**0.015**
5M.FR.PO	3.53	0.529	2.384	**0.017**
1M.MF.NS	3.39	0.557	2.194	**0.028**
3M.MF.NS	3.01	0.534	2.065	**0.039**
3M.MF.PO	2.36	0.521	1.651	0.099
3M.MF.PR	2.26	0.505	1.618	0.106
3M.DF.PR	2.25	0.490	1.659	0.097
5M.RT.PR	2.12	0.480	1.562	0.118
3M.DF.NS	1.92	0.483	1.348	0.178
1M.MF.PO	1.85	0.464	1.322	0.186
5M.DF.PR	1.82	0.498	1.201	0.230
5M.DF.NS	1.82	0.474	1.261	0.207
5M.FR.PR	1.79	0.486	1.193	0.233
1M.FR.PO	1.68	0.467	1.108	0.268
3M.FR.NS	1.62	0.468	1.033	0.302
3M.FR.PO	1.31	0.460	0.585	0.558
1M.RT.PR	1.17	0.482	0.334	0.738
1M.DF.PO	1.16	0.451	0.319	0.749
3M.FR.PR	0.99	0.482	-0.027	0.978
5M.MF.NS	0.98	0.459	-0.035	0.972
1M.DF.PR	0.92	0.477	-0.172	0.863
5M.MF.PR	0.77	0.472	-0.554	0.579
1M.FR.PR	0.73	0.451	-0.706	0.480
3M.RT.PR	0.69	0.472	-0.797	0.426
5M.MF.PO	0.68	0.479	-0.792	0.429
1M.MF.PR	0.61	0.472	-1.038	0.299
1M.DF.NS	0.47	0.459	-1.667	0.096

Table A6: Likelihood of *F. campestris* germination to be improved by different storage treatment combinations. Logistic regression output, with estimates exponentiated to show odds ratios.

Treatment Group	**Estimate (odds ratio)**	**SE**	**z-value**	**p-value**
(Intercept)	0.05	1.024	-2.975	**0.003**
5M.FR.PO	10.50	1.161	2.026	**0.043**
5M.FR.NS	9.69	1.136	1.999	**0.046**
1M.MF.PO	7.50	1.148	1.754	0.079
1M.DF.NS	7.00	1.146	1.697	0.090
3M.MF.NS	5.83	1.142	1.545	0.122
3M.FR.PR	5.25	1.210	1.370	0.171
1M.MF.PR	5.04	1.120	1.444	0.149
5M.MF.PR	4.77	1.137	1.374	0.169
5M.DF.PR	4.00	1.160	1.195	0.232
1M.RT.PR	3.65	1.158	1.119	0.263
C.RT.PO	3.50	1.157	1.083	0.279
1M.MF.NS	3.23	1.275	0.920	0.358
3M.RT.PR	3.23	1.275	0.920	0.358
1M.DF.PR	3.11	1.155	0.982	0.326
3M.MF.PO	2.80	1.271	0.810	0.418
5M.MF.NS	2.74	1.193	0.844	0.399
1M.FR.PR	2.52	1.192	0.775	0.438
3M.DF.NS	2.52	1.192	0.775	0.438
5M.DF.NS	2.47	1.267	0.714	0.475
5M.RT.PR	2.47	1.267	0.714	0.475
1M.FR.NS	2.21	1.265	0.627	0.531
3M.FR.PO	1.83	1.261	0.477	0.633
3M.FR.NS	1.62	1.259	0.381	0.703
5M.MF.PO	1.50	1.258	0.322	0.747
3M.DF.PO	1.11	1.449	0.069	0.945
5M.DF.PO	0.88	1.445	-0.092	0.926
3M.DF.PR	0.78	1.444	-0.174	0.862
1M.FR.PO	0.70	1.443	-0.247	0.805
3M.MF.PR	0.00	2930.944	-0.006	0.996
5M.FR.PR	0.00	2610.939	-0.006	0.995
1M.DF.PO	0.00	2570.016	-0.007	0.995

Appendix B

Table B1: Proportion of variance in site characteristics explained by each of the five principal components in the PCA. PCAs 1 and 2 cumulatively explain over 65% of the variation in site characteristic data

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Standard deviation	1.446	1.125	1.016	0.714	0.322
Proportion of Variance	0.418	0.253	0.207	0.102	0.021
Cumulative Proportion	0.418	0.671	0.877	0.979	1.000

Table B2: Principal component loadings show that the proportion of increaser species and proportion of fescue at each site contribute most strongly to PC1. Values in bold are considered strong contributors to that principal component.

	PC1 (42%)	**PC2 (25%)**	**PC3 (21%)**	**PC4 (10%)**	**PC5 (2%)**
Proportion Increaser spp.	**0.590**	0.387	-0.115	-0.209	**-0.667**
Functional Diversity (1-D)	0.276	**-0.627**	-0.394	**-0.598**	0.135
Proportion Invasive spp.	-0.100	0.279	**-0.900**	0.287	0.140
Average Litter (lbs/acre)	-0.429	**0.526**	0.005	**-0.719**	0.150
Proportion Fescue	**-0.618**	-0.320	-0.144	-0.014	**-0.704**

Table B3: All species identified across 33 sites in Glenbow Ranch Provincial Park. Growth form, size, and grazing response information was used to calculate site metrics.

Genus	**Species**	**Common name**	**7-letter Code**	**Growth form**	**Size**	**Grazing response**	**Lifespan**
Achillea	*borealis*	yarrow	Achibor	Forb	Medium	Increaser	Perennial
Agoseris	*glauca*	false dandelion	Agosgla	Forb	Medium	Increaser	Perennial
Agrostis	*stolonifera*	creeping bentgrass	Agrosto	Graminoid	Medium	Increaser	Perennial
Allium	*cernuum*	nodding onion	Allicer	Forb	Medium	NA	Perennial
Amelanchier	*alnifolia*	saskatoon	Amelaln	Shrub	Tall	Decreaser	Perennial
Anemonastrum	*canadense*	Canada anemone	Anemcan	Forb	Medium	Increaser	Perennial
Anemone	*cylindrica*	long-fruited anemone	Anemcyl	Forb	Medium	Increaser	Perennial
Anemone	*multifida*	cutleaf anemone	Anemmul	Forb	Medium	Increaser	Perennial
Antennaria	*microphylla*	little-leaved pussytoes	Antemic	Forb	Ground cover	Invader	Perennial
Antennaria	*sp.*	pussytoes sp.	Antensp	Forb	Ground cover	Invader	Perennial
Anticlea	*elegans*	white camas	Antiele	Forb	Tall	Increaser	Perennial
Apocynum	*androsaemifolium*	spreading dogbane	Apocand	Forb	Medium	NA	Perennial

Arctostaphylos	*uva-ursi*	bearberry	Arctuva	Shrub	Ground cover	Increaser	Perennial
Artemisia	*campestris*	field wormwood	Artecam	Forb	Medium	Increaser	Perennial
Artemisia	*frigida*	pasture sage	Artefri	Forb	Medium	Increaser	Perennial
Artemisia	*ludoviciana*	prairie sage	Artelud	Forb	Tall	Increaser	Perennial
Astragalus	*agrestis*	field milkvetch	Astragr	Forb	Medium	Increaser	Perennial
Astragalus	*flexuosus*	flexible milkvetch	Astrfle	Forb	Medium	Increaser	Perennial
Astragalus	*laxmannii subsp. robustior*	prairie milkvetch	Astrlax	Forb	Medium	Increaser	Perennial
Astragalus	*sp.*	milkvetch sp.	Astrasp	Forb	Medium	Increaser	Perennial
Bromus	*pumpellianus*	Pumpelly's brome	Brompum	Graminoid	Tall	Decreaser	Perennial
Bromus	*inermis*	smooth brome	Bromine	Graminoid	Tall	Invader	Perennial
Bromus	*riparius*	meadow brome	Bromrip	Graminoid	Tall	Invader	Perennial
Calamagrostis	*montanensis*	plains reedgrass	Calamon	Graminoid	Medium	Increaser	Perennial
Campanula	*rotundifolia*	harebells	Camprot	Forb	Medium	Increaser	Perennial
Carex	*duriuscula*	needleleaf sedge	Caredur	Graminoid	Ground cover	Increaser	Perennial
Carex	*filifolia*	thread-leaved sedge	Carefil	Graminoid	Ground cover	Increaser	Perennial

Carex	*inops subsp. heliophila*	sun sedge	Careino	Graminoid	Ground cover	Increaser	Perennial
Carex	*scirpoidea*	bulrush sedge	Caresci	Graminoid	Ground cover	Increaser	Perennial
Carex	*sp.*	undifferentiated sedge	Carexsp	Graminoid	Ground cover	Increaser	Perennial
Cerastium	*arvense*	field chickweed	Ceraarv	Forb	Medium	NA	Perennial
Chenopodium	*album*	lamb's-quarters	Chenalb	Forb	Medium	Invader	Annual
Cirsium	*arvense*	creeping thistle	Cirsarv	Forb	Tall	Invader	Perennial
Cirsium	*flodmanii*	Flodman's thistle	Cirsflo	Forb	Tall	NA	Perennial
Comandra	*umbellata*	bastard toadflax	Comaumb	Forb	Medium	NA	Perennial
Cynoglossum	*officinale*	houndstongue	Cynooff	Forb	Medium	Increaser	Biennial
Dalea	*purpurea*	purple prairie clover	Dalepur	Forb	Medium	NA	Perennial
Danthonia	*parryi*	Parry's oatgrass	Dantpar	Graminoid	Medium	Increaser	Perennial
Dasiphora	*fruticosa*	shrubby cinquefoil	Dasifru	Shrub	Medium	Increaser	Perennial
Elaeagnus	*comutata*	silverberry	Elaecom	Shrub	Tall	Increaser	Perennial
Elymus	*lanceolatus subsp. lanceolatus*	northern wheatgrass	Elymlan	Graminoid	Tall	Decreaser	Perennial
Elymus	*sp.*	wheatgrass sp.	Elymusp	Graminoid	Tall	Decreaser	Perennial

Elymus	*trachycaulus subsp. subsecundus*	awned wheatgrass	Elymtra	Graminoid	Tall	Increaser	Perennial
Elymus	*repens*	quackgrass	Elymrep	Graminoid	Medium	Invader	Perennial
Erigeron	*caespitosus*	tufted fleabane	Erigcae	Forb	Medium	Increaser	Perennial
Erigeron	*grandiflorus*	large-flowered fleabane	Eriggra	Forb	Medium	Increaser	Perennial
Erigeron	*sp.*	fleabane sp.	Erigesp	Forb	Medium	Increaser	Perennial
Erysimum	*inconspicuum*	shy wallflower	Erysinc	Forb	Medium	NA	Biennial
Festuca	*scabrella*	rough fescue sp.	Festsca	Graminoid	Tall	Decreaser	Perennial
Festuca	*idahoensis*	Idaho fescue	Festida	Graminoid	Medium	Increaser	Perennial
Festuca	*campestris*	foothills rough fescue	Festcam	Graminoid	Tall	Decreaser	Perennial
Festuca	*hallii*	plains rough fescue	Festhal	Graminoid	Tall	Decreaser	Perennial
Festuca	*saximontana*	mountain fescue	Festsax	Graminoid	Medium	Increaser	Perennial
Fragaria	*virginiana*	wild strawberry	Fragvir	Forb	Ground cover	NA	Perennial
Gaillardia	*aristata*	common gaillardia	Gailari	Forb	Medium	Increaser	Perennial
Galium	*boreale*	northern bedstraw	Galibor	Forb	Medium	NA	Perennial

Geranium	*viscosissimum*	sticky purple geranium	Geravis	Forb	Medium	Decreaser	Perennial
Geum	*triflorum*	three-flowered avens	Geumtri	Forb	Medium	Increaser	Perennial
Gutierrezia	*sarothrae*	broom snakeweed	Gutisar	Forb	Medium	Invader	Perennial
Hedysarum	*americanum*	alpine sweetvetch	Hedyame	Forb	Tall	Increaser	Perennial
Hedysarym	*boreale*	boreal hedysarum	Hedybor	Forb	Tall	Increaser	Perennial
Helianthus	*pauciflorus subsp. subrhomboideus*	rhombic-leaved sunflower	Helipau	Forb	Medium	NA	Perennial
Helictochloa	*hookeri*	Hooker’s oatgrass	Helihoo	Graminoid	Medium	Increaser	Perennial
Hesperostipa	*curtiseta*	western porcupinegrass	Hespcur	Graminoid	Tall	Decreaser	Perennial
Hesperostipa	*sp.*	porcupinegrass sp.	Hespesp	Graminoid	Tall	Decreaser	Perennial
Hesperostipa	*comata*	needle-and-thread grass	Hespcom	Graminoid	Tall	Increaser	Perennial
Heterotheca	*villosa*	hairy golden aster	Hetevil	Forb	Medium	Increaser	Perennial
Heuchera	*richardsonii*	Richardson’s alumroot	Heucric	Forb	Medium	NA	Perennial
Hordeum	*jubatum*	foxtail barley	Hordjub	Graminoid	Medium	Invader	Perennial

Juniperus	*communis*	common juniper	Junicom	Shrub	Medium	NA	Perennial
Juniperus	*horizontalis*	creeping juniper	Junihor	Shrub	Ground cover	NA	Perennial
Koeleria	*macrantha*	prairie junegrass	Koelmac	Graminoid	Medium	Increaser	Perennial
Lappula	*squarrosa*	bluebur	Lappsqu	Forb	Medium	NA	Annual
Linaria	*vulgaris*	common toadflax	Linavul	Forb	Medium	Invader	Perennial
Linum	*lewisii*	wild blue flax	Linulew	Forb	Medium	NA	Perennial
Lithospermum	*ruderale*	woolly gromwell	Lithrud	Forb	Tall	Increaser	Perennial
Maianthemum	*stellatum*	star-flowered solomon's seal	Maiaste	Forb	Medium	NA	Perennial
Medicago	*sativa*	alfalfa	Medisat	Forb	Tall	Invader	Perennial
Melilotus	*sp.*	sweetclover sp.	Melilsp	Forb	Tall	Invader	Biennial
Monarda	*fistulosa*	wild bergamot	Monafis	Forb	Medium	NA	Perennial
Muhlenbergia	*cuspidata*	plains muhly	Muhlcus	Graminoid	Medium	Increaser	Perennial
Muhlenbergia	*richardsonis*	mat muhly	Muhlric	Graminoid	Medium	Invader	Perennial
Nasella	*viridula*	green needle grass	Nasevir	Graminoid	Tall	Decreaser	Perennial
Oatgrass	*sp.*	Hooker's or Parry's oatgrass	Oatgrsp	Graminoid	Medium	Increaser	Perennial

Oxytropis	*campestris var. spicata*	late yellow locoweed	Oxytcam	Forb	Medium	Increaser	Perennial
Oxytropis	*deflexa*	reflex locoweed	Oxytdef	Forb	Medium	Increaser	Perennial
Oxytropis	*splendens*	showy locoweed	Oxytspl	Forb	Medium	Increaser	Perennial
Pascopyrum	*smithii*	western wheatgrass	Pascsmi	Graminoid	Tall	Increaser	Perennial
Pediomelum	*esculentum*	breadroot scurf-pea	Pediesc	Forb	Medium	NA	Perennial
Phleum	*pratense*	timothy	Phlepra	Graminoid	Medium	Invader	Perennial
Poa	*compressa*	Canada bluegrass	Poacom	Graminoid	Medium	Invader	Perennial
Poa	*pratensis*	Kentucky bluegrass	Poaprat	Graminoid	Medium	Invader	Perennial
Poa	*glauca*	glaucous bluegrass	Poaglau	Graminoid	Medium	NA	Perennial
Poa	*sp.*	bluegrass sp.	Poasp	Graminoid	NA	NA	NA
Populus	*tremuloides*	trembling aspen	Poputre	Tree	Tall	Invader	Perennial
Potentilla	*concinna*	early cinquefoil	Potecon	Forb	Medium	Increaser	Perennial
Potentilla	*hippiana*	woolly cinquefoil	Potehip	Forb	Medium	Increaser	Perennial
Potentilla	*litoralis*	coast cinquefoil	Potelit	Forb	Medium	Increaser	Perennial
Potentilla	*sp.*	cinquefoils	Potensp	Forb	Medium	Increaser	Perennial

Ranunculus	*cardiophyllus*	heart-leaved buttercup	Ranucar	Forb	Medium	Increaser	Perennial
Ribes	*oxyacanthoides*	Canada gooseberry	Ribeoxy	Shrub	Medium	Increaser	Perennial
Rosa	*sp.*	wild rose sp.	Rosasp	Shrub	Medium	Increaser	Perennial
Rubus	*sp.*	wild raspberry sp.	Rubussp	Shrub	Medium	NA	Perennial
Rumex	*triangulivalvis*	narrow-leaved dock	Rumetri	Forb	Tall	NA	Perennial
Salix	*sp.*	willow sp.	Salixsp	Shrub	Tall	Increaser	Perennial
Schizachyrium	*scoparium*	little bluestem	Schisco	Graminoid	Medium	Increaser	Perennial
Shepherdia	*canadensis*	buffaloberry	Shepcan	Shrub	Tall	Increaser	Perennial
Solidago	*glutinosa*	spike goldenrod	Soliglu	Forb	Medium	Increaser	Perennial
Solidago	*missouriensis*	low goldenrod	Solimis	Forb	Medium	Increaser	Perennial
Solidago	*sp.*	goldenrod sp.	Solidsp	Forb	Medium	Increaser	Perennial
Sonchus	*arvensis*	perennial sow-thistle	Soncarv	Forb	Tall	Invader	Perennial
Sporobolus	*rigidus*	sandgrass	Sporrig	Graminoid	Tall	Decreaser	Perennial
Symphoricarpos	*occidentalis*	buckbrush	Sympocc	Shrub	Medium	Increaser	Perennial
Symphyotrichum	*falcatum*	white prairie aster	Sympfal	Forb	Medium	Increaser	Perennial

Symphyotrichum	*laeve*	smooth blue aster	Symplae	Forb	Tall	Increaser	Perennial
Taraxacum	*officinale*	common dandelion	Taraoff	Forb	Medium	Invader	Perennial
Thalictrum	*venulosum*	veiny meadowrue	Thalven	Forb	Tall	Increaser	Perennial
Thermopsis	*rhombifolia*	buffalo bean	Therrho	Forb	Medium	NA	Perennial
Thlaspi	*arvense*	stinkweed	Thlaarv	Forb	Tall	Invader	Annual
Tragopogon	*dubius*	goatsbeard	Tragdub	Forb	Tall	Invader	Biennial
Urtica	*gracilis*	slender stinging nettle	Urtigra	Forb	Tall	NA	Perennial
Vicia	*americana*	American vetch	Viciam	Forb	Ground cover	Decreaser	Perennial

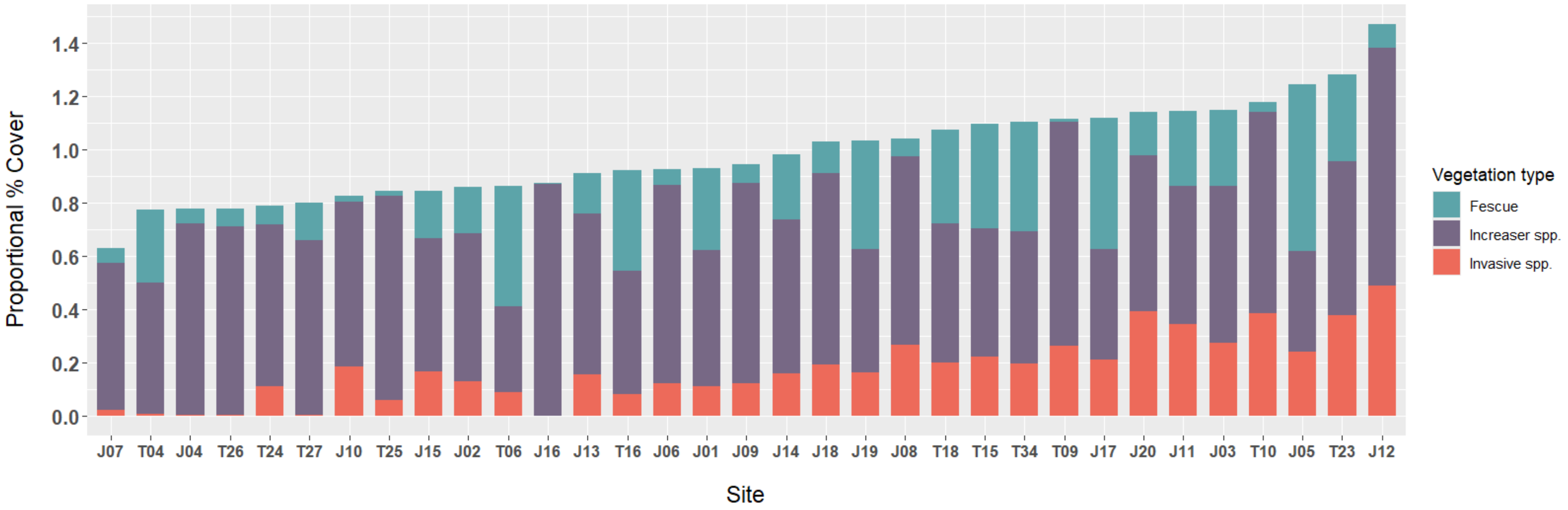

Figure B1: The distribution of different influential vegetation types at each of 33 sites at Glenbow Ranch Provincial Park. "Fescue" cover refers to both *Festuca campestris* and *F. hallii*. "Increaser spp." are native species that are tolerant of disturbance, and will increase in prevalence compared to more sensitive species as disturbance intensifies. "Invasive spp." are non-native species which may actively and aggressively displace native grassland communities, even in the absence of other disturbances. Because different vegetation layers can overlap, percent cover totals may exceed 100%