Characterizing the Habitat Requirements of Rare and

Hard-to-Establish Puget-Trough Prairie Forbs

By

John James Messina

A Thesis

Submitted in partial fulfillment

of the requirements for the degree

Master of Environmental Studies

The Evergreen State College

September 2019

This Thesis for the Master of Environmental Studies Degree

By

John James Messina

Has been approved for

The Evergreen State College

By

_____

Thesis Reader

_____

Date

ABSTRACT

The prairies of the Puget-Trough and Willamette Valley represent some of the most fragmented landscapes in the Pacific Northwest, while also providing critical habitat to a wide array of rare and threatened species. This thesis asks three basic questions: 1.) What is the simplest and most accurate way to describe the characteristics that differentiate microsites on the prairie landscape? 2.) Which microsites yield the strongest germination performance of locally rare species, *Balsamorhiza deltoidea* and *Gaillardia aristata*? 3.) How do microsite characteristics influence germination of *Balsamorhiza deltoidea* and *Gaillardia aristata*? Looking across three different sites, this study finds that *Balsamorhiza deltoidea* and *Gaillardia aristata* diverge from each other in both their preferred microsite type and how environmental parameters and biotic interactions influence germination rates. *Balsamorhiza deltoidea* has a stronger preference for mounds and highland sites, which can be characterized by lower soil bulk densities and more vigorous plant growth. Conversely, *Gaillardia aristata,* has a stronger preference for intermound and lowland sites, characterized by higher soil bulk densities and less dense plant growth. Microsites offer land managers and restoration ecologists a valuable scale by which restoration activities can be carried out without specialized tools or knowledge.

Table of Contents

List of Figures

List of Tables

Acknowledgements

Chapter One: Literature Review

I begin this literature review with a broad survey of grassland restoration strategies and processes. Next, I will turn to the issue of biodiversity in restoration and what role biodiversity has in ecosystem functioning. After I establish some specific and broad roles for increased biodiversity, I will turn to a discussion of plant rarity and the specific benefits that may come with restoration and conservation of rare species. This literature review serves the purpose of orienting the reader to the field of restoration ecology and the challenges of this work in grasslands. Ecological restoration can be tricky business; this review provides examples why some environmental disturbances, restoration treatments, and ecological theories do not always provide clear guidance for restoration of rare and hard-to-establish grassland species.

Restoration History and Strategy

Native grasslands continue to decline globally due to a myriad of causes, including cattle grazing, desertification, and intensive agriculture (Ceballos et al. 2010, Isselstein et al. 2005, Millennium Ecosystem Assessment 2005). In one study researchers found that of 468 articles published in the journal *Restoration Ecology*, 16% were concerned with the ongoing restoration of grasslands (Ruiz-Jaen and Aide 2005). The widespread loss of native grassland diversity and ecosystem services thus requires well developed restoration strategies, informed by research, long-term monitoring, and identification of concrete goals (Kaye 2009).

Since the nascent field of restoration ecology grew out of an ecological restoration project at the University of Wisconsin, our understanding of prairie restoration has increased an appreciable amount (arboretum.wisc.edu/about-us/history/). Restoration ecology, like conservation biology, is very action/goal-oriented: working with stakeholders is just as important as the ecological theories that underpin management recommendations. Bridging the gap between ecology and land management is at the core of the Society for Ecological Restoration's mission (http://www.ser.org/ page/MissionandVision). Academic research has improved our ability to predict restoration outcomes (Laughlin et al. 2017), our understanding of community assembly processes for both aboveground and belowground communities (Kraft and Ackerly 2014, Vályi et al. 2016), and, through these advances, greatly informed the practices of land managers. Emphasis on exotic species removal, active seeding/installation of native species and restoration of historic disturbance regimes are key insights following decades of research (Rowe, 2010).

Invasive Species Theory and Management

The presence of endangered species has driven much of the science that has informed the restoration of fragmented and degraded prairies. One of the largest obstacles to reviving some endangered species and associated native diversity is the constant unrelenting competition of non-natives. The former range of the South Sound prairies is often by overrun by Scotch broom (*Cytisus scoparius*), when left unmaintained. Other non-natives, such as hairy cat's ear (*Hypochaeris radicata*) or oxeye daisy (*Leucanthemum vulgare*) thrive even when

prairies are burned and actively seeded. Key to restoring native diversity and understanding the habitat needs of hard-to-establish species is the management of invasive species.

Typical Scotch broom-invaded prairie just a few miles from one of the study sites.

Invasive plants typically must undergo some management plan, often requiring intensive efforts in the initial stages (Solecki 1997). Reoccurring manual and chemical treatment of exotic species is often required to create space for natives to reestablish. Removal of these non-native plants is necessitated due to insidious strategies utilized by exotic species that result in loss of native biodiversity and associated deleterious impacts to ecosystem services (Greipsson and DiTommaso 2006, Pimentel et al. 2000).

While the challenges non-native species present to restoration practices may seem obvious, some restoration ecologist argue for a management approach that focuses on establishing novel communities accompanied with a high degree a

functional diversity (Jackson and Hobbs 2009). Embracing a restoration paradigm that leaves room for constructing novel ecosystems with a mix of native and exotic species means acknowledging that humans have exerted some type of influence on many native landscapes (Vale 2002) as well as recognizing that the future likely brings dramatic shifts in historic climactic regimes (IPCC Climate Change Synthesis Report, 2014). Further, some have argued that because non-native species often represent a significant contribution to the total abundance and richness of plant communities, they should be considered a part of the local diversity, not something that 'removes' diversity (Schlaepfer 2018). A common and abundant exotic in the South Sound prairies, English plantain (*Plantago lanceolata*), is the oviposition plant of choice for Taylor's Checkerspot butterfly (*Euphydryas editha taylori*) when golden paintbrush (*Castilleja levisecta*) or other host plants are not present (Kaye et al. 2011).

Further, pollinator network analysis shows that exotic species do provide some facilitation of pollination for native species that should be taken into consideration when invasive treatment plans are devised (Waters et al. 2014). Treatment of exotic species is therefore not always as easy as native vs exotic; care must be taken to focus efforts on species that reduce biodiversity and provide few resources for pollinators or other grassland fauna.

For nearly as long as naturalists have been observing native plants, they have been documenting plant invasions into those native environments. Seminal works like Charles Elton's 1933 *The Ecology of Invasions by Animals and Plants* and extensive research by invasion ecologist Daniel Simberloff and others lead us

to today where we have a well-developed understanding of non-native species ecology and competitive dynamics (Kuebbing and Meyerson 2018).

While much has been written on the means by which non-native plants succeed, this review only aims to briefly address a few concepts. Mechanisms for exotic species success are often explained by the 'Escape from Enemies' and 'Novel Weapons' hypotheses (Hierro et al. 2005). Escape from Enemies has its roots in Darwin's theory of naturalization which states that closely related species will likely be similar in functionality and thus will compete intensely, resulting in neither species being dominant (Darwin 1859). When an exotic species is introduced, lower genetic relatedness to co-occurring plants will allow the introduced species to outcompete co-evolved natives.

Modern molecular techniques allow ecologists to utilize comparative phylogenetics to tease out how big of a role the relatedness of species can play regarding whether a non-native plant's invasion bid is successful or not. A 2009 study in Australia looked at the phylogenetic clustering of nonnative plants in national parks to determine if a trend of successful, phylogenetically distinct invasives, could be identified. Researchers found that successful invasives in Asteraceae, Caryophyllaceae, Poaceae and Solanaceae all seemed to be phylogenetically clustered at larger spatial scales (Cadotte et al. 2009). Further, grass invasion in California can also be partially explained by the phylogenetic distance of native grasses from nonnative ones (Strauss et al. 2006). It follows logically then that phylogenetically diverse environments would be more resistant to invasion, which is what researchers found when examining the invasion

success of alien plants on Robben Island, South Africa (Yessoufou et al. 2019). When a plant community is more phylogenetically diverse, i.e. the species in the community are less closely related, then the probability that an invasive species will encounter a plant that it is phylogenetically near to increases, and so too does the chance that those species will be stronger competitors.

The Novel Weapons Hypothesis purports that exotic and invasive species use various means of changing the chemical and/or microbial environment to a state that inhibits the growth and fitness of native species. Often this is done through allelopathy, the process by which biochemicals are released by one species to the benefit or detriment of neighboring plant species. These can be active processes like root exudation or passive ones like decomposition and leeching. Many exotic species weaken the mycorrhizal mutualism of native species via allelopathy, including the local exotic *Cytisus scoparius* (Grove et al. 2011).

In both Escape from Enemies and Novel Weapons hypotheses, exotics succeed due to anachronistic effects, meaning that invasive species enter a system without a co-evolved history with native competitors. While nothing about plant invasion is simple, the challenge presented by nonnative species requires careful study and deliberate methods.

Fire History and Management

Puget Trough Prairies existed naturally from approximately 10,000 years ago to around 5,000 years ago during a period of warming that came after the

return of glacial conditions characteristic of the Younger Dryas (Barnosky 1985).

During this warming period, conifer encroachment diminished, and a frequent fire

regime was established. Prairies thrived naturally throughout this warm period.

Beginning around 5,000 years ago, however, the brief period of dry and warm

weather succumbed to the current cooler and wetter conditions. These new

conditions were much less conducive to natural maintenance of prairie structure

typified by sparse tree growth and plant communities dominated by grasses and

forbs. However, prairies continued to persist thanks to indigenous burning

practices (Boyd 1999). Prairies were incredibly valuable landscapes for native

peoples, offering excellent hunting grounds and gathering opportunities for food

and medicine (Storm and Shebitz 2006). Thanks to indigenous practices, we still

have intact Puget Trough prairies today.

Fire is a critical component of grassland persistence; species that have co-

evolved histories with fire have been well documented, especially the iconic

indigenous food plant, *Camassia quamash* (Storm and Shebitz 2006). Gillespie

and Allen (2006) found that a rare California grassland forb, *Erodium*

macrophyllum, had higher fecundity and germination/ survival in burned plots.

This was likely due to a decreased abundance of exotic grasses, which inhibited

persistence of *E. macrophyllum*. Prairie restoration, as examined through

ethnographic methods, is often successful when a low intensity frequent fire

regime is re-introduced (Rowe 2010).

Fire, in addition to promoting the persistence of rare prairie endemics, has

another critical job: to help prevent conifer encroachment, something native

peoples understood well. Without an established fire regime, conifers easily invade prairies (Haugo 2010, South Puget Sound Prairies Site Conservation Plan 2002), leading to altered soil moisture and nutrient regimes which may eliminate the requirements for many prairie species to persist. Today fire, along with invasive species treatment, constitute the most important restoration actions in the South Sound Prairies.

While used as a restoration tool, fire can also be manipulated by some noxious invasives to their great advantage. *Bromus tectorum* (cheatgrass), a systemic invasive in the Great Basin, grows rapidly between the well-spaced sagebrush - bitterbrush scrub that have historically characterized the Great Basin (Billings 1992). Once established in dense meadows, the cheatgrass rapidly dries out and burns annually. Native vegetation reestablishes much less aggressively than cheatgrass, completing the feedback loop (Billings 1992). Moving to the South Puget Sound prairies, an invasive forb, *H. radicata*, often flushes after a fire (personal observation 2017, Buschmann 2000). Without follow-up chemical treatments, prescribed burning of the prairies would likely result in large populations of *H. radicata*, leading to less space for more desirable species to establish.

Soil Disturbance

In addition to fire, another important disturbance is found on the prairie: mechanical disruption of soil. Endemic to many prairies in the South Puget Sound, especially on Joint Base Lewis McChord, are gophers - including the endangered Mazama Pocket Gopher (*Thomomys mazama*). Pocket gophers are

prolific burrowers, creating constant soil turnover, both below the soil and on the surface. This soil disturbance has been shown to create micro-conditions more conducive to increased plant cover, higher species richness, and greater variation in species composition among disturbed soils (Jones et al. 2008). Further, in a 1997 study, soil disturbance and mound building by gophers also seemed to increase the presence of a rare forb, *Aster curtis*, (Hartway and Steinburg 1997).

While the micro-disturbances caused by pocket gophers have helped promote a more heterogeneous landscape, they can also increase the number of exotic forbs if the prairie is already invaded to a moderate degree (Hartway and Steinburg 1997). The degree to which the undisturbed soils in proximity to gopher burrow sites are invaded strongly influences how well invasive species outcompete natives in the disturbed soils (Hartway and Steinburg 1997); essentially, the gopher disturbed soils magnify the native-exotic dynamics found in nearby undisturbed soils. The success of the most ruthless Puget Trough prairie invasive, Scotch broom (*Cytisus scoparius*), is greatly enhanced by soil disturbance. A study on Scotch broom in the Northern Californian grasslands, found that soil disturbance (meant to mimic that of a gopher) resulted in a significantly higher number of *C. scoparius* recruits in comparison to reference plots (Bossard 1991). Indeed, many forms of soil disturbance resulting from anthropogenic sources such as road building, cattle ranching, and agriculture have increased presence of alien exotic species (Hobbs and Huenneke 1992).

Restoration of the South Sound prairies may include reintroduction of historic disturbance regimes including soil tillage via gophers and fire, both

coupled with targeted removal of exotic species and problematic native species (i.e., Douglas fir, *Pseudotsuga menziesii*). Juggling these tasks can be tricky given that invasive species can take advantage of many of the same disturbances that natives rely on. In addition to management surrounding the maintenance of disturbance, native species need to be actively managed. Active, not passive, restoration that emphasizes planting and seeding native species, is often needed to restore high levels of native diversity.

Native Species Restoration and Impacts on Ecosystem Functioning

Restoration ecology informs us of the methods needed to achieve more native diversity, however, why go through all the trouble and money to strive for near historic levels of native diversity, especially when passive regeneration has been fruitful in other systems (Crouzeilles et al. 2017, Prach and Moral 2015). For experimental and applied ecologists, many would argue that increased native biodiversity can be linked to an increase in ecosystem functioning (Balvanera et al. 2006, Cardinale et al. 2006, Frainer et al. 2013, Tilman and Downy 1994); although some theoreticians are less than convinced on the positive link between species diversity and ecosystem function and stability (Schwartz et al. 2000).

Ecosystem functioning itself can be complex to measure experimentally but can be thought of as the cumulative biotic and abiotic processes of an ecosystem that contribute to its inherent sustainability, resilience, and resource transfer dynamics. Ecosystem functions are basically mechanisms that help deliver ecosystem services. Ecosystem services encompass every ecosystem product that promotes a sustainable system for humanity. Clean air and water are

classic examples of ecosystem services. Ecosystem functions can be as large scale as the spawning salmon's role in riparian nutrient cycling to something as small as a single vole digging for a truffle, which turns organic matter into the soil, resulting in higher soil fertility.

Native plant diversity, when examined through various diversity indices such as richness, evenness, Shannon's diversity, etc., contributes strongly to ecosystem functioning by creating redundancy, an important component of a system's resilience (Meadows 2009). Redundancy can improve the strategic use of resources and, hence, ecosystem resiliency. For instance, diverse plant assemblages may exploit water more efficiently than lower diversity assemblages when water is a limiting resource, which occurs regularly in grassland ecosystems (Harpole, Potts, & Suding, 2007).

Guderle et al. (2017) carried out a large field study where water uptake was measured by several biophysical and soil abiotic methods. Species richness and functional richness were manipulated to low and high levels while depth of soil penetrated by roots and aboveground biomass were controlled for. Leaf area size was found to be related to increased water uptake and high diversity assemblages maintained greater total leaf area than low diversity areas. Diverse plant assemblages would be more resilient and desirable if increases in water uptake efficiency are correlated with an increase in species richness. Many regions in North America can expect longer and hotter summers resulting from changing climatic regimes (IPCC Climate Change Synthesis Report 2014), making strategic use of water resources paramount.

In addition to facilitation of water uptake, increases in biodiversity also lead to greater overall primary production (Hector et al. 1999). Primary production is essential for many ecosystem processes as it supplies the core of terrestrial food webs (Hui 2012). Diverse suites of plants may lead to greater water uptake and greater overall primary productivity by taking advantage of positive plant to plant facilitation processes – especially as environmental conditions harshen (Brooker et al. 2008). A useful example of this interaction can be found in alpine plant communities; as elevation increases, interspecific species competition tends to lessen as harsher conditions are buffered by mutual plant facilitation. Plant to plant facilitation allows for more specialized resource utilization as well as more amenable microhabitat abiotic conditions, such as temperature buffering and enhanced soil moisture (Anthelme et al. 2014, Brooker et al. 2008). Maximizing resource utilization and primary productivity are yet more ways in which increased biodiversity builds resiliency.

Another oft cited role for biodiversity of native plant species is found in the biotic resistance hypothesis. This hypothesis addresses the question of why invasion succeeds or fails at a given site. It proposes two outcomes dependent on diversity: communities with high levels of biodiversity have very little niche space that can be exploited, resulting in competitive exclusion of an introduced invasive species. On the other hand, a community depauperate of species will have more unexploited niche space and be vulnerable to alien species gaining a foothold (Elton 1958). In a meta-analysis by Levine et al. (2004), the notion that communities with high levels of diversity will expel invaders requires a more

nuanced understanding. It seems that invasion is rarely completely repelled, rather diversity is negatively correlated with the abundance of invader species (i.e. higher diversity leads to lower invader abundance). In an examination of four possible mechanisms underpinning biotic resistance (competition, species diversity, herbivory, and soil fungal composition) Levine et al. (2004) found that all factors except soil fungal communities contributed to biotic resistance.

Pollinator services have been shown to increase when grasslands retain high floristic diversity (Collinge et al. 2003, Potts et al. 2009). Pollinator species, like many groups of vulnerable species, are experiencing global declines (Potts et al. 2010). Drivers of these declines include the strong effects of habitat loss and changing environmental conditions, including changes in seasonal weather patterns. The presence of harmful chemical classes used in pesticides in the environment is also threatening to pollinator health (Potts et al. 2010) and persistent non-lethal effects to pollinators remain a cause for concern (Morandin et al. 2005). Restoring native grasslands is a necessary action to help mitigate some of the difficult challenges pollinators face in the Anthropocene.

While most of the conversation here has revolved around the effect of increasing biodiversity, there is some debate as to the utility of emphasizing species diversity (often measured as species richness) over functional diversity. Functional diversity is defined as follows in the excellent review by Diaz and Cabido (2001):

"(Functional diversity) is the value and range of functional traits of the organisms present in a given ecosystem. The value of traits refers to the presence and relative abundance of certain values (or kinds) of leaf size, nitrogen content, canopy heights, seed dispersal and dormancy characteristics, vegetative and reproductive phenology, etc. The range of traits refers to the difference between extreme values of functional traits, for example, the range of leaf sizes, canopy heights, or rooting depths deployed by different plants in an ecosystem."

Using this definition, it is possible that adding plants with very redundant traits may increase *species* diversity, but not *functional* diversity. While in most circumstances, species richness may be a good surrogate for functional diversity, functional diversity and species diversity do have subtle differences. Short-term fluxes in energy and primary production are more strongly influenced by functional diversity while species diversity within functional traits helps to increase more long-term sustainability (Diaz and Cabido 2001). Researchers may find value in using species diversity as a broad measurement of 'biodiversity,' however, considering both functional and species diversity may paint a more holistic picture of ecosystem dynamics.

At its core, restoration ecology is the restoration of ecosystem functions. Understanding what functions are lost and/or desired is a critical step in the restoration process. Insights from experimentation and theory can help to inform how native species improve functioning of water usage, resistance to invasion, pollination, etc. While different methods to measure and evaluate the effects of diversity on ecosystem functioning are available, the broader conceptual ties between diversity and ecosystem functioning are key to the underpinnings of any successful restoration project.

Species Rarity: Causes and Conservation

There are two types of rare plants in a restoration setting. First, there are plants that have become rare due to disturbances they have not co-evolved with (excessive habitat fragmentation, conversion to agriculture, invasive species, etc.), and second, plants that are naturally rare across the landscape. This thesis is concerned with restoring plants whose rarity is natural and whose persistence is more fragile. Abundant species are habitat generalists, whereas rare species tend to be more habitat specialists (Pärtel 2002). Rare species conservation poses unique challenges due to the nature of habitat specialists. When a species is a habitat specialist, degradation of that habitat disproportionately harms that species compared to a generalist that will thrive in several different habitat types (Reinartz 1997). Before delving into the conservation and ecology of rare plants, understanding what causes plant rarity needs be addressed.

One way to determine the cause of rarity is to ask if a rare plant is seed or habitat limited (Candeias and Warren 2016). A species may not be releasing enough propagules into ideal habitat to be able to compete interspecifically (seed limitation), or conversely a species may be dispersing in inappropriate habitat, resulting in loss of local populations (habitat limitation). In a study on rare plant persistence in gravelly glacial outwash soils in New York State, Candeias and Warren (2016) found three prairie forbs whose rarity on the landscape was better explained by habitat limitation than seed limitation. Further, the researchers discussed how competition from abundant species was a leading cause of failure to survive after germination.

As mentioned before, plant species can be split into two groups: generalists and habitat specialists. The Douglas Fir (*Pseudotsuga menziesii*) illustrates the generalist species well; this impressive conifer is one of the most iconic and recognizable species in the Pacific Northwest. This partially has to do with the cultural values attached to this tree, as well as the fact that it is highly abundant. In every ecosystem there are species like the Douglas Fir, those that are numerous and successful. Now consider the Matsutake mushroom (*Trichloma matsutake*); while it is difficult to throw a rock in a PNW forest and not hit a Douglas Fir, chances are most people living in the PNW have not found a Matsutake mushroom in a wild setting. These fungi form a symbiotic relationship with only a handful of tree species (Yamanaka et al. 2014). The lifecycle and symbiotic requirements of Matsutake mushrooms makes them rare in the landscape. Species abundance curves (whether plants, animals, or fungi) in ideal circumstances are comprised of a few very abundant and successful species, a group of moderately abundant species, and an abundance of rare species (McGill et al. 2007, Mouillot et al. 2013, Preston 1948). This has implications for the conservation of biodiversity; when restoration seeks to restore historic, or near historic, levels of biodiversity, this often means restoring rare species.

Care should also be taken to not conflate sites with high species richness with sites where rare and/or threatened species are found. Species rich sites and the presence of rare species do not always go together; Prendergast et al. (1993), found that more often species-rich sites do not contain rare species. To further complicate the issues surrounding the conservation of rare plants is the fact that

rare plants are often poor competitors against native habitat generalists (Candeias and Warren 2016, Lloyd et al. 2002).

As discussed above, rare plant restoration has the potential to boost overall biodiversity, which theoretically improves ecosystem functioning. In addition to the increase in biodiversity, there is experimental evidence for unique contributions made by rare plant restoration. Researchers removing plants from experimental plots can control for consistent biomass and level of soil disturbance, while manipulating the species richness by pulling rare species out of experimental plots and pulling the equivalent biomass of abundant species out of control plot. Using this approach, Hector et al. (1999) found that plots with rare species left intact had increased survival of several native species that were sown as a restoration treatment.

While rare plants are often thought to be poor competitors, evidence shows this isn't always the case. In a study looking at plant competition between rare and common plants of the families *Rosaceae* and *Poaceae*, the common *Poaceae* species outcompeted the rare ones, while four of the five rare *Rosaceae* species were highly competitive both in monoculture with the common plants and in a mixed plot invaded by the grass *Agrostis capillaris* (Lloyd et al. 2002). Competitive outcomes between plants thus can't be boiled down to the commonness or rarity of a given species.

One useful exercise for evaluating the importance of rare species is grounded in quantifying species functional traits and the redundancy of those traits (Mouillot et al. 2013). First, we can ask if a rare species goes extinct, are the

functional traits of that species restricted to just that individual or may those traits be found in other, more common, species? Another way to ask this: Is the loss of rare specie's functional traits (given local extinction) insured against by more common species also maintaining similar functional traits? When examining three species-rich ecosystems (marine coral reefs, alpine plant communities, and tropical forests), researchers found that species with the lowest levels of functional redundancy tend to be the rarest species in the species pool (Mouillot et al. 2013). One third of alpine plants, for example, that exhibited the low functional redundancy, were locally rare. Scaling up, over three quarters of alpine plants with low levels of functional redundancy are regionally rare (Mouillot et al. 2013). Rare plants are thus shown to possess rare functional traits (for example lifecycle, leaf area, vegetative height, leaf persistence, dispersion mode, flowering phenology, etc.) that are not common in their more abundant neighbors. Mouillot et al. (2013) give an example of the cliff dwelling Pyramidal Saxifrage (*Saxifraga cotyledon*), a slow growing rare forb with uniquely long stems which help to make the flowers detectable to pollinators.

Extant grassland species work both as individual plants and as a community to help build ecosystem resiliency and deliver ecosystem services. Attention to rare species and their conservation is an important component of these processes. A diverse grassland with few specialists will likely lack resilience and functioning when compared to a diverse grassland supportive of rare species and habitat specialists (Mouillot et al. 2013). While intensifying climate change has been shown to be extremely disruptive of plant species distributions,

abundances, and interactions – often to the increased benefit of generalists and detriment to specialists (Van der Putten et al. 2010), conservation of rare species should remain a goal of restoration ecologists and managers, especially given their unique contributions. This means that active and persistent restoration work will be needed in the future to ensure the health and viability of our rare species and the environments to which they belong.

Literature Review Works Cited

Anthelme, F., Cavieres, L. A., & Dangles, O. (2014). Facilitation among plants in alpine environments in the face of climate change. *Frontiers in Plant Science*, 5.

Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., & Schmid, B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, *9*(10), 1146–1156.

Barnosky, C. W. (1985). Late Quaternary vegetation near Battle Ground Lake, southern Puget Trough, Washington. *Geological Society of America Bulletin, 96*,263–271.

Billings, W. D. (1992). Ecological Impacts of Cheatgrass and Resultant Fire on Ecosystems in the Western Great Basin. Paper presented at the Symposium on Ecology, Management, and Restoration of lnter-mountain Annual Rangelands, 9.

Bossard, C. C. (1991). The Role of Habitat Disturbance, Seed Predation and Ant Dispersal on Establishment of the Exotic Shrub *Cytisus scoparius* in California. *American Midland Naturalist, 126*(1), 1.

Boyd, R. (1999). Indians, Fire, and the Land in the Pacific Northwest. Oregon State University Press, Corvallis.

Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., … Michalet, R. (2008). Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, 96(1), 18–34.

Buschmann, G. (2000). Weeds of South Puget Sound. Cascadia Prairie Oak Partnership.

Cadotte, M. W., Hamilton, M. A., & Murray, B. R. (2009). Phylogenetic relatedness
and plant invader success across two spatial scales. *Diversity and Distributions,
15*(3), 481–488.

Candeias, M., & Warren, R. J. (2016). Rareness starts early for disturbance-dependent
grassland plant species. *Biodiversity and Conservation, 25*(13), 2771–2785.

Cardinale, B. J., Srivastava, D. S., Emmett Duffy, J., Wright, J. P., Downing, A. L.,
Sankaran, M., & Jouseau, C. (2006). Effects of biodiversity on the functioning of
trophic groups and ecosystems. *Nature, 443*(7114), 989–992.

Ceballos, G., Davidson, A., List, R., Pacheco, J., Manzano-Fischer, P., Santos-Barrera,
G., & Cruzado, J. (2010). Rapid Decline of a Grassland System and Its Ecological
and Conservation Implications. *PLoS ONE, 5*(1).

Collinge, S. K., Prudic, K. L., & Oliver, J. C. (2003). Effects of local habitat
characteristics and landscape context on grassland butterfly diversity.
Conservation Biology, 17(1), 178–187.

Crouzeilles, R., Ferreira, M. S., Chazdon, R. L., Lindenmayer, D. B., Sansevero, J. B.
B., Monteiro, L., … Strassburg, B. B. N. (2017). Ecological restoration success is
higher for natural regeneration than for active restoration in tropical forests.
Science Advances, 3(11), e1701345.

Darwin, C., (1859). On the origin of species by means of natural selection, or
preservation of favored races in the struggle for life. London: John Murray.

Díaz, S., & Cabido, M. (2001). Vive la difference: Plant functional diversity matters to
ecosystem processes. *Trends in Ecology & Evolution, 16*(11), 646–655.

Dunwiddie, P. W., & Martin, R. A. (2016). Microsites Matter: Improving the Success of Rare Species Reintroductions. *PLoS ONE, 11*(3), e0150417.

Elton, C. S., (1958). *The Ecology of Invasions by Animals and Plants*. Springer Netherlands.

Frainer, A., McKie, B. G., & Malmqvist, B. (2013). When does diversity matter? Species functional diversity and ecosystem functioning across habitats and seasons in a field experiment. *Journal of Animal Ecology*, 83(2), 460–469.

Gillespie, I. G., & Allen, E. B. (2006). Fire and competition in a southern California grassland: impacts on the rare forb *Erodium macrophyllum*. *Journal of Applied Ecology*, 41(4), 643–652.

Greipsson, S., & DiTommaso, A. (2006). Invasive non-native plants alter the occurrence of arbuscular mycorrhizal fungi and benefit from this association. *Ecological Restoration*, *24*(4), 236–241.

Grove, S., Parker, I., Haubensak, K. (2011). Soil legacy effects of Scotch broom invasion on Douglas-fir mycorrhizae. Paper Presented at 96th ESA Annual Convention. Austin, TX.

Guderle, M., Bachmann, D., Milcu, A., Gockele, A., Bechmann, M., Fischer, C., … Hildebrandt, A. (2017). Dynamic niche partitioning in root water uptake facilitates efficient water use in more diverse grassland plant communities. *Functional Ecology*. 32(1), 214-227.

Harpole, W. S., Potts, D. L. and Suding, K. N. (2007), Ecosystem responses to water and nitrogen amendment in a California grassland. Global Change Biology, 13: 2341-2348.

Hartway, C and E.K. Steinberg. (1997). The influence of pocket gopher disturbance on the distribution and diversity of plants in western Washington prairies, p. 131–139. In: P. Dunn and K. Ewing (eds.). *Ecology and conservation of the south Puget Sound prairie landscape*. The Nature Conservancy of Washington, Seattle, U.S.A

Haugo, R. D. (2010). *Causes and Consequences of Conifer Invasion into Pacific Northwest Grasslands*. University of Washington.

Haveren, B. P. V. (1983). Soil Bulk Density as Influenced by Grazing Intensity and Soil Type on a Shortgrass Prairie Site. *Journal of Range Management*, 36(5), 586.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M., Diemer, M., Dimitrakopoulos, P., … Lawton, J. (1999). Plant Diversity and Productivity Experiments in European Grasslands. *Science*, *286*.

Hierro, J. L., Maron, J. L., & Callaway, R. M. (2005). A biogeographical approach to plant invasions: The importance of studying exotics in their introduced and native range. *Journal of Ecology*, 93(1), 5–15.

Hobbs, R. J., & Huenneke, L. F. (1992). Disturbance, Diversity, and Invasion: Implications for Conservation. *Conservation Biology*, 6(3), 324–337.

Hobbs and Harris (2001) Restoration Ecology: Repairing the Earth's Ecosystems in the New Millennium. *Restoration Ecology*, 9(2), 239–246

Hui, D. (2012) Food Web: Concept and Applications. *Nature Education Knowledge* 3(12):6

IPCC. – (2014): Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel

on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)].
IPCC, Geneva, Switzerland, 151pp.

Isselstein, J., Jeangros, B., & Pavlu, V. (2005). Agronomic aspects of biodiversity
targeted management of temperate grasslands in Europe – A review. *Agronomy
Research*. 3(2).

Jackson, S. T., & Hobbs, R. J. (2009). Ecological Restoration in the Light of
Ecological History. *Science*, 325(5940), 567–569.

Jones, C. C., Halpern, C. B., & Niederer, J. (2008). Plant Succession on Gopher
Mounds in Western Cascade Meadows: Consequences for Species Diversity and
Heterogeneity. *The American Midland Naturalist*, *159*(2), 275–286.

Kaye, T. N., Stanley, A. G., & Ross, D. (2011). *Dispersal behavior and habitat
variation of Taylor's checkerspot butterfly* (p. 27). Institute for Applied Ecology.

Kaye, T. N. (2009). Toward successful reintroductions: The combined importance of
species traits, site quality, and restoration technique. Proceedings of the CNPS
Conservation Conference, 17, 99–106

Kraft, N. J. B., & Ackerly, D. D. (2014). Assembly of Plant Communities. In R. K.
Monson (Ed.), *Ecology and the Environment* (pp. 67–88). New York, NY:
Springer New York.

Kuebbing, S. E., & Meyerson, L. A. (2018). Honoring Daniel Simberloff: an
unwavering champion of invasion biology. *Biological Invasions*, 20(12), 3379–
3383.

Laughlin, D. C., Strahan, R. T., Moore, M. M., Fulé, P. Z., Huffman, D. W., & Covington, W. W. (2017). The hierarchy of predictability in ecological restoration: are vegetation structure and functional diversity more predictable than community composition? *Journal of Applied Ecology*, 54(4), 1058–1069.

Levine, J. M., Adler, P. B., & Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, 7(10), 975–989.

Lloyd, K., Lee, W., & Wilson, J. (2002). Competitive Abilities of Rare and Common Plants: Comparisons Using Acaena (Rosaceae) and Chionochloa (Poaceae) from New Zealand. *Conservation Biology*, 16(4), 975-985.

McGill, B. J., Etienne, R. S., Gray, J. S., Alonso, D., Anderson, M. J., Benecha, H. K., Dornelas, M., Enquist, B. J., Green, J. L., He, F., Hurlbert, A. H., Magurran, A. E., Marquet, P. A., Maurer, B. A., Ostling, A., Soykan, C. U., Ugland, K. I. and White, E. P. (2007),- Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecology Letters*, 10: 995–1015.

Meadows, Donella H. (2009). Thinking in systems: a primer. London; Sterling, VA: Earthscan

Millennium Ecosystem Assessment. Ecosystems and human well-being: desertification synthesis. Washington, D. C.: World Resources Institute; 2005.

Morandin, L. A., Winston, M. L., Franklin, M. T., & Abbott, V. A. (2005). Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Management Science*, 61(7), 619–626.

Mouillot, D., Bellwood, D. R., Baraloto, C., Chave, J., Galzin, R., Harmelin-Vivien, M., … Thuiller, W. (2013). Rare species support vulnerable functions in high-diversity ecosystems. *PLoS biology*, 11(5).

Newsham, K. K., Fitter, A. H., & Watkinson, A. R. (1995). Arbuscular Mycorrhiza Protect an Annual Grass from Root Pathogenic Fungi in the Field. *Journal of Ecology*, *83*(6), 991–1000.

Öpik, M., Moora, M., Liira, J. and Zobel, M. (2006), Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology*, 94: 778–790

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology*, *6*(10), 763–775.

Pärtel, M. (2002), Local Plant Diversity Patterns and Evolutionary History at the Regional Scale. *Ecology*, 83: 2361–2366.

Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and Economic Costs of Nonindigenous Species in the United States. *BioScience*, 50(1), 53.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, *25*(6), 345–353.

Potts, S. G., Woodcock, B. A., Roberts, S. P. M., Tscheulin, T., Pilgrim, E. S., Brown, V. K., & Tallowin, J. R. (2009). Enhancing pollinator biodiversity in intensive grasslands. *Journal of Applied Ecology*, *46*(2), 369–379.

Prach, K., & Moral, R. del. (2015). Passive restoration is often quite effective:
Response to Zahawi et al. (2014). *Restoration Ecology*, 23(4), 344–346.

Prendergast, J. R., Quinn, R. M., Lawton, J. H., Eversham, B. C., & Gibbons, D. W.
(1993). Rare species, the coincidence of diversity hotspots and conservation
strategies. *Nature*, 365(6444), 335–337.

Preston, F. W. (1948), The Commonness, And Rarity, of Species. *Ecology*, 29, 254–
283.

Schlaepfer, M. A. (2018). Do non-native species contribute to biodiversity? *PLOS
Biology*, 16(4), e2005568.

Schwartz, M., Brigham, C., J. D. Hoeksema, Lyons, K., Mills, M., & P. J. van
Mantgem. (2000). Linking Biodiversity to Ecosystem Function: Implications for
Conservation Ecology. *Oecologia*, 122(3), 297-305.

Solecki, M. K. (1997). Controlling Invasive Plants. In The Tallgrass Restoration
Handbook (pp. 251–278). Island Press.

Stinson, D. W. (2005). Washington State Status Report for the Mazama Pocket
Gopher, Streaked Horned Lark, and Taylor's Checkerspot. Washington
Department of Fish and Wildlife, Olympia. 129+ xii pp.

Storm, L., & Shebitz, D. (2006). Evaluating the Purpose, Extent, and Ecological
Restoration Applications of Indigenous Burning Practices in Southwestern
Washington. *Ecological Restoration*, 14(4), 256-268.

Reinartz, J. (1997). Restoring Populations of Rare Plants. In *The Tallgrass Restoration
Handbook* (pp. 89–95).

Rowe, H. I. (2010). Tricks of the Trade: Techniques and Opinions from 38 Experts in Tallgrass Prairie Restoration. Restoration Ecology, 18, 253–262.

Ruiz-Jaen, M. C., & Aide, T. M. (2005). Restoration Success: How Is It Being Measured? *Restoration Ecology*, 13(3), 569-577.

Schultz, C. B. (2001). Restoring resources for an endangered butterfly. *Journal of Applied Ecology*, 38(5), 1007–1019.

Strauss, S. Y., Webb, C. O., & Salamin, N. (2006). Exotic taxa less related to native species are more invasive. *Proceedings of the National Academy of Sciences*, 103(15), 5841–5845.

Vale, T. (2002). Fire, native peoples, and the natural landscape. Washington, DC: Island Press.

Vályi, K., Mardhiah, U., Rillig, M. C., & Hempel, S. (2016). Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. *The ISME Journal*, *10*(10), 2341–2351.

Van der Putten, W. H., Macel, M., & Visser, M. E. (2010). Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 365(1549), 2025–2034.

Waters, S., Fisher, S., and Hille Ris Lambers, J. (2014). Neighborhood-contingent indirect interactions between native and exotic plants: multiple shared pollinators mediate plant reproductive success during invasions. *Oikos* 123: 433-440

Washburn, A. L. (1988). Mima Mounds: An Evaluation of *Proposed Origins with special Reference to the Puget Lowlands* (p. 59). Washington DNR.

Yamanaka, T., Ota, Y., Konno, M., Kawai, M., Ohta, A., Neda, H., … Yamada, A. (2014). The host ranges of conifer-associated Tricholoma matsutake, Fagaceae-associated *T. bakamatsutake* and *T. fulvocastaneum* are wider in vitro than in nature. *Mycologia*, 106(3), 397–406.

Yessoufou, K., Bezeng, B. S., Gaoue, O. G., Bengu, T., & van der Bank, M. (2019). Phylogenetically diverse native systems are more resistant to invasive plant species on Robben Island, South Africa. *Genome*, 62(3), 217–228.

Chapter Two: Thesis Research

Introduction

Loss of grassland biodiversity may not tug on the heartstrings as much as loss of pristine tropical rainforest, however grasslands provide niche habitats for many rare and threatened species and, like tropical rain forests, are consistently threatened by a myriad of anthropogenic forces (Bond and Parr 2010). Our very own Puget Trough prairies, along with their cousins to the south - the Willamette Valley prairies, are situated in areas where the human population is forecast to grow dramatically in the next decade, which will inevitably carry with it more intensive land use modification (Thurston Regional Planning Council 2017). Further, our prairies are highly fragmented, which reduces dispersal of many rare and hard-to-establish native prairie plants (Soons and Heil 2002). This fragmented landscape with reduced opportunity for species dispersal is subject to the forces of extinction as determined by the theory of island biogeography (MacArthur and Wilson 1967). This ultimately means that land managers must actively manage these landscapes by removing invasive species, reintroducing historic fire regimes, and augmenting both extant and locally extinct native plant populations through seeding and plug installation (Dunwiddie and Bakker 2011). Understanding the complex ecology of our Puget Trough prairies will allow for these management techniques to be fine-tuned so that the most cost-effective and impactful methods can be used.

Puget Balsamroot (*Balsamorhiza deltoidea,* hereafter Balsamroot) is a perennial forb in the aster family that is listed on the Washington Natural Heritage Rare Species list for 2018 (www.dnr.wa.gov/nhplists). In Sarah Krock's thesis, which looked at the effect of sowing time and site diversity on native germination and establishment, Krock (2016) found that Balsamroot had extremely low establishment rates, often less than 1%. While Balsamroot is rare on the landscape and has experienced low field establishment, it does grow very well at the Center for Natural Management's (CNLM) seed farm, suggesting some field-linked parameter limits Balsamroot establishment (S. Hamman, personal communication). Balsamroot yields large, sunflower-like flowers that bloom throughout the late spring and early summer, providing a high-quality nectar source to species such as the Taylor's checkerspot butterfly (Linders et al. 2015).

Blanket flower (*Gaillardia aristata*), another perennial forb in the aster family, is not listed as endangered or threatened in the state of Washington and is common east of the cascades in dry and open locales (www.pnwherbaria.org). West of the Cascades, however, it is rare or absent in many of the prairies in the South Sound (personal observation, 2017). Like Balsamroot, Blanket flower yields large sunflower-like flowers and is an excellent late-season nectar source for many pollinator species (Lee-Mäder et al. 2016). Given its preference for well-drained soils, Blanket flower represents a good candidate to help increase native diversity, thus improving habitat for many pollinators in the South Sound prairies.

Both Balsamroot and Blanket flower represent species that have not been successfully restored on a large scale and provide quality native nectar sources for rare and endangered invertebrates. The Taylor's checkerspot butterfly (*Euphydryas editha taylori*), federally listed as endangered in 2013, is at extreme risk of extinction, primarily due to the loss of habitat (Stinson 2005, U.S. Fish and Wildlife Service 2013).

Taylor's checkerspot butterfly populations vary significantly year by year due to changing weather patterns. During the larval stages of the butterfly, a host plant provides vital shelter and nutrition. Larvae must reach an adequate level of development before plants become desiccated with the arrival of summer and a long diapause period lasting throughout winter (Stinson 2005). This means many sites become locally extinct if local conditions aren't amenable to the fragile butterfly life-cycle. Maintenance of Taylor's checkerspot populations is thus highly dependent on dispersing adults who may recolonize sites that were unsuitable in previous seasons. Loss of habitat represents a huge challenge to the persistence of Taylor's checkerspot and other sensitive pollinators. The story of the Taylor's checkerspot is not too dissimilar to that of the spotted owl; both species are quite rare with their threatened status partially due to the loss of sensitive habitats (old-growth forest and diverse grasslands able to support numerous metapopulations to cope with stochastic pressures).

Given the frequent stochastic pressures butterflies face, along with the loss of habitat due to anthropogenic forces, it follows that augmenting current habitat and creating new habitat are critical strategies for the recovery of the Taylor's

checkerspot butterfly. This strategy means that seeding/ plug installation of native

forbs to establish populations of both ideal host plants and spring nectar sources

that are available through the various stages of the pollinator's lifecycle are

essential. Available densities of both host plants and adult nectar sources have

been shown to be strongly linked to population densities of the Fender's blue

butterfly, a sensitive butterfly species in Oregon prairies (Schultz and Dlugosch

1999).

Unfortunately, restoring many native species to the prairies is no easy task

due to many of the challenges facing restoration of native forbs to the South

Sound prairies. However, one key issue warrants mentioning here: low

germination rates. While this is typical for many restoration projects, low

germination presents a major challenge, especially when seed stock is limited or

expensive to procure. Wilson et al. (2004) monitored seeding establishment in the

Willamette Valley prairies for two years looking at one forb from the Borage

family and three forbs from the Aster family. The researchers found that all the

study forbs were marred by extremely low germination and none of the forbs had

a cover over 1.6% after two years. Another study by Applestein et al. (2018)

monitored seeding establishment, as affected by seeding method (seed-drill,

hydro-mulch, and broadcast) and seeding rate, in the South Sound prairies over

three years. Intuitively, the most predictive measure of establishment for year one

of monitoring was seeding rate, while the most predictive measure for

establishment in year three was year one establishment. Even at the highest

seeding rate, plant density (measured by cover) never topped 10% and was often

less than 5% (Applestein et al. 2018). This suggests that establishment of three study species (families *Rosaceae*, *Asteraceae*, and *Poaceae*) is very limited by seed availability in the field.

Compounding the issue of low germination is that after years of invasion and fire suppression, prairie soils are impoverished of native seed. An expansive study of the Puget Trough – Willamette Valley prairies by Stanley et al. (2011) found that no level of invasive removal treatment increased native plant cover. The only treatment that increased plant cover even moderately was seed addition, emphasizing how seed limited prairies in this ecoregion are and the importance of native seed additions as part of the restoration process. One way to address the issue of low germination is identifying microsites where seeds have the highest probability of germination and survival.

The Puget Trough prairies historically contained a varied and diverse mix of soil types, but most of the deeper and more productive soils have been converted to agriculture. Today many of the remnant prairies consist of 'Mima Mounds,' named for a prominent mounded South Sound prairie. The origin of these mounds has been contested in the past; however most recent research has coalesced around the opinion that these mounds are a result of glacial outwash processes. This idea was first formulated as the Sun cup hypothesis by J. Bretz in 1913. Bretz proposed that coarse sediments accumulating in the melting glaciers may have collected in pits called sun cups. As these cups melted the ice-captured soil and gravel would have settled in a mounded shape (Bretz 1913).

The various prairie microsite types are defined by two things: elevation and soil class. Elevation affects the movement of water to and from a microsite. Low-lying swales, for example, are composed of more mesic soils as water tends to pool in low lying areas. Pedogenesis at these sites can be attributed to glacial outwash by the Vashon ice stade, which reached the southernmost point approximately 17,000 BP, afterwards retreating an average of 340 meters per year (Porter and Swanson 1998). Mounded and swale microsites typically consist of a Nisqually-Spanaway soil complex that is differentiated by deeper horizons of more fine sediments than surrounding shallow and sandy/ gravelly intermounded and upland microsites consisting of Spanaway soils (Bretz 1913, Dunwiddie and Martin 2016).

While microsites are inherently defined by abiotic processes (soil sediment composition, glacial legacies), there is also a biotic understanding regarding the multiple ways that microsites differ. Looking out on a mounded prairie there is a general sense that the mounds foster more vigorous plant growth characterized by denser growing forbs, an abundance of non-native blackberry, and more bracken ferns. Researching any microsite effect on the restoration of rare plants requires an examination of both the aboveground and belowground biota that characterize a given microsite.

Understanding the micro-site preference of rare prairie forbs will allow for research to help inform management and to get their most bang-for-buck in restoration work (Dunwiddie and Martin 2016, Falk et al. 1996). Microsites offer distinct, somewhat undefined, niches that plants seem to be selected for. Past

research shows that mounded topographies foster greater plant diversity and higher survival of *Castilleja levisecta,* (i.e. golden paintbrush) than either the intermounded areas or low-lying swales (Dunwiddie and Martin 2016). Further, this finding has stronger predictive power than both functional group richness and indicator species– both very relevant due to the hemi-parasitic nature of *C. levisecta* (Dunwiddie and Martin 2016). Work by Guerrant and Kaye (2007) in the Willamette Valley prairies showed that microtopographic position strongly influenced native forb survival; *Lomatium* sp. preferred lower topographies with higher soil moisture while *Sericocarpus*, *Erigeron*, and *Horkelia* spp. found greater survival in higher, drier microsites.

The microsite effect is noticeable simple by eye, as well. After a wildfire burned the south parcel of the Scatter Creek Prairie in summer 2017, a noticeable pattern in the fall regrowth could be seen. Much more vigorous regrowth of prairie vegetation occurred on the mounds as opposed to swales or intermounded areas that were all equally burned (personal observation, 2017). Indeed, in mounded prairies that haven't been burned, one can still pick out the darker shaded mounds across the landscape due fewer grasses and more ferns/shrubs.

Mound microsites are prominent to the eye due to dense vegetation (Image taken at Mima Mounds Prairie).

Evaluating prairie microsites as a method for identifying appropriate habitat for species of conservation concern may yield promising results for rare plant restoration. I hope to address both the practical implication of this strategy (identifying suitable microsites is a cost-effective means to improve germination/survival and add more pollinator habitat) and potential mechanistic controls on native plant establishment in a restoration context (the underlying reasons as to why a microsite may yield better restoration outcomes). Through this thesis, I address the following questions:

1.) What is the simplest and most accurate way to describe the characteristics that differentiate microsites on the prairie landscape?

2.) Which microsites yield the strongest germination performance of locally rare species, *Balsamorhiza deltoidea* and *Gaillardia aristata*?

3.) How do microsite characteristics influence germination of *Balsamorhiza deltoidea* and *Gaillardia aristata*?

Preservation of biodiversity is often seen as an 'insurance policy' in the face of constant change, characteristic of the current Anthropocene (Diaz and Cabido 2001). While biodiversity is generally thought to increase ecosystem functioning/ services, this research selected the study forbs due to their status as late season and high-quality nectar sources for endangered species, not 'diversity simply for diversity's sake.' A common critique of restoration is that it often lacks concrete goals that link restored habitat to specific species (Kaye 2009). By linking restoration of specific plant species with endangered invertebrates this

study engages a discipline-wide effort to have concrete restoration goals that promote the viability of target endangered species (Kaye 2009).

Methods

Site Layout and Description

Sites for this study were chosen to minimize differences in disturbance regimes and to capture a representative diversity of microsites typical to the South Sound prairies. Glacial Heritage Preserve [46.865128, -123.040876] and two sites at Joint Base Lewis McChord (Johnson Prairie [46.927283, -122.734468] and Training Area 15 [47.012644, -122.440316]) all received a prescribed burn the year before seeding with *B. deltoidea* and, in the case of Glacial Heritage, *B. deltoidea* and *G. aristata*. Plot arrangement differed between sites, as the Joint Base Lewis McChord (JBLM) plots had been established as part of a larger study. Plot layout at Glacial Heritage consisted of three transects with between 7 and 8 1-m^2 quadrats on each transect for a total of 24 quadrats, evenly split between two microsite types. JBLM sites each consisted of three 150 meter long transects. Along each transect 10 4 m^2 quadrats were placed, generating a total of 30 quadrats. The transects at JBLM and Glacial Heritage were all on soils of the Spanaway series (Washburn 1998, Dunwiddie and Martin 2016). All three sites have been actively managed for high quality prairie habitat with ongoing burning and invasive removal treatments over the past 15-20 years.

Seeding strategy also differed between sites, with the goal of maximizing seed to ground contact. Plots at Glacial Heritage were mowed with string trimmer, with the cuttings being raked off before seeds were hand scattered. Each plot at

GH received 50 *B. deltoidea* and 72 *G. aristata* seeds. The plots at JBLM had 20 'scratches' in each plot. In the corners and middle of each quadrat a hand cultivator was used to scratch the soil surface, after which two seeds of *B. deltoidea* were dropped into each scratch by hand.

Microsite identification was primarily based on visual criteria, as easy identification of the microsite is the most useful to management based on microsite preferences. Glacial Heritage microsites were placed into two bins: mounds and the intermounds. JBLM microsites were initially categorized into four bins: mounds, uplands, slopes, and swales. Visually speaking the differences between these four microsites was much less stark than the mounds and intermounds of Glacial Heritage. For the purposes of this thesis, the original four microsites have been condensed into two categories based on the topographic position of each microsite for some of the analyses. The 'highland' microsites, i.e. the two highest microsites, consist of the mounds and uplands. The 'lowland' microsites, i.e. the two lowest microsites, consist of the slopes and swales. This was done after finding no significant difference in microsite metrics between the mounds and the uplands and no significant difference between the slopes and swales.

Data Collection

Data on seed germination and soil moisture were collected for each site: once in March, early April, late April, and May. Total germinants were recorded at each visit, without marking individual plants. Moisture was collected in the center of each quadrat with a moisture probe at the same time germination was

monitored for GH and within one week of monitoring JBLM. Bulk density was collected in March from all plots at all sites. Bulk density was measured as fine-earth bulk density, i.e. the amount of fine (<2 mm) sediments for a given volume.

In addition to collecting data on abiotic parameters, biotic parameters were also considered in so far as they influenced germination rates. Within each plot species richness, percent coverage of functional types (grass, forb, bryophyte), and species status as native or exotic was recorded. For this, a point-intercept method was used by constructing a meter-squared quadrat with legs and 16 equidistant intersections using twine strung around the frame. A pin was then dropped at each intersect, perpendicular to the ground. Each plant part that touched the pin was recorded as a 'hit.' The total number of 'hits' per plot was used as a surrogate for plant density. Each hit was also documented as either a native or non-native and as a grass, forb, or bryophyte. For example, to calculate the native percent, the number of native hits was divided by the total number of hits for that plot, then multiplied by 100.

Statistical Methods and Rationale

A variety of different tests were used to explore the central questions of this thesis. Non-parametric tests were used, as both the count data and data characterizing the microsites were non-normal, determined most often by a Shapiro-Wilk test (Ghasemi and Zahediasl 2012). When a simple difference in data was sought, such as for differences in plant density between microsites, either a Kruskal Wallace test or Wilcoxon Rank Sum test was used, depending on the number of microsite-types being considered. Both tests are appropriate for the

non-normal nature of these datasets. A negative binomial regression was chosen to model the influence that microsites themselves and the belowground and aboveground parameters exert on the count data.

The GLM with a link function designating the negative binomial family was used here because the negative binomial distribution handles overdispersion (an issue of higher than expected variance) well, which was the case with zero-inflated count data. A model was considered a good fit when the deviance residual (based on deviance of the model's residuals) was less than the five percent critical chi-squared value (based on the residual for the model's degrees of freedom) (Table 6). To determine differences between microsites or sampling periods, an Estimated Marginal Means (EMM) was used as a post hoc test. It is important to recognize that an EMM is a prediction based on a model of weighted averages and not the raw data. Lastly, a negative binomial regression does not provide a R^2 as in traditional ordinary least squares (OLS) regression, however the negative binomial does provide coefficient estimates. Coefficient estimates are equivalent to one unit of change in the independent variable. In this case, the difference in the logs of expected values of the dependent variable is expected to change by the respective coefficient, given other dependent variables in the model are held constant (stats.idre.ucla.edu).

P-values will be referred to 'weakly significant' if $p < 0.1$ or simply 'significant' if $p < 0.05$. All data were analyzed, and figures created using R Studio version 3.5.1. Tables reporting the test statistic and other statistical test information are found in the Tables Appendix.

Results

Microsite Characteristics

Bulk Density

Results at Glacial Heritage offer some of the clearest microsite differences in terms of both germination rates and microsite characteristics. This is likely the case due to an experimental design that defined only two microsite types for GH. When considered in abiotic terms, mounds (M) and intermounds (IM) at GH differ by both soil moisture and bulk density, although the disparity was much larger for bulk density (Figure 1). Average bulk density for mounds (0.25 g/cm³, sd = 0.25), was significantly lower than intermounds (0.36 g/cm³, sd = 0.36) ($F_{(191,190)} = -0.106$, $p < 0.01$) (Table 1).

Results at JBLM show less of clear difference in microsite bulk density, although one trend stayed consistent throughout the two sites. In both Training Area 15 (TA15) and Johnson Prairie (JP) mounds had a lower bulk density than either of the other three microsite types. Using a GLM, the disparity in bulk density for JP was significant between mounds and slopes ($p < 0.05$), while weakly significant between mounds and swales ($p < 0.1$), and mounds and upland microsites ($p < 0.1$) (Table 2). At TA15 mound bulk density exhibited a weakly significant difference from the upland microsites ($p < 0.1$) (Table 3).

Moisture

Disparities in moisture between the two microsites at GH were less pronounced than differences in bulk density, although a clear trend throughout the spring showed that intermounded areas retained a higher soil moisture (Figure 2).

Mounds at Glacial Heritage throughout the season on average maintained a soil moisture of 19.14% (sd = 11.92), while intermounded areas maintained an average soil moisture of 24.96% (sd = 14.87) (Table 1). High standard deviations for these values may be due to near zero percent moisture in May once soils had largely dried up. A Wilcoxon rank sum test confirmed that moisture differed by microsite (w = 0.6148, $p < 0.05$). Further, an EMM post-hoc test found there was a significant difference in moisture between mounds and intermounds during the March and early April sampling periods (Table 4).

Both TA15 and JP showed almost no difference in moisture between microsites; moisture stayed constant through the season until soils dried out in May (Figure 3). In TA15 upland sites did have a slightly higher soil moisture content than other microsites, however, not enough to register as significant using a Kruskal Wallace test (h(2) = 3.4037, $p > 0.1$). Moisture values in May for TA15 were much higher for JP, however this is likely due to a rain event that happened between sampling the two sites.

Aboveground Biotic Parameters

Considering all the aboveground biotic responses, the strongest difference between GH mounds and intermounds was that of plant density: mounds were characterized by a higher number of hits (avg: 35.17, sd: 7.41) than intermounds (avg: 22.83, sd: 4.62) due to more vigorous plant growth (Wilcoxin rank sum test, w = 2.5, $p < 0.05$) (Figure 4, Table 5). The values for nonnative cover also showed mild, but not statistically significant, differences between GH microsites with exotic cover on mounds averaging 53.03% (sd = 29.09), compared to that of

intermounds, which averaged 44.8% (sd = 7.04) cover (Figure 5, Table 5). These differences in exotic cover, however, were not detectable by a Wilcoxon rank sum test (w = 15, p > 0.1). Species richness (w = 12.5, p > 0.1, Figure 6), native cover (w = 21, p > 0.1, Figure 7), forb cover (w = 17, p > 0.1, Figure 8), and grass percent cover (w = 22 , p > 0.1, Figure 9), also did not significantly differ by microsite.

While differences did exist between the JBLM highland (upland and mound sites) and lowland (slope and swale) microsites for functional and native vs. nonnative cover, these differences were not consistent between JP and TA15. At JP a Wilcoxon rank sum test showed a significant difference in species richness between highland and lowland sites where the lowlands harbored a greater number of species (w = 4, p = 0.0115) (Figure 6). This difference, however, was not found at TA15 (Figure 6). No significant differences were found between microsites for plant density (Figure 4), nonnative cover (Figure 5), or either forb or grass cover (Figures 8 and 9).

Impacts on Germination

Germination Influenced by Microsite

Glacial Heritage was seeded with two perennial forbs, *B. deltoidea* and *G. aristata*. *B. deltoidea* emerged earlier in the season, however both *G. aristata* and *B. deltoidea* hit peak germination in late April (Figures 10 & 11). The negative binomial model (count data treated as the dependent variable and microsite category as a factored independent variable) was found to be a good fit for both *B. deltoidea* and *G. aristate* (Table 6).

While a pattern showing a consistent difference in germination between GH mounds and intermounds was found for *B. deltoidea* (Figure 11), the difference was only predicted by the model to be statistically significant in May (negative binomial GLM, EMM post-hoc, $p < 0.05$) (Table 7). This difference and the overall pattern demonstrate a clear *B. deltoidea* germination preference for mounds. Interestingly a stronger preference for intermounds was found for *G. aristata* (Figure 10). While the general pattern shows a *G. aristata* preference for the intermounds, the difference was weakly significant only in late April (negative binomial GLM, EMM post-hoc, $p < 0.1$) (Table 8).

Peak *B. deltoidea* germination for JP occurred in late April, while germination at TA15 was surprisingly consistent throughout the season (Figure 12). Average percent germination of *B. deltoidea* differed throughout the season between the highland microsites (mounds and uplands) and the lowland microsites (slopes and swales) for both JBLM sites. At JP the differences in germination between highland and lowland sites, was weakly significant during the late April sampling period (negative binomial GLM, EMM post-hoc, $p < 0.1$) (Table 9). For TA15, on the other hand, the model predicted significant differences in germination between highland and lowland sites for early April (negative binomial GLM, estimated marginal means post-hoc, $p < 0.1$), late April (negative binomial GLM, EMM post-hoc, $p < 0.05$), and May (negative binomial GLM, EMM post-hoc, $p < 0.05$) (Table 10).

Negative binomial models were also used to elucidate any influences on germination from the abiotic parameters used to help characterize the microsites. Evaluating bulk density and moisture impacts on GH *B. deltoidea* germination, the model predicted a significant negative effect of bulk density on germination (negative binomial, $p < 0.05$) (Table 11), however no significant influences exerted by moisture were found (Table 11).

Using the same approach for the *G. aristata* counts, the model predicted a significant positive influence of bulk density on germination (negative binomial, $p < 0.05$), while no effect of moisture on germination was found (Table 12). Soil moisture was not a significant influence on *B. deltoidea* germination at either JP or TA15. Bulk density, however, did have a significant effect on germination at JP (negative binomial GLM, $p < 0.05$), but not at TA15. Tracking with the GH results, lower bulk density values at JP yielded a positive influence on *B. deltoidea* germination.

Aboveground parameters (functional group cover, species richness, plant density, and native vs nonnative cover) were also evaluated using a negative binomial model to find whether an influence on germination exists. Plant density, grass cover and forb cover all positively impacted GH *B. deltoidea* germination ($p < 0.05$ for all) (Table 13) while *G. aristata* germination was only weakly positively affected by forb cover ($p < 0.1$) (Table 14). The influence of the biotic parameters (functional cover, native vs nonnative cover) on germination yielded no significant influence on the germination of *B. deltoidea* at either JBLM site.

Discussion

In 1992, famed ecologist E. O. Wilson declared that the coming century will be the era of restoration in ecology (E. O. Wilson 1992). Indeed, as the field of ecology has developed new and powerful methods to demonstrate the importance of diverse and resilient ecosystems, the need to restore landscapes that have been heavily degraded has increased greatly. In western Washington state, native prairies have been subject to fragmentation, species invasion, and a changing climate. In order to preserve the rich diversity and associated services of our prairies, active and persistent restoration is needed (Bakker and Dunwiddie 2011). One of the key goals of the restoration of these prairies is establishing and boosting populations of native forbs.

Native perennial forbs offer some of the strongest habitat for endangered invertebrates due to the high-quality nectar resources and a long blooming period – characteristics important to facilitating more resilience in a prairie where warmer climates threaten the timing of critical pollinator-host interactions (Hegland et al. 2009, Memmott et al. 2007, Potts et al. 2010, Schweiger et al. 2010). Restoration of several high-quality prairie forbs in the Puget-Trough prairies is often hindered by extremely low germination. To address this issue the three critical questions driving this thesis work were: 1.) What is the simplest and most accurate way to describe the characteristics that differentiate microsites on the prairie landscape? 2.) Which microsites yield the strongest germination performance of *B. deltoidea* and *G. aristata*? 3.) How do microsite characteristics influence germination of *B. deltoidea* and *G. aristata*?

Research on the microsites in the South Sound has largely been driven by efforts to understand the habitat requirements of rare species. Reestablishing this habitat for Taylor's checkerspot butterfly and other pollinators whose populations and associated ecosystem services are threatened by habitat fragmentation and degradation is a critical step. Ultimately, the microsite is a convenient way to define 'habitat.' Microsites have long been an important conceptual tool for ecologists to understand how species-specific recruitment might be stifled, especially when seed limitation is not the only culprit (Eriksson and Ehrlén 1992). Even when seed limitation is shown to not be an issue, many species seem to be microsite limited (Ingersoll and Wilson 1993).

The nature of the microsite and how it becomes defined influences the microsite's measurable characteristics, which is ultimately how microsites manifest effects on germination. Characteristics like light (Severns 2008, Tang et al. 1992), temperature (Rice 1985), co-occurring vegetation (Donath et al. 2007) or, in the case of this study, bulk density and moisture (Thill et al. 1979) and vegetation structure (Ryser 1993) are all variables known to influence plant germination. Further, all these variables are likely to be differentiated by different microsite types whether it be patches in grasslands (Rose and Frampton 2010, Tang et al. 1992), stature of grasses (Rose and Frampton 2010, Severns 2008) or sites with gopher mounds (Rice 1985).

The role of microsites in a restoration setting is also important to acknowledge relative to the scale of restoration work. Conducting research on a scale that is too small or too skewed as to not represent the greater landscape will

not yield helpful data to inform restoration actions just as research on too large a scale can lack in action driven restoration prescriptions. In addition to issues surrounding scale, microsites are easily recognizable and offer a valuable unit of observation for land managers, as they do not require specialized knowledge or instruments to identify them. Mounds are prominent and abundant at Glacial Heritage while Johnson Prairie and Training Area 15 have fewer mounds but more dramatic slopes transitioning into upland sites.

While all three sites differed in study design, there were a few parameters that stayed consistent across GH, JP, and TA15. In particular, the mounds at all three sites were characterized by a bulk density lower than either the intermounds at GH or the slopes, swales, and uplands at the JBLM sites. This lower bulk density is often indicative of less compacted soil (Haveren 1983), and more organic matter (USDA/NRCS – Soil Quality Indicators). The lowest average bulk density was found at GH, which was not surprising since GH boasts the most notable mounds of the three sites. The characterization of mounds across sites as hosting deeper, finer soils than surrounding soil types conforms with other descriptions of the South Sound prairie microsites and their soil makeup (Dunwiddie and Martin 2016).

The 'mima mounds' found in all three sites are found in prairies outside of the Puget-Trough – Willamette Valley prairie complex as well. 'Mima mound' structures have been described in the literature as occurring in San Diego county (Cox 1984), Argentina (Cox and Roig 1986), Minnesota (Ross et al. 1968), Kenya (Cox and Gakahu 1985), and Missouri (Horwath and Johnson 2006).

Interestingly the differences in soil moisture by microsite were not found to be as strong as the bulk density signal. While microsites were not found to harbor notable different moisture regimes throughout the spring, they illuminate another aspect of the South Sound Prairies. Native prairie soils are often thought to be relatively harsh due to low nutrients and low water holding potential (Ugolini and Schlichte 1997). The fact that JP microsites in March had an average moisture of 20.8%, which subsequently dropped to zero percent the next month is a testament to how excessively drained these soils are. This trend was also found at GH, and, to a lesser extent due to localized rain right before sampling, TA15.

While the data do demonstrate the harshness of prairie soils, they do not show how an elongated period of moisture would improve germination of desired natives. Field observations seemed to suggest that hot and dry conditions in late May were a major component of germinant stress and death; it is possible more moist conditions may improve performance. Soil moisture does have clear links to seedling mortality in grassland systems (Morgan 1995).

While high seedling mortality may be attributed to May's hot and dry conditions, native and non-native plants may not respond to soil moisture in the same way. For example, drought conditions in California grasslands have been demonstrated to favor native perennial grasses over non-native annual grasses (Hamilton et al. 1999). Soil moisture, like many other variables, is difficult to see as a simple good or bad effect. When augmenting soil moisture through irrigation or other means, restoration practitioners should take note of the level of plant

invasion; increasing soil moisture may have unintended consequences on native vs. non-native competition.

Microsites can easily be differentiated by eye due to another factor aside from topography, which is that of plant density. Glacial Heritage showed the clearest difference here where plant growth was noticeably denser on the mounds than the surrounding intermounded areas. The cause of the denser growth could possibly be attributed to higher total nutrients in the mounded areas, as higher nutrients are often associated with lower bulk densities. While this study did not look at soil nutrients, given the differences found in bulk density between microsites, analysis of soil nutrients in future studies could provide promising insights into the conditions that characterize microsites and their associated plant communities.

Higher aboveground plant density in mounded areas may also be providing thermal refugia during the late spring and early summer when a lack of forest cover leads to hot and dry conditions on the prairie. For Johnson Prairie and Glacial Heritage, a higher plant density on the highland and mound microsites resulted in greater germination of *B. deltoidea*. Towards the end of monitoring for germination, most of the seedling die-off seemed to be related to heat stress as many of the germinants that survived through the final sampling period had denser growth surrounding them. Not all forbs responded in the same way; for example, *G. aristata* did better in areas with less dense growth. For plants that are more easily stressed out by late season heat, seeding into areas that have allowed

some growth after a burn would be preferred compared to seeding immediately after a burn when there are few plants to provide a thermal refugia.

Recognizing the connection between differing forb phenologies and their preferred habitat is an important component of the conservation of rare and threatened species. The later emergence of *G. aristata* in the more exposed microsites follows what would be expected; species that emerge in hotter conditions are likely to prefer habitats that are more exposed to heat stress but offer the benefit of reduced plant competition. Conversely the earlier emergence of *B. deltoidea* conforms to a preference for microsites that provide more cover and thermal refuge. While *B. deltoidea* avoids the environmental stress of a later season emergence, it must contend with increased plant competition in the early spring. Exploring the link between phenological traits of individual species and their preferred microhabitats, such as was done in Galen and Stanton (1991), is an important avenue for future research that focuses on the restoration of rare and ecologically important species.

Vigorous growth of non-native plants is generally thought of as being deleterious to establishment of desirable native species, however this study found no effect of differences in native vs. non-native cover on the germination of the study forbs. As far as germination goes it seems that both native and non-native plants provide the same quality thermal refugia. The study sites are actively managed with herbicide, so no site had an overwhelming presence of non-native species. Past a certain threshold it would be expected that non-native growth would suppress germination of natives (Fabbro et al. 2014, Mangla and Callaway

2008) but given the current makeup of the prairie there is no clear evidence that the current level of invasion is strongly inhibiting the germination of desirable native forbs, at least when considered on the microsite scale.

Understanding the habitat requirements of rare and hard-to-establish grassland species is tricky business, as what is ideal habitat for one species may be detrimental to another. In the context of the South Sound prairies, microsites offer an easily identifiable and feasible way to manage for features that support the re-establishment of stubborn species. Further, prairie microsites are differentiated by multiple biotic and abiotic features, providing niche space for which target species are selected. Understanding both which microsites yield strongest growth and what mechanisms underpin that pattern are valuable insights for researchers and managers alike.

Principle Conclusions

- Microsites can be differentiated by several parameters, most noticeably soil bulk density. While many parameters measured by this study were not consistent across all three sites, mounds consistently had lower bulk densities than the other microsites.

- While the different microsites showed no detectable difference in moisture regime, this study agrees with previous assessments of Puget-Trough prairie soils being harsh and excessively drained.

- Germination of both *G. aristata* and *B. deltoidea* showed a preference for different microsite types across all three sites. *B. deltoidea* germinated at higher rates in the mounds at Glacial Heritage and in the highland sites (mounds + uplands), whereas *G. aristata* germinated at higher rates in the inter-mounds of Glacial Heritage.

- Density of aboveground growth differed strongly between mounds and inter-mounds at Glacial Heritage. Although denser plots had slightly more non-native species, it was not a higher presence of alien species *per se* that influenced germination.

- *B. deltoidea* and *G. aristata* should be kept in different seed mixes to be sown in their preferred microsites/ soil type preference.

- For plants that are more easily stressed out by late season heat, seeding into areas that have allowed some growth after a burn would be preferred compared to seeding immediately after a burn when there are few plants to provide a thermal refugia.

- Testing for microsite preference is a viable approach for hard-to-establish native forbs. Defining too many microsites may dilute the effect a microsite has on any given desirable outcome.

Thesis Works Cited

Applestein, C., Bakker, J. D., Delvin, E. G., & Hamman, S. T. (2018). Evaluating

Seeding Methods and Rates for Prairie Restoration. *Natural Areas Journal*, 38(5),

347–355.

Barnosky, C. W. (1985). Late Quaternary vegetation near Battle Ground Lake,

southern Puget Trough, Washington. Geological Society of America Bulletin

96:263–271.

Bond, W. J., & Parr, C. L. (2010). Beyond the forest edge: Ecology, diversity and

conservation of the grassy biomes. *Biological Conservation*, 143(10), 2395–2404.

Boyd, R. (1999). Indians, Fire, and the Land in the Pacific Northwest. Oregon State

University Press, Corvallis.

Bretz, J. H. (1913). *Glaciation of the Puget Sound region.* University of Chicago.

Cox, G. W. (1984). The Distribution and Origin of Mima Mound Grasslands in San

Diego County, California. *Ecology*, 65(5), 1397–1405.

Cox, G. W., & Roig, V. G. (1986). Argentinian Mima Mounds Occupied by

Ctenomyid Rodents. *Journal of Mammalogy*, 67(2), 428–432.

Díaz, S., & Cabido, M. (2001). Vive la difference: plant functional diversity matters to

ecosystem processes. *Trends in Ecology & Evolution*, 16(11), 646–655.

Donath, T. W., Bissels, S., Hölzel, N., & Otte, A. (2007). Large scale application of

diaspore transfer with plant material in restoration practice – Impact of seed and

microsite limitation. *Biological Conservation*, 138(1), 224–234.

Dunwiddie, P. W., & Bakker, J. D. (2011). The Future of Restoration and Management
of Prairie-Oak Ecosystems in the Pacific Northwest. *Northwest Science*, 85(2),
83–92.

Dunwiddie, P. W., & Martin, R. A. (2016). Microsites Matter: Improving the Success
of Rare Species Reintroductions. *PLoS ONE, 11*(3), e0150417.

Endangered and Threatened Wildlife and Plants; Designation of Critical Habitat for
Taylor's Checkerspot Butterfly and Streaked Horned Lark. (2013). Federal
Registrar.

Eriksson, O., & Ehrlén, J. (1992). Seed and microsite limitation of recruitment in plant
populations. *Oecologia*, 91(3), 360–364.

Fabbro, C., Güsewell, S., & Prati, D. (2014). Allelopathic effects of three plant
invaders on germination of native species: A field study. *Biological Invasions*,
16(5), 1035–1042.

Falk, D., Millar, C., & Olwell, M. (1996). Restoring Diversity: Strategies for
Reintroduction of Endangered Plants. Island Press, Washington DC.

Galen, C. and Stanton, M. L. (1991), Consequences of Emergence Phenology for
Reproductive Success in *Ranunculus adoneus* (Ranunculaceae). *American
Journal of Botany*, 78: 978-988.

Ghasemi, A., & Zahediasl, S. (2012). Normality Tests for Statistical Analysis: A Guide
for Non-Statisticians. *International Journal of Endocrinology and Metabolism*,
10(2), 486–489.

Guerrant Jr, E. O., & Kaye, T. N. (2007). Reintroduction of rare and endangered

plants: common factors, questions and approaches. *Australian Journal of Botany*,

55(3), 362.

Hamilton, J. G., Holzapfel, C., & Mahall, B. E. (1999). Coexistence and interference

between a native perennial grass and non-native annual grasses in California.

Oecologia, 121(4), 518–526.

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A.-L., & Totland, Ø. (2009). How

does climate warming affect plant-pollinator interactions? *Ecology Letters*, 12(2),

184–195.

Horwath, J. L., & Johnson, D. L. (2006). Mima-type mounds in southwest Missouri:

Expressions of point-centered and locally thickened biomantles. *Geomorphology*,

77(3–4), 308–319.

Ingersoll, C. A., & Wilson, M. V. (1993). Buried propagule bank of a high subalpine

site: Microsite variation and comparisons with aboveground vegetation. *Canadian

Journal of Botany*, 71(5), 712–717.

Krock, S. (2016). Effects of sowing time and relative prairie quality on first year

establishment of 23 native prairie species. MES Thesis, The Evergreen State

College, Olympia, WA.

Lee-Mäder, E., Fowler, J., Vento, J., & Hopwood J. (2016). 100 Plants to Feed the

Bees. The Xerces Society, Storey Publishing, LLC, Portland, OR.

Linders, M. J., Hynson, L., Lewis, K., and Hamilton, L. (2015). Captive rearing and
    translocation of Taylor's checkerspot in south Puget Sound: 2014-2015. Annual
    Progress Report to U.S. Fish and Wildlife Service and Joint Base Lewis-McChord
    Army Compatible Use Buffer Program. Washington Department of Fish and
    Wildlife. Olympia, Washington.

Macarthur, R., & Wilson, E. (1967). The Theory of Island Biogeography. Princeton
    University Press.

Mangla, S., & Callaway, R. M. (2008). Exotic invasive plant accumulates native soil
    pathogens which inhibit native plants. *Journal of Ecology*, 96(1), 58–67.

Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and
    the disruption of plant–pollinator interactions. *Ecology Letters*, 10(8), 710–717.

Morgan, J.W., (1995) Ecological Studies of the Endangered Rutidosis
    leptorrhynchoides. II. Patterns of Seedling Emergence and Survival in a Native
    Grassland. *Australian Journal of Botany*. 43, 13-24.

Porter, S. C., & Swanson, T. W. (1998). Radiocarbon age constraints on rates of
    advance and retreat of the Puget Lobe of the Cordilleran ice sheet during the last
    glaciation. *Quaternary Research*, *50*(3), 205–213.

Rice, K. J. (1985). Responses of Erodium to Varying Microsites: The Role of
    Germination Cueing. *Ecology*, 66(5), 1651–1657.

Rose, A.B., Frampton, C.M. (1999). Effects of microsite characteristics
    on Hieracium seedling establishment in tall- and short-tussock grasslands,
    Marlborough, New Zealand, New Zealand *Journal of Botany*, 37:1, 107-118.

Ross, B. A., Tester, J. R., & Breckenridge, W. J. (1968). Ecology of Mima-Type Mounds in Northwestern Minnesota. *Ecology*, 49(1), 172–177.

Ryser, P. (1993), Influences of neighboring plants on seedling establishment in limestone grassland. *Journal of Vegetation Science*, 4: 195-202.

Schultz, C. B., & Dlugosch, K. M. (1999). Nectar and hostplant scarcity limit populations of an endangered Oregon butterfly. *Oecologia*, 119(2), 231–238.

Schweiger, O. , Biesmeijer, J. C., Bommarco, R. , Hickler, T. , Hulme, P. E., Klotz, S. , Kühn, I. , Moora, M. , Nielsen, A. , Ohlemüller, R. , Petanidou, T. , Potts, S. G., Pyšek, P. , Stout, J. C., Sykes, M. T., Tscheulin, T. , Vilà, M. , Walther, G. , Westphal, C. , Winter, M. , Zobel, M. & Settele, J. (2010). Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. *Biological Reviews*, 85: 777-795.

Severns, P. M. (2008). Seedling population size and microhabitat association in *Lupinus oreganus* A. Heller var. Kincaidii C.P. Sm. (Fabaceae) a threatened plant of western Oregon grasslands. *Native Plants Journal*, 9(3), 358–365.

Soons, M. B., & Heil, G. W. (2002). Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. *Journal of Ecology*, 90(6), 1033–1043.

Stanley, A. G., Dunwiddie, P. W., & Kaye, T. N. (2011). Restoring Invaded Pacific Northwest Prairies: Management Recommendations from a Region-Wide Experiment. *Northwest Science*, 85(2), 233–247.

Stinson, D. W. (2005). Washington State Status Report for the Mazama Pocket

    Gopher, Streaked Horned Lark, and Taylor's Checkerspot. Washington

    Department of Fish and Wildlife, Olympia. 129+ xii pp.

Storm, L., & Shebitz, D. (2006). Evaluating the Purpose, Extent, and Ecological

    Restoration Applications of Indigenous Burning Practices in Southwestern

    Washington. *Ecological Restoration*, 14(4), 256-268.

Tang, Y., Washitani, I., & Iwaki, H. (1992). Effects of microsite light availability on

    the survival and growth of oak seedlings within a grassland. *The Botanical*

    Magazine, 105(2), 281–288.

Tilman, D., & Downing, J. A. (1994). Biodiversity and stability in grasslands. *Nature*,

    367(6461), 363–365.

Thill, D., Schirman, R., & Appleby, A. (1979). Influence of Soil Moisture,

    Temperature, and Compaction on the Germination and Emergence of Downy

    Brome (Bromus tectorum). *Weed Science*, 27(6), 625-630.

Thurston Regional Planning Council, WA. (2017). Population, Housing &

    Employment Data Tables

Ugolini, F.C and K. Schlichte. (1973). The effect of Holocene environmental changes

    on selected western Washington soils. *Soil Science* 116:218-227.

Wilson, M. V., Ingersoll, C.A., Wilson, M.G., and Clark, D. L. (2004). Why pest plant

    control and native plant establishment failed: a restoration autopsy. *Natural Areas*

    Journal. 24:23-31.

Wilson, E. O. (1992). The Diversity of Life. *Bulletin of Science, Technology & Society*,

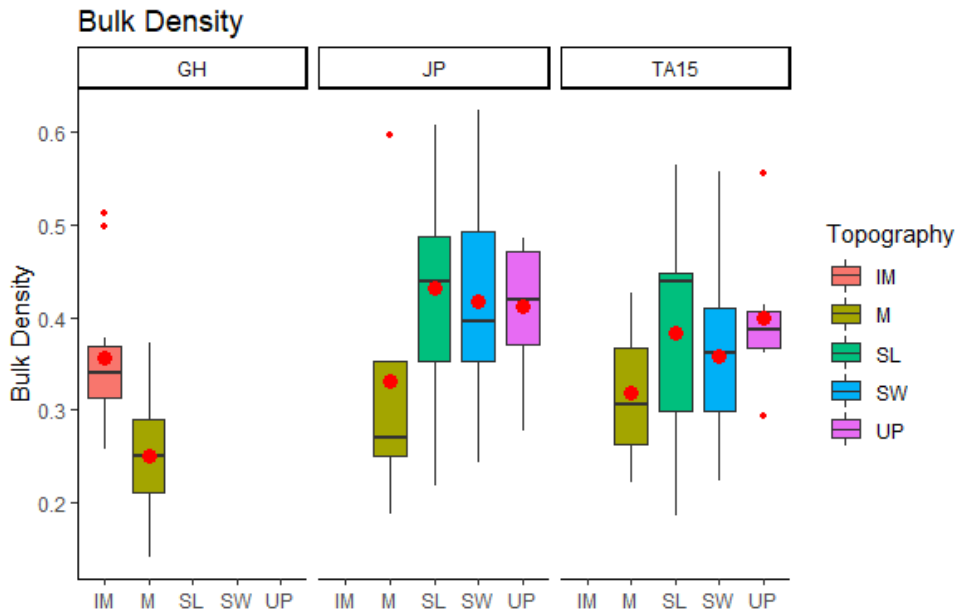14(1), 51–51.

Appendix 1: Figures

Bulk Density

Figure 1 – boxplot of soil bulk densities (g/cm^3). IM = intermound, M = mound, SL = slope, SW = swale, UP = upland.
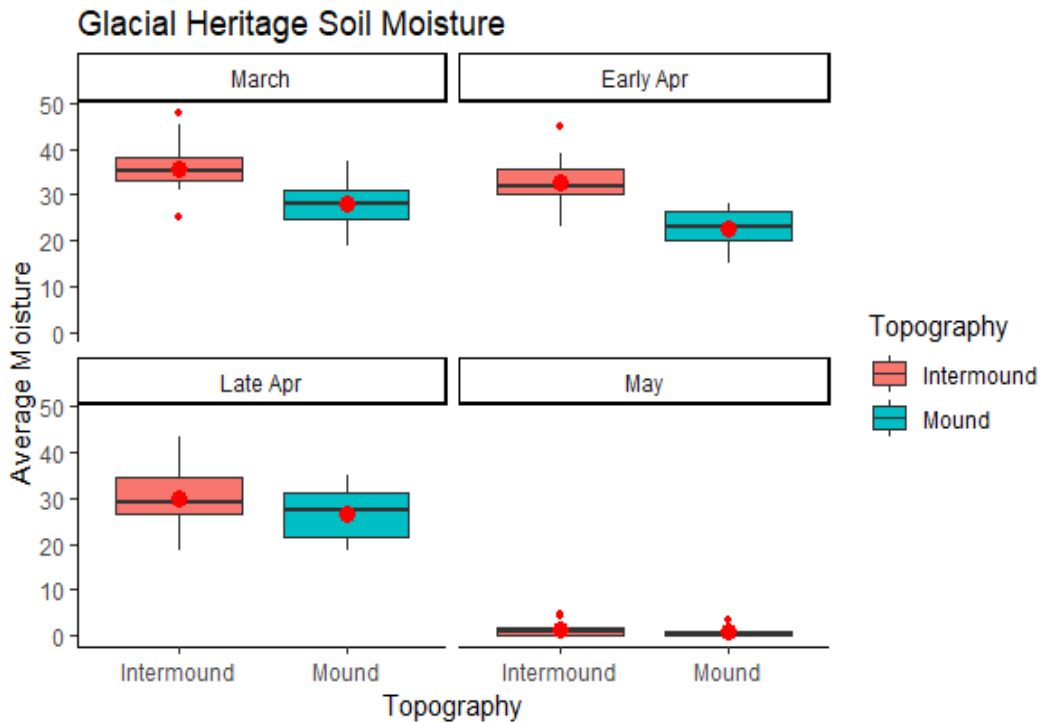

Figure 2 – boxplot of avg. % soil moisture at Glacial Heritage broken down by date and microsite type.
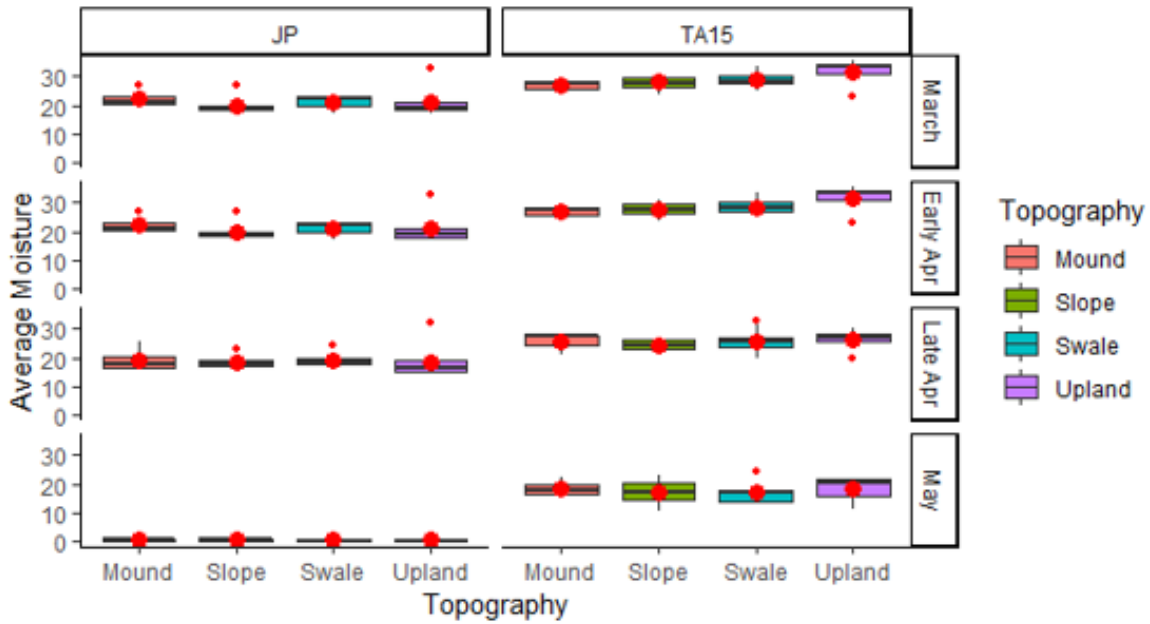
Figure 3 - boxplot of avg. % soil moisture at JBLM broken down by date and microsite type.
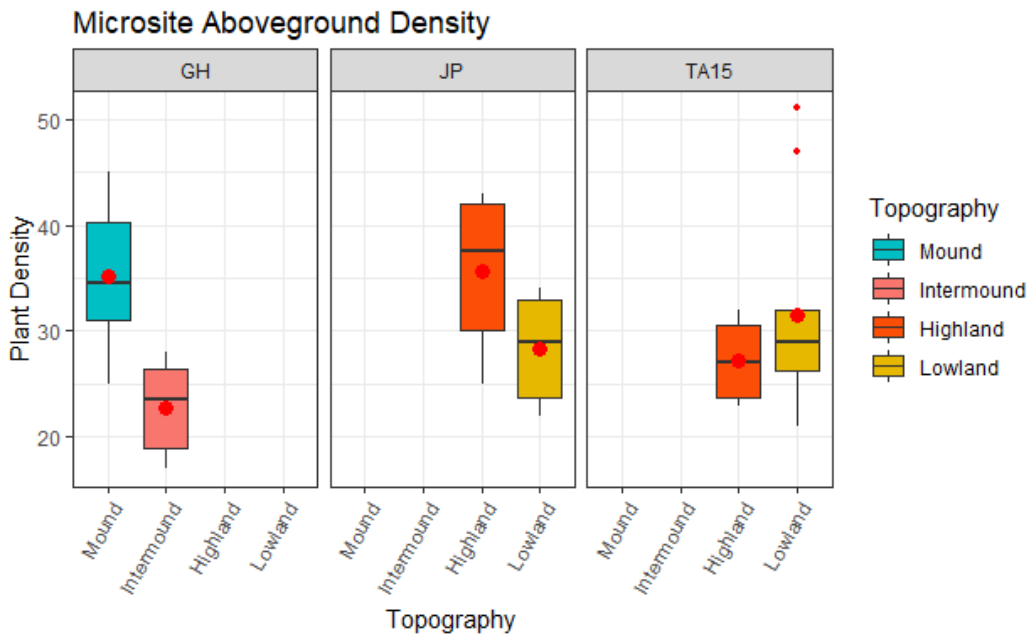
Figure 4 – boxplot of plant density, broken down by microsite type and site. Plant density represents the number of hits in a point-intercept grid, as described in methods.

Figure 5 – boxplot of microsite exotic cover broken down by microsite type and site.
Cover values of 1 indicate 100% cover while values of 0 indicate 0% cover.
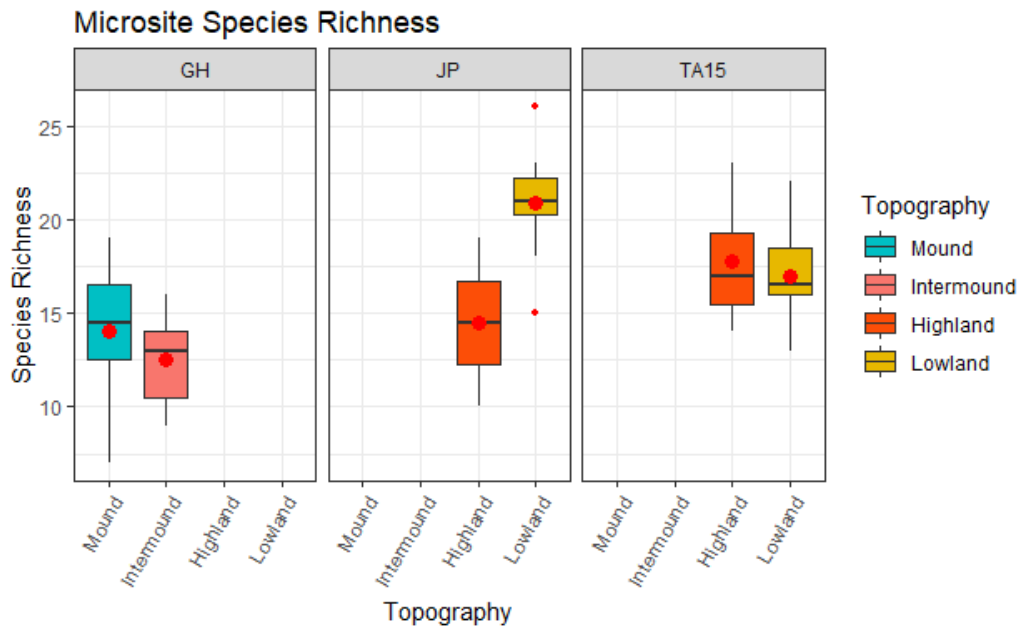
Figure 6 – boxplot of microsite species richness broken down by microsite type and site.
Cover values of 1 indicate 100% cover while values of 0 indicate 0% cover.
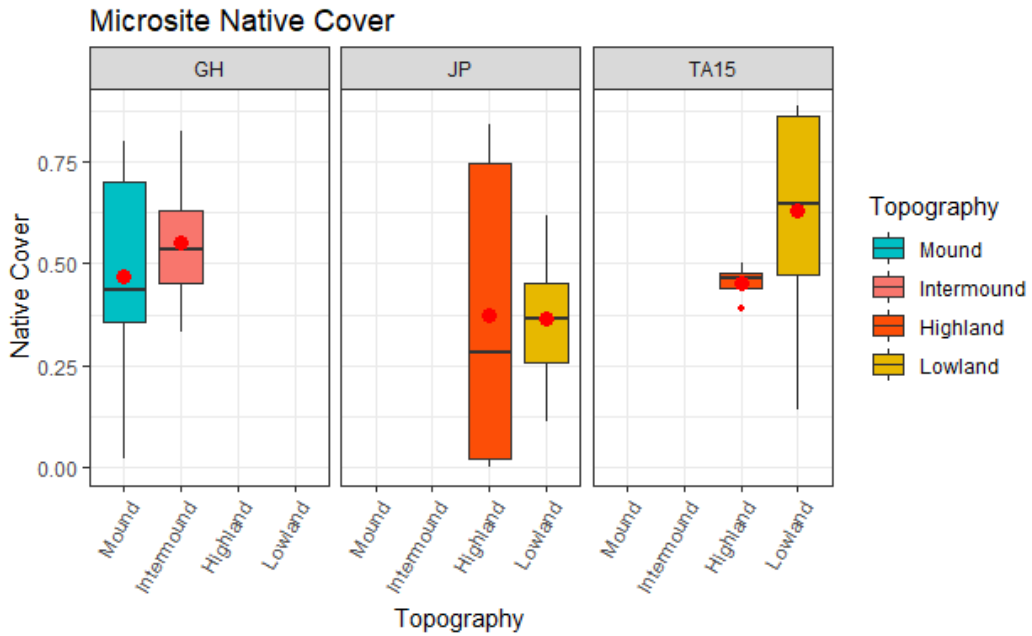
Figure 7 - boxplot of native cover broken down by microsite type and site. Cover
values of 1 indicate 100% cover while values of 0 indicate 0% cover.

Figure 8 - boxplot of forb cover broken down by microsite type and site. Cover
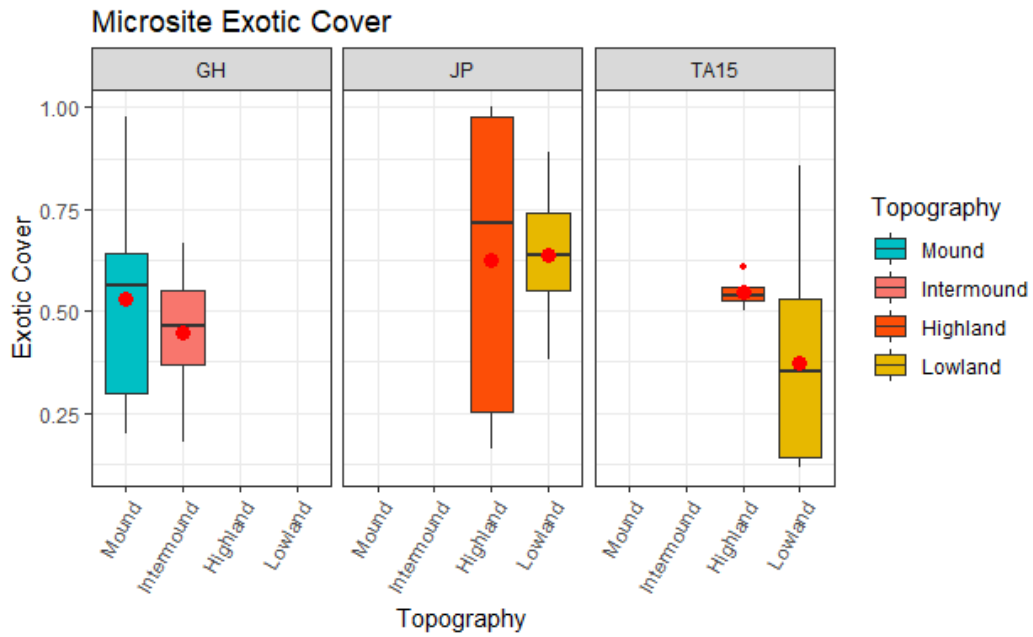values of 1 indicate 100% cover while values of 0 indicate 0% cover.

Figure 9 - boxplot of grass cover broken down by microsite type and site. Cover values of 1 indicate 100% cover while values of 0 indicate 0% cover.

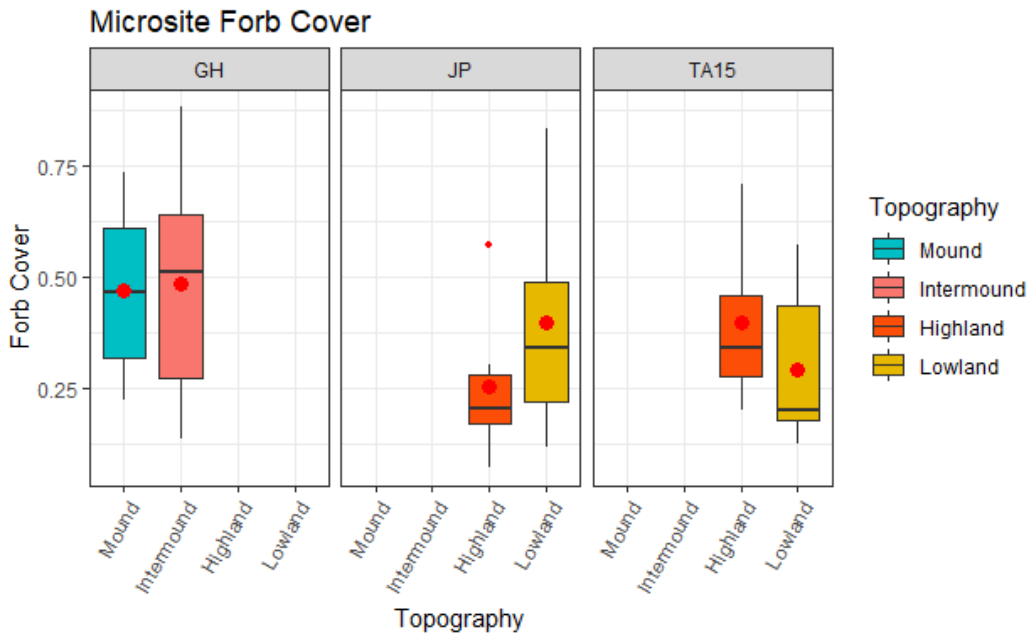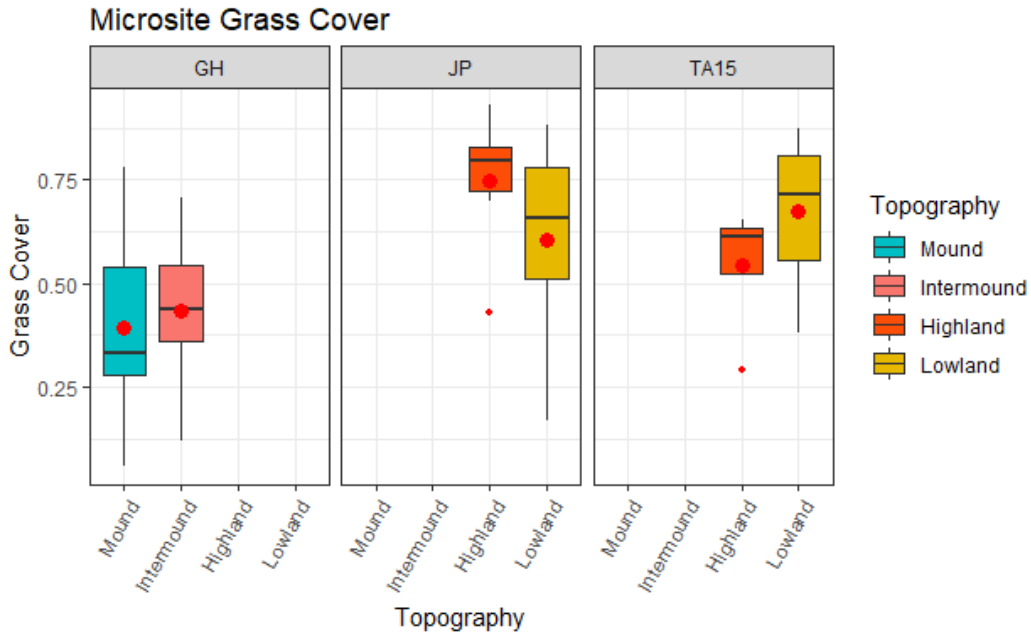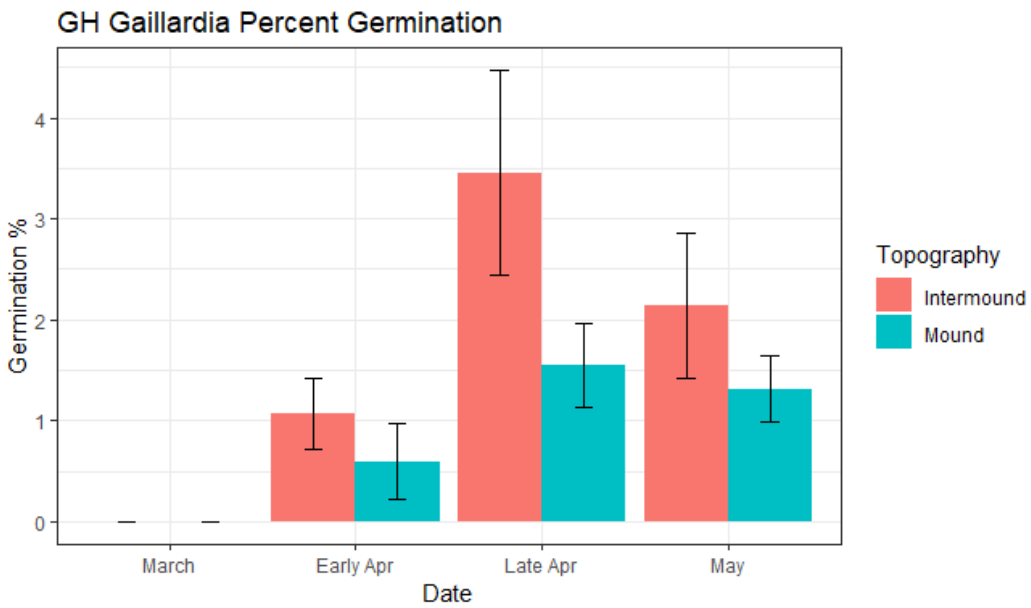Figure 10 – avg. gaillardia germination % by microsite with standard deviation error bars.
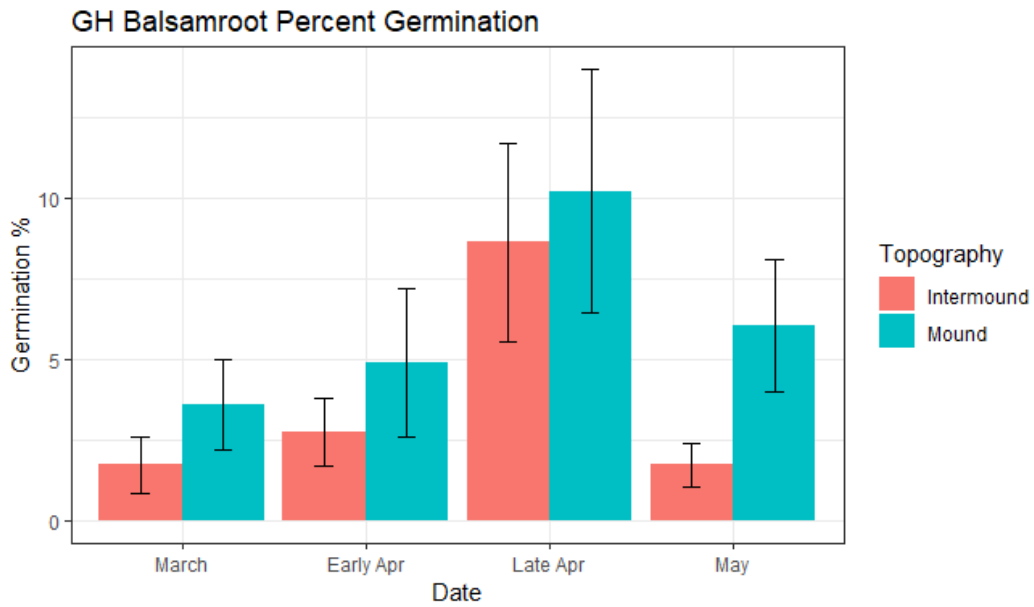
Figure 11 – avg. GH balsamroot germination % by microsite with standard deviation error bars.
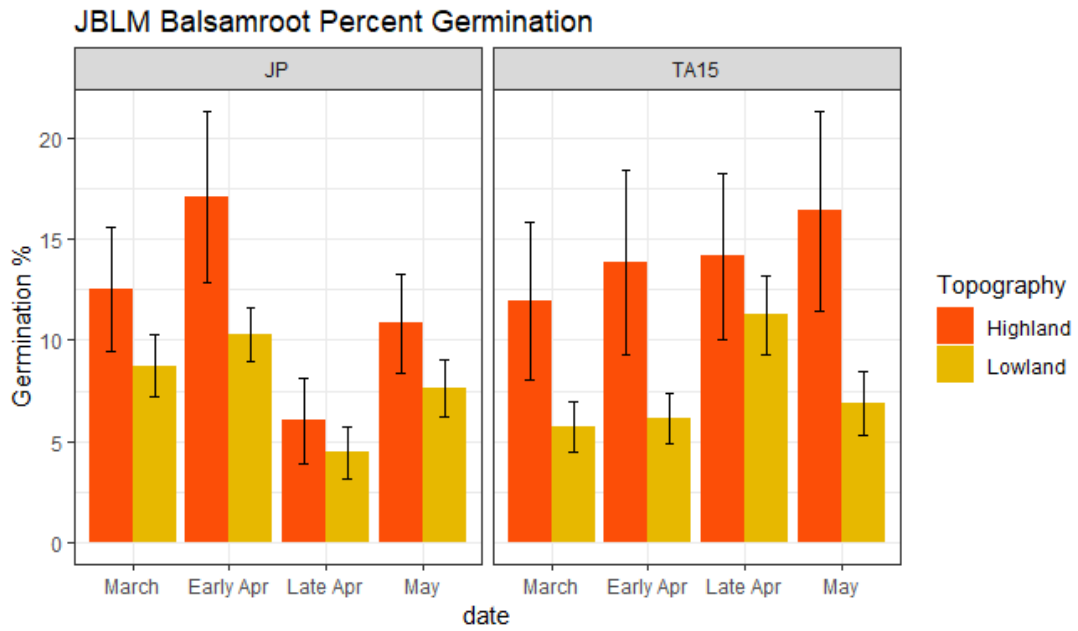
Figure 12 – avg. JBLM balsamroot germination % by microsite with standard deviation error bars.

Appendix 2: Tables

Table 1 – Descriptive statistics for soil moisture and bulk
density at different microsites at GH over time.

Site	Topography	Date	n	Moisture mean	Moisture sd	BD mean	BD sd
GH	Intermound	March	12	35.58%	6.88	0.36	0.08
GH	Intermound	Early Apr	12	32.67%	5.82	0.36	0.08
GH	Intermound	Late Apr	12	30.16%	6.31	0.36	0.08
GH	Intermound	May	12	1.43%	1.65	0.36	0.08
GH	Mound	March	12	27.96%	5.62	0.25	0.07
GH	Mound	Early Apr	12	22.46%	4.84	0.25	0.07
GH	Mound	Late Apr	12	26.59%	5.71	0.25	0.07
GH	Mound	May	12	0.93%	1.03	0.25	0.07

Table 2 – Estimated marginal means for GLM post-hoc test for
differences in bulk density between microsites at JP.

EMM post-hoc for JP bulk density

Contrast	Estimate	SE	Z ratio	P-value
Mound - Slope	-0.09959	0.0332	-2.997	0.0145
Mound - Swale	-0.08626	0.0344	-2.507	0.0588
Mound - Upland	-0.08031	0.0344	-2.334	0.0903
Slope - Swale	0.01333	0.0266	0.5	0.959
Slope - Upland	0.01929	0.0266	0.724	0.8877
Swale - Upland	0.00595	0.0281	0.212	0.9966

Table 3 – Estimated marginal means for GLM post-hoc test for
differences in bulk density between microsites at TA15.

EMM post-hoc for TA15 bulk density

Contrast	Estimate	SE	Z ratio	P value
Mound - Slope	-0.0652	0.0337	-1.936	0.2129
Mound - Swale	-0.0395	0.0312	-1.263	0.5864
Mound - Upland	-0.0812	0.0345	-2.355	0.086
Slope - Swale	0.0257	0.0229	1.124	0.6747
Slope - Upland	-0.016	0.0271	-0.591	0.9347
Swale - Upland	-0.0418	0.0241	-1.734	0.3058

Table 4 – Estimated marginal means for GLM post-hoc test for
differences in moisture between microsites at GH.

EMM post-hoc for GH moisture

Contrast	Estimate	SE	Z ratio	p-value
Intermound, March - Mound, March	7.625	1.45	5.241	p < 0.0001
Intermound, Early April - Mound, Early April	10.208	1.45	7.017	p < 0.05
Intermound, Late April - Mound, Late April	3.567	1.45	2.452	p = 0.2166
Intermound, Early May - Mound, May	0.508	1.45	0.349	p = 1.000

Table 5 – mean species richness, plant density, native cover, exotic cover, forb cover, and grass cover with standard deviation for microsites within each site.

Site	Topography	n	M richness	SD richness	M density	SD density	M native	SD native	M exotic	SD exotic	M forb	SD forb	M grass	SD grass
GH	Intermound	6	12.50	2.66	22.83	4.62	0.55	0.17	0.45	0.17	0.49	0.28	0.43	0.20
GH	Mound	6	14.00	4.20	35.17	7.41	0.47	0.29	0.53	0.29	0.47	0.20	0.39	0.26
JP	Mound	2	12.50	0.71	35.50	9.19	0.00	0.00	1.00	0.00	0.14	0.10	0.86	0.10
JP	Slope	4	19.50	3.70	32.75	1.26	0.49	0.11	0.51	0.11	0.26	0.13	0.74	0.13
JP	Swale	4	22.25	2.50	24.00	2.16	0.23	0.09	0.77	0.09	0.53	0.28	0.47	0.28
JP	Upland	4	15.50	3.87	35.75	8.46	0.56	0.36	0.44	0.36	0.31	0.18	0.69	0.18
TA15	Slope	4	16.00	0.82	24.50	4.36	0.66	0.35	0.34	0.35	0.35	0.21	0.63	0.21
TA15	Swale	6	17.67	3.08	36.17	10.23	0.60	0.20	0.40	0.20	0.26	0.14	0.70	0.15
TA15	Upland	4	17.75	3.86	27.25	4.43	0.45	0.05	0.55	0.05	0.40	0.22	0.54	0.17

Table 6 – GH count model, goodness of fit. Models were considered a good fit if the model residual deviance was less than the 5% critical chi-squared model deviance value.

GH count model goodness of fit

Species data	Model	Model d.f.	Model residual deviance	5% Critical chi-sq deviance
B. deltoidea	count (dependent) ~ microsite (independent)	88	100.3225	110.898
G. aristata	count (dependent) ~ microsite (independent)	88	75.82066	110.898

Table 7 – Balsamroot germination GLM by microsite at GH.

GH balsamroot germination

estimated marginal means post-hoc

Contrast	Date	Estimate	SE	Z - ratio	P - value
Mound - Intermound	March	-0.734	0.589	-1.245	0.213
Mound - Intermound	Early April	-0.582	0.553	-1.052	0.2928
Mound - Intermound	Late April	-0.168	0.505	-0.334	0.7387
Mound - Intermound	May	-1.253	0.575	-2.177	0.0295

Table 8 – Gaillardia germination GLM by microsite at GH.

GH gaillardia germination

estimated marginal means post-hoc

Contrast	Date	Estimate	SE	Z - ratio	P - value
Mound - Intermound	March	na	na	na	na
Mound - Intermound	Early April	0.588	0.614	0.958	0.3382
Mound - Intermound	Late April	0.802	0.421	1.908	0.0564
Mound - Intermound	May	0.492	0.46	1.07	0.2848

Table 9 – Johnson Prairie balsamroot germination GLM.

JP germination by microsite

estimated marginal means post-hoc

contrast	date	estimate	SE	z.ratio	p-value
Highland - Lowland	Early April	0.356674944	0.285556909	1.249050302	0.211647
Highland - Lowland	Late April	0.508119262	0.273334413	1.858965566	0.063032
Highland - Lowland	March	0.307025035	0.33872677	0.906409126	0.364719
Highland - Lowland	May	0.349375641	0.293965395	1.188492413	0.234639

Table 10 – Training Area 15 balsamroot germination GLM.

TA15 germination by microsite

estimated marginal means post-hoc

contrast	date	estimate	SE	z ratio	p-value
Highland - Lowland	Early April	0.731066	0.391641	1.866673	0.061947
Highland - Lowland	Late April	0.81871	0.385741	2.122435	0.033801
Highland - Lowland	March	0.230524	0.372971	0.618075	0.536526
Highland - Lowland	May	0.868712	0.378839	2.29309	0.021843

Table 11 – GH balsamroot GLM on bulk density and moisture predictors.

GH BALDEL negative binomial: count ~ abiotic	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.929808	0.546924	3.528474	0.000418
bulk density	-4.30705	1.705991	-2.52466	0.011581
moisture	0.014547	0.010747	1.353653	0.175847

Table 12 – GH gaillardia GLM on bulk
density and moisture predictors.

GH GAIARI negative binomial: count ~ abiotic	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.17501	0.608889	-1.92976	0.053637
bulk density	4.084012	1.785779	2.286964	0.022198
moisture	-0.01161	0.011451	-1.0143	0.31044

Table 13 – Biotic parameters influence on balsamroot
germination using negative binomial GLM.

Biotic GH BALDEL negative binomial: count ~ biotic	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-7.98144	3.180735	-2.50931	0.012097
Richness	-0.01417	0.061841	-0.22912	0.818779
Density	0.070466	0.027169	2.593647	0.009496
Exotic	0.041494	0.99594	0.041663	0.966767
Forb	7.169098	2.927689	2.448722	0.014336
Grass	7.672913	2.929281	2.619384	0.008809

Table 14 – Biotic parameters influence on gaillardia
germination using negative binomial GLM.

GH GAIARI negative binomial: count ~ biotic	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.73164	3.702057	-0.73787	0.460593
Richness	0.110107	0.088473	1.244525	0.213306
Density	-0.05626	0.035829	-1.57022	0.116364
Exotic	-1.70108	1.396615	-1.218	0.223224
Forb	5.071007	2.935217	1.727643	0.084052
Grass	3.369795	3.261795	1.033111	0.301552

fin.