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Remnant assessment and soil inoculation to inform large-scale prairie restoration at Eastern Washington University

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**REMNANT ASSESSMENT AND SOIL INOCULATION TO INFORM
LARGE-SCALE PRAIRIE RESTORATION AT EASTERN
WASHINGTON UNIVERSITY**

A Thesis

Presented To

Eastern Washington University

Cheney, Washington

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Biology

By

Erik E. Peterson

Spring 2021

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Abstract

The Palouse Prairie of Eastern Washington and Western Idaho, characterized by rolling hills of deep loess soil, is one of the most endangered ecosystems in the world, with more than 99% converted to tilled farmland. To mitigate this loss, Eastern Washington University has begun prairie restoration on a tilled wheat field adjacent to campus, in the northern extent of the Palouse Prairie Ecoregion. However, effective restoration requires understanding reference conditions, and there are relatively few studies of remnant prairie plant communities or soils, particularly in the Northern Palouse. From north to south in the Palouse Prairie Ecoregion, there are gradients in precipitation and temperature that affect plant communities. In addition, the Prairie is intersected by Channeled Scablands, which were formed when the ancient Missoula Floods washed away some of the loess hills, leaving exposed basalt. In deeper soil pockets, Channeled Scablands have plant communities overlapping with Palouse Prairie. In prairies, both the plants and the soil microbiome can play crucial structural roles in supporting the ecosystem. Successful restoration of degraded plant communities may rely on restoration of the original soil microbiome. Therefore, my goal was to identify and survey remnant prairie vascular plant communities in the region surrounding Eastern Washington University to understand how they vary from north to south and differ from Channeled Scabland, and to understand the role that intact prairie soils, with their complement of microbial species, can play in native plant growth. This resulted in a two-part thesis, with Chapter 1 documenting remnant plant communities, and Chapter 2 studying the effect of whole soil inoculation with native prairie soils on plant growth. To document how remnant prairie plant communities near EWU differ from sites in the

southern Palouse and from Channeled scablands, I identified over 100 remnants from aerial imagery across Whitman and Spokane Counties, and conducted vegetation surveys at thirteen sites, including both Palouse Prairie and Channeled Scabland locations.

Palouse Prairie and Channeled Scabland plant communities, while overlapping, had significant differences as indicated by PERMANOVA analysis. Palouse Prairie remnants had relative more native species, such as *Symphoricarpos albus* and *Balsamorhiza sagittata*, as indicators, while Channeled Scablands tended to have more invasive species, including invasive annual grasses such as *Bromus tectorum* and *Poa bulbosa*. There were also distinctive differences between northern and southern Palouse sites, with northern sites having more *Pinus ponderosa* and *Geum triflorum* while southern sites had more *Ventenata dubia* and *Lomatium dissectum*. Unlike when all sites were analyzed, Palouse site community composition was correlated with aspect and solar radiation.

To determine the effect of the native prairie soil microbiome on native plant growth, I inoculated native and nonnative grass species with soil from native prairies and the restoration site in a greenhouse experiment. The three inoculum sources were Turnbull National Wildlife Refuge, the EWU restoration site, and Kamiak Butte. Plants were grown in unsterilized background soil collected from the EWU restoration site and sterile or unsterile soil inoculum. In general, inoculum source had no effect on either grass species but sterilization of inoculum resulted in increased growth of the native grass especially in soil collected from the EWU restoration. Invasive grass was unaffected by treatment. Results indicate possible nutrient limitation or altered soil microbiome at the EWU restoration site. Overall, my study results provide a better understanding of reference plant and soil communities for the EWU Prairie restoration site.

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Chapter 1. Palouse Prairie and Channeled Scabland Plant Communities

Background

Worldwide, grassland ecosystems are being degraded by invasive species, land-use change, and over-exploitation (Lindborg, 2007; Nyamai et al., 2011; Sielaff, Polley, Fuentes-Ramirez et al., 2019) with some grasslands experiencing up to 99% loss (Black et al., 1998; Donovan et al., 2009). Consequently, native grassland restoration has been a goal worldwide to increase biodiversity and ecosystem function of degraded habitats (Barak et al., 2015; Hanson et al., 2008). Many approaches to grassland restoration have been applied (Koziol & Bever, 2015; Mack et al., 2019), with variable results. As with other systems, long-term restoration results are rarely monitored and restoration may be incomplete (Margules & Pressey, 2000; Middleton et al., 2010). Due to the specificity of an area in restoration, an understanding of a reference system is first needed to understand how restoration may take place (Palmer et al. 2005). These references may be formed from extant areas that represent the undegraded target ecosystem (McDonald et al. 2016) and are useful in highly fragmented landscapes.

The Palouse Prairie, found in Eastern Washington, Northern Idaho, and Northeastern Oregon, is characterized by rolling hills of deep, loess soil, and is one the most endangered grassland ecosystems of the United States (Hanson et al, 2008; Noss et al., 1996). The original Palouse Prairie ecosystem was host to a large diversity of perennial bunchgrasses, forbs, and low shrubs (Weddell 2001) as well as various soil microorganisms and invertebrates which increase plant species diversity and resist invasive species (Callaway et al., 2004).

Since the first European settlement of the region in the mid 1800s (Black et al., 1998), over 99% of the original Palouse Prairie range has been converted to agricultural uses (Daubenmire 1942, Noss et al. 1996, Weddell 2001). The remaining prairies consist of small isolated remnant patches, commonly found on steep and untilled slopes, roadside eyebrows, or field margins (Hanson et al. 2008). These remnants are further threatened by rural development and encroaching invasive species (Donovan et al. 2009). Nevertheless, species composition in these remnants can help guide prairie restoration, and may serve as source species.

Due to large scale land conversion to agriculture, the remaining Palouse Prairie is highly fragmented across the landscape. These remaining fragments can be important sources of species refugia as well as dispersal sources for native plant species (Lindgren and Cousins 2017). The ability to disperse between these remnant areas is key to maintaining species diversity and these remaining areas effectively become isolated islands in the midst of agricultural fields (Lindborg 2007, Lindgren and Cousins 2017).

In the Northern Palouse, the deeper loess soils were originally fragmented by the Missoula Floods that occurred approximately 13,000 years ago (O'Connor et al. 2020). Wind-blown loess soil once covered the basalt layer which created the iconic rolling hills of the Palouse (Bretz et al. 1956). The rolling hills silt layer was lost in many areas of the north during the last glacial retreat when the extensive Glacial Lake Missoula was released by the Columbia Lobe of the Cordilleran Ice Sheet (O'Connor et al. 2020). The resultant catastrophic flood removed huge tracts of silt, exposing the underlying bedrock and creating what is known as the Channeled Scablands of Eastern Washington (Larsen and Lamb 2016). Differences in topography, such as soil depth, aspect, or slope, have

often been cited for changes in plant community (Boeken and Shachak 1994, Grman et al. 2013, Bernards and Morris 2017).

The Channeled Scablands' much shallower soil means drier conditions for plants and may not support the variety of plants that the rolling hills once did. In addition, these communities may be composed of much more drought tolerant species or those that only form shallow root structures such as annual grasses. Although much of the soil was washed away during the Missoula floods, some patches of deeper soil were left and may resemble the rolling hills prairies on isolated soil islands (Bryant et al. 2013).

Physical site characteristics are important drivers of species richness and composition. For example, Hanson et al. (2008) investigated the effect of remnant physical attribute traits on plant diversity using twelve prairie remnants spread across Whitman and Latah counties in Washington and Idaho, respectively. Aspect was found to be a significant predictor of species composition while actual slope was not. Although not a significant predictor, the authors explain that remnants within agricultural fields tended to have lower invasive species abundance than those on field edges (Hanson et al. 2008). In contrast, researchers at The Nature Conservancy found that remnants adjacent to roads and abandoned fields had the greatest number of invasive species (Rowe et al., 2013).

Although understanding the arrangement, size, and individual characteristics of prairie remnants is important, locating small widely spread remnants is often difficult. Little work has been done in the Northern Palouse Prairie region to identify prairie remnants; work that has occurred mainly focused on identifying areas with rare plant species. In the southern Palouse, there have been more studies, including extensive studies of prairie remnants within the Nez Perce Reservation further south (Robins and

Sondenaa 2015). The Nez Perce Tribe research team used one-square mile gridded aerial imagery to identify 500 unknown prairie remnants, which were then prioritized for site visits and restoration (Robins and Sondenaa 2015).

In an effort to mitigate local prairie reductions, Eastern Washington University is currently undertaking a 120-acre reconstruction of original prairie adjacent to campus. The site is located on deeper Palouse-like rolling hills and few low seasonally wetlands but is nearby expansive Channeled Scabland areas. Wheat cultivation has occurred on the restoration site for over fifty years and in 2021 planting will begin in a staged approach to full site restoration. The overall project seeks to reflect the diversity of the original prairie while accomplishing other goals such as carbon sequestration and a working laboratory.

However, without a firm understanding of the local prairie communities, species selection and restoration goals can be difficult to set. Intact prairie remnants are widely spread and small, usually occurring in areas where tilling was not possible. In addition, many of these individual sites are owned by private landowners or farming corporations requiring individual permissions to access.

The aim of my study is to locate prairie remnants in the Northern portion of the Palouse ecoregion and document their plant composition. I sought to determine how different northern Palouse plant communities are from nearby Channeled Scabland plant communities and the more southern portions of the Palouse Prairie. I hypothesized that Palouse Prairie sites would have different plant communities than Channeled Scabland, and sites in the north will have different species composition than sites in the south. I predicted soil depth, aspect, slope, and precipitation would be significant predictors for community composition.

Methods

My study took place on Palouse Prairie remnants within Spokane and Whitman Counties. This area experiences between 16-20 inches of precipitation annually (“PRISM Climate Group, Oregon State U” 2004) with most precipitation occurring during winter and spring, with summers being hot and dry (Black et al., 1998). In contrast, further south, Whitman County experiences more precipitation: 20- 24 inches annually, and slightly higher winter temperatures. The surrounding areas of Pullman, WA have much larger contiguous areas of rolling loess soil.

I identified possible prairie sites using the ESRI world imagery layer available through ArcMap supplemented with Google Earth satellite imagery as needed (Figure 1.1). Without a firm understanding of northern Palouse Prairie plant community composition, sites were chosen with no obvious signs of plowing or buildings from the deeper soil regions associated with Palouse Prairie. I used Agricultural Land Use 2019 layers from the Washington State Department of Agriculture and “Washington Natural Heritage Program Element Occurrences” from Washington State Department of Natural Resources to identify places where plowing may have occurred recently but signs of plowing were not visible on aerial imagery. Sites were digitized in ArcMap 10.6.1 at a scale less than 1:1000 m. Finally, parcel ownership was determined through the Spokane County Scout interactive property viewing map (Spokane County 2019) and the 2017 Spokane County parcel layer provided by Dr. Stacy Warren, professor of geography at EWU, which had more complete contact information in certain cases. All reference layers were projected in HARN Washington State Plane South to avoid any discrepancies

between layer locations. Contact letters were sent to land owners requesting permission to access sites for field visits.

Palouse Prairie ecosystems are also known to occur within a few larger reserves within Whitman County such as: Rose Creek Nature Preserve, Steptoe Butte, Kamiak Butte, Whelan Cemetery, and Smoot Hill. These sites were also surveyed to compare to more northerly Palouse remnants. Finally, sites in Spokane County that are considered Channeled Scabland were chosen to understand differences between these communities and prairie sites. The proximity of the Channeled Scabland to the restoration site could indicate important local plant species, both native and invasive.

To assess plant community composition in each remnant, one square meter plots were randomly placed across locations with as much distance between plots as sites would allow. Ten of these plots were placed at each remnant location. Survey at Steptoe Butte was restricted to only private lands which are more northerly facing than the lands of Washington State Parks. Surveys at Kamiak Butte were restricted to southerly sites because prairie sites are isolated on northern slopes. At Turnbull National Wildlife Refuge, ten plots were randomly placed and an additional five were randomly located within deeper soil areas on the tops of Mima mounds, which are small hills of loess soil approximately 10-20 m across and 1-2 m tall (Bryant et al. 2013). Mima mounds are deeper soil islands which; due to the greater soil depth, these areas are likely to hold more moisture and support a more diverse plant community (Hanson et al. 2008).

At each plot, soil depth, aspect, slope, location, topographic type, and location were all recorded. Soil depth was measured by inserting a 115 cm probe to refusal at each corner of the square plot frame; corner depths were averaged for individual plot soil

depth. Aspect was measured using a compass placed over the center of the plot. Slope was determined by clinometer readings from 5 m upslope and 5 m downslope, differences in readings were measured again for accuracy and averaged if any discrepancy remained. Location was determined using Garmin etrex G.P.S. handheld device with an average accuracy of +/- 4 m.

In each plot, percent cover of all vascular plant species present was recorded, with identifications based on “Flora of the Pacific Northwest” (Hitchcock and Cronquist 2018) and the University of Washington Burke Herbarium Image Collection. Species names were updated using “Integrated Taxonomic Information System” (ITIS, 2020). Plant species that were not field identified were collected as voucher specimens and taken back to the lab for identification.

Following physical plot measurements, percent cover was assigned for each of eight groundcover types: moss, wood, litter, gravel, soil, rock, lichen, and other. Plant species were identified as native or non-native using the USDA Plants National Database (USDA, 2020) and combined for each plot to produce a total native cover and total non-native cover.

Elevation, precipitation, solar radiation, and glacial flood history (Palouse vs. Channeled Scablands) were determined for each plot using GIS. Elevation was determined using the “National Elevation Dataset” interactive map Provided by the US Geological Service using 1/3 arc second resolution to determine an estimated elevation for individual plots. Precipitation for each remnant location was determined from NOAA NCDC climate station 30 year normals if a weather station was within 10 km of the remnant location; otherwise, precipitation was determined from the PRISM map zone

average. I categorized each remnant location as either Palouse Prairie or Channeled Scablands, based on appearance, soil depths, locations, and glacial flood history. Finally, solar radiation was determined using ArcGIS Desktop 10.7.1 points solar radiation tool in Spatial Analyst tools. Ten-meter resolution USGS quad digital elevation models for each plot were downloaded from WAGDA and used to complete solar radiation analysis with time configuration set for the entire year of 2019.

For community composition analysis, rare plant species appearing in less than four plots were removed from further analysis to avoid outlier effects. The reduced vegetation matrix was then used to create a Bray Curtis dissimilarity index using `vegdist` in R. The dissimilarity data was then used with the `NbClust` command to determine the optimal cluster amount. These clusters were then used to create a dendrogram using the `cutree` function in `dendextend` package.

Clusters were then used to define groups for species dominance analysis and indicator species analysis. Dominance and indicator species analysis was then used in conjunction with clusters to determine important plant associations. Dominance analysis was done using the `aggregate` function in R to determine the average cover of each species in each cluster. Indicator species were determined using the function `multipatt` in the R package `indicspecies` version 1.7.9. The analysis was run on each cluster individually, as a comparison of Palouse and Channeled Scabland, and northern and southern Palouse Prairie sites.

Patterns in plant community composition within and among sites were determined using non-metric multidimensional scaling (NMDS) and PERMANOVA. NMDS was run with a stress maximum of 0.2 determined before analysis and Bray-Curtis dissimilarity

index. Data were transformed using a Wisconsin double standardization. Five hundred and eighty two runs were completed with a max iteration of 999.

To determine whether community composition differed significantly among sites, and between Channeled Scabland and Palouse Prairie, PERMANOVA was used. To determine whether community composition differed among sites in the northern and southern portion of the Palouse Prairie, I used PERMANOVA analysis on Palouse Prairie Sites only using county as my grouping variable with all Channeled Scabland sites removed. Spokane county represented northern sites and Whitman County represented southern sites.

Results

Over a hundred possible remnants were identified from aerial investigation representing approximately 63 total private landowners. Of those 63, thirteen approved site visits, ten refused, and forty landowners did not reply. A total of thirteen prairie remnant sites (Figure 1.2) were selected in Spokane and Whitman Counties, with a total of 135 individual plots assessed. Six of these sites were located in Channeled Scablands and seven were located in rolling Palouse Prairie. A total of 245 plant species were identified, of which 161 were native, 73 were non-native, and 11 were identified to genus only (Appendix A).

I identified 15 distinct plant community associations based on cluster analysis. The dendrogram showed a split between Channeled Scabland and Palouse Prairie sites for most sites (Figure 1.3). Names for plant community associations were determined by indicator analysis (Table 1.1) and dominance analysis (Table 1.2).

While overlapping, plant community composition was different among study sites ($p=0.001$), and between Channeled Scabland and Palouse sites ($p=0.001$; Table 1.4). Overlap of Palouse and Channeled Scablands sites was evident as shown by ordination analysis (stress = 0.195, runs = 582; Figure 1.4). Specific survey site explained more of the overall variation in community composition than Channeled Scabland vs. Palouse ($R^2 = 0.235$ and $R^2 = 0.071$, respectively). Slope, precipitation, elevation, and longitude were correlated with plant community composition (Table 1.3). Channeled Scabland communities tended to have greater non-native plant cover, northern latitudes, and solar radiation.

Plant community composition differed between northern and southern Palouse Prairie remnants ($p = 0.001$; Table 1.7), although there was overlap in species composition as shown by the ordination analysis (stress = 0.191, runs 20, Figure 1.6). When Channeled Scabland sites were excluded, aspect, elevation, and solar radiation were significantly correlated with community composition in Palouse Prairie Remnants (Table 1.8).

Palouse Prairie sites were indicated by two native forbs *Symphoricarpos albus* and *Balsamorhiza sagittata*, while Channeled Scabland sites were indicated by the invasive annual grasses *Bromus tectorum* and *Poa bulbosa* (Table 1.1). When only Palouse Prairie sites were used, northern sites were indicated by *Geum triflorum* and *Pinus ponderosa*, southern sites were indicated by *Ventenata dubia* and *Lomatium dissectum* (Table 1.1). In Palouse sites four native species had the highest dominance: *Symphoricarpos albus*, *Balsamorhiza sagittata*, *Geranium viscosissimum*, and *Geum triflorum*. Channeled Scabland sites had the highest dominance of the native forbs

Eriogonum heracleoides, *Madia gracilis*, *Geranium viscosissimum* and the invasive annual grass *Ventenata dubia* (Table 1.2). When only Palouse Prairie sites were analyzed: northern sites had highest dominance of *Symphoricarpos albus*, *Balsamorhiza sagittata*, *Potentilla gracilis*, and *Geum triflorum*, and southern sites had highest dominance of *Symphoricarpos albus*, *Balsamorhiza sagittata*, *Geranium viscosissimum*, and *Lomatium dissectum* (Table 1.2).

Slope and average soil depth had the strongest correlation with community composition ($p = 0.001$ in both cases; Table 1.3), followed by precipitation, elevation, native and non-native cover, and site. Aspect and solar radiation were not correlated with community composition.

Discussion

My study adds to existing literature on Palouse Prairie plant communities because it is the first assessment of Palouse Prairie remnants in the northern extent of its range (Bowlick et al. 2015). These Palouse Prairie remnants are rare and difficult to find. The Northern Palouse has a much more fragmented geologic landscape, due to the presence of the Channeled Scablands, than in the South. Northern Palouse remnants varied in species composition, and had some slight differences from the southern remnants, such as having greater abundance of *Geum triflorum* and less *Lomatium dissectum*. Because sites are highly variable, the combination of many sites in the area will provide the most valuable reference for prairies in the Northern Palouse.

Palouse Prairie and Channeled Scablands had significantly different community composition, although many species overlapped. Palouse prairie sites had higher

dominance of *Symphoricarpos albus* and *Balsamorhiza sagittata*. Channeled Scabland sites had higher cover of *Eriogonum heracleoides* and *Ventenata dubia*. This overlap may be due to the presence of deeper soil areas, such as the Mima mounds on the Channeled Scablands.

Aspect and solar radiation were related to species composition in Palouse Prairie remnants, consistent with other studies (Hanson et al. 2008) that have shown northern slopes supporting more diversity and native species while southern slopes contain less diversity and often more exotic species. When Channeled Scablands were included, aspect may not have been significant because many of our remnants were small and did not contain all aspects within the remnant.

Invasive species were more frequent and had greater abundance in Channeled Scablands sites than in Palouse Prairie but Channeled Scabland plots, at times, did have a high diversity of species. Middleton et al. (2010) found a reduction in diversity as the age of a restoration increased but this effect was attributed to a gradual loss of annual species as perennials became the dominant species. Such annual species can be the largest threat to native remnants and restorations (Nyamai et al. 2011), but have been shown to be effectively reduced by dense perennial bunchgrass cover by reducing water and nutrient availability (Reisner et al. 2013). Channeled Scablands maintained high diversity and had high invasive cover indicating some other factor such as site history or disturbance playing a role in Channeled Scabland communities. Further analysis into site history could identify the underlying cause to these patterns.

Invasive annual grasses, such as *Poa bulbosa*, *Bromus commutatus*, and *Ventenata dubia*, were very common in the surrounding areas of the restoration site.

Their close proximity, and the resulting increased susceptibility of the remnant site to invasion, indicate that an active restoration approach should be used which utilizes manual removal, soil manipulation, or high-density native plantings (Humphries et al. 2021). These native plantings should include local native species identified by this project such as: *Pseudoroegneria spicata*, *Symphoricarpos albus*, and *Geum triflorum*. The dominance of these species in local remnants illustrates their ability to compete with invasive species and may be necessary to maintain restoration integrity (Nyamai et al. 2011). In northern areas, *Pinus ponderosa* and *Geum triflorum* were identified as an important plant community association.

This study was limited by our inability to visit sites multiple times during the summer of 2019. One visit at each site most likely led to our investigation missing some species that were early or late blooming and may bias our results towards mid-season species (Miller et al. 2017). We were limited by ownership access and in some cases, such as Steptoe Butte, only able to assess a limited range of aspects. Finally, we observed a great deal of heterogeneity in plant communities at individual sites, the one m² plots used in this study may not have been large or numerous enough to capture the breadth of the diversity at each site.

Future analyses should focus on repeated visits of remnants to obtain a broader understanding of these communities and how they change through time. These repeated visits could be combined with local wetland studies to understand and develop goals for the entire EWU reconstruction site. Understanding of northern prairie and southern prairie was limited by the amount of northern prairie sites available. Surveys should use larger and more numerous plots at each location.

My study provides the most detailed analysis of the variation in Palouse Prairie plant communities from north to south, and how they differ from Channeled Scabland plant communities, to date. My study provides evidence that local remnants are Palouse Prairie plant communities, making them ideal reference sites for campus prairie restoration. Further, my study shows that the Channeled Scablands plant communities at Turnbull National Wildlife Refuge have many Palouse species, making them a good potential source for genetically local seeds and plant material. The data provided by the study adds to our current understanding of this endangered ecosystem and will help determine which species and sources to use for restoration at Eastern Washington University.

Tables

Table 1.1 The species for each plant community association, Palouse Prairie, and Channeled Scabland based on indicator analysis. The “A” value refers to the positive predictive value of the species and “B” value refers to fidelity of species. Spokane and Whitman counties show species from only Palouse Prairie sites.

INDICATOR SPECIES ANALYSIS				
Cluster	Species	A	B	p
1	<i>Poa secunda</i> *	0.981	1	0.005
	<i>Poa bulbosa</i>	0.980	1	0.005
2	<i>Lagophylla ramosissima</i> *	0.983	1	0.005
	<i>Centaurea cyanus</i>	0.980	1	0.005
3	<i>Epilobium brachycarpum</i> *	0.801	1	0.005
	<i>Bromus tectorum</i>	0.686	0.807	0.005
4	<i>Pseudoroegneria spicata</i> *	0.971	1	0.005
	<i>Clarkia pulchella</i> *	0.970	0.75	0.005
5	<i>Eriogonum heracleoides</i> *	0.985	1	0.005
	<i>Clarkia pulchella</i> *	0.805	0.714	0.005
6	<i>Taeniatherum caput-medusae</i>	0.994	1	0.005
	<i>Lomatium simplex</i> *	0.943	0.5	0.025
7	<i>Madia gracilis</i> *	0.985	1	0.005
	<i>Ventenata dubia</i>	0.927	0.917	0.005
8	<i>Geranium viscosissimum</i> *	0.985	0.931	0.005
	<i>Hieracium scouleri</i> *	0.952	0.655	0.005
9	<i>Balsamorhiza sagittata</i> *	0.971	0.933	0.005
	<i>Lomatium dissectum</i> *	0.944	0.6	0.005
10	<i>Madia exigua</i> *	0.957	1	0.005
	<i>Chondrilla juncea</i>	0.999	0.727	0.005
11	<i>Symphoricarpos albus</i> *	0.948	1	0.005
	<i>Galium boreale</i> *	0.954	0.429	0.005
12	<i>Phlox longifolia</i> *	0.983	1	0.005
	<i>Bromus hordeaceus</i>	0.962	1	0.015
13	<i>Potentilla gracilis</i> *	0.942	1	0.005
	<i>Balsamorhiza sagittata</i> *	0.732	1	0.005
14	<i>Lupinus leucophyllus</i> *	0.934	1	0.05
	NA			
15	<i>Alopecurus pratensis</i>	0.997	1	0.01
	NA			
Palouse Prairie	<i>Symphoricarpos albus</i> *	0.813	0.700	0.005
	<i>Balsamorhiza sagittata</i> *	0.973	0.414	0.005
Channeled Scabland	<i>Bromus tectorum</i>	0.750	0.662	0.005
	<i>Poa bulbosa</i>	0.973	0.385	0.005
	<i>Ventenata dubia</i>	0.984	0.640	0.001

Whitman County	<i>Lomatium dissectum</i> *	1.000	0.320	0.016
Spokane County	<i>Geum triflorum</i>	0.863	0.500	0.006
	<i>Pinus ponderosa</i>	0.804	0.400	0.016

Table 1.2 Species with highest average cover in each plant community association, Palouse Prairie, and Channeled Scabland grouping. Spokane and Whitman counties show species from only Palouse Prairie sites.

DOMINANCE ANALYSIS		
Cluster	Plant Species	Average Cover
1	<i>Poa bulbosa</i>	47.5
	<i>Lomatium triternatum</i> *	10
	<i>Poa secunda</i> *	4.5
	<i>Taeniatherum caput-medusae</i>	4
2	<i>Lagophylla ramosissima</i> *	20
	<i>Centaurea cyanus</i>	12
	<i>Epilobium brachycarpum</i> *	4.5
	<i>Lomatium simplex</i> *	3
3	<i>Epilobium brachycarpum</i> *	5.03
	<i>Festuca idahoensis</i> *	3.94
	<i>Bromus japonicus</i>	3.47
	<i>Ventenata dubia</i>	3.18
4	<i>Pseudoroegneria spicata</i> *	30
	<i>Clarkia pulchella</i> *	10.13
	<i>Lupinus sericeus</i> *	8.75
	<i>Epilobium brachycarpum</i> *	4
5	<i>Eriogonum heracleoides</i> *	52.86
	<i>Lagophylla ramosissima</i> *	3
	<i>Clarkia pulchella</i> *	2.14
	<i>Bromus tectorum</i>	2.14
6	<i>Taeniatherum caput-medusae</i>	37.5
	<i>Bromus japonicus</i>	3.5
	<i>Festuca idahoensis</i> *	3
	<i>Bromus tectorum</i>	2
7	<i>Madia gracilis</i> *	28.08
	<i>Ventenata dubia</i>	20.42
	<i>Amsinckia menziesii</i> *	7.67
	<i>Poa bulbosa</i>	7.58
8	<i>Geranium viscosissimum</i> *	19.14
	<i>Geum triflorum</i> *	5.98
	<i>Symphoricarpos albus</i> *	5.09
	<i>Hieracium scouleri</i> *	4.1
9	<i>Balsamorhiza sagittata</i> *	25.73
	<i>Lomatium dissectum</i> *	7.67

	<i>Symphoricarpos albus</i> *	4.4
	<i>Ventenata dubia</i>	2.83
10	<i>Madia exigua</i> *	38.57
	<i>Chondrilla juncea</i>	5.21
	<i>Danthonia californica</i> *	3.61
	<i>Pinus ponderosa</i> *	3.5
11	<i>Symphoricarpos albus</i> *	38.57
	<i>Crataegus douglasii</i> *	5.21
	<i>Poa pratensis</i> *	3.61
	<i>Geum triflorum</i> *	3.5
12	<i>Phlox longifolia</i> *	40
	<i>Achillea millefolium</i> *	3
	<i>Bromus japonicus</i>	2
	<i>Epilobium brachycarpum</i> *	2
13	<i>Potentilla gracilis</i> *	21.33
	<i>Balsamorhiza sagittata</i> *	9.33
	<i>Geum triflorum</i> *	5.33
	<i>Pseudoroegneria spicata</i> *	4
14	<i>Pseudoroegneria spicata</i> *	4
	<i>Lupinus leucophyllus</i> *	4
	<i>Poa bulbosa</i>	2
	<i>Pinus ponderosa</i> *	1
15	<i>Alopecurus pratensis</i>	95
	<i>Achillea millefolium</i> *	3
	<i>Galium aparine</i> *	1
	<i>Epilobium brachycarpum</i> *	0.5
Palouse Prairie	<i>Symphoricarpos albus</i> *	9.35
	<i>Balsamorhiza sagittata</i> *	6.68
	<i>Geranium viscosissimum</i> *	4.51
	<i>Geum triflorum</i> *	3.75
Channeled Scabland	<i>Eriogonum heracleoides</i> *	7.25
	<i>Ventenata dubia</i>	5.34
	<i>Madia gracilis</i> *	5.34
	<i>Geranium viscosissimum</i> *	4.15
Spokane County	<i>Symphoricarpos albus</i> *	16.75
	<i>Geum triflorum</i> *	9.40
	<i>Balsamorhiza sagittata</i> *	7.45
	<i>Potentilla gracilis</i> *	5.30
Whitman County	<i>Symphoricarpos albus</i> *	6.39

	<i>Balsamorhiza sagittata</i> *	6.37
	<i>Geranium viscosissimum</i> *	4.88
	<i>Lomatium dissectum</i>	3.4

Table 1.3 Correlations between ordination axes and measured environmental variables for all sites.

	NMDS1	NMDS2	NMDS3	r ²	Pr(>r)
Northing	-0.932	0.184	0.311	0.188	0.001
Easting	0.582	-0.247	-0.775	0.096	0.006
Aspect	0.007	-0.590	0.807	0.049	0.106
Slope	0.889	-0.227	-0.397	0.416	0.001
Average Soil Depth	0.319	0.806	0.499	0.360	0.001
Moss Cover	-0.815	0.246	0.525	0.084	0.015
Wood Cover	0.598	0.781	0.181	0.078	0.012
Litter Cover	0.572	0.430	0.698	0.134	0.001
Gravel Cover	-0.471	-0.285	0.835	0.090	0.007
Soil Cover	-0.279	-0.627	-0.727	0.107	0.001
Rock Cover	-0.451	-0.148	-0.880	0.045	0.098
Lichen Cover	-0.493	-0.639	-0.591	0.137	0.002
Other Cover	-0.583	-0.505	0.637	0.026	0.347
Native Cover	0.921	0.099	0.373	0.429	0.001
Non-Native Cover	-0.530	0.841	0.113	0.280	0.001
Elevation	0.460	-0.089	-0.883	0.153	0.001
Precipitation	0.706	0.685	-0.180	0.062	0.038
Solar Radiation	-0.841	0.284	0.457	0.050	0.081

Table 1.4 PERMANOVA results table analyzing differences in plant community composition among sites and between Channeled Scabland and Palouse Prairie.

	Df	SumsOfSqs	MeanSqs	F.Model	R ²	Pr(>F)
Channeled vs Palouse	1	3.758	3.758	12.196	0.071	0.001
Site	11	12.449	1.132	3.673	0.235	0.001
Residuals	119	36.663	0.308		0.693	
Total	131	52.87			1	

Table 1.5 Characteristics and community designations for each site including average cover and species counts determined from individual plot data. Cover and species counts for each site are averaged across ten plots for each site.

Site	Community	Northing	Easting	Size (km ²)	Non-native Cover	Non-native Species	Native Cover	Native Species
TUR	Channeled	5249611	461642	64.95	51.51	4.53	63.9	8.73
SMO	Palouse	5185418	481833	3.24	9.75	3.7	68.95	11
STE	Palouse	5209049	478109	2.3	9.65	3.6	88.55	11.7
GEG	Channeled	5273730	455844	1.97	15.3	3.7	32.15	3.9
KAM	Palouse	5190461	488436	1.21	5.25	3.9	36.35	9.4
BEL	Channeled	5237646	449232	1	21.25	5.2	46.55	8.8
FIS	Channeled	5247476	438598	0.72	29.95	4.8	44.15	10.1
SIL	Palouse	5268639	451953	0.49	5.3	2.9	62.65	10.3
FEL	Channeled	5251379	480731	0.11	24.21	5.3	32.52	6
CUL	Channeled	5254411	469956	0.84	11.05	4.8	30.45	6.2
ROS	Palouse	5185733	484172	0.06	11.85	4.3	39.7	9.7
MCG	Palouse	5248703	465888	0.03	6.5	2.1	102.35	11.4
WHE	Palouse	5180599	491020	0.01	10.25	3.2	56.9	13.4

Table 1.6 Average values for environmental variables found at each study site location. Values averaged across all plots of the given locations.

Site	Slope	Average Soil Depth (cm)	Elevation (m)	Precipitation (in/yr)	Solar Radiation (WH/m ² /yr)
GEG	1.1	24.1	728.2	16.6	1108317
BEL	6.4	43.8	651.6	18.0	1114055
FEL	4.6	25.7	751.6	18.0	1111972
CUL	3.8	31.8	744.9	19.2	1116605
FIS	2.6	67.6	668.0	14.0	1075398
SIL	10.5	47.5	801.3	16.6	848936
WHE	12.0	31.0	766.8	16.9	1135239
STE	17.5	56.8	879.8	22.0	1102068
SMO	15.2	25.9	866.6	20.4	1001755
KAM	16.7	35.4	996.8	22.0	987823
ROS	12.0	35.2	715.9	20.4	1118272
MCG	17.9	51.2	754.7	19.2	1081114
TUR	2.4	59.8	712.4	19.2	1103719

Table 1.7 Difference between survey site county and community composition of Palouse Prairie sites only determined by PERMANOVA.

	Df	SumsOfSqs	MeanSqs	F.Model	R ²	Pr(>F)
County	1	1.0614	1.06143	2.9856	0.04206	0.001
Residuals	68	24.1753	0.35552		0.95794	
Total	69	25.2367			1	

Table 1.8 Correlations between ordination axes and measured environmental variables for all only Palouse Prairie sites.

	NMDS1	NMDS2	NMDS3	r ²	Pr(>r)
Northing	0.853	-0.089	-0.515	0.083	0.120
Easting	-0.906	0.272	0.325	0.053	0.307
Aspect	0.312	-0.332	0.890	0.266	0.001
Slope	0.758	0.232	-0.610	0.055	0.306
Average Soil Depth	0.405	0.769	-0.495	0.124	0.032
Moss Cover	-0.043	0.164	0.985	0.024	0.661
Wood Cover	0.219	0.967	0.133	0.196	0.001
Litter Cover	0.488	-0.696	0.527	0.075	0.179
Gravel Cover	-0.524	0.346	0.778	0.084	0.105
Soil Cover	0.112	0.920	-0.377	0.073	0.179
Rock Cover	-0.727	0.279	0.627	0.096	0.077
Lichen Cover	-0.861	-0.499	0.102	0.037	0.486
Other Cover	0.149	-0.783	-0.604	0.039	0.482
Native Cover	0.982	0.163	0.101	0.381	0.001
Non-Native Cover	-0.473	0.748	0.466	0.330	0.001
Elevation	-0.507	-0.043	-0.861	0.506	0.001
Precipitation	-0.492	0.710	-0.504	0.223	0.003
Solar Radiation	0.238	0.489	0.839	0.166	0.012

Figures

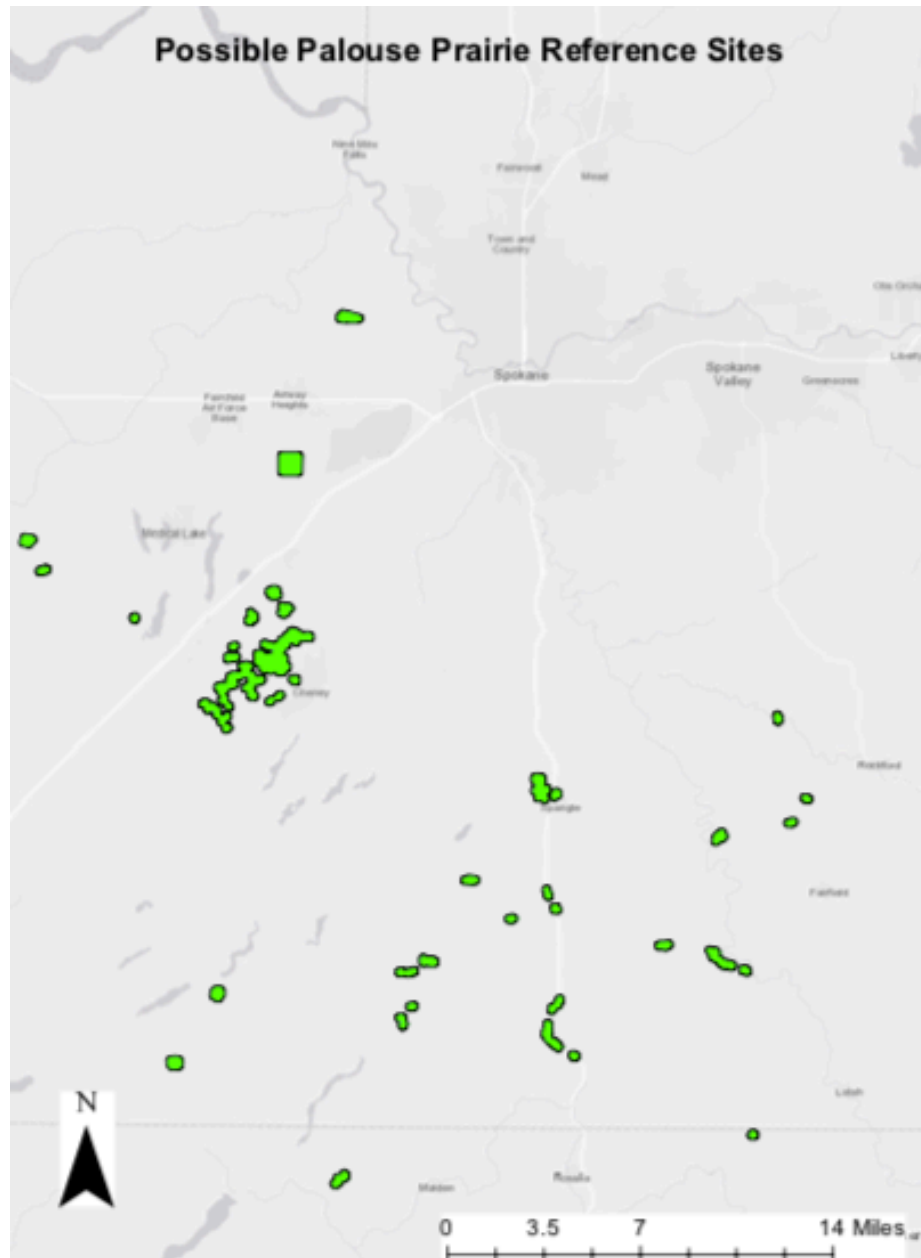


Figure 1.1 Overview of possible Palouse Prairie remnants determined through aerial imagery search in the northern edge of the Palouse Prairie.

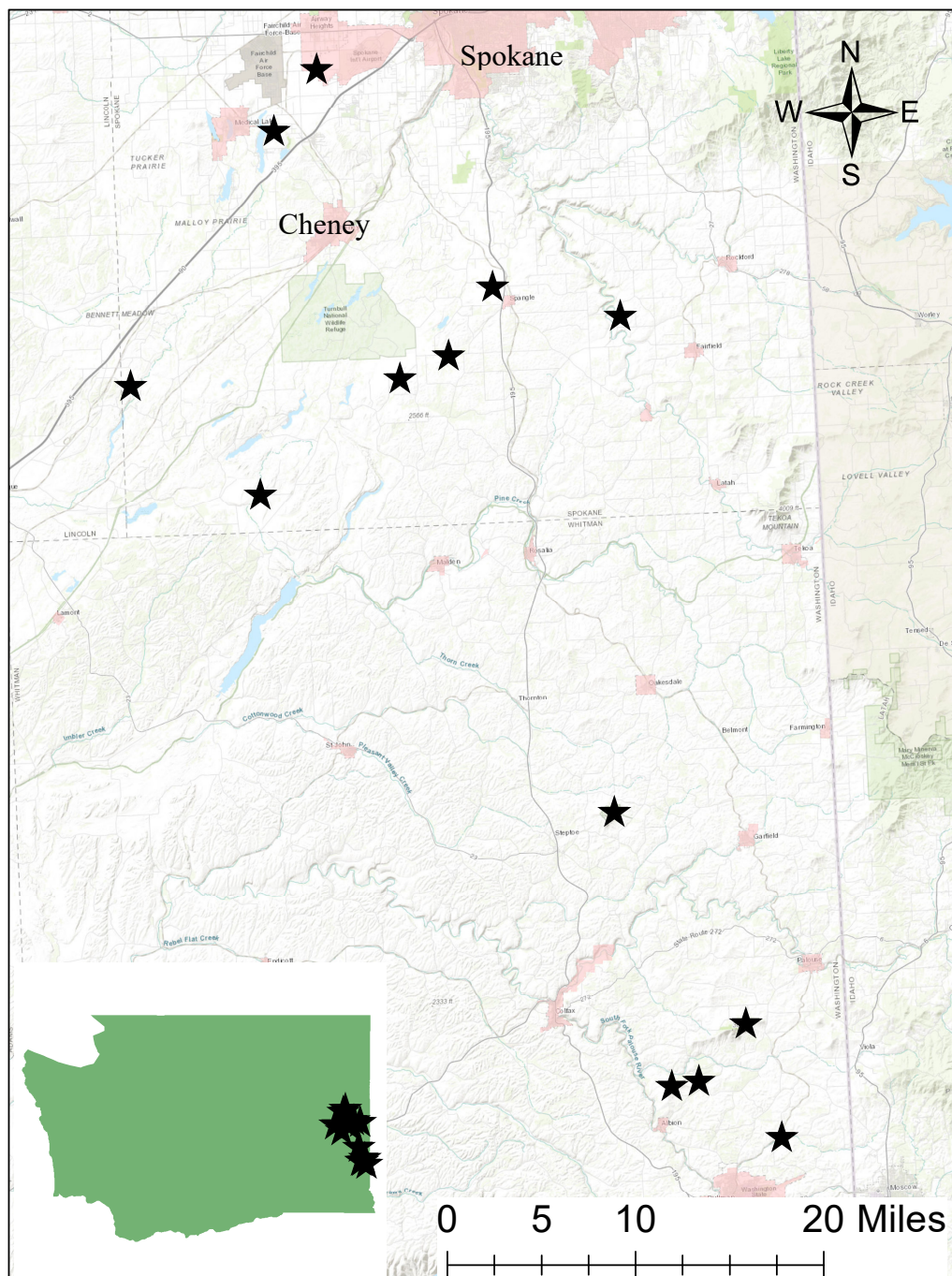


Figure 1.2 Locations of sites surveyed across Spokane and Whitman County for the study. Inset map shows relative location to Washington State. Pink areas represent cities with Spokane WA visible along the upper edge of the map and Pullman WA visible along the lower edge. Stars represent individual sites.

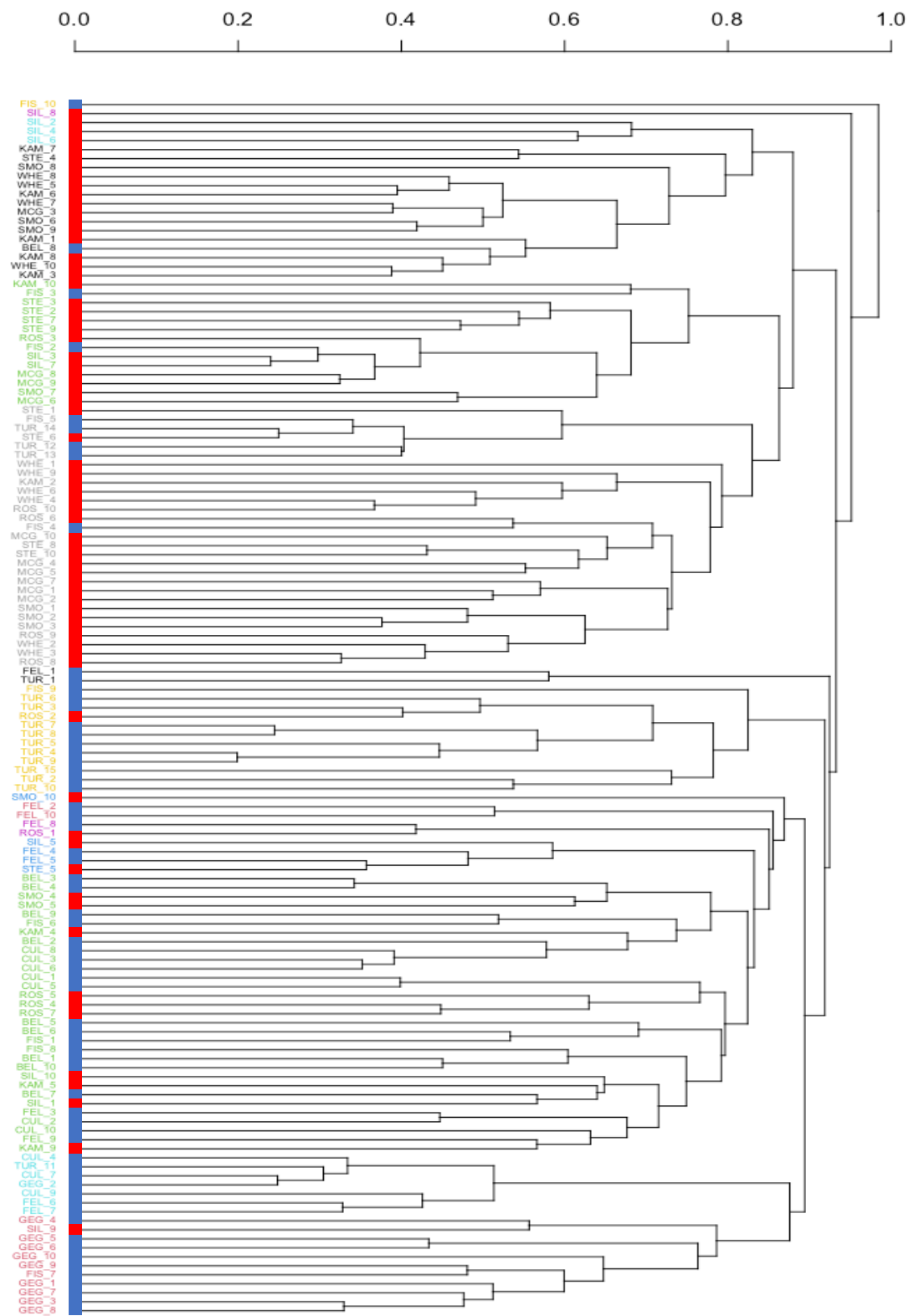


Figure 1.3 Dendrogram of individual plots grouped by cluster analysis (colored plot locations). Red line ends indicate plots assigned to Palouse Prairie, while blue indicates Channeled Scabland plots.

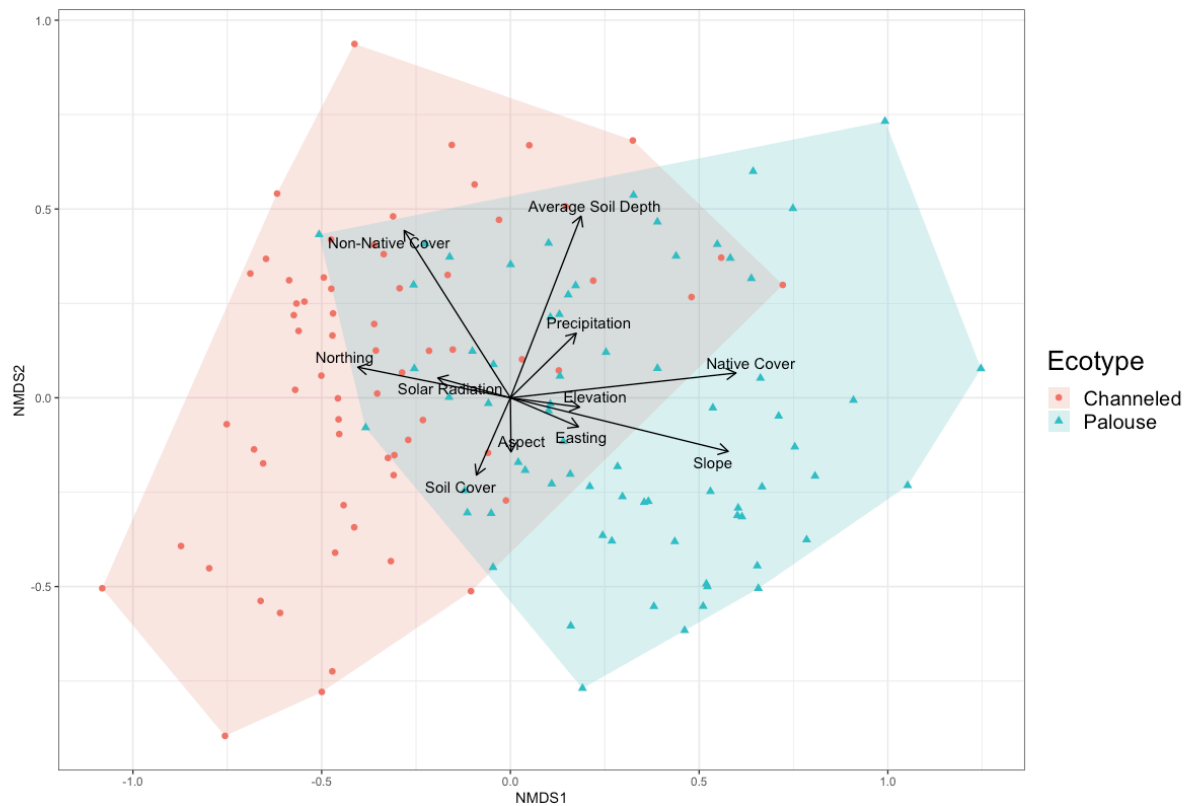


Figure 1.4 Ordination of community similarity in Channeled Scabland (red) and Palouse Prairie (blue) communities based on non-metric multidimensional scaling. Each point is a 1m² plot placed randomly at each site.

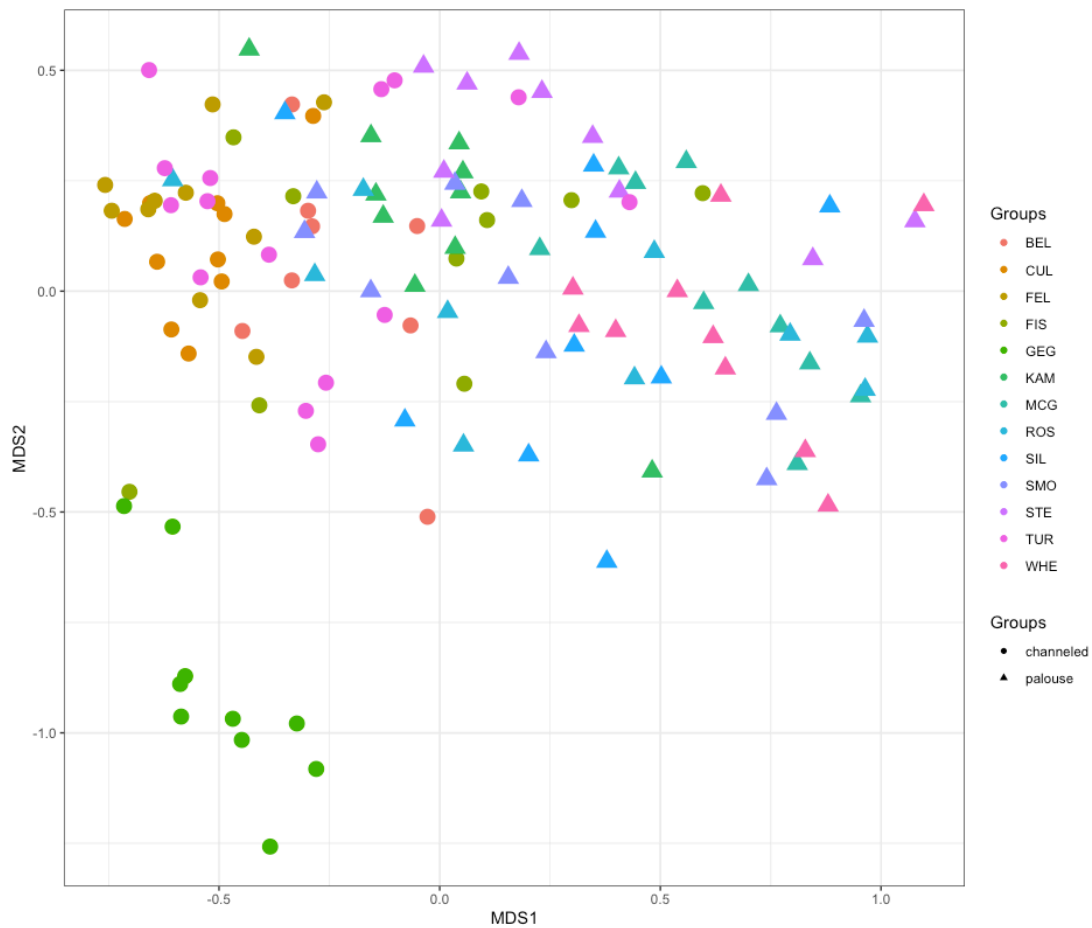


Figure 1.5 Ordination of individual survey plots for all sites grouped by site based on non-metric multidimensional scaling. Colors represent different survey sites. Circles are Channeled Scabland sites and triangles are Palouse Prairie sites.

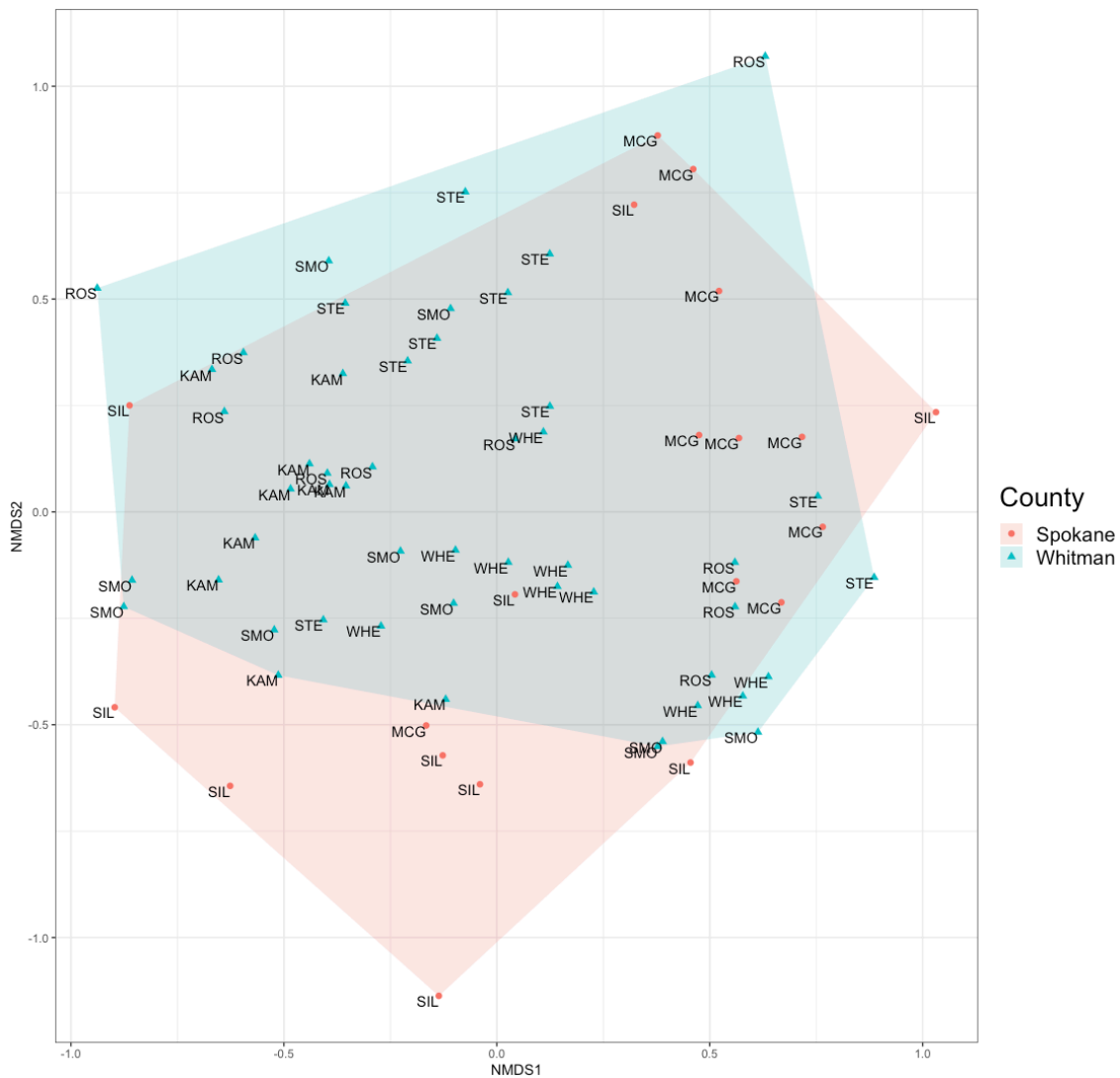


Figure 1.6 Ordination of plots from only Palouse sites by county.

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Appendix

Full Species List

Achillea millefolium L.
Achnatherum nelsonii (Scribn.) Barkworth
Acmispon americanus (Nutt.) Rydb. var. *americanus*
Agastache urticifolia (Benth.) Kuntze var. *urticifolia*
Agoseris glauca (Pursh) Raf.
Agoseris grandiflora (Nutt.) Greene
Agoseris heterophylla (Nutt.) Greene
Agropyron cristatum (L.) Gaertn.
Agrostis exarata Trin.
Agrostis gigantea Roth
Allium acuminatum Hook.
Alopecurus aequalis Sobol.
Alopecurus pratensis L.
Amelanchier alnifolia (Nutt.) Nutt. Ex M. Roem.
Amsinckia menziesii (Lehm.) A. Nelson & J.F. Macbr.
Antennaria luzuloides Torr. & A. Gray
Anthemis cotula L.
Anthriscus caucalis M. Bieb.
Apera interrupta (L.) P. Beauv.
Apocynum androsaemifolium L.
Arnica sororia Greene
Arrhenatherum elatius (L.) P. Beauv. Ex J. Presl & C. Presl.
Artemisia absinthium L.
Artemisia douglasiana Besser
Artemisia tripartita Rydb.
Asteraceae sp.
Astragalus sp.
Balsamorhiza sagittata (Pursh) Nutt.
Berberis aquifolium Pursh
Berberis repens Lindl.
Blepharipappus scaber Hook.
Bromus briziformis Fisch. & C. A. Mey.
Bromus commutatus Schrad.
Bromus hordeaceus L.
Bromus inermis Leyss.
Bromus japonicus Houtt.
Bromus sitchensis Trin

Bromus squarrosus L.
Bromus tectorum L.
Bromus vulgaris (Hook.) Shear
Bryonia alba L.
Buglossoides arvensis (L.) I.M. Johnst.
Calamagrostis canadensis (Michx.) P. Beauv.
Calamagrostis rubescens Buckley
Calochortus elegans Pursh
Camassia quamash (Pursh) Greene
Carex filifolia Nutt.
Carex laeviculmis Meinsh.
Carex praeceptorum Mack.
Carex praegracilis W. Boott
Castilleja cusickii Greenm.
Castilleja hispida Benth.
Castilleja lutescens (Greenm.) Rydb.
Castilleja sp.
Castilleja tenuis (A. Heller) T. I. Chuang & Heckard
Centaurea cyanus L.
Centaurea solstitialis L.
Centaurea stoebe L.
Cerastium arvense L.
Cerastium fontanum Baumg. ssp. *vulgare*
Cerastium sp.
Chaenactis douglasii (Hook.) Hook. & Arn. var. *douglasii*
Chamaenerion angustifolium (L.) Scop.
Chondrilla juncea L.
Chrysothamnus viscidiflorus (Hook.) Nutt.
Cichorium intybus L.
Cirsium arvense (L.) Scop.
Cirsium brevifolium Nutt.
Cirsium undulatum (Nutt.) Spreng.
Clarkia pulchella Pursh
Claytonia perfoliata Donn ex Willd. ssp. *intermontana*
Claytonia rubra (Howell) Tidestr. ssp. *depressa*
Clematis hirsutissima Pursh var. *hirsutissima*
Collinsia parviflora Lindl.
Collomia grandiflora Douglas ex Lindl.
Collomia linearis Nutt.
Comandra umbellata (L.) Nutt.
Conium maculatum L.

Convolvulus arvensis L.
Crataegus douglasii Lindl.
Cryptantha affinis (A. Gray) Greene
Cynoglossum officinale L.
Dactylis glomerata L.
Danthonia californica Bol.
Danthonia spicata (L.) P. Beauv. Ex Roem & Schult.
Delphinium distichum Geyer ex A. Gray?
Delphinium nuttallianum Pritz.
Deschampsia danthonioides (Trin.) Munro
Descurainia pinnata (Walter) Britton
Dianthus armeria L. ssp. *armeria*
Dipsacus fullonum L.
Draba verna L.
Drymocallis glandulosa (Lindl.) Rydb.
Echinocystis lobata (Michx.) Torr. & A. Gray.
Elymus elymoides (Raf.) Swezey
Elymus glaucus Buckley ssp. *glaucus*
Elymus repens (L.) Gould
Epilobium brachycarpum C. Presl.
Epilobium sp.
Equisetum laevigatum A. Braun
Equisetum sp.
Erigeron compositus Pursh
Erigeron corymbosus Nutt.
Erigeron filifolius (Hook.) Nutt.
Erigeron pumilus Nutt. var. *intermedius*
Eriogonum compositum Douglas ex Benth.
Eriogonum heracleoides Nutt.
Eriogonum niveum Douglas ex Benth.
Erodium cicutarium (L.) L'Her. Ex Aiton ssp. *cuticularium*
Erysimum repandum L.
Erythronium grandiflorum Pursh
Euphorbia cyparissias L.
Fallopia convolvulus (L.) Á. Löve
Festuca campestris Rydb.
Festuca idahoensis Elmer
Fragaria virginiana Mill. ssp. *glauca*
Frasera albicaulis Griseb. var. *albicaulis*
Fritillaria pudica (Pursh) Spreng.
Gaillardia aristata Pursh

Galium aparine L.
Galium boreale L.
Galium trifidum L.
Gentiana affinis Griseb.
Geranium viscosissimum Fisch. & C. A. Mey.
Geum triflorum Pursh
Grindelia hirsutula Hook. & Arn.
Helianthella uniflora (Nutt.) Torr. & A. Gray
Heracleum maximum W. Bartram
Heuchera cylindrica Douglas
Hieracium scouleri Hook.
Holodiscus discolor (Pursh) Maxim. Var. *discolor*
Holosteum umbellatum L. ssp. *umbellatum*
Hordeum jubatum L. ssp. *jubatum*
Hordeum vulgare L.
Hypericum perforatum L.
Iris missouriensis Nutt.
Iris sp.
Juncus dudleyi Wiegand
Juniperus scopulorum Sarg.
Koeleria macrantha (Ledeb.) Schult.
Lactuca serriola L.
Lagophylla ramosissima Nutt.
Lathyrus pauciflorus Fernald var. *pauciflorus*
Lepidium perfoliatum L.
Leymus cinereus (Scribn. & Merr.) Á. Löve
Linaria dalmatica (L.) Mill.
Linum lewisii Pursh
Lithophragma parviflorum (Hook.) Nutt.
Lithospermum ruderale Douglas ex Lehm.
Lomatium ambiguum (Nutt.) J. M. Coult. & Rose
Lomatium dissectum (Nutt.) Mathias & Constance
Lomatium leptocarpum (Torr. & A. Gray) J.M. Coult. & Rose
Lomatium macrocarpum (Nutt. ex Torr. & Gray) J.M. Coult. & Rose
Lomatium simplex (Nutt. ex S. Watson) J.F. MacBr.
Lomatium triternatum (Pursh) J.M. Coult. & Rose
Lupinus leucophyllus Douglas ex Lindl.
Lupinus sericeus Pursh
Madia exigua (Sm.) A. Gray
Madia glomerata Hook.
Madia gracilis (Sm.) D.D. Keck

Maianthemum stellatum (L.) Link
Malus pumila Mill.
Medicago lupulina L.
Medicago sativa L.
Mentha sp.
Mertensia longiflora Greene
Microsteris gracilis (Hook.) Greene
Myosotis micrantha Pall. Ex Lehm.
Myosurus minimus L.
Navarretia intertexta (Benth.) Hook.
Nepeta cataria L.
Olsynium douglasii (A. Dietr.) E.P. Bicknell
Perideridia montana (Blank.) Dorn
Phleum pratense L.
Phlox longifolia Nutt.
Physocarpus malvaceus (Greene) Kuntze
Pinus ponderosa P. Lawson & C. Lawson
Plantago lanceolata L.
Plantago patagonica Jacq.
Poa bulbosa L.
Poa compressa L.
Poa interior Rydb.
Poa pratensis L.
Poa secunda J. Presl
Poa sp.
Polemonium sp.
Polygonum douglasii Greene
Polygonum majus (Meisn.) Piper
Polygonum polygaloides Meisn.
Potentilla gracilis Douglas ex Hook.
Potentilla recta L.
Potentilla rivalis Nutt.
Poteridium annuum (Nutt.) Spach.
Poterium sanguisorba L.
Prunella vulgaris L.
Prunus mahaleb L.
Prunus virginiana L.
Pseudoroegneria spicata (Pursh) Á. Löve
Pyrrocoma carthamoides Hook.
Pyrrocoma liatrifomis Greene
Ranunculus sp.

Ribes aureum Pursh
Ribes viscosissimum Pursh
Rosa canina L.
Rosa gymnocarpa Nutt.
Rosa nutkana C. Presl
Rosa woodsii Lindl.
Rumex crispus L.
Sambucus cerulea Raf.
Senecio hydrophiloides Rydb.
Senecio integerrimus Nutt.
Sidalcea oregana (Nutt. ex Torr. & A. Gray) A. Gray
Silene douglasii Hook.
Silene latifolia Poir.
Sisymbrium altissimum L.
Solidago lepida DC.
Solidago missouriensis Nutt.
Sonchus arvensis L.
Sonchus oleraceus L.
Spiraea lucida Douglas ex Greene
Symphoricarpos albus (L.) S.F. Blake
Symphyotrichum spathulatum (Lindl.) G.L. Nesom
Synthyris rubra (Douglas ex Hook.) Benth.
Syringa vulgaris L.
Taeniatherum caput-medusae (L.) Nevski.
Tanacetum vulgare L.
Taraxacum officinale F.H. Wigg.
Thlapsi arvense L.
Toxicoscordion venenosum (S. Watson) Rydb.
Tragopogon dubius Scop.
Trifolium microcephalum Pursh
Triteleia grandiflora Lindl.
Turritis glabra L.
Valerianella locusta (L.) Laterr.
Ventenata dubia (Leers) Coss.
Veratrum sp.
Veratrum viride Aiton
Verbascum thapsus L.
Verbena bracteata Lag. & Rodr.
Veronica peregrina L.
Veronica sp.
Vicia americana Muhl. ex Willd.

Vicia cracca L.

Vicia villosa Roth

Viola adunca Sm.

Wyethia amplexicaulis (Nutt.) Nutt.

Chapter 2. The Effect of Whole Soil Inoculation on a Native Perennial and Invasive Annual Grass

Background

Soil communities play pivotal roles in maintaining and determining plant community function, biodiversity, and reproduction in native ranges (Heinze et al, 2016; Hoeksema et al., 2010; Middleton & Bever, 2012). These soil communities can consist of plant pathogens, fungi, algae, and various micro-fauna and are important to ecosystem function. Each of these organisms may act in positive or negative feedback loops which cause increased or decreased plant growth, respectively through interactions between plants and their soil microbiome components (Bever, 2002; Heinze et al., 2015). As plant communities shift during succession, individual species within these communities will shift as well but when land is converted to agriculture many of these soil communities are lost. These losses can be due to mechanical disruption through tilling or complete loss due to conversion from native prairie to an agricultural monocrop and can be long lasting in soil legacies (Smith et al., 2018).

The soil microbiome can have important effects on the soils and the resident plant species and vice versa. These feedbacks can usually be broken down into positive and negative feedback loops (Callaway et al. 2004). Positive feedback loops are thought of as providing beneficial effects to those species in which they interact and although beneficial to a specific species, are usually thought to decrease biodiversity through fostering of one species within a community (Callaway et al. 2004). Examples of a positive feedback loop are arbuscular mycorrhizal fungi (AMF) communities. These

communities promote the growth of a symbiotic plant species thus increasing opportunity for AMF colonization which further benefits the growth of that particular plant species (Bever et al., 2012). The negative feedback loops inhibit the growth or reproduction of a certain species and are thought to increase biodiversity within a local community (Callaway et al. 2004). A negative feedback loop involves the accumulation of pathogenic microbes that build up in the soil as a specific plant species becomes more dense in a community. These pathogens become more abundant and inhibit that plants growth, allowing other plant species to thrive in the area, increasing biodiversity in the locale (Bever et al., 2012).

Experiments have shown that soil inoculations can increase the growth of target species. Work done in 1895 isolated the nitrogen fixing bacteria *Bacillus ellenbachiensis*, which increased crop yield 10-20% when used to inoculate soil (Matics 2015). Although specific to agriculture, these early experiments showed that introduced organisms could positively affect the growth of target species. Within the last few decades, increasing amounts of work have focused on soil inoculation as a way to restore belowground communities derived from remnant locations (Middleton and Bever 2012). Evidence suggests that these inoculations can increase successional status of plant communities, slow or resist invasion, increase plant co-existence, and resist herbivory (Middleton & Bever, 2012; Middleton et al., 2015; Seifert et al., 2009).

Experiments done in the Mid-Western tallgrass prairies have shown the greatest effect of inoculation using the most diverse inoculant material (Fitzsimons and Miller 2010). In these prairies, while adding solely AMF communities was beneficial for plant growth, those sites inoculated with whole soil communities were better able to maintain

plant diversity over longer periods (Bever, 2002; Fitzsimons & Miller, 2010). In a review of 306 targeted inoculation studies, it was found that the highest plant growth response was in studies that inoculated with whole soil (Hoeksema et al. 2010). Middleton et al. (2015) also describes whole soil inoculum sourced from local remnant locations producing a higher diversity prairie restoration (Middleton et al. 2015). These results indicate that while introduction of a negative feedback microbe may seem counterintuitive to the goal of restoration, these microbes are important for the long-term success of a restoration and species biodiversity maintenance.

Many of these positive and negative feedbacks can be lost in agricultural systems due to reduced soil communities from high fertilizer use and tillage (Jones et al. 2018). This reduced condition can persist for many years even after the agricultural practices have ceased (Smith et al. 2018). These shifted soil communities can be ideal hosts for invasive species of plants as these species are less reliant on native soil conditions for growth and reproduction than those early native colonizers (Seifert et al. 2009, Smith et al. 2018). Invasive species are one of the major causes to biodiversity losses across the world and have numerous economic impacts (Callaway et al. 2004). It has been suggested that the pervasiveness of exotic species in new landscapes involves a release from the biotic controls of its native landscape (Bever et al., 2012). In their native ranges, exotic species are subject to negative feedbacks by host specific microbes discussed earlier but, in a new range the soil does not contain these inhibitory microbes (Seifert et al. 2009). The release from negative feedback as well as life history traits such as fast growth and large reproduction efforts allows invasive species to become overgrown in a local area while excluding those native species (Koziol and Bever 2015). Once

introduced, these invasive species can shift soil microbiome to favor their own growth further excluding native species (Jordan et al., 2011).

Invasive plant species have been found to be negatively affected by intact AMF communities (Koziol and Bever 2015). Researchers at the Water Research Laboratory near Temple, TX used both native and exotic plants grown in AMF monoculture pots to determine how AMF colonization varied between native and exotic species (Sielaff et al., 2019). Surprisingly, AMF root colonization was found to be higher on invasive species than the native species tested but colonization was negatively correlated with aboveground biomass in invasive species (Sielaff et al. 2019). Thus, while invasive species had higher colonization, there was no benefit to AMF association. The opposite was true for native species whose root colonization has shown a strong positive correlation with above-ground biomass (Sielaff et al. 2019). Therefore a restored soil microbiome may be able to resist introduction of invasive species by increasing native plant vigor (Middleton and Bever 2012). Results from these studies are promising and may be effective in resisting exotic plant species in the restoration of Palouse Prairie communities.

Locally adapted soil from native prairie remnants near the restoration may contain the most closely related soil communities to the late-successional stage communities that are desired (Middleton and Bever 2012). Using this method requires a large amount of soil to be spread across the site and disked to mix to a useful depth or localized in each planting (Yelenik and Levine 2011). Unfortunately, transferring large amounts of native prairie soil is usually impossible because intact prairie communities are so rare (and endangered). Although the original technique would be impossible, a modified form of

this could be done using a technique developed in Midwest tallgrass prairie. Soil is taken from local prairies and is used to inoculate “nurse plants,” plants designed to facilitate microbial introductions in order to “nurse” AMF inoculations and allow these AMF communities to spread throughout the restoration site (Middleton and Bever 2012). Research has shown positive growth effects of inoculation on nurse plants as well. Measuring nurse plant response to inoculation may be the first step in understanding how soil inoculation could be used for a large-scale restoration.

In northeastern Washington, the deeper loess soils, traditionally associated with Palouse Prairie, were originally fragmented by the Missoula Floods that occurred approximately 13,000 years ago (O’Connor et al. 2020). Wind-blown loess soil covered a basalt layer creating the iconic rolling hills of the Palouse (Bretz et al. 1956). This silt layer was washed away during the last glacial retreat (O’Connor et al. 2020) when the resultant catastrophic flood removed huge tracts of silt, exposing the underlying bedrock and creating the Channeled Scablands of Eastern Washington (Larsen and Lamb 2016). In the Channeled Scablands, Mima mound prairies can be found, which have overlapping plant species composition with Palouse Prairie, as documented in Chapter 1 of this thesis. However, the soil profile is more shallow, and there are different disturbance regimes with more grazing and northern pocket gopher (*Thomomys talpoides*) activity (with soil turnover) in Channeled Scablands (Reyneck 2012), and it is not clear how comparable the soil microbiome is between these two areas. Differences in topography, such as soil depth, aspect, or slope, have often been cited for changes in plant community (Boeken and Shachak 1994, Grman et al. 2013, Bernards and Morris 2017). Due to the Channeled Scablands much shallower soil it is unknown how reliant plants of the Channeled

Scablands may be on their soil communities or how well those soil communities may be used to inoculate restoration species.

At Eastern Washington University, a 120-acre restoration of original prairie is currently under way. The restoration site is located on deeper Palouse rolling hills but is near an expansive Channeled Scabland area at Turnbull National Wildlife Refuge (TNWR).

The overarching goal of my study was to determine whether whole soil inoculation from native prairie remnants would benefit Palouse Prairie restoration by helping native perennial species at the expense of invasive annual species. To address this question, I conducted a greenhouse experiment testing effects of inoculation of whole soils from a native Palouse Prairie remnant, a Channeled Scabland prairie remnant, and a tilled wheat field, as well as sterilized soil on two plant species with varying life histories. I predicted that the native perennial species, *P. spicata*, would show the largest growth inoculated with soil sourced from intact natural areas, while the invasive annual species, *V. dubia*, would be unaffected by inoculation source location. I further predicted that sterilization of inoculum would reduce its beneficial effect on *P. spicata* by removing beneficial soil organisms but would not affect *V. dubia*. Finally, I predicted that soil sourced from the native Palouse Prairie remnant would produce a larger growth effect than soil sourced from a Channeled Scablands prairie remnant.

Methods

I collected soil inoculum from Kamiak Butte County Park and TNWR, as well as from the peripheries of the EWU restoration site. Kamiak Butte is a county park located

11 miles north of Pullman, WA in Whitman County. Kamiak Butte reaches an elevation of about 3600 feet and receives approximately 22 inches of precipitation annually.

TNWR is located five miles South of Cheney WA, in Spokane County. TNWR experiences approximately 19 inches of precipitation annually and is associated with the Channeled Scabland with much shallower soil than Kamiak Butte. Both areas receive most precipitation during winter months and have long dry periods during the summer months. Seed for *P. spicata* was obtained from the U.S. Forest Service supplied to Ethan Bean and *V. dubia* was collected by Jared Lamm at TNWR.

Two grass species were used to represent a combination of life history traits. These include: *Pseudoroegneria spicata* (perennial grass) and *Ventenata dubia* (invasive annual grass). *P. spicata* is a perennial native grass often used in restoration for its resistance to invasion, drought tolerance, and palatability by grazing wildlife (Larson et al. 2004, Harvey et al. 2020). *Ventenata dubia*, an invasive annual grass, pushes out native species and reduces soil stability due to shallow roots (Ingwell and Bosque-Pérez 2015). *V. dubia* has grown to dominate many of the drier areas of Eastern Washington grassland and is a major threat to restoration (Jones et al. 2018).

My study was fully factorial with seedlings from each of the two plant species inoculated with six different treatments: soil from EWU restoration site, soil from Kamiak Butte, and soil from TNWR. Each of these soil collections were then split in half and one half left unaltered and one half sterilized. This results in a total of 12 treatments (2 plant species * 3 sites * 2 for sterile and unsterile inoculum). Each treatment had ten replicates for a total of 120 plants in the study.

I collected inoculum using an Oakfield Soil Core (dimensions 10 cm height x 2 cm diameter) with a total volume of 31 mL (0.13 US cup) of soil removed for each sample. A total of 400 samples were collected for a total of 12.4 L (3.3 US Gal.) soil removed from each source location. Soil samples were collected at regular intervals and adjusted for individual site characteristics to minimize site impact. Collected soil was sieved on site through a one-centimeter sieve and homogenized before being used for inoculation. All equipment used for sample extraction, storage, and mixing was sterilized through the use of twenty-minute baths in a 20% bleach solution and rinsed with deionized water. Sterilization of equipment occurred between each source location as well as background soil collection to avoid contamination between sites. Background soil was collected from the EWU restoration site from field margins to avoid crop interruption and was collected using a larger shovel since site impact was not a concern but only the top 10 cm of soil was taken. Background soil was then mixed using a bleach-sterilized concrete mixer in a 1:2 ratio with autoclave-sterilized sand, allowing for proper soil drainage in individual pots. Concrete mixer drum was sterilized using a 15% bleach solution that was used to fill inside the drum and the outer surface was sprayed with the same solution. Background sand and inoculum used in sterile treatments were sterilized by autoclave with one 90-minute cycle, a 24-hour rest period, and a second 90-minute cycle.

P. spicata, and *V. dubia* were germinated in bleach sterilized open trays with autoclaved Jiffy Seed Starting Mix as the growth media. Following germination, each seedling was transplanted by hand to an individual Deepot D40L 0.656 L cone-tainer. *P. spicata* and *V. dubia* were transplanted at a rate of one seedling per pot. Pots were

inoculated with the specific soil treatment at a rate of 20% by volume or 0.132 L per pot. 0.396 L of background soil was added to the bottom of the pot, followed by a 0.132 L band of inoculum, the seedling was then planted in the remaining top 0.132 L band of background soil. This soil arrangement (Figure 2.1) allowed roots to grow through the inoculum layer while controlling for contamination during watering. Plants were given unique identifier codes and starting plant height was measured. Plants that died within the first week were replaced and new initial heights were recorded.

Plants were grown out in the soil treatments at the Eastern Washington University research greenhouse between July 2019 and February 2020. Pots were randomly arranged across one table in the greenhouse and were randomly re-arranged every two weeks to avoid any placement effects during the study. Raised pot racks were used to ensure pots remained high enough off the table to avoid contamination between plants. Plants were top watered regularly to maintain growth over the course of the study with non-chlorinated water to avoid any adverse effects on soil microbial communities. Greenhouse temperature was maintained within a range of 60° to 80° and light was supplemented from 06:00 to 18:00 daily throughout the study period.

Plant height was measured from the soil surface to the tallest point of the plant for each individual. Following the conclusion of the growth experiment, all surviving individuals were harvested for dry biomass measurements. All plants were cut at the soil surface and saved for above-ground biomass. Soil was then gently washed away from the roots using non-chlorinated water. Above and below-ground structures were put into individual envelopes and dried at 40° C for 5 days. Following drying, each individual was removed from its envelope and weighed.

I evaluated the effect of inoculum source and sterilization on plant height over time for each species using a repeated measures analysis in SAS statistical software (SAS for Windows version 9.4) using the PROC mixed procedure with unstructured (type = UN) covariance structure. Plant height data were evaluated for normal distribution and log transformed as necessary. I tested the effect of inoculum and sterilization on final biomass of all surviving plants using ANOVA in R (R version 4.0.0). Figures for all analyses were graphed in R using the effects package (4.2-0).

Results

Soil inoculum source location did not affect plant height for *P. spicata* ($p = 0.18$; Figure 2.2) or *V. dubia* ($p=0.71$; Figure 2.3), however inoculum sterilization increased plant height in *P. spicata* ($p=0.03$, Figure 2.2) and *V. dubia* $p=0.07$; Figure 2.3; Table 2.1). *V. dubia* height and biomass data were not normally distributed so were log transformed for all analyses. *P. spicata* data were not transformed.

P. spicata stem biomass was increased slightly in sterilized restoration site soil, however the effect was not statistically significant (sterilization $p=0.06$ and soil source $p = 0.07$, interaction NS, Table 2.2). Root biomass and total biomass of *P. spicata* were significantly affected by the interaction of soil source and sterilization ($p = 0.014$ and $P=0.027$, respectively; Table 2.2). Plant biomass was unaffected sterilization or soil source in the annual grass *V. dubia* (p values all >0.15 , Figure 2.7).

Discussion

My hypothesis that soil inoculum from remnant prairies would benefit native perennial plant growth was not supported. Soil inoculum had no effect on overall plant height for either the native or nonnative species in my study. There are several potential explanations for these results, including the presence of microbes in the unsterilized background soil used across all the treatments, or sterilization increasing nutrient levels in soils.

Adding inoculum to unsterilized background soil may have affected the results due to priority effects, in which the first species to colonize an area becomes the most abundant (Verbruggen et al. 2013). In an inoculation study of two AMF species, Werner and Kiers (2015) found that simultaneous inoculation of the two species led to equal root abundance, until the stronger AMF species took over. When a lag in time between inoculations occurred, the invading species consistently showed lower colonization. In my experiment, the unsterilized background soil, which comprised 80% of the total soil in the pot, may have contained soil biota that colonized test plants before the inoculum biota could establish. Priority effects would lead to the background soil biota then becoming the dominant community, reducing any effect of between site variation.

Contrary to my hypotheses, soil sterilization increased the height and biomass of *P. spicata* but had no significant effect on *V. dubia*. Soil sterilization through the use of an autoclave has been found to lower pH and increase extractable nutrients such as phosphorus, potassium, and nitrogen (Skipper and Westermann 1973). These nutrients are often thought to limit plant growth so the release of labile nutrients by sterilization

could explain the increased growth observed in this study. Differences in soil nutrients have been found to alter the soil microbial community (Heinze et al. 2015) with higher proportions of AMF in nutrient limited soils and higher proportions of bacteria in nutrient rich soils. Under agricultural practices, soils are expected to be high in nutrients, while plowing would further reduce AMF populations. Variation between communities could lead to a variable release of nutrients between the remnant soils (Kamiak Butte and TNWR) and the restoration site soil (Williams-Linera and Ewel 1984).

Reduced microbial populations may have also increased the competitive advantage of plants grown in sterile soil. Without microbial populations, plants had no competition for nutrients and were able to utilize a larger portion of soil nutrients (Gebhardt et al. 2017). In addition, Lotrario et al. (1995) found that complete inhibition of biotic components required at least three cycles of 30 minutes at 121 degrees Celsius in an autoclave chamber but enzyme activity can continue although reduced (Gebhardt et al. 2017). These findings indicate the possibility that enzymes could have continued working further increasing available nutrients in sterile treatments.

Like increased plant height, increased biomass may be related to nutrient volatilization or release from detrimental soil biota (Skipper and Westermann 1973, Gebhardt et al. 2017). Interestingly, sterilization of the EWU soil did seem to result in increased growth of both species. These results may indicate that our restoration site is limited in essential nutrients needed for plant growth. It may also be an indication that the EWU site contains harmful microbial populations and sterilization reduces their populations to a point where plants were able to grow unrestricted (Williams-Linera and Ewel 1984).

Based on my study, it is not clear whether soil inoculation would be beneficial in large-scale restoration in Eastern Washington as it has been in other locations (Koziol et al. 2018). But before coming to any conclusion, it would be helpful to study a larger group of plant species, different background soils, and longer time periods. It is possible that the species I studied may not respond to soil microbiome changes as well as other species. In her preliminary work, Dr. Tanya Cheeke found that *P. spicata* was more responsive to increased soil nutrients than microbial soil communities (T. Cheeke, personal communication, June 2021). In addition, my experiment took place in the greenhouse which has a very different environment than our field restoration site. For example, soil source may have a much more pronounced effect under drought conditions or with plant species competition which did not occur in my experiment. Plant responses are also highly variable, so ten replicates may not be enough to capture significant effects between groups (Wubs et al. 2019). In addition, my study focused on only two response variables, plant height and dry biomass. Further research could focus on other characteristics of health such as flower production, germination, or survival.

Further work should be done testing effects of inoculation on sterile background soil for vigorous plug planting. In the many previous studies that have shown the efficacy of native sourced soil inoculation on plant growth, many, if not all, used sterilized background soil (Middleton et al. 2015, Koziol et al. 2018). Soil inoculation may be effective in only sterilized soil, may be dependent on the microbiome of the site it is being used upon, or may take longer time periods for effects to manifest. Further research is needed to show the best use of inoculation in the future.

Although the initial findings of this study are still inconclusive, we have shown that varying soil treatments do have an effect on the growth of the resident plant. Further exploration of this topic could include more diverse test plants, a deeper understanding of soil microbial components, and detailed nutrient analysis of restoration soils. A deeper understanding of these soil communities and their impacts could lead to a more diverse and effective prairie restoration at EWU..

Tables

Table 2.1 Repeated Measures results showing effect of soil source and sterilization on plant height over time.

Dependent Variable	Effect	Num DF	Den DF	F Value	Pr > F
P. spicata Height	Sterile	1	53.6	4.98	0.03
	Soil Source	2	53.6	1.8	0.18
	Sterile*Soil Source	2	53.6	0.88	0.42
V. Dubia Height	Sterile	1	51.4	3.34	0.07
	Soil Source	2	50.2	0.34	0.71
	Sterile*Soil Source	2	50.2	1.93	0.16

Table 2.2 Dry biomass results showing effect of soil source and sterilization on final plant biomass determined through ANOVA.

Dependent Variable		Df	Sum Sq	Mean Sq	F value	Pr(>F)
<i>P. spicata</i> stem biomass	Sterile	1	0.1488	0.149	3.343	0.07
	Soil Source	2	0.2704	0.135	3.038	0.06
	Sterile*Soil Source	2	0.1447	0.072	1.625	0.21
	Residuals	51	2.2703	0.045		
<i>P. spicata</i> root biomass	Sterile	1	0.035	0.0346	0.09	0.76
	Soil Source	2	0.434	0.2171	0.567	0.57
	Sterile*Soil Source	2	3.545	1.7726	4.631	0.01
	Residuals	51	19.522	0.3828		
<i>P. spicata</i> total biomass	Sterile	1	0.33	0.327	0.514	0.48
	Soil Source	2	1.39	0.6946	1.093	0.34
	Sterile*Soil Source	2	4.95	2.4744	3.893	0.03
	Residuals	51	32.42	0.6357		
Log <i>V. dubia</i> total biomass	Sterile	1	1.381	1.3811	2.003	0.17
	Soil Source	2	0.244	0.1222	0.177	0.84
	Sterile*Soil Source	2	0.175	0.0875	0.127	0.88
	Residuals	28	19.307	0.6895		

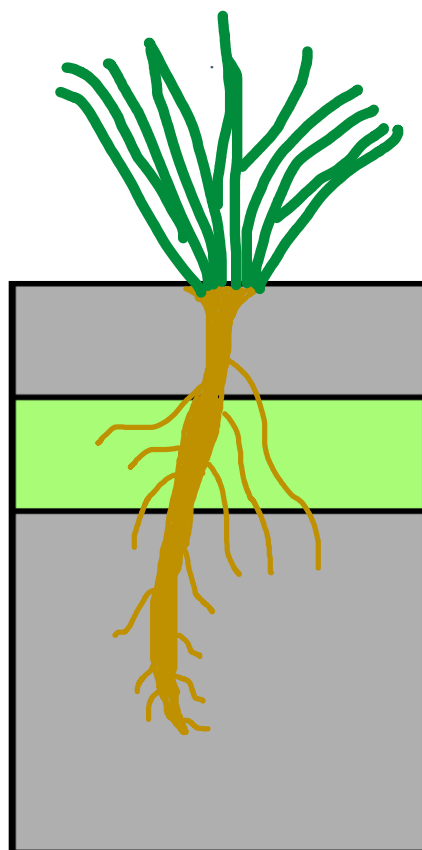
Figures

Figure 2.1 Illustration of the soil arrangement used for individual pot inoculation.

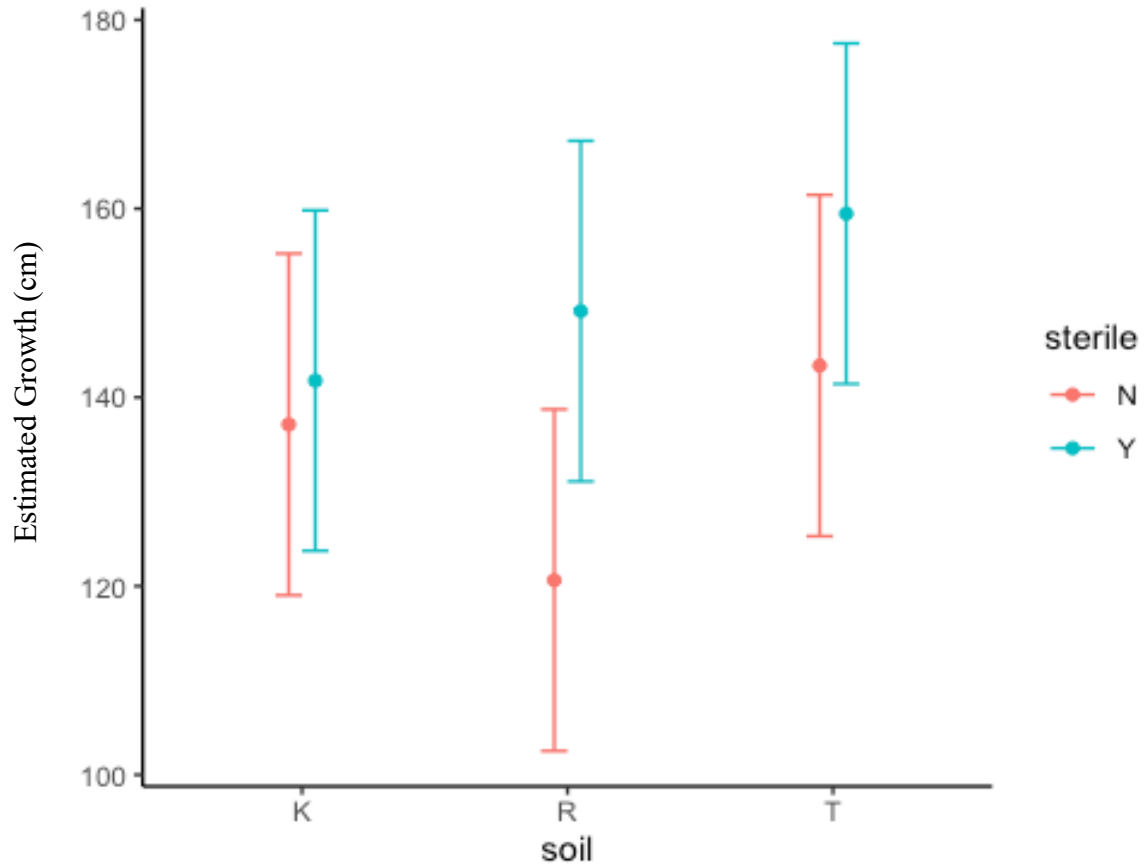


Figure 2.2 Repeated measures ANOVA results for *P. spicata* showing effect of sterilized or unsterilized soil inoculum source (K for Kamiak Butte, R for restoration site, and T for TNWR) on estimated growth.

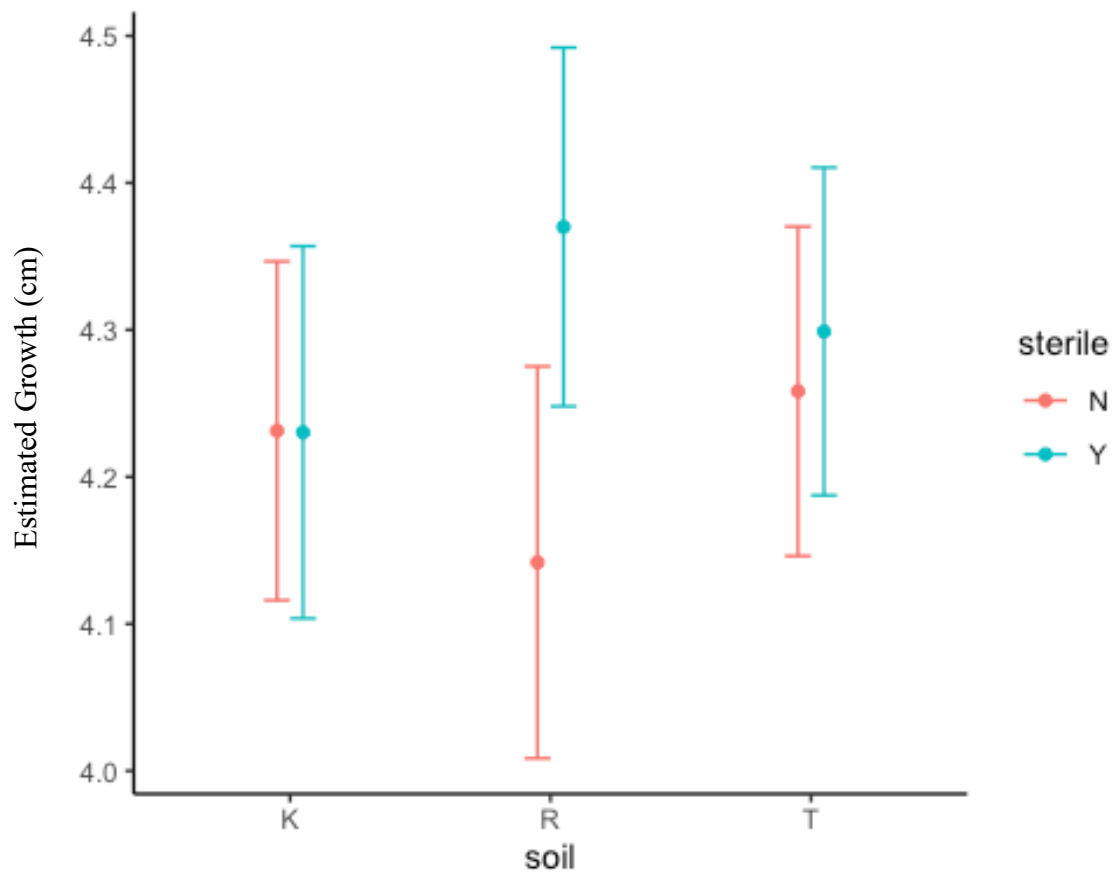


Figure 2.3 Repeated measures ANOVA results for *V. dubia* showing effect of sterilized or unsterilized soil inoculum source (K for Kamiak Butte, R for restoration site, and T for TNWR) on estimated growth.

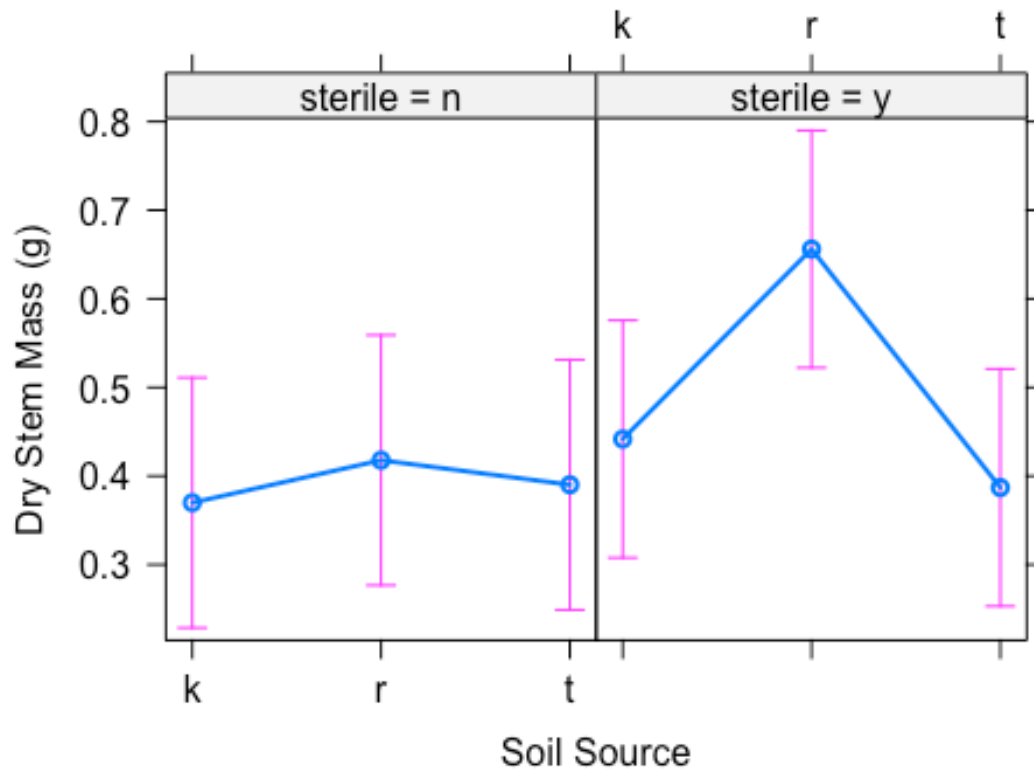


Figure 2.4 Effect of soil source (K for Kamiak Butte, R for restoration site, and T for TNWR) and sterilization on final above-ground biomass of *P. spicata*.

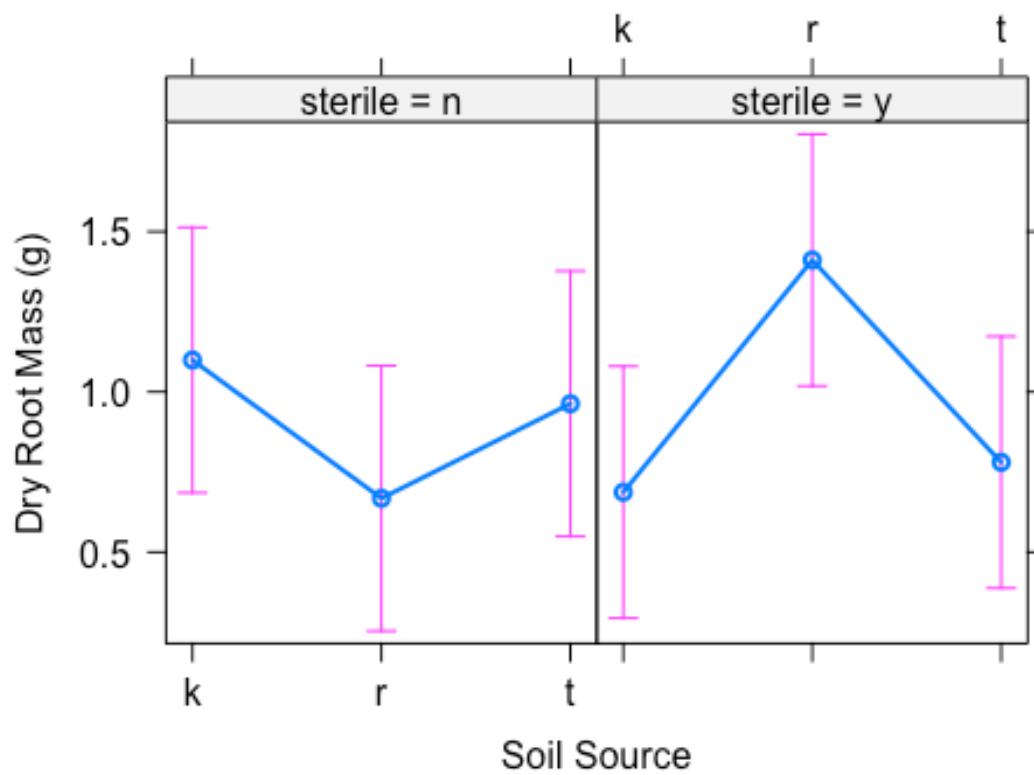


Figure 2.5 Effect of soil source (K for Kamiak Butte, R for restoration site, and T for TNWR) and sterilization on final below-ground biomass of *P. spicata*.

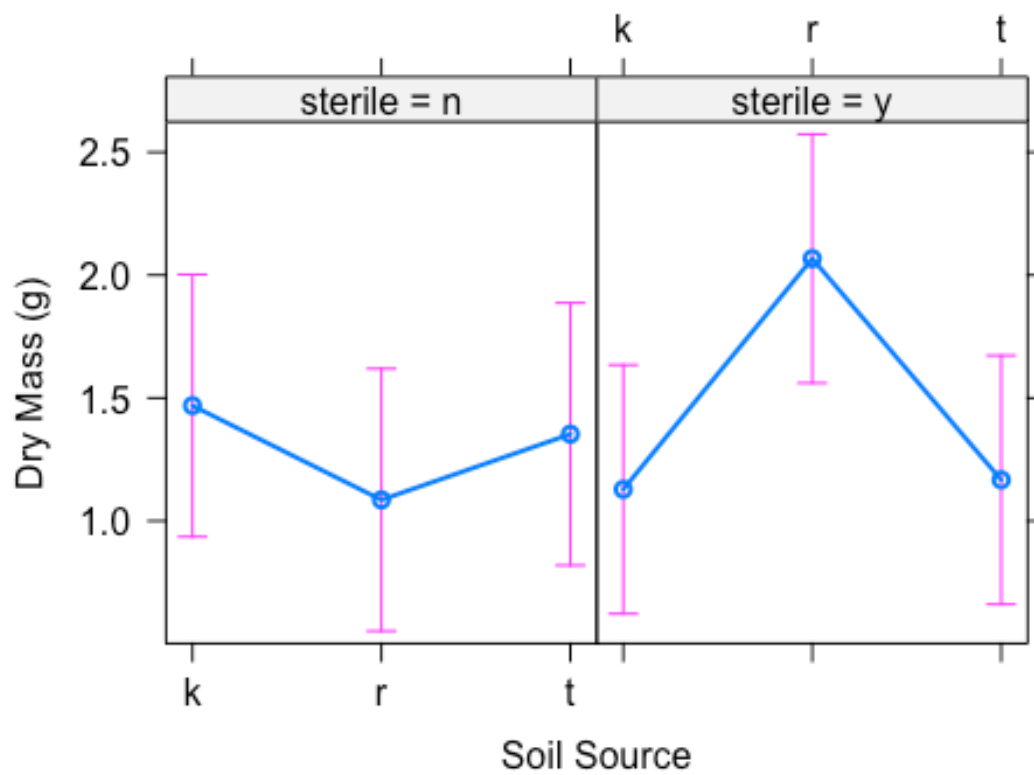


Figure 2.6 Effect of soil source (K for Kamiak Butte, R for restoration site, and T for TNWR) and sterilization on final total biomass of *P. spicata*.

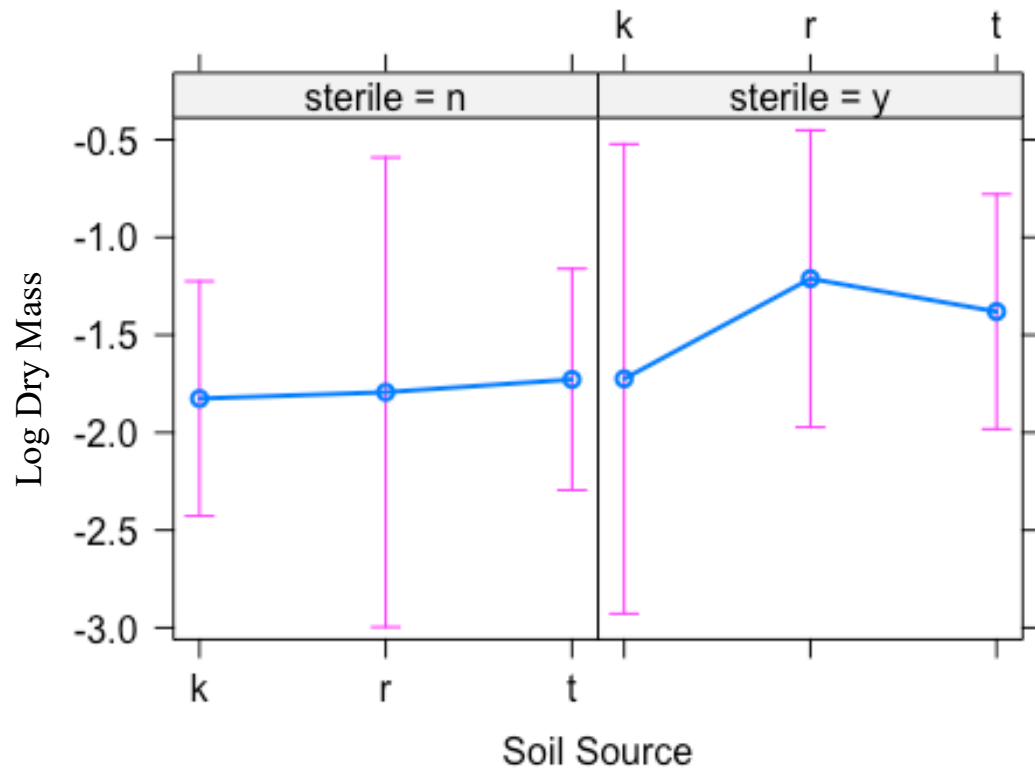


Figure 2.7 Effect of soil source (K for Kamiak Butte, R for restoration site, and T for TNWR) and sterilization on log transformed total biomass of *V. dubia*.

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VITA

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Education

Master of Science <i>Ecology/Restoration Ecology</i>	Eastern Washington University	2021
Geographic Informations Systems <i>Graduate Certificate</i>	Eastern Washington University	2021
Bachelor of Science <i>Biology</i>	Washington State University	2017 <i>Magna Cum Laude</i>
Associate of Arts and Sciences <i>General Studies</i>	Columbia Basin Community College	2014 <i>High Honors</i>

Teaching Experience

Graduate Fellow Teaching Assistant Eastern Washington University
Fall 2019 – Spring 2020

- Setup and execute labs for general biology, experimental design, macro-invertebrate, and botany classes. Graded and submitted scores for lab documents, research papers, and general assignments.

Assistant Director Washington State University Drumline Washington State University
Fall 2015 – Spring 2018

- Teach marching basics and music fundamentals as they relate to athletic band performance. Coordinate with spirit, dance, and athletic coaches for break and performance timing.

Percussion Caption Head Richland High School
A.C. Davis High School
Fall 2009 – Fall 2015 Naches Valley High School

- Write and design music and visual aspects of marching band shows related to percussion performance. Educate students in music and marching fundamentals. Coordinate individual staff members to achieve percussion and full ensemble goals.

Fellowships, Honors, and Memberships

Eastern Washington University Biology Graduate Fellowship	2019
Finalist Outstanding Biology Senior WSU	2017
Phi Beta Kappa member	2017

Washington Native Plant Society member	2021
Idaho Native Plant Society member	2021

Funding

EWU Mini-Grant	Spring 2019
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Presentations

Brown, R., Bean, E., Peterson, E. E. “Native Plant Restoration on the Palouse Prairie”

EWU Prairie Restoration Brown Bag Series. 2019.

Peterson, E. E. “ Palouse Prairie Community Assessment to Inform Large-Scale

Restoration at Eastern Washington University” Powerpoint Presentation. Eastern

Washington University Research and Creative Works Symposium. 2020.

Hill, S., Peterson, E. E. “Prairie Reconstruction Research at EWU” Powerpoint

Presentation. Eastern Washington University Geology Club. 2020.