# Forward-looking Approaches to Rangeland Restoration in a Variable World

by

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# DISSERTATION ABSTRACT

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Doctor of Philosophy in Environmental Sciences, Studies, and Policy

Title: Forward-looking Approaches to Rangeland Restoration in a Variable World

Ecological restoration is a powerful tool for repairing degraded ecosystems and promoting biodiversity and ecosystem functions. As global change drivers shift baseline conditions, forward-looking restoration approaches aim to establish resilient communities that can buffer and adapt to existing and future conditions. Genetic diversity and functional trait diversity of seeds are important considerations when restoring ecosystems that are resilient to multiple stressors. Restoration outcomes vary depending on the environmental conditions and interactions with neighboring species, both of which can change over time. Incorporating the role of variability in restoration frameworks is important to guide and assess efforts in a variable and changing world.

Here, I examine forward-looking approaches to restoration on semi-arid rangelands of the western United States. Specifically, I focused on rangelands in the Great Basin and California. Biodiversity and ecosystem functions of these rangelands are threatened by the interacting effects of wildfires, invasive annual grasses, and drought. Each of the chapters presented in my dissertation ask a question that seeks to improve rangeland restoration in an era of climate change. In Chapter II, I conduct a field survey after a mega-fire and examine how the current practice of post-seeding with native seeds affects the genetic diversity of wildland populations. In Chapter III, I test how an emerging seed sourcing strategy called climate-adjusted provenancing – supplementing local provenance with nonlocal provenances biased towards future climatic conditions – improves seedling establishment under drought. In Chapter IV, I consider how

intraspecific variability in trait plasticity influence predictability of seedling performance under water stress and cheatgrass competition. In Chapter V, I explore the above- and belowground relationships between community-level functional diversity and biomass production across variable rainfall conditions. In Chapter VI, I apply the concepts from modern coexistence theory to assess restoration trajectories and guide restoration actions. Throughout, I collaborate with scientists from multiple disciplines and use wide ranging methods and concepts to provide practical solutions to contemporary restoration challenges.

This dissertation includes previously published co-authored material.

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# CHAPTER I

# INTRODUCTION

As human impact on Earth’s ecosystems increases, ecological restoration is a powerful tool to repair disturbed or degraded ecosystems (Dunn et al., 2019). Restoration activities may be designed to replicate a pre-disturbance ecosystem or to create a novel ecosystem where it had not previously occurred (Hobbs et al., 2009). The former largely draws on ecological theories from ecological succession and community assembly, which assume a stable reference state (Hilderbrand et al., 2005) and a linear recovery trajectory (Brudvig, 2011). The latter is gaining traction as shifts in baseline conditions from climate change, altered disturbance regimes, and introduction of invasive species make interventions that worked in the past not work under novel conditions (Perring et al., 2015; Breed et al., 2018, 2019; Gurgel et al., 2020). Explicitly incorporating the role of variability in restoration frameworks is important to guide and assess efforts in a variable and changing world (Wainwright et al., 2017).

Forward-looking restoration approaches aim to achieve a resilient community that can buffer and adapt to existing and future conditions (e.g., Butterfield et al., 2017). From an ecological standpoint, adaptive capacity (i.e., the ability to buffer environmental stressors) of an community is influenced by underlying genetic properties (genetic diversity, composition of genes and alleles; Wernberg et al., 2018) and representation of key functional traits and diversity (Wang & Loreau 2014; Angeler et al., 2019). Genetic diversity ensures that populations have the evolutionary potential when conditions change in the future (Hoffmann & Hercus 2000). While functional diversity enables communities to perform the same ecosystem function while responding differently to environmental change (Ives & Carpenter 2007; Hautier et al., 2014). In contemporary restoration science, determining provenance and choosing an historic versus unknown future baseline conditions are key considerations as what seed we sow now have impacts on future persistence of desirable plant communities (Breed et al., 2018).

Current best practice recommends local provenance for restoration to maintain locally adapted genotypes and avoid maladaptation and genetic pollution (McKay et al. 2005), but locally sourced seeds may not survive or persist in future climate conditions (Broadhurst et al., 2008; Havens et al., 2015). To increase the adaptive capacity of restored populations, alternate provenancing strategies have been proposed. In particular, climate-adjusted provenancing supplements local seeds with nonlocal provenances from a climatic gradient biased toward projected conditions (Prober et al., 2015). Understanding how seed source affects functional traits – especially the largely unexplored belowground traits - their plasticity, and their influences on seedling performance are needed to improve seed selection.

Once restoration interventions are implemented, restoration outcomes need to be evaluated to determine the next steps. When the environment is variable and changing, assessing nonlinear restoration trajectories from abundances could be challenging (Wainwright et al., 2017). At the same time, modern coexistence theory has highlighted the idea that environmental variation and associated fluctuations in species abundances are not only common, but at times essential for maintaining species populations (Adler et al., 2007; Chesson, 2000; HilleRisLambers et al., 2012). Assessing restoration outcomes through the lens of modern coexistence theory may improve our ability to identify the factors governing success or failure, ultimately improving the adaptive management process (Hallett et al., 2023).

Rangelands (uncultivated, non-forested lands such as grasslands, savannas, shrublands) in western United States have high biodiversity and provide important ecosystem functions and services (Yahdjian et al. 2015). In my dissertation, I focused on two systems: Great Basin rangelands (Chapter II-IV) and California rangelands (Chapter V & VI). The sustainability of these ecosystems are threatened by the interacting effects of wildfires, invasive species, and climate change (Davies et al., 2011). In Great Basin rangelands, large scale seed-based restoration efforts are implemented on public lands after wildfires to halt and reverse the annual grass-fire cycle (Pilliod et al., 2017). The question in hand is whether restored populations will persist into the future with increasing pressures from invasive annual grasses and drought. In California rangelands, species-diverse native plant communities with high endemism are co-occurring with naturalized annual grasses (Bartolome et al., 2014). Fluctuating plant responses to temporal rainfall variability create a challenge for land managers to manage for reliable forage for livestock (Shaw et al., 2022) and determine restoration success (Williams, 2011). Here I seek to answer how functional diversity can predict plant community response to variable rainfall conditions, and how environmental and competitive variability could be incorporated in restoration assessments.

In Chapter II of my dissertation, “Post-fire seeding did not adversely affect the genetic diversity of bluebunch wheatgrass (*Pseudoroegneria spicata*) populations in Soda Fire,” I examined how the current practice of post-fire seeding with native seeds affects the genetic diversity of wildland populations. This chapter was co-authored with Matthew J. Germino, Lauren M. Hallett, and Matthew A. Streisfeld. In the aftermath of Soda Fire – a wildfire that burned at the border of southeastern Oregon and southwestern Idaho in 2015 – the Bureau of Land Management drill-seeded the burned areas with a cultivar of *P. spicata*. Using this fire as a natural experiment, I surveyed *P. spicata* populations within the fire perimeters of Soda Fire, and I asked whether post-fire seeding homogenizes or maintains the genetic diversity of *P. spicata* populations across a burned landscape.

In Chapter III, “Interannual variation in effects of climate-adjusted provenancing on seedling recruitment in Great Basin rangelands,” I examined how an alternative seed sourcing strategy called climate-adjusted provenancing improves restoration outcomes in future climatic conditions. This chapter was co-authored with Lucas Silva, Stella M. Copeland, Rory C. O’Connor, and Lauren M. Hallett. In collaboration with the USDA ARS Eastern Oregon Agricultural Research Center, I conducted a common garden experiment at the Northern Great Basin Experimental Range in Harney County, Oregon, testing the effects of climate-adjusted provenancing on seedling recruitment in three drought scenarios. I asked whether seeding seeds sourced from warmer and drier climate increases seedling performance under drought.

In Chapter IV, “Intraspecific variation in native grass seedling response to water stress depends on the annual grass invasion context,” I examined the influence of trait plasticity in predicting seedling performance under variable environmental conditions. This chapter was co-authored with Emily J. Cook and Lauren M. Hallett. While links between traits and seedling performance have been developed in the Great Basin, the efforts to understand the predictability of seedling establishment from seedling traits had been limited to plastic responses to one variable factor: either water or cheatgrass, but not both. I conducted a greenhouse experiment with a native perennial grass, Sandberg bluegrass (*Poa secunda*) and manipulation of both water conditions and cheatgrass competition. I asked whether seedling traits are plastic to interacting effects of drought and cheatgrass competition, and whether that shift in traits represent an adaptive plasticity.

In Chapter V, “Functional diversity buffers biomass production across variable rainfall conditions through different processes above- versus belowground,” I examined the plant community response to variable rainfall conditions. This chapter was co-authored by E. Ashley

Shaw, Caitlin T. White, Katherine N. Suding, and Lauren M. Hallett. Functional diversity, defined as the diversity of functional trait values within a community, promotes ecosystem functioning (e.g., biomass). In California annual grasslands, where seasonal drought is common, drought-related shifts in species composition affect functional trait diversity, which indirectly affects biomass production. This relationship between functional diversity and biomass is understudied belowground. I combined field-collected biomass data and greenhouse trait data to test the role of mass ratio and niche complementarity hypotheses in shaping above and belowground biomass in California annual grasslands. I asked which mechanism operated above- and belowground across seasonal drought conditions.

In Chapter VI, "Application of modern coexistence theory to rare plant restoration trajectories and management actions," I demonstrated how to apply concepts from the modern coexistence theory to assess restoration trajectories and guide restoration actions. This chapter was a joint effort of the sToration working group hosted by sDiv, the Synthesis Centre of the German Centre for Biodiversity Research (iDiv) Helle-Jena-Leipzig. I leveraged the long-term monitoring data of an endangered forb species, Contra Costa goldfields (*Lasthenia conjugens*) from a restoration project in California vernal pools. Vernal pools are seasonal pools of water that provide critical habitats for rare plant diversity, which are threatened by habitat loss and invasion of annual grasses. Because of the inherent environmental fluctuations of this system, determining restoration success solely from abundance is difficult. By parameterizing population models with monitoring data and partitioning growth rates with modern coexistence theory, I asked whether short-term abundances are sufficient to determine long-term re-establishment success, and what factors contribute to unpredictable restoration outcomes.

Chapter VII summarizes the results of these studies.

# CHAPTER II

# POST-FIRE SEEDING DID NOT ADVERSELY AFFECT THE GENETIC DIVERSITY OF BLUEBUNCH WHEATGRASS (*PSEUDOROEGNERIA SPICATA*) POPULATIONS IN SODA FIRE

## Contributions

LA and LMH conceived the research idea; LA, LMH and MJG designed the research; LA and MJG collected samples; LA collected and analyzed data with input from MAS; LA wrote the manuscript with contributions from all authors.

## Introduction

The number and scale of restoration projects using native seeds are increasing globally to repair degraded lands and to halt biodiversity loss (as highlighted in the International Standards for the Practice of Ecological Restoration; Gann et al., 2019). Genetic diversity is the foundation of biodiversity, and it is important for species adaptation to a local environment (Frankham 2005; Randall Hughes et al., 2008). As species adapt to changing environments, the maintenance of genetic diversity is an important goal for ecological restoration in the face of climate change (Holl et al., 2022; Kettenring et al. 2013). While genetic diversity is often considered in the planning stages of seed-based restoration (Pedrini & Dixon 2020), especially when choosing seed sources (e.g., Massatti et al., 2020), it is often ignored during the evaluation or monitoring of restoration success.

In the sagebrush ecosystems of the Great Basin, United States, climate change and the presence of invasive species are increasing the scale, frequency, and intensity of wildfires (Balch et al., 2013; Coates et al., 2016; Crist et al., 2023), necessitating successful implementation of landscape-scale restoration and rehabilitation projects. In response to massive wildfires, federal agencies, such as the Bureau of Land Management (BLM), apply large quantities of native and non-native seeds, mostly grasses, to hundreds of thousand acres of burned areas to resist annual grass invasion and minimize the probability of recurring fires (Davies et al., 2011; Kulpa et al., 2012; Pilliod et al., 2017). Initially, post-fire seed mixes contained predominantly non-native species (e.g., crested wheatgrass), but this shifted to include native species during the latter half of the 1900s (Svejcar et al., 2017). Genetically, however, native seed mixes are not diverse because they largely contain commercially produced cultivars that were sourced from a handful of origins and have been selected for seedling vigor and drought tolerance (Aubry et al., 2005). The effects of using a limited number of cultivars in post-fire seeding on genetic diversity of natural populations is unknown but potentially significant. If seeding homogenizes allele frequencies, for example, seeded populations would have lower genetic diversity than unseeded populations, potentially reducing the adaptive capacity of the restored populations.

Natural seed dispersal from surrounding seed-source patches is thought to be a key driver of plant population recovery in some disturbed areas (Corbin & Holl, 2012), and an important process for maintaining genetic diversity in fragmented landscapes (Aavik & Helm, 2018). In burned landscapes after megafires, seed dispersal is presumably more restricted farther away from the fire edge (i.e., seed-source populations; Busby & Holz, 2022; Kim et al., 2022). Active restoration (direct seeding) aims to supplement seeds where seeds are limited (Palma & Laurance 2015). However, we have little understanding of how post-fire seeding impacts plant genetic diversity across space. Post-fire seeding may be key to regaining genetic diversity where natural dispersal is low, but it may also result in the homogenization of local gene pools (Holl et al.,

2022) that excludes additional dispersal-generated diversity where dispersal is high. Furthermore, dispersal is not the only mechanism of post-fire recovery; resprouting of surviving individuals or germination of seedbank (Nolan et al., 2021) could also maintain genetic diversity in burned areas. Understanding the genetic responses to post-fire seeding is critical for identifying where to prioritize restoration when the next wildfire occurs.

To understand the effects of seeding on genetic diversity of wildland populations in burned landscapes, we focused on the Soda Fire, a megafire that burned nearly 280,000 acres of sagebrush steppe in southeastern Oregon and southwestern Idaho in 2015 (Germino et al., 2022). Soon after the fire, seeding treatments were applied primarily to break the annual grass-fire cycle. Since then, the effectiveness of these treatments to reduce invasion of annual grasses (Anthony & Germino, 2023; Kluender & Germino, 2024) and fire risks (Price & Germino 2024) have been investigated, but none have addressed patterns of genetic diversity in the target species. Soda Fire has been seeded with bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] Á.Löve ssp. *spicata*), an outcrossing, wind-pollinated, cool-season, native perennial grass species (Ogle et al., 2010) - one of the most commonly seeded native species following disturbance in the Great Basin. Here, we conducted a genetic survey of *P. spicata* populations in three areas that were drill-seeded with a cultivar of *P. spicata* known as Anatone. Specifically, we asked the following questions: Does post-fire seeding lead to a reduction in genetic diversity of wildland populations (individuals in the population that survived, seeded and not seeded)? If so, are there also corresponding patterns associated with the distance to the fire's edge? We hypothesized that seeded populations have lower genetic diversity than unseeded populations, which would suggest that seeding homogenizes wildland populations. Also, we hypothesized that genetic diversity will decline with the distance to the nearest fire edge, which would suggest that seeding would be more important in the center of a fire where seed dispersal is limited.

## Methods

### *Study Area*

This study took place in the fire boundary of the Soda Fire, which burned nearly 1,133 $km^2$ of sagebrush rangelands from August 10 to 23, 2015, along the border of southwestern Idaho and southeastern Oregon in Owyhee County, Idaho, U.S.A. (-116.89, 43.33). The Soda Fire affected 41 grazing allotments, recreation areas, a wild horse management area, and important sagebrush habitat for wildlife across boundaries of federal, private, and state-owned lands. Most of the area affected were public lands administered by the Bureau of Land Management (BLM). Elevation of the site ranges from 701 to 2,054 m. Higher elevation sites are dominated by big sagebrush (*Artemisia tridentata*) shrubs, intermixed with native perennial bunchgrasses (e.g., *P. spicata*, *Elymus elymoides, Poa secunda*) and forbs (e.g., *Collinsia parviflora*). Lower elevation sites are dominated by invasive annual grasses, such as medusahead (*Taeniatherum caput-medusae*) and cheatgrass (*Bromus tectorum*). The climate of the site is characterized by cold, wet winters and hot, dry summers with highly variable rainfall patterns.

### *Survey Design and Sampling Procedure*

In June 2020 and 2021, we surveyed a total of 40 populations of *P. spicata* across three burning and seeding treatments within the fire perimeter of Soda Fire: burned-unseeded (n = 15), burned-seeded (n = 15), and unburned-unseeded (n = 10). We replicated the sampling design across three areas that were drill seeded with Anatone, a cultivar of *P. spicata* (Fig. 2.1): Salmon Butte

(8.74 km2) was seeded in fall of 2015; and West (8.56 km2) and Rockville (17.00 km2) were seeded in fall of 2016. Anatone is a natural-origin, non-manipulated *P. spicata* commercial germplasm source originating from Asotin County in southeastern WA that demonstrates superior seed germination and seedling vigor compared to other accessions and cultivars (Monsen et al., 2003). No other *P. spicata* cultivar was seeded in these areas. We sampled areas that had been drill seeded and not aerial seeded, because previous studies have shown that drill seeded perennial grasses are more likely to successfully establish than aerial seeded individuals (Nelson et al., 1970; Svejcar et al., 2023). We sampled burned-unseeded and burned-seeded plots across a gradient of distances from the nearest fire edge (unburned patches or outer burn perimeter) from 922 m to 6020 m. We calculated the distance from each plot to the nearest fire edge by overlaying fire perimeter data from the Monitoring Trends in Burn Severity database (Eidenshink et al., 2007) and potential sampling plots in ArcMap 10.4, and using the proximity tool. Locations of burned-unseeded and burned-seeded plots were selected from an existing array of plots established for prior studies (Germino et al. 2018, 2022). We used the basal diameter data of *P. spicata* from their surveys – in addition to the visual confirmation of drill seeded individuals in distinct rows (see Figure 2.1e) – to infer the differences between populations that survived the fire and populations that newly established after the fire. Larger basal diameter in 2016 would be indicative of plants that survived the fire. We prioritized burned-unseeded plots in sites with pre-fire populations (median basal diameter > 2.5 cm in 2016 and > 7 cm in 2019) and no record of seeding before 2015 to sample individuals that survived the fire. We prioritized burned-seeded plots in sites with post-fire populations (median basal diameter < 7 cm in 2019) to sample individuals that were seeded. Burned-seeded plots had significantly higher density of *P.*

*spicata* (3.31 ± 0.31 individual/m$^2$) than burned-unseeded (2.29 ± 0.27) or unburned-unseeded (2.00 ± 0.61) plots.

At each plot, we collected ten fresh, green leaves of *P. spicata* from 20 individuals within a 50 m radius from the center of the plot. *P. spicata* do not have rhizomatous roots, but we chose individuals at least 2 m apart to ensure that we did not sample the same individual more than once. Some samples were damaged or lost during fieldwork, resulting in 752 unique individuals (an average of 18.8 individuals x 40 populations). We clipped the whole leaf from the base of the blade, and we avoided collecting dead or discolored leaves. We stored leaves from each individual separately in coin envelopes labeled with plot name and individual number. The samples were air-dried for 24 hours and kept in sealed plastic bags with silica gel in a cooler for 1-7 days. Upon returning from fieldwork, samples were immediately stored at -80ºC.

In addition to wildland-collected samples, we obtained leaf samples from Anatone plantings - the commercial germplasm source of *P. spicata* - grown at the Orchard Experimental Research Site near Boise, Idaho. The seeds produced from this site are considered the original seed stock of Anatone that is commercially available and commonly used in restoration treatments (Monsen et al., 2003). Fresh leaf samples were collected from eight individuals in January 2023 and stored following the same protocol as the wildland-collected samples.

### *DNA Extraction and Microsatellite Genotyping*

DNA was extracted from each of the 760 samples (n = 752 wildland-collected + 8 Anatone) following a modified CTAB protocol with a PVP lysing solution (Doyle and Doyle, 1990).

We initially tested 102 PCR primer pairs developed for *P. spicata* by (Bushman et al. 2008) using genomic DNA from a panel of 40 samples (eight individuals each from five populations, UU13, BU18, BS18, BU19, BS19). PCR amplifications  were performed in 20 µL reactions containing 13.7 µL of Nano water, 0.1 µL of Taq DNA polymerase, 2.0 µL of standard Taq buffer, 2.0 µL of Bovine Serum Albumin, 0.4 µL each of the reverse primer and fluorescently labeled forward primers (either 6-FAM or 5HEX), 0.4 µL of dNTP, and 1.0 µL of template DNA sample. PCR temperature cycling conditions (Eppendorf Mastercycler proS) were as follows: (I) 3 min at 94 C, (II) denaturation for 45 s at 94 C, (III) annealing for 45 s at 60 C, (IV) extension for 45 s at 72 C, (V) 20 repetitions for steps 2-4, and (VI) a final elongation at 54 C for 27 min. We selected 10 polymorphic loci with consistent amplification and easily scorable peaks (Table S1; FF343025.1, FF347040.1, FF343209.1, FF340262.1, FF344307.1, FF344338.1, FF344396.1, FF347548.1, FF342618.1, FF340831.1).

To assess patterns of genetic structure and diversity, we genotyped each of the 760 sampled individuals at the 10 polymorphic microsatellite loci. All genotyping was carried out using capillary electrophoresis on an Applied Biosystems 3730 at the DNA analysis facility at Oregon State University. Fragment analysis and scoring were conducted in Geneious Prime version 2023.1.2, and then the data were checked manually.

### *Analysis*

To investigate patterns of genetic structure in *P. spicata* within the fire perimeter, we used Principal Component Analysis (PCA) of the genotype data with the ‘princomp’ function in base R 4.3.0 (R Core Team, 2023). We analyzed all 10 loci because there was only a marginal difference in the percentage of variance explained when we removed loci where >50% of individuals failed to amplify (Fig. S2.1). We used PERMANOVA with the ‘adonis’ function in the ‘vegan’ package (Oksanen et al., 2022) to determine statistical differences between areas. To determine the most likely number of genetic clusters (K), STRUCTURE 2.3.4 (Pritchard et al., 2000) was used on the complete microsatellite dataset without a priori groupings of individuals into populations. We ran analyses following Gilbert et al. (2012); after preliminary analyses to determine the adequate burn-in and number of iterations, we performed 10 separate runs at each K from 1 to 6, with a burn-in of 100,000 generations followed by 100,000 sampling generations. To determine the appropriate value of K, we plotted the average natural logarithmic probability (-ln prob) of the data between successive K values (Evanno et al., 2005) using Structure Harvester (Earl & von Holdt, 2012), and selected the value of K at which the (-ln prob) began to plateau. For the selected K value, the output of the clustering analysis was visualized using the 'pophelper' package (Francis, 2017) in R.

To investigate the patterns of genetic diversity, we calculated four genetic diversity metrics: total number of alleles per locus (A), mean effective allelic richness (Ae), mean observed heterozygosity (Ho), mean expected heterozygosity (He), and inbreeding coefficients (Fis) with the 'gstudio' package (Dyer, 2023) in R. Total number of alleles is the observed number of alleles per locus. Effective allelic richness is the number of equally frequent alleles that it would take to achieve the expected heterozygosity. Observed heterozygosity is the fraction of individuals in the population that are heterozygous at a given locus calculated based on genotype frequencies ($Ho = 1 - \sum_{i=1}^{n} f[AiAi]$), whereas expected heterozygosity is the expected fraction of heterozygotes in the population under the Hardy-Weinberg model calculated based on allele frequencies ($He = 1 - \sum_{i=1}^{n} p_i^2$). According to the Hardy-Weinberg equilibrium, natural populations may show less observed heterozygosity than expected due to non-random mating, genetic drift, mutation, and natural selection. We then used ANOVA followed by Tukey tests to compare these genetic diversity metrics across areas and treatments within each area: Anatone, burned-seeded, burned-unseeded, unburned-unseeded. To determine if distance from the fire's edge affects patterns of genetic diversity, we used generalized linear regression models, with mean effective allelic richness, expected and observed heterozygosity as response variables and distance from the fire edge as an explanatory variable; we ran these models separately for burned-seeded and burned-unseeded treatments in each area.

## Results

A total of 355 genotypes were found across 10 loci. Successful PCR amplification ranged from 21 to 76%. The populations of *P. spicata* we sampled within the Soda Fire group genetically according to their geography rather than the burn/seeding treatments (Fig. 2.2a). The Rockville populations were more genetically divergent from the Salmon and West populations (PERMANOVA, $p = 0.01$). The seeded *P. spicata* cultivar, Anatone, was distinct from wildland-collected individuals, even in burned-seeded treatment areas (Fig. 2.2b, c, d). STRUCTURE analysis identified three genetic clusters as the most likely number of groups associated with these data (denoted as $K = 3$; Fig. S2.2). Consistent with the PCA, individuals from Anatone and Rockville largely formed one cluster, while samples from Salmon and West populations showed more mixed ancestry across the other two clusters (Fig. 2.2e). Anatone did not form a different cluster from Rockville, even at higher values of K (Fig. S2.3).

The total number of alleles per locus (A) ranged from 1-3 in Anatone and 7-20 in wildland-collected populations (Fig. 2.3a). Anatone had a fixed allele in 5 out of 10 loci examined (Fig. S2.4). Effective allelic richness (Ae) ranged from 1.00-9.64 (mean ± SE; Anatone = 1.44 ± 0.19, Rockville = 3.94 ± 0.13, West = 2.31 ± 0.07, Salmon = 1.94 ± 0.06; Fig 3b). The Ho (observed heterozygosity) ranged from 0-1.00 (Anatone = 0.31 ± 0.15, Rockville = 0.51 ± 0.02, West = 0.22 ± 0.02, and Salmon = 0.13 ± 0.01; Fig 2.3 c), and the He (expected heterozygosity) ranged from 0-0.89 (Anatone = 0.21 ± 0.08, Rockville = 0.66 ± 0.02, West = 0.47 ± 0.02, Salmon = 0.36 ± 0.02; Fig 2.3 d). There were no significant differences in Ae, Ho, or He across burned-seeded, burned-unseeded, or unburned-unseeded populations in all three areas (Table S2.1). Ae, Ho, and He were significantly higher in Rockville than in the other two areas or among Anatone samples (Table S2.1). The mean inbreeding coefficient (Fis) was 0.24 ± 0.03 in Rockville, 0.57 ± 0.04 in West, to 0.72 ± 0.02 in Salmon (overall mean = 0.53 ± 0.02). No significant relationship was found between genetic diversity (Ae, Ho, or He) and distance from the nearest fire edge in either burned-seeded or burned-unseeded populations in all three areas (Fig. 2.4; Table S2.2).

## Discussion

Our study on the restoration genetics of *Pseudoroegneria spicata* within the perimeter of the Soda Fire in the Intermountain West, USA is one of the first studies that assessed the effects of post-fire seeding on the genetic diversity of native grass species. Other similar studies have focused on either natural post-fire regeneration (Uchiyama et al., 2006; von Takach Dukai et al., 2020) or post-fire planting in forest ecosystems (Rajora & Pluhar 2003). We found that post-fire seeding in the Soda Fire did not homogenize the genetic diversity of *P. spicata* populations. This result is encouraging since post-fire seeding of sagebrush steppe is one of the largest uses of native seeds for restoration (NASEM 2023), and there have been recent efforts to ensure the use of genetically appropriate seeds in restoration projects via seed certification and procurement protocols (McCormick et al. 2021). Though we found that Anatone was lower in genetic diversity than wildland populations, we could not distinguish the identities of seeded individuals in seeded plots. Further, we found no pattern between genetic diversity and distance from the fire edge, likely illustrating the complex nature of post-fire recovery owing to wind-mediated dispersal and persistence of viable seeds on heterogeneous landscapes.

Contrary to our prediction that seeding would have a homogenizing effect on the genetic diversity of the target species, we found that genetic diversity in seeded populations was similar to the surrounding unseeded populations in burned or unburned areas. Other studies have shown varying results in restoration projects. For example, a global meta-analysis of 48 studies on impacts of revegetation plantings on the genetic diversity of plant species found that about half (46%) of the case studies demonstrated higher genetic diversity in restored populations, while the other half (52%) demonstrated lower genetic diversity (Jordan et al. 2019). Another meta-analysis of 83 studies showed that seeding tends to maintain - but planting reduces - genetic diversity in restored populations (Wei et al., 2023). The variation in the effects of seeding on genetic diversity of the target species is probably caused by many factors, such as seeding density and the size of restoration efforts, functional groups of target species, and the spatiotemporal variability in gene flow to and from surrounding habitats (Wei et al., 2023). While these context dependencies exist, seeding in the Soda Fire serves as an example of how a contemporary post-fire restoration approach can increase vegetation cover without negatively impacting the genetic diversity of native grass populations.

We observed lower or similar genetic diversity in the seeded cultivar of *P. spicata*, Anatone, than wildland-collected populations. This is consistent with patterns of genetic diversity in other developed restoration materials (e.g., Broadhurst et al. 2017). A genecology study of *P. spicata* found that genetic diversity within six commercial germplasm sources - including Anatone - was, on average, similar to or greater than the wildland populations, but some wildland populations had higher genetic diversity than the commercial germplasm sources (Massatti et al., 2018). Moreover, Massatti et al. (2018) found that commercial germplasm sources are most genetically similar to the wildland populations in Palouse/Wallawa and the

Western Great Basin where the seeds were originally collected. In our study, the alleles found in Anatone were also present in wildland populations in all but one locus (Fig. S4), suggesting that Anatone is likely maintaining the genetic integrity of natural populations of this area. As for the one locus (FF347548.1) that was distinct in Anatone, it is possible that this locus mutated during the agronomic process of seed production (Dryer et al. 2016; but see Conrady et al. 2022). Because of this mismatch in genetic markers at this one loci, we were not able to genetically identify which individual was sown in the seeded populations. It is possible that some of the individuals we sampled were not sown from Anatone, but rather from natural seed or resprouts, although our focus on small plants should have minimized risks of the latter.

Our genetic cluster analyses revealed that the population structure of *P. spicata* within the fire perimeter is mainly shaped by geography rather than burn or seeding treatments. We sampled three areas that were equidistant (approximately 20 km) from each other, and found that the populations in Rockville were genetically more distinct from those in West and Salmon Butte, while populations in West and Salmon Butte were genetically similar. Similarly, Davidson & Germino (2020) found genetic differentiation in natural populations of sagebrush, a wind-dispersed native shrub species across the same region. Given that *P. spicata* is wind-dispersed, its dispersal is probably limited by geographical barriers and proximity to neighboring populations (Jones, 2003). We generally lack information on seed dispersal distance of native species, but a synthesis of plant dispersal kernels found that the median dispersal distance of tall, wind-dispersed graminoids (only 3 studies total) is 46 m (Bullock et al. 2017). We presume the highway between Rockville and West/Salmon Butte may have disrupted dispersal between these habitats. Alternatively, the observed genetic differentiation could be a legacy effect of past land management treatments. For example, the Vale Program intended to increase forage cover in southeast Oregon by plowing and seeding non-native perennial grasses, such as crested wheatgrass in Rockville in 1964 (Heady & Bartolome 1977), which may have fragmented the *P. spicata* populations in Rockville and changed their genetic structure from populations in the surrounding area.

We predicted that genetic diversity would decline with distance from the nearest fire edge because seedling recruitment after disturbance is often related to proximity to seed sources (Webber et al. 2010; Leirfallom et al. 2015). However, we found no pattern between genetic diversity and distance from the fire edge in both seeded and unseeded areas. Applestein et al. (2022)'s study on sagebrush - another common wind-dispersed restoration species - has shown a high degree of variability in seed dispersal from unburned remnant patches. Perhaps, there are other factors like rugged topography and vegetation structure that affect the pattern of seed dispersal of wind-dispersed species (Nathan et al. 2009). Besides seed dispersal from surrounding populations, respouting of pre-existing individuals that survived the fire and/or recruitment from the seedbank (Tangney et al. 2022) may have maintained a similar level of genetic diversity across unseeded populations in burned areas regardless of distance to fire edge, but the exact mechanism remains unknown and warrants future research.

One of the limitations of our study is that we only captured a snapshot of genetic structure and diversity of *P. spicata* populations in one window of time (i.e., four to six years after seeding). Because perennial grasses generally take two to three years to produce reproductive units (James et al. 2011), planted individuals have only gotten a maximum of four years of seed production - we may need more time than that to detect changes in genetic diversity following seeding. Another limitation is that we were not able to distinguish the genetic identities of seeded Anatone individuals in seeded populations using the microsatellite loci we chose. If we were to increase the number of microsatellites or use newer molecular techniques like RADseq, we could increase genetic resolution within populations (Sunde et al., 2020) and answer questions pertinent to restoration, such as how much the post-fire recruitment comes from seeding vs natural recovery and how much gene flow is occurring among populations. While we worked with a foundational species with known ploidy and genetic markers, many native plant species targeted for restoration have never been sequenced before. As such, applying genetic approaches more broadly to non-model species, especially those with complex genomes caused by heterozygosity, polyploidy, repetitive sequences, and/or gene duplication, remains a challenge for restoration genetics (Williams et al., 2014).

In a world of changing climate, successful landscape-scale restoration projects are urgently needed to allow native species to adapt to environmental conditions (von Holle et al. 2020). It is important to verify whether restoration activities, like seeding, capture the genetic variation of remnant populations because this provides insight into their future resilience and evolutionary potential (Sgro et al. 2011; Broadhurst et al. 2008). With the current advancement of molecular technologies and computational capabilities to process large genomic and environmental data, restoration genetics has untapped potential to improve the outcomes of restoration activities (Broadhurst et al. 2023). For example, genetic information could be used to guide pre-restoration decision-making processes, such as seed sourcing (provenance delineation and adaptive provenancing), and to assess and monitor restoration outcomes (Mijangos et al., 2015; Breed et al. 2019). Here, we demonstrated the utility of genetic surveys to evaluate the effects of large-scale post-fire seeding on native grass populations in the sagebrush steppe of the Intermountain West, USA. The management implications include gaining confidence in the plant material used in post-fire treatments and identifying areas on the landscape to prioritize for seeding *before* the next wildfire occurs. However, we acknowledge that genetic surveys are not always feasible due to limited access to genomic facilities or technical support for restoration practitioners. Strategic collaboration between restoration and conservation genetics is one of many ways to break down this barrier (Breed et al. 2019).

## Figures

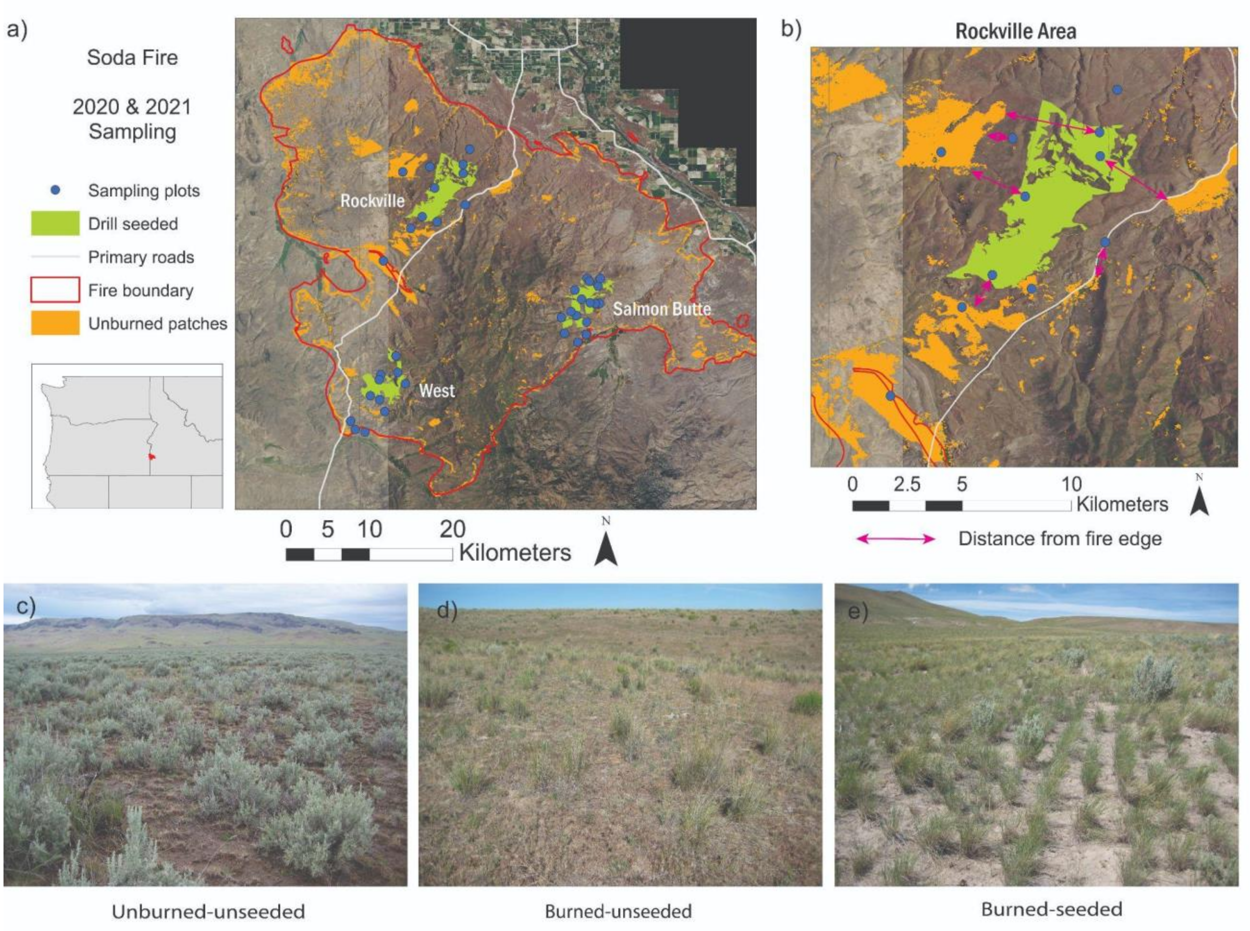

**Figure 2.1.** a) Map of sampling plots within the Soda Fire boundary located in southeastern Oregon and southwestern Idaho, USA. Plots (blue points) distributed across three areas: Rockville, West, and Salmon Butte. Highway (grey line) separates Rockville from West and Salmon Butte. b) Zoomed in the Rockville Area drill-seeded (green polygons) with bluebunch wheatgrass (*P. spicata*). Plots stratified by treatments: unburned-unseeded (UU), burned-unseeded (BU), and burned-seeded (BS). Pink arrows represent distance between plots and the nearest fire edge (outer fire boundary or unburned patches (orange polygons)). c) Unburned-unseeded plots are dominated by sagebrush interspersed with native grasses and forbs. d) Burned-unseeded plots are dominated by annual grasses with sparse perennial grasses. e) Burned-seeded plots have distinct rows of bluebunch wheatgrass (*Pseudoroegneria spicata*).

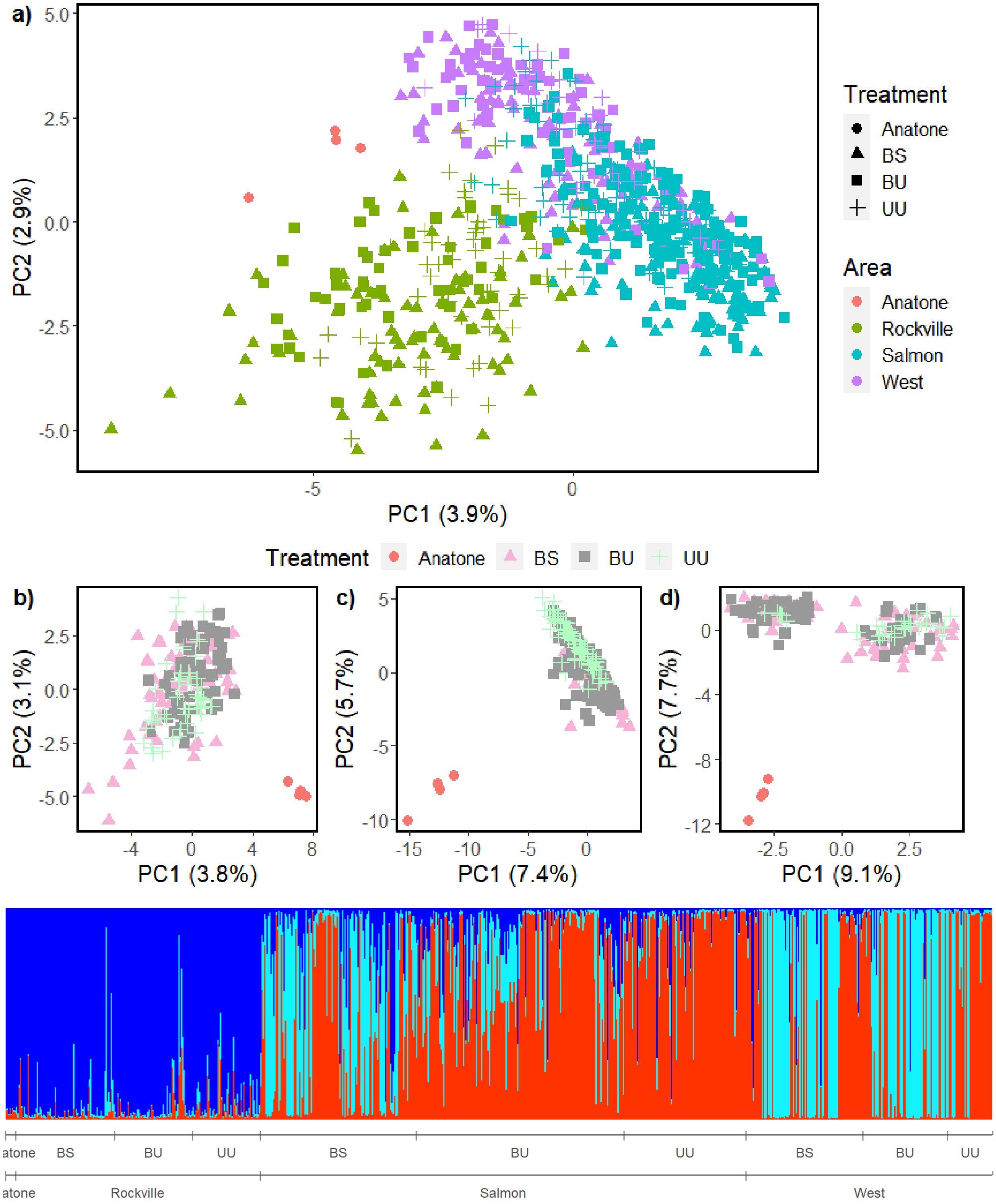

**Figure 2.2.** Genetic structure of *P. spicata* in Soda Fire. a) PCA of whole microsatellite data. b) PCA of subset of microsatellite data from Rockville, c) Salmon, and d) West. e) STRUCTURE bar plot of ancestry coefficients with K = 3. The first 8 samples are Anatone.

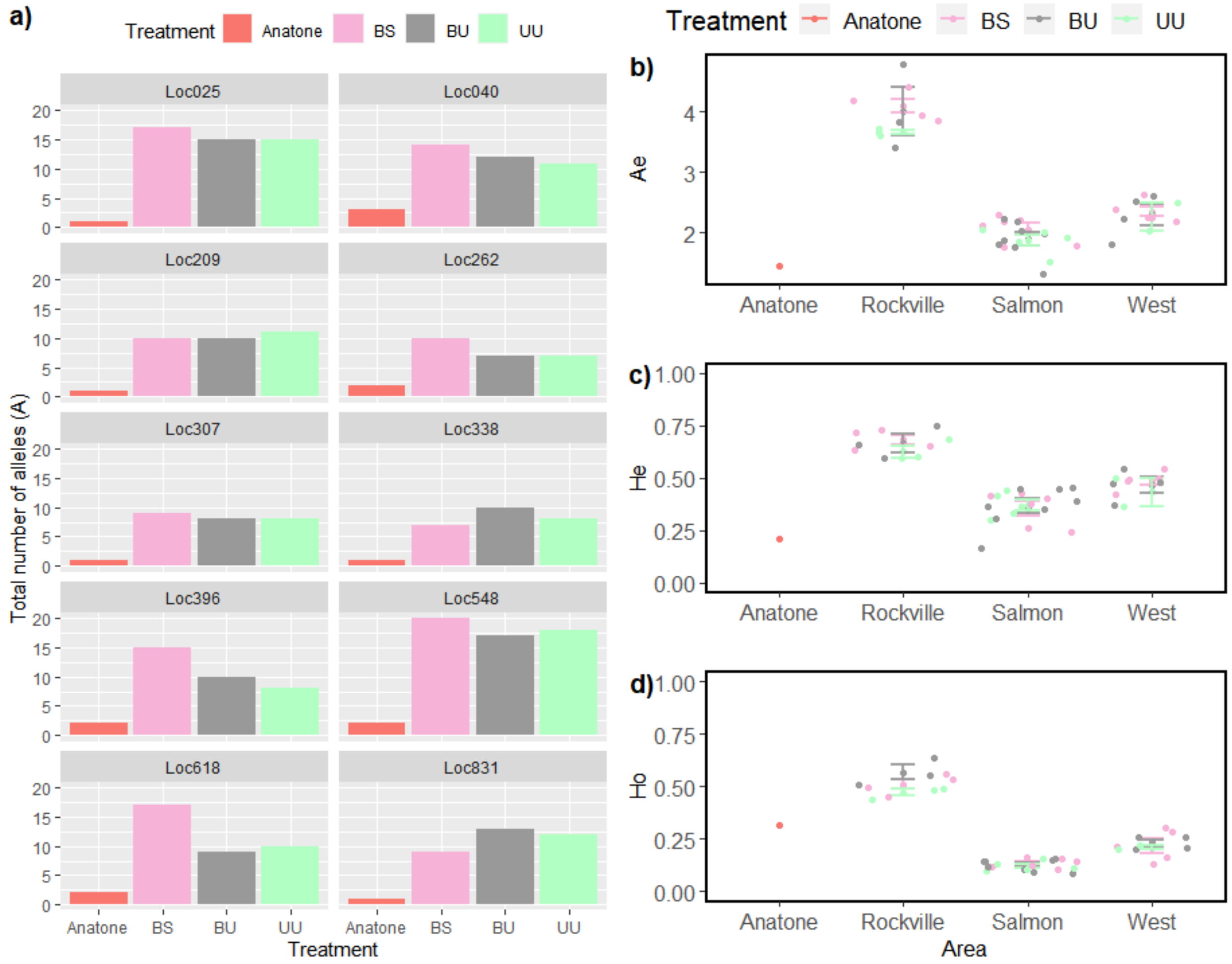

**Figure 2.3.** Genetic diversity of Anatone cultivar is lower than wildland-collected populations of *P. spicata* in Soda Fire. a) Total number of alleles (A) per locus for each treatment, b) mean allelic richness (Ae), c) mean expected heterozygosity (He), d) mean observed heterozygosity (Ho) across treatments and sampling areas. Each point is a mean of each population. Anatone = seeded cultivar, BS = burned-seeded, BU = burned-unseeded, UU = unburned-unseeded.

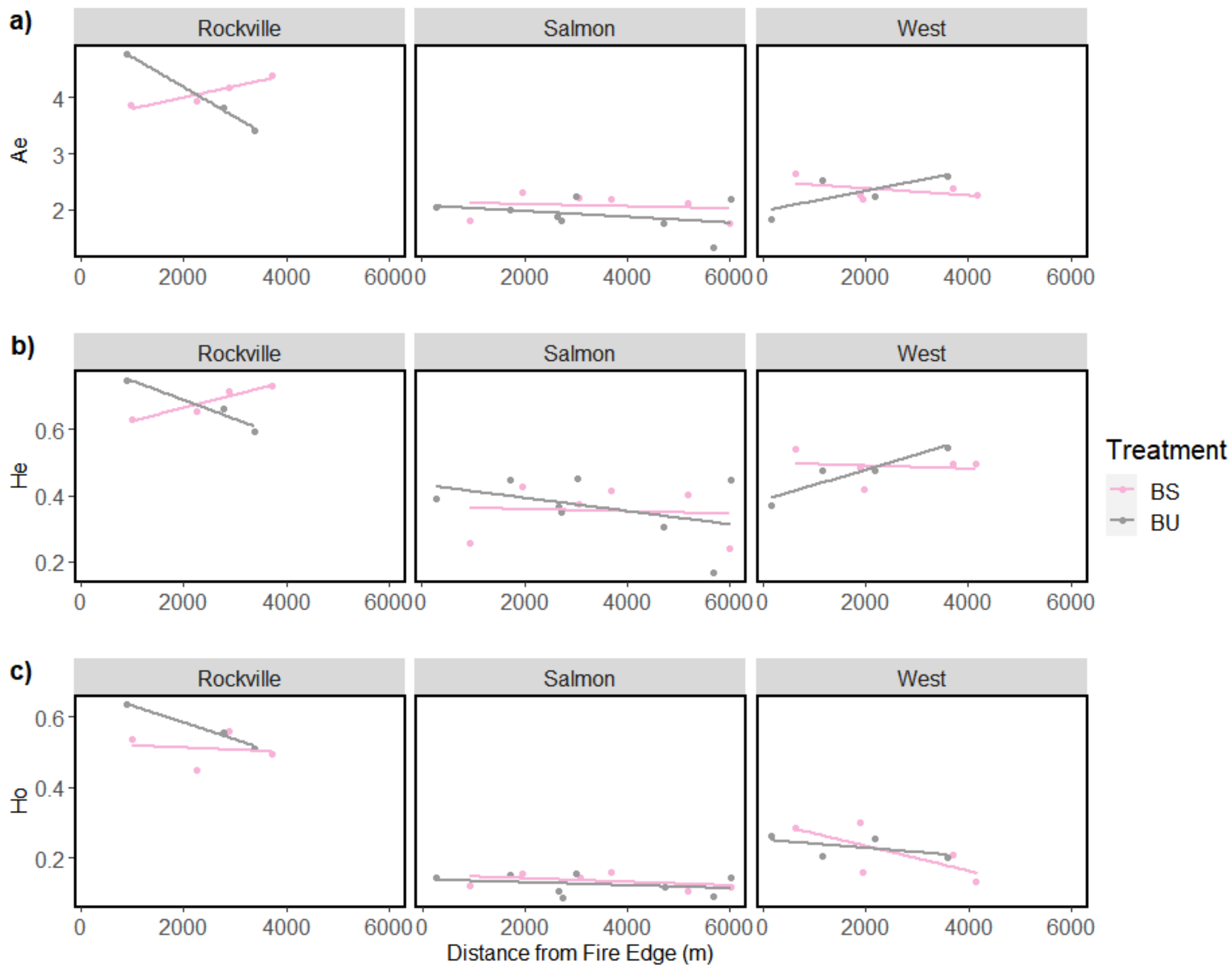

**Figure 2.4.** Relationships between genetic diversity and distance from fire edge. a) Mean allelic richness (Ae), b) mean expected heterozygosity (He), c) mean observed heterozygosity (Ho). Each point is a mean of each population. BS = burned-seeded, BU = burned-unseeded.

# CHAPTER III

# INTERANNUAL VARIATION IN CLIMATE-ADJUSTED PROVENANCING ON SEEDLING RECRUITMENT IN GREAT BASIN RANGELANDS

From Aoyama, L., L. Silva, S. M. Copeland, R. C. O’Connor, L. M. Hallett. *In review*. Interannual variation in effects of climate-adjusted provenancing on seedling recruitment in Great Basin rangelands. *Restoration Ecology*.

## Contributions

LA and LH conceived the ideas and designed the methodology. LA collected and analyzed the data, and led the writing of the manuscript with input from all authors.

## Introduction

Climate change is shifting the baseline conditions of restoration, especially in water-limited, dryland ecosystems that occupy about 41% of the earth’s terrestrial surface (Mortimore et al. 2009). Seeding native species is crucial in drylands because plant community recovery following disturbance can be slow (Estruch et al., 2018) and hindered by invasive species (Garbowski et al., 2021). Restoration practitioners are challenged by how to make decisions about seed provenance – the geographic origin of seeds – in face of climate change (Havens et al., 2015). In drylands, local provenancing is a common practice, assuming local adaptation (McKay et al. 2005), but locally sourced seeds may not survive or persist in future climate conditions (Broadhurst et al., 2008; Havens et al., 2015). In an era of climate change, forward-looking seed provenancing that anticipates changes in climatic conditions of the restoration site may improve restoration success.

To increase the adaptive capacity of restored populations, alternate provenancing strategies have been proposed. These include strategies to increase the genetic diversity of the seed mix (e.g., relaxed local, composite, and admixture provenancing; Breed et al. 2013; Broadhurst et al. 2008; Bucharova et al., 2019) and strategies to match geographic origin with anticipated future environmental conditions (e.g., predictive and climate-adjusted provenancing; Carvalho et al., 2021; Prober et al., 2015; Woolridge et al., 2023). In particular, climate-adjusted provenancing mixes local seeds with nonlocal provenances from a climatic gradient biased toward projected conditions (Prober et al., 2015). As climate models forecast more frequent, severe droughts in drylands (Huang et al., 2017; Scholes, 2020), long-term species persistence may depend on successful seedling survival in drier conditions. We expect populations from warmer/drier sites to perform better in drought because they generally have functional traits associated with drought resistance such as small leaves, low osmotic potential, and high integrated water use efficiency (Blumenthal et al., 2021). Furthermore, intraspecific variation in thermal performances reflect adaptation to the spring environments in which the seeds are found (Angert et al., 2011; Gremer et al., 2020). For example, populations from cooler sites with higher germination temperature optima are more likely to delay germination and avoid freezing, while populations from warmer sites with lower germination temperature optima are more likely to germinate early and escape drying (Hardegree et al., 2008; Zhang et al., 2020). By mixing provenances, we could incorporate these diverse strategies to overcome stressful conditions. Despite the theoretical underpinnings of climate-adjusted provenancing, experimental field-tests are limited (but see Nolan et al., 2023).

We tested climate-adjusted provenancing in sagebrush steppe rangelands of the Great Basin in the western United States, where successful re-establishment of native perennial plants is urgently needed to reverse the positive feedback of wildfire and annual grass invasion (Chambers et al., 2014) but native seedling establishment is very low (only 2 to 7%; James et al., 2011; Larson et al., 2015). Stressors include freezing and drying in spring during emergence (Boyd & Lemos, 2013; James et al., 2019) and drought in summers during establishment (Pyke 1990). More frequent and longer drought conditions forecasted in this region (Snyder et al., 2019) may further hamper seeding success in the future. Therefore, understanding how climate-adjusted provenancing affects early seedling development across years is important for long-term species persistence in the Great Basin.

In this study, we conducted a common garden experiment with manipulative rainfall treatments: ambient, moderate and severe drought. We used bottlebrush squirreltail (*Elymus elymoides* (Raf.) Swezey), a native perennial bunchgrass with known genecology (Larson et al. 2003) and high intraspecific trait variation (Baughman et al., 2019; Leger et al., 2019), as a focal species. This species is widely available and commonly seeded after wildfires due to its ability to colonize in early seral conditions, resist invasive species, and tolerate fire (Parsons et al., 2011). We sourced seeds from six sites along an aridity gradient spanning 486 km latitudinally across the Great Basin. Second-year seedling survival is a critical benchmark for perennial grass establishment as they take two years to transition to adults (James et al., 2011). We hypothesized that provenances from warmer/drier sites have higher second-year survival under drought from higher water-use efficiency and lower germination temperature optima than those from cooler/wetter sites.

## Methods

### *Study Site*

We conducted our study at the Northern Great Basin Experimental Range (NGBER hereafter; 43.4727° N, -119.6933° W) in Harney County, OR, U.S.A from 2020-2022. The site is reflective of the sagebrush steppe vegetation type in the Northern Basin and Range Ecoregion. Big sagebrush (*Artemisia tridentata* Nutt.) shrubs are dominant and intermixed with native perennial bunchgrasses (e.g. *Achnatherum thurberianum* [Piper] Barkworth, *Poa secunda* J. Presl) and annual forbs (e.g., *Collinsia parviflora* Lindl., *Microsteris gracilis* [Hook.] Greene). Elevation of the site is 1396 m above sea level. Soil type of the site is Gradon gravelly fine sandy loam (Lentz 1986). The climate at the site has cold-wet winters and hot-dry summers, with the growing season constrained by freezing and drying periods. Studies at this site have shown that native perennial grass seedlings emerge between March and June (James et al., 2011, 2019), and established seedlings survive as juveniles through winter and transition to adults by the second growing season (James et al., 2011; Larson et al., 2015). The 17-year average annual rainfall of water year (begins on October 1st and ends on September 30th) from 2004 to 2020 was 264.4 mm, and mean annual temperature over the same period was 7.3 °C (NOAA, 2022). Water year precipitation in 2021 and 2022 were 166.6 mm and 257.5 mm, respectively (Fig. S3.1). Based on the Palmer Drought Severity Index, the region was affected by moderate to severe drought for most of the study period (between September 2020 to April 2022) and extreme drought between June to October in 2021 and February to March in 2022 (Fig. S3.2; NOAA National Centers for Environmental Information, 2023).

### *Experimental Design*

*Elymus elymoides* seeds were sourced from six locations across an aridity gradient in the Great Basin (Table S3.1; Fig. 3.1a). Vale and Susanville were wild-collected in 2016 and 2018, respectively. Elko, Roaring Springs, Little Sahara, and Norcross were farm-grown and harvested in 2018. Seeds were stored in tightly sealed bags in a cool, dry room.

At NGBER, we established a common garden experiment in a sagebrush steppe in October 2019. We chose a flat site with low abundance of cheatgrass (*Bromus tectorum* L.), a common invasive annual grass in Great Basin rangelands. We set up 1.5 x 3 m plots in a randomized block design with eight blocks and three rainfall treatments (n = 24 plots; Fig. S3.3): 0% reduction for ambient rainfall, 50% reduction for moderate drought, 80% reduction for severe drought. Each plot was subdivided into twelve 25 x 25 cm subplots for nested seeding treatments with six provenances and two replicates. We broadcasted 300 *E. elymoides* seeds in each subplot in September 2020, and reseeded the same density with the same seed stock in September 2021. We initially nested cheatgrass treatments within rainfall treatments; the average densities of cheatgrass were 145.0 ± 4.0 individuals/$m^2$ in competition plots and 7.8 ± 1.0 individuals/$m^2$ in non-competition plots. We combined those plots as replicates because there was no effect of competition on seedling survival (Fig. S3.4) or growth (Fig. S3.5). To reduce microsite variability from shrubs, we first manually removed aboveground biomass to prepare the site. We constructed fixed rainout shelters that passively reduce precipitation by a constant percentage (Yahdjian & Sala, 2002; Gherardi & Sala, 2013; Fig. 3.1c). Roofs were made with 3.1 mm acrylic sheets (Multicraft Plastics, Inc., Eugene, OR), cut to 0.12 m x 1.8 m strips, and bent to 120 degree angle down the middle. Aluminum flashings were inserted 35 cm deep around the shelters to reduce surface and lateral soil flow on to the plots and to limit plant roots from accessing water outside the treatment. Plots were fenced with 4-ft high wire mesh that excluded cattle but not native herbivores (e.g. rabbits, gophers). Defoliation from rodent herbivory was evident but not detrimental to seedling survival during the study period. In October 2019, we burned each plot with a propane blow torch to mimic post-fire conditions because seeds are commonly sown after fire in this region. In September 2020, we raked the annual plants and dug out the perennial forbs and grasses with a shovel before seeding. To account for microclimates across rainfall treatments, we deployed soil moisture sensors (TEROS 10 and ZL6 Data Logger, Meter Group) and air and soil temperature sensors (iButton, Maxim Integrated), one of each in twelve plots. Soil moisture and temperature sensors were installed at 5 cm below the soil surface; air temperature sensors protected by radiation shields (following design by Holden et al., 2013) were installed at 10 cm above the soil surface. These sensors logged data every two hours year-round.

### *Plant Demography*

We censused *E. elymoides* seedling recruitment within in each 25 x 25 cm subplot from March to July of 2021 and 2022. The first cohort seeded in 2020 was tracked for two growing seasons, while the second cohort seeded in 2021 was tracked for only one year because its first-year survival was so low that treatment effects would be uninterpretable in the second year. *E. elymoides* is generally considered ‘non-dormant’ (Young et al. 2003), suggesting low numbers of new recruits from the first cohort in the 2022 census. Nonetheless, the numbers in the 2022 census should be interpreted as the recruitment from the second cohort and possible residual seeds from the first cohort. We distinguished first and second-year seedlings by using visible phenology cues (i.e., new recruits had seed glumes still attached or no standing dead biomass), and marked live first cohort with aluminum rings and second cohort with paper clips (Fig. 3.1d).

We revisited all subplots each month, marked new seedlings, removed markings from dead seedlings, and counted number of live and dead individuals.

### *Plant Growth*

In July 2022, we measured two metrics of growth potential: plant height (cm) and dried biomass (g). We measured height to the nearest 0.1 cm of two second-year seedlings per subplot where available. We selected individuals without evidence of defoliation. For biomass, we clipped all live second-year seedlings, oven dried them at 60 °C for 72 hours, and weighed them individually to the nearest 0.01 mg.

### *Thermal performance*

Thermal performance of each provenance was determined by conducting germination trials in November 2021. We stratified seeds in a freezer at 0 °C for four weeks prior to growing them in a growth chamber (Winslow, 2002). We sowed each provenance (25 seeds/provenance, five replicates) separately on a wet filter paper in a petri dish for two weeks in a constant temperature of 8, 10, 12, 15, and 20 °C. We added 2 mL of water to each petri dish at the start, and watered every two days as needed to keep the filter moist. Lights were on from 6 am to 6 pm daily. Numbers of germinated seeds were counted on the 14th day. All the provenances were over 90% viable. Mean proportion of germination was calculated by the dividing number of seeds germinated by the number of seeds sown. Maximum germination optima were the highest observed germination on nonlinear thermal performance curves.

### *Leaf traits*

In July of 2022, we measured specific leaf area (SLA, $cm^2/g$) and leaf $^{13}C$ isotopic composition, or $^{13}C/^{12}C$ ratios ($\delta\ ^{13}C$, ‰), an integrated proxy for intrinsic water-use efficiency. SLA is associated with the leaf economic spectrum (low SLA = slow growing, resource-conservative). Leaf stable isotope ratios reflect rates of photosynthesis relative to stomatal conductance integrated over the lifespan of the leaf (Cernusak et al., 2013).

We sampled fresh leaves from two non-defoliated individuals of second-year seedlings per subplot, scanned within 12 hours to capture fresh leaf area, oven dried at 60 °C for 72 hours, and weighed. We calculated SLA by dividing fresh leaf area by dried leaf weight. Dried leaves were ground to powder in a ball mill, rolled in aluminum tins, and analyzed for stable isotope ratios using a thermal conversion elemental analyzer-continuous flow isotope ratio mass spectrometry system (Nu Horizon 2, EuroVector Elemental Analyzer, Nano-CF interface) at the Stable Isotope Lab at the University of Oregon. Carbon isotopic abundance ratios were converted to δ notation using:

$$\delta = \left[\frac{R_{sample}}{R_{standard}} - 1\right] * 1000$$

where R is a ratio of heavy to light isotopes for the leaf carbon sample and the international carbon isotope standard, Vienna Pee Dee Belemnite, respectively.

### *Analyses*

All analyses were conducted in R version 4.2.1. (R Core Team, 2022). To describe the climate gradient amongst sites, we ordinated the 30-year average (1991-2020) monthly climate data from the nearest weather station to the seed origin site (Table S3.1); some weather stations had fewer number of years of data available (NOAA, 2022; Fig S3.4). We selected climate variables we thought were most relevant to seedling emergence and survival: mean annual precipitation, mean air temperature in early growing season (October to March) and late growing season (April to June), number of days below 0 °C, and mean vapor pressure deficit in July. We conducted a principal component analysis using the *rda* function in *vegan* package (Oksanen et al., 2022), but not integrating predictor or explanatory variables. To evaluate the effects of drought severity on first-year emergence across provenances and years, we built separate mixed effect models with rainfall treatment, provenance, year, and their interactions as fixed factors and block and collection type (wild vs farm-grown) as random effects for cumulative emergence and final density at the end of the first growing season. We further evaluated the effects of drought severity on second-year seedling survival and growth by building separate mixed effect models with rainfall treatment, provenance, and their interactions as fixed factors and block and collection type as random effects. To compare thermal optima and leaf traits across provenances, we built mixed effect models with provenance as a fixed factor and collection type as a random effect. All mixed models were built with the *lme* function in the *nlme* package (Pinheiro et al., 2022) followed by post-hoc Tukey tests (*emmeans* package Lenth, 2023). We checked for normality in model residuals visually (*qqnorm*) and with Shapiro tests (*shapiro.test*), and transformed the outcome if they were not normally distributed.

## Results

### *Treatment Effects on Microclimate*

Rainout shelters effectively reduced soil moisture in top 5 cm of the soil surface by 36% and 62% in moderate and severe drought plots, respectively (Fig. 3.2). Soil temperatures in top 5 cm of the soil surface were on average 0.7°C and 1.5°C warmer in moderate and severe drought plots compared to ambient plots (Fig. 3.2). The 2021 growing season began with a wet and particularly cold spring (below zero daily min soil temp in February to May) followed by a dry heat dome in the summer (above 40 °C daily max soil temp in June and July; Fig. 3.2). Soil temperature continuously increased throughout the growing season in 2021, while it fluctuated in 2022. The 2022 growing season was also abnormally dry until two rainfall events occurred in May and June (Fig. 3.2).

*Demography*

The cumulative emergence of the 2020 and 2021 cohorts were 80.8 ± 3.8 and 11.6 ± 1.0 seedlings/$m^2$, respectively, out of a seeding rate of 4,800 seeds/$m^2$. We found significant effects of rainfall treatment ($F_{2, 521} = 67.55$, $p < 0.001$), provenance ($F_{5, 521} = 15.11$, $p < 0.001$), and year ($F_{1, 521} = 532.28$, $p < 0.001$), and combined interaction effect ($F_{10, 521} = 2.78$, $p = 0.002$) on cumulative emergence ($R^2_m = 0.64$; $R^2_c = 0.64$; Fig. 3.3a, c). Of the 2020 cohort, Vale and Susanville had significantly higher cumulative emergence than other provenances in moderate (Vale: 135.5 ± 12.9; Susanville: 116.7 ± 12.7) and severe drought (Vale: 168.7 ± 16.0; Susanville: 180.5 ± 23.4; Fig. 3.3a). For the 2021 cohort, we found no significant difference in cumulative emergence across provenances (Fig. 3.3c). We observed higher cumulative emergence in moderate (86.7 ± 5.0) and severe (119.5 ± 7.6) drought than ambient condition (36.0 ± 2.9) in 2021 (Fig. 3.3a), but lower cumulative emergence in moderate (11.0 ± 1.7) and severe (8.4 ± 1.6) drought than ambient condition (15 ± 1.8) in 2022 (Fig. 3.3c).

The final densities of first-year seedlings of the 2020 and 2021 cohorts were 12.2 ± 0.3 and 1.5 ± 0.1 seedlings/$m^2$, respectively. We found significant effects of rainfall treatment ($F_{2, 521} = 35.18$, $p < 0.001$), provenance ($F_{5, 521} = 3.90$, $p = 0.001$), and year ($F_{1, 521} = 213.95$, $p < 0.001$), as well as all combined interaction effect ($F_{10, 521} = 1.86$, $p = 0.04$) on first-year survival ($R^2_m =$

0. 41; $R^2_c = 0.41$; Fig. 3.3a, c). Of the 2020 cohort, Vale (41.0 ± 4.5) had significantly higher first-year survival than Susanville (26.7 ± 3.1) in moderate drought ($p = 0.07$; Fig. 3.3a). For the 2021 cohort, we found no significant difference in first-year survival across provenances (Fig. 3.3c). First-year survival rates in 2021 and 2022 were half in severe drought (0.17 ± 0.02; 0.17 ± 0.04) compared to moderate drought (0.33 ± 0.02; 0.34 ± 0.07).

The final densities of second-year seedlings of the 2020 cohort in ambient rainfall, moderate drought, and severe drought conditions were 0.4 ± 0.2, 4.2 ± 0.6, and 1.4 ± 0.4 seedlings/m$^2$, respectively. We found significant effects of rainfall treatment ($F_{2, 251} = 23.92$, $p < 0.001$) and provenance ($F_{5, 251} = 3.72$, $p = 0.002$), but no interaction effect ($F_{10, 251} = 1.34$, $p = 0.21$) on second-year survival ($R^2_m = 0.\ 22$; $R^2_c = 0.25$; Fig. 3.3b). In moderate drought, Vale (7.5 ± 1.6) had the highest second-year survival than other five provenances, including the more 'local' source (Roaring Springs; 3.5 ± 1.2) in terms of both geographic distance and climate (Fig. 3.3b).

### *Plant Growth*

We found no difference in average per capita biomass across seed sources ($F_{5, 129} = 1.66$, $p = 0.14$) or rainfall treatments ($F_{2, 129} = 0.11$, $p = 0.88$; Fig. S3.7a). Likewise, average height did not differ across provenances ($F_{5, 61} = 0.44$, $p = 0.81$) or rainfall treatments ($F_{2, 61} = 2.07$, $p = 0.13$; Fig. S3.7b).

### *Thermal Performance*

Germination temperature optima varied by provenances (Fig. 3.4a; $F_{5, 4287} = 11.40$, $p < 0.001$): Little Sahara at 10 °C, Norcross, Elko, and Roaring Springs at 12 °C, and Vale and Susanville at 15 °C.

*Leaf Traits*

SLA did not differ across provenances ($F_{5, 54} = 0.74$, $p = 0.59$) or rainfall treatments ($F_{2, 54} = 3.38$, $p = 0.05$; Fig. S3.7c). Water use efficiencies measured by stable carbon isotopic compositions ($\delta\ ^{13}C$) differed across provenances ($F_{5, 17} = 11.68$, $p < 0.001$; $R^2_m = 0.\ 40$; $R^2_c = 0.94$; Fig. 3.4b). Larger $\delta\ ^{13}C$ values were interpreted as evidence of higher water use efficiency. In particular, Vale was the most water use efficient, while Little Sahara was the least water use efficient (Fig. 3.4b).

## Discussion

We conducted an empirical test of climate-adjusted provenancing (mixing local seeds with seeds 'pre-adapted' to future conditions; Prober et al., 2015) in Great Basin rangelands and found partial evidence for this strategy improving seedling recruitment under moderate drought. We observed a strong interannual variation in seedling recruitment depending on the year seeds were sown, as typically observed in this region (e.g., Svejcar et al. 2023). While we observed differences in seedling recruitment by provenances for the first cohort, recruitment in the second cohort was so low across provenances that the effect of climate-adjusted provenancing was undetected in another year. Intraspecific variation in functional traits reflected different strategies to cope with abiotic stressors such as freezing and drying. Specifically, one source with high germination temperature optimum and high water use efficiency avoided freezing and resisted drought tolerance, while another with low germination temperature optimum and low water use efficiency capitalized on two water events that occurred in the second growing season. Understanding these differences in stress coping strategies among populations within species is critical for creating seed mixes that increases the adaptive capacity of the restored population.

Seedling recruitment was significantly variable across years. The first cohort's cumulative emergence was on average 6.9 times higher than the second cohort's. The year-to-year difference in seedling recruitment is probably attributed to the amount and timing of rainfall and fluctuating temperature during the first growing season (James et al., 2019; Muñoz-Rojas et al., 2016; Pyle et al. 2021). Other factors such as legacy effects of seed storage and seed age may have also contributed to the strong interannual differences in seedling recruitment (De Vitis et al., 2020). Our study occurred during two drought years: in 2021, a freezing, moderately dry spring was followed by a heat dome in the summer; in 2022, a warmer but exceptionally dry spring was followed by two rainfall events in the summer. The second cohort's recruitment was presumably limited by the lack of soil moisture early in the growing season. We do not have the second-year survival data of the second cohort to compare it to the first, but the second cohort's near-zero first-year survival suggests that even climate-adjusted provenancing may not buffer against seeding failures in extremely severe drought years.

Our results demonstrate that there are multiple ways to reach the same outcome; intraspecific trait variation suggest diverse strategies to survive the challenges of stressful conditions. In this study, Susanville and Vale with high germination temperature optima had high cumulative emergence in the first year, which agrees with other studies that seeds that germinate in warmer temperatures successfully emerge by avoiding frost mortality (Hardegree et al., 2008; James et al., 2019; Roundy & Madsen, 2016). In addition to freezing, drought is another prominent stressor to seedling recruitment in drylands (Larson et al., 2020). Previous studies found higher drought resistance traits in drier environments (Baughman et al., 2019; Blumenthal et al., 2021). Accordingly, the driest provenance, Vale, in this study was most drought resistant as evidenced by high water use efficiency, which may explain the differences in first-year survival between Vale and Susanville despite having similar emergence rates. Then, why did seeds from Little Sahara with low water use efficiency have equally high second-year seedling survival as Vale? Low drought resistance is associated with a more resource acquisitive strategy (higher productivity, higher specific root length, higher SLA; e.g., Balachowski & Volaire, 2018). Little Sahara possibly escaped drought by emerging early (as suggested by low germination temperature optima) and establishing long roots that tapped into deep soil moisture. While we observed similar aboveground biomass, height, and SLA across provenances (Fig. S3.7), rapid root growth is an adaptation of deep-rooted perennial species for young seedlings to survive summer drought in drylands (Hanslin et al., 2019; Leger et al., 2019). In context of climate change, lower elevation sites that are generally warmer and drier in the spring may experience extreme summer temperatures. To buffer plant vulnerabilities to climate change, further research on intraspecific variation in frost and heat tolerance and how they trade-off with drought resistance is needed to select seeds precisely suitable for future environmental conditions.

We found no consistent pattern between climate of origin and seedling survival in drought conditions, which further supports the case for prescreening seedling traits rather than simply selecting seeds from hotter and drier climates. No pattern may suggest that, as climate changes, plant populations are not always optimally adapted to the climate in which they are growing (Anderson et al., 2012; Shaw & Etterson, 2012). Alternatively, climate alone is not a sufficient proxy for seedling performance. Similarly, a study on predictive provenancing in tallgrass prairie in Illinois, USA, found that climate of origin did not predict overall fitness of three forb species sourced from three sites across a latitudinal gradient (Woolridge et al., 2023). Another explanation is that the artificial selection from agricultural seed production altered the phenotypes in commercial seeds, thereby seedling survival. Commercial seeds are generally larger, fast growing, and possess less potentially adaptive traits than wild-collected seeds (Leger et al., 2021). The retention of adaptive traits in wild-collected seeds might explain our results on high water use efficiency in Vale and Susanville compared to other provenances. Given that agronomic seed increase is a critical process in seed-based restoration, future research on seedling performance of wild-collected vs. commercial seeds in future climatic conditions is warranted.

Field experiments that manipulate climatic conditions are a powerful approach toward mechanistically understanding and predicting the effects of changing climate (e.g., drought events) on plant communities and ecosystems (Beier et al., 2012), but they are not perfect. For example, rainout shelters used in drought experiments simulate rain reduction but do not control for increased evaporative demand associated with high temperatures, low humidity, and clear skies (Kröel-Dulay et al., 2022). In addition, rainout shelters may cause lower air temperature due to the interception of radiation (Yahdjian & Sala, 2002), or a greenhouse effect, enhanced by reduced air flow under shelters (Vogel et al., 2013). In our study, rainout shelters reduced soil moisture as intended, but they also made the drier plots warmer due to high specific heat capacity of water. Increased early-season soil temperatures in our drought plots may have artificially increased emergence by reducing frost impacts, which explains why first-year success increased with drought compared to ambient in 2021. Furthermore, when drought experiments occur during a natural drought, the drought effects are accentuated in drier years, as we observed in 2022. Despite these limitations, drought experiments are useful in comparing seedling recruitment under variable climatic conditions. As environmental change accelerates, an experimental approach to strategic seed sourcing is invaluable to prevent large seedling failure and resource loss.

Forecasted climate change trends toward increased frequency and severity of ecological drought in dryland ecosystems (Bradford et al., 2020) will likely have a large effect on future restoration efforts. To increase the adaptive capacity of restored populations, we need to rethink about the ‘local is best’ paradigm and pursue alternative provenancing approaches (Havens et al., 2015; Nolan et al., 2023; Dupré la Tour et al., 2020). We provided a proof-of-concept for the climate-adjusted provenancing (Prober et al. 2015) as a management strategy to improve restoration outcomes in warmer and drier environments in the future. Our results suggest that identifying the ‘pre-adapted’ source, even for one stressor (drought), is more complex than using a simple climate-gradient, perhaps because sources vary in their drought response. We caution practitioners not to use climate-adjusted provenancing for every restoration species (Jones, 2013). For example, local provenancing is still the best option for species with small population size and/or low gene flow in fragmented habitats to avoid inbreeding, increase genetic diversity, and minimize outbreeding depression in restored populations (Broadhurst et al. 2008; Hufbauer et al., 2015; Mijangos et al., 2015). That said, we recommend climate-adjusted provenancing for widespread, foundational species such as *E. elymoides* that occur in large area that is likely to undergo significant shifts in climate. Because policymakers and practitioners ultimately make the decision about seed sourcing, development of evidence-based provenance policies and establishment of stronger research-practitioner collaborations are required to facilitate the adoption of forward-looking provenancing approaches (Breed et al. 2018).

## Figures

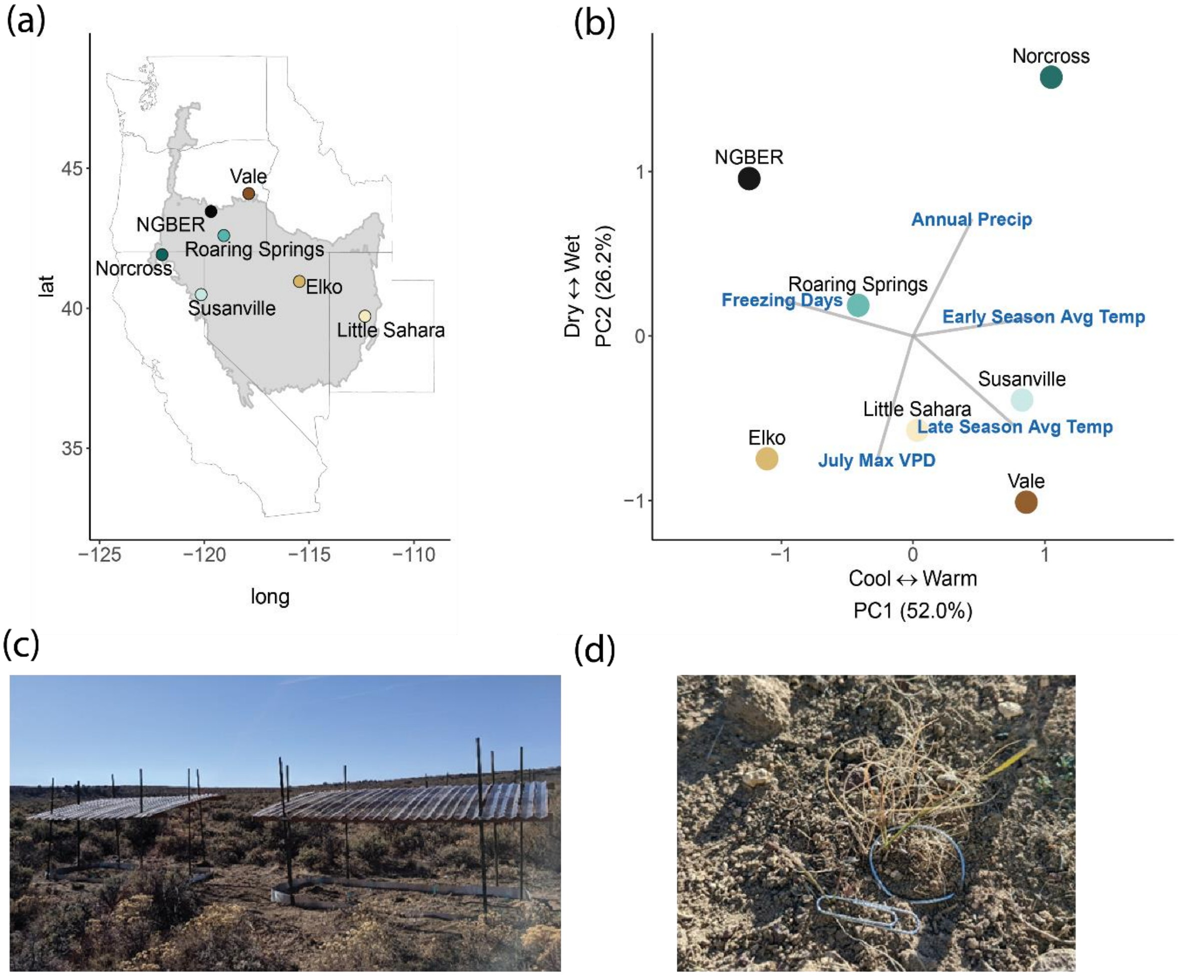

**Figure 3.1.** (a) Map of *Elymus elymoides* provenances in the Great Basin (grey). (b) Principal component analysis of climate of origin with 30-year normal climate data (1991-2020). Color of dots rank the sites by their overall moisture levels. (c) Rainout shelters at the study site reduced rain by 50 % (left) and 80 % (right). (d) Marked live seedlings from the first cohort with aluminum rings and the second cohort with paper clips.

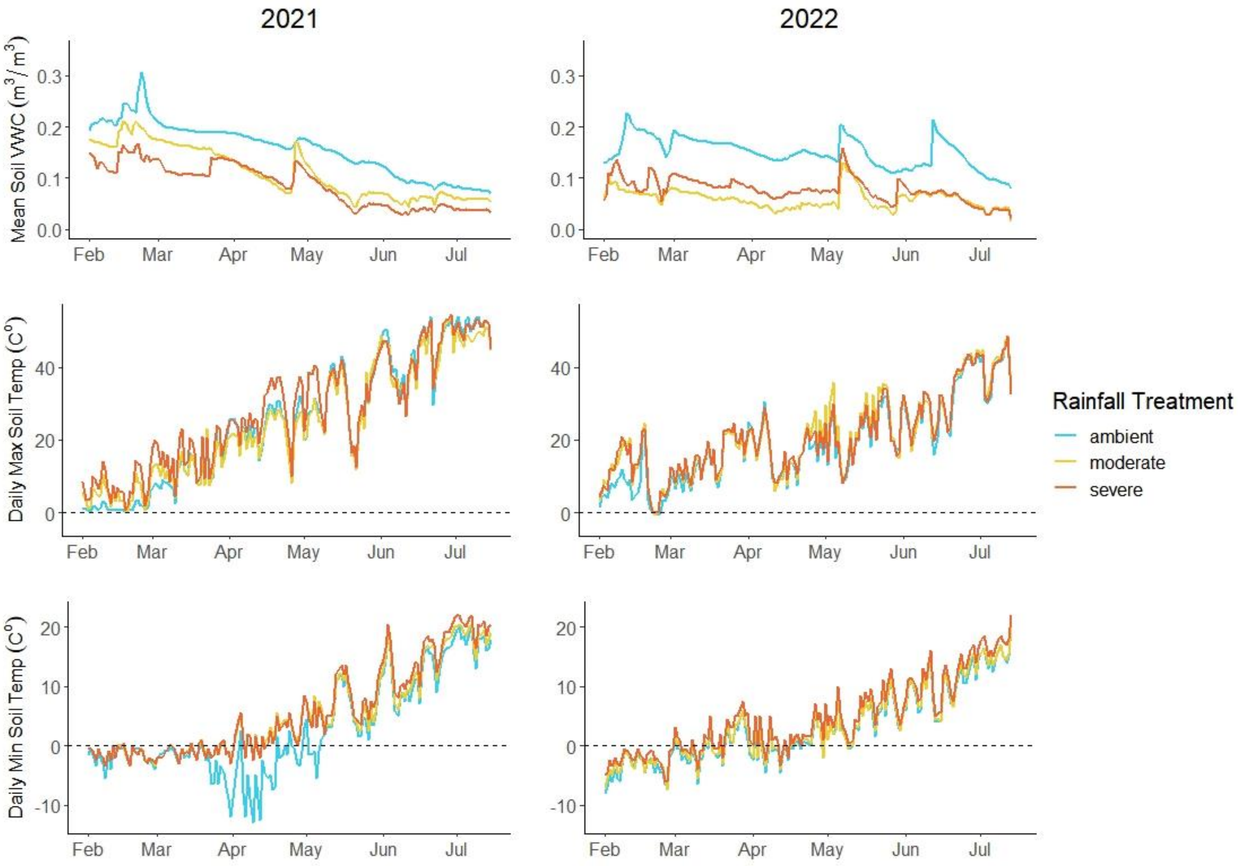

**Figure 3.2.** Rainfall treatment effects on mean soil volumetric water content (m$^3$/m$^3$) and daily max/min soil temperature (°C) in 5 cm below soil surface at the study site during growing seasons of 2021 and 2022. Dashed lines indicate freezing temperature.

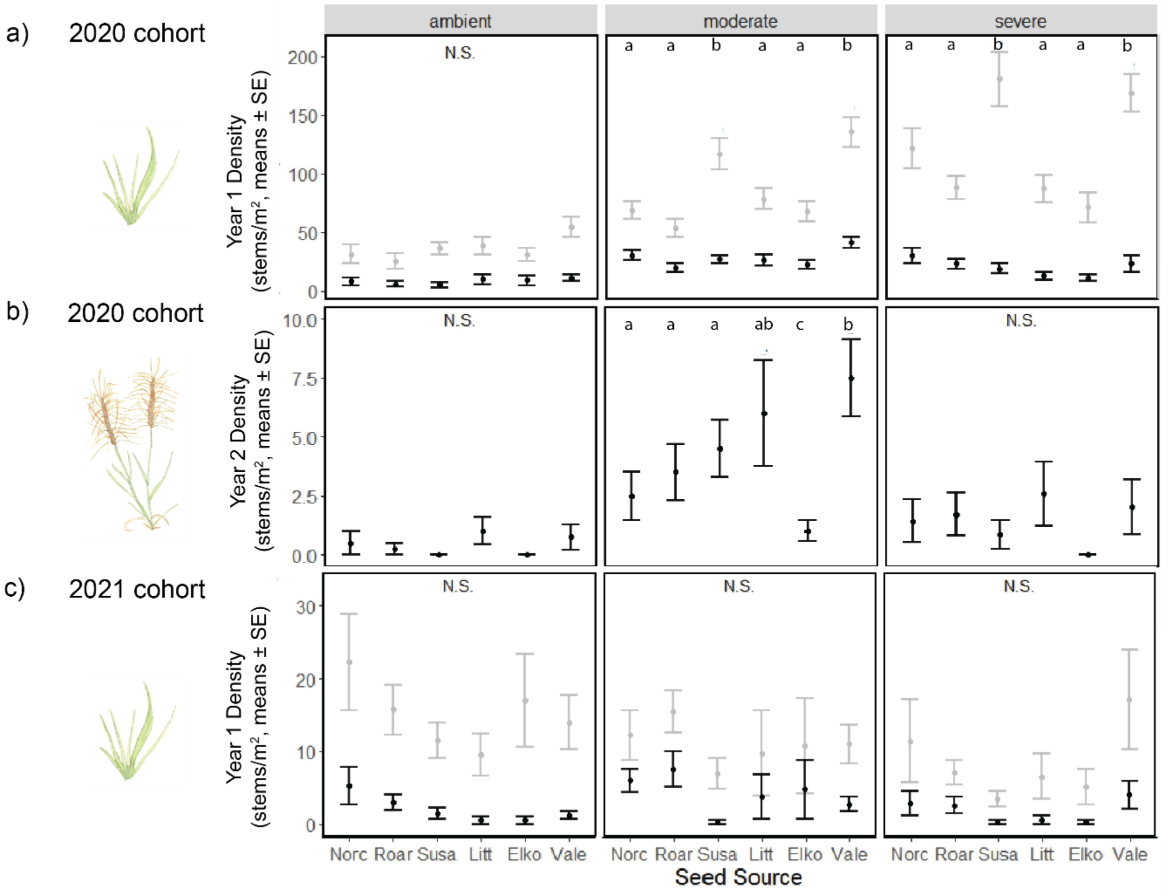

**Figure 3.3.** Rainfall treatment effects on (a) the first-year cumulative emergence (grey) and survival (black) of the 2020 cohort ; (b) the second-year survival of the 2020 cohort; and (c) the first-year cumulative emergence (grey) and survival (black) of the 2021 cohort (means ± SE). Rainfall treatments: ambient, moderate drought, and severe drought. Provenances sorted along the wet-dry gradient (according to the PC2 axis of Fig. 1). Letters above bars indicate significant difference ($p < 0.05$) in densities across provenances within rainfall treatment.

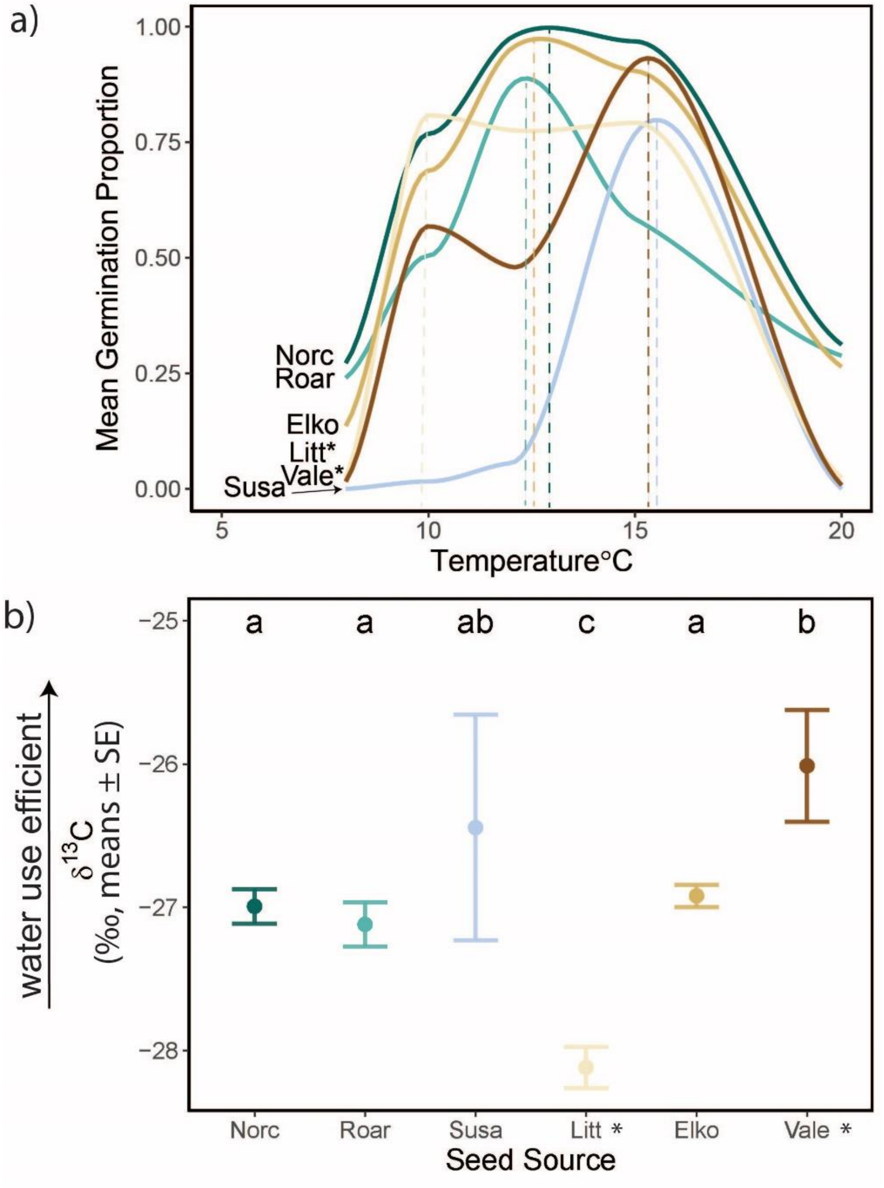

**Figure 3.4.** (a) Thermal-performance of *E. elymoides* seeds by provenance. Dashed lines indicate the temperature optima for each provenance. (b) Leaf $^{13}C$ isotopic composition ($\delta\ ^{13}C$, ‰) of second-year seedlings (means ± SE). Statistical significance ($p < 0.05$) of water use efficiency indicated by letters above bars. Asterisks (*) represent two most successful provenances (see Fig. 3c).

# CHAPTER IV

# INTRASPECIFIC VARIATION IN NATIVE GRASS SEEDLING RESPONSE TO WATER STRESS DEPENDS ON THE ANNUAL GRASS INVASION CONTEXT

From Aoyama, L., E. Cook, and L. M. Hallett. 2022. Intraspecific variation in native grass seedling response to water stress depends on the annual grass invasion context. *Restoration Ecology* e13816.

## Contributions

LA, LH conceived and designed the research; LA, EC performed the experiments and collected the data; LA analyzed the data; LA, EC, LH wrote and edited the manuscript.

## Introduction

A demand for successful seed-based restoration is increasing globally (e.g., Broadhurst et al. 2016; Torok et al. 2018). However, establishing plants from seeds can be challenging, especially in semiarid ecosystems, where drought and competition with invasive species hamper seedling establishment (Garbowski et al. 2021). While seed provenance – the geographic source of seeds used in restoration – and plant traits are often carefully selected to assure that seeds are well suited for the restoration site condition (Funk et al. 2008; Bucharova et al. 2017), an unresolved question remains: how do we select for seeds that meet these dual challenges? Seeds selected for drought tolerance could also be beneficial in resisting invasive species, or alternatively the trade-offs in drought tolerant traits and invasive resistant traits may have detrimental effects on the seedling performance. Here, we explore this question focusing on the large-scale, post-fire restoration of native perennial grasses in the Great Basin Desert of North America.

Understanding the interacting effects of water stress and invasive species on native perennial grass traits and seedling establishment is critical for one of the world's largest seed-based restoration efforts (Pilliod et al. 2017).

Local provenancing, or the use of locally-sourced seed within seed transfer zones, has long been the tradition of restoration efforts (McKay et al. 2005). This seeding practice is grounded in ecological theory, such that dispersal, abiotic, and biotic factors 'filter' species into local communities from regional species pools based on their traits (Weiher & Keddy 1999). Many seed transfer zones are delineated by a few abiotic filters that influence seedling establishment, such as aridity and mean annual temperature. For example, seed transfer zones for native perennial grass species in the Great Basin are developed from these two factors, as seeds from warmer, arid populations show more drought-adapted traits (smaller in size, narrower leaves, and earlier phenology) compared to seeds from cooler, wetter populations (St. Clair et al. 2013). However, source populations that match the climatic conditions of the restoration site are not necessarily optimally adapted to the restoration site due to genetic drift, fluctuating selection, gene flow, or lack of variation for adaptive traits (e.g., Lenormand 2002; Kawecki & Ebert 2004; McKay et al. 2005). Further, biotic factors such as competition with invasive species impose additional filters to seeds that fall within the same seed transfer zone.

A trait-based approach to restoration has highlighted that trait expression, perhaps more than source provenance, is central to predict plant performance (e.g., growth, seedling establishment, survival; Funk et al. 2017). Previous studies in the Great Basin have identified that early emergence, longer root length, higher root allocation and seed mass are strong predictors of native perennial grass establishment and persistence in restoration settings (e.g., Atwater et al. 2015; Leger & Goergen 2017; Leger et al. 2019). These resource-conservative traits reflect a myriad of drought strategies to cope with low water availability common in semiarid environments (Funk 2013). For example, by emerging early, a plant could ‘escape’ dry periods by growing larger and having deeper roots before surface soil dries out (Leger et al. 2019). Deeper or longer roots enhance drought ‘avoidance’ by allowing plants continued access to water under drying conditions (Harrison & LaForgia 2019). Separately, low specific leaf area (SLA) and high leaf dry matter content (LDMC) promote drought ‘tolerance’ by limiting water loss and maintaining hydraulic conductance under extreme water deficit (e.g., Kursar et al. 2009). Further, larger seeds support seedling persistence under drought by providing resources and slowing metabolism (Lebrija-Trejos et al. 2016).

In addition to drought tolerance, resistance to invasive species is highly desirable in seeds used for restoration (Leger & Baughman 2015). Resistance to invasive annual grasses, particularly cheatgrass (*Bromus tectorum* L.), is important in the Great Basin Desert because the invasion of annual grasses are rapidly transforming native perennial plant communities to low diversity annual grasslands (Bradley et al. 2018). However, we lack tests of whether the drought-tolerant resource-conservative traits are predictive of seedling establishment rate under water stress and invasive species competition. Cheatgrass germinates early in the growing season after rain in fall or early spring, and grows fast-growing shallow roots for rapid resource uptake (Melgoza et al. 1990; Relchenberger & Pyke 1990). Native seedlings that germinate earlier and shift their morphological and physiological traits to more resource-acquisitive traits (coarser roots; higher SLA; taller) can establish in invaded sites by increasing resource competition with invasive species (e.g., van Kleumen et al. 2010; Funk 2013). Alternatively, native seedlings that germinate later and capitalize on their resource-conservative traits (finer roots; lower SLA; shorter) further differentiate their niches from invasive species to avoid competition (Gioria and

Osborne 2014). There is evidence that native perennial grasses in competition with *B. tectorum* put greater allocation in root biomass and increase proportion of fine roots (Rowe & Leger 2011; Phillips & Leger 2015; Foxx 2021), but these studies measured traits in one soil moisture condition. When competition effects interact with drought effects, seedling traits may have a trade-off between growing quickly but costly, and growing slowly but efficiently. Understanding the directionality of trait responses to multiple filters and their impact on seedling establishment is important for designing seed mixes for a variable environment.

Individual trait variation in response to environmental change, or phenotypic plasticity, if adaptive, could increase the success of establishment and persistence of an organism (Pigliucci 2001). Several studies have shown that environmental variation promotes higher plasticity (e.g., Baythavong 2011; Lazaro-Nogal et al. 2015; Vizcaino-Palomar et al. 2020). As such, plasticity related to drought tolerance, as well as resistance to invasive annual grasses, may enhance seedling establishment in the Great Basin. However, phenotypic plasticity may not always be advantageous. For example, high plasticity is sometimes associated with low fitness such as survival and reproduction (e.g., Kreyling et al. 2019). Individuals experience lower fitness either by expressing a suboptimal phenotype in a given environment or by expending extra energy to simply possess the ability to be plastic (Auld et al. 2010). As the Great Basin and other semiarid ecosystems are expected to increase in inter-annual precipitation variability (D'Odorico & Bhuttachan 2012), and intensification of drought events (Lloret et al. 2012), the question of whether plasticity in seedling traits is an adaptive plasticity or a growth-limiting plasticity is important to consider for seed selection that is adapted to future conditions.

Here, we conducted a greenhouse experiment with Sandberg bluegrass (*Poa secunda*), a primarily self-pollinating, early-seral native perennial grass that is widespread in the Great Basin.

*P. secunda* has shorter tillers, shallower roots, and earlier phenology than other native perennial bunchgrasses in the region, which makes it a particularly strong competitor against cheatgrass (Leger & Baughman 2015; Leger & Goergen 2017). Specifically, we looked to answer the following questions: 1) Does seed provenance influence seedling performance (i.e., emergence and growth) under variable water availability and *B. tectorum* competition? We expected that seeds from drier sites are stress tolerant and can tolerate water stress and cheatgrass competition better than those from wetter sites. 2) How does *B. tectorum* competition mediate the native grass trait response to water stress, and does that affect the trait-fitness relationships? *P. secunda* seedlings in competition with cheatgrass under drought could express either resource-acquisitive traits (lower proportion of fine roots; high SLA) to compete for resources or resource-conservative traits (higher proportion of fine roots; low SLA) to tolerate water stress. If there is a trade-off in traits, the directionality of trait-fitness relationship is contingent on the presence of cheatgrass. 3) Is there any evidence that the trait shifts represent adaptive plasticity? We expected trait plasticity to positively correlate with drought tolerance. Intraspecific variation of traits and their plasticity provide opportunities for restoration practitioners to select specific seeds suited to the conditions in the area to be restored.

## Methods

### *Experimental Design*

We collected Sandberg bluegrass (*Poa secunda*) seeds from five wild populations along an aridity gradient across the Great Basin from June to July 2019 (Figure 4.1). We extracted mean Aridity Index, calculated as mean annual precipitation over mean annual potential evapo-transpiration of 1950-2000, from Consortium for Spatial Information (CGIAR-CSI;

https://cgiarcsi.community/data/global-aridity-and-pet-database/?amp). We selected sites that were flat, at least 100 meters from roads, with well-drained soil, and elevation ranging from 1290-1670 m (Table 4.1). Dominant vegetation at these sites were sagebrush (*Artemisia tridentata* Nutt) and native perennial grass species such as squirreltail (*Elymus elymoides* Raf. Swezey) and Thurber's needle grass (*Stipa thurberiana* Piper). We collected cheatgrass (*Bromus tectorum*) seeds and soil (top 5-15 cm) in June 2019 from the Northern Great Basin Experimental Range in Riley, Oregon. We checked the viability of seed from different seed sources by conducting a germination trial in a growth chamber at the University of Oregon in February 2020. Our greenhouse experiment took place at the University of Oregon Greenhouse Facility in Eugene, Oregon from November 2020 to January 2021.

To evaluate the establishment rates of *P. secunda* seedlings in relation to seed provenance, water availability and *B. tectorum* competition, we implemented a fully-factorial randomized block design with source populations, competition (with and without cheatgrass), and watering (wet and dry) as main factors. Each combination of population, competition treatment, and watering treatment was replicated eight times (n = 160 pots; 5 populations x 2 competition treatments x 2 watering treatments x 8 blocks). We sowed 0.0125g of *P. secunda* seeds by each population ¼ inches deep in 7 cm x 7 cm x 7 cm pots 90% filled with mixed soil (3:1 ratio of field-collected soil:vermiculite). We chose the seeding density of *P. secunda* seeds (~5,100 seeds/m$^2$) based on the full broadcast rate recommended by the USDA Natural Resources Conservation Service (Majerus et al. 2009). For the competition treatments, we sowed 0.165g of *B. tectorum* seeds mixed together with *P. secunda* seeds in competition pots, and no *B. tectorum* seeds in non-competition pots. We chose the seeding density of *B. tectorum* seeds (~ 11,000 seeds/m$^2$) based on its mid-range field observations (Beckstead & Augspurger 2004). In competition pots, on average 27 *B. tectorum* seedlings and 6 *P. secunda* seedlings established per pot. In non-competition pots, on average 6 *P. secunda* seedlings established per pot. For the watering treatment, the “wet” pots were watered 160 ml twice a week, while the “dry” pots were watered 40 ml twice a week. We chose the amount of water applied based on the average monthly precipitation from November to April in 1981-2010 in Weed, CA (approximately 3 inches; U.S. Climate Data 2019) and Reno, NV (approximately 0.8 inches; U.S. Climate Data 2019). To have a consistent watering treatment but randomized micro-environment within the greenhouse, we labeled each tray containing 10 pots either “wet” or “dry,” and reshuffled them every Monday. Soil moisture levels of 10 pots were tracked weekly using a soil moisture probe (TERROS 10, METER Group, Inc). Temperature within the greenhouse was constantly set to 18.3 °C. Artificial lights were on daily from 6 am to 6 pm.

### *Trait Measurements*

We measured the following *P. secunda* aboveground traits following the trait handbook (Perez-Harguindeguy et al. 2013): emergence, average height (cm), average specific leaf area (cm/g), and average leaf dry matter content (g/g). Early emergence is a drought ‘avoidance’ strategy and a *B. tectorum* competition strategy. Greater height and specific leaf area and lower leaf dry matter content are associated with the resource-acquisitive strategy. We also measured the following belowground traits: total root length (cm), surface area (cm2), average root diameter (mm), number of root tips and forks, fine root length (cm), coarse root length (cm). Longer and finer roots with higher surface area and root tips and forks are associated with the resource-conservative strategy. We weighed five replicates of 10 *P. secunda* seeds per population to calculate the average seed mass (g) per seed. Seed mass data for the Reno population is not available because we used up all the seeds from that population before measuring the seed weight.

We tracked the emergence date (when the first *P. secunda* seedling germinates in each pot) from the first day of seeding until all the pots germinated. On week 8 following germination of *P. secunda*, we counted the number of *P. secunda* established seedlings to calculate establishment rates (number of established seedlings/number of seeds sown). In the same week, we measured all aboveground traits and harvested above- and belowground biomass of *P. secunda*. We clipped the aboveground biomass of each individual, oven-dried it at 60 °C for 72 hours, and weighed it at room temperature (20 °C). As for roots, we hand-washed them under running water over a 2 mm sieve, and stored them in 50% ethanol solution at 4 °C. We kept the *B. tectorum*'s shoots and root intact, which helped us separate long and fibrous *P. secunda* roots from short and coarse *B. tectorum* roots by hand. We picked out detritus from root samples with tweezers 5 minutes per sample, then scanned and analyzed them using WinRhizo software to measure belowground traits (Regent Instruments, Siante-Foy, Quebec, Canada). After scanning, we oven dried the roots at 60 °C for 72 hours and weighed them. Root biomass was used to calculate total biomass (g), specific root length (SRL; cm/g), and root mass ratio (RMR; g/g). Lower SRL and higher RMR are associated with the resource-conservative strategy. Long and thin roots (high SRL) are belowground equivalent of thin leaves, which are less expensive to produce than thick leaves (Withington et al. 2006).

### *Analyses*

All analyses were carried out in R 4.1.2 (R Core Team 2021). We used a p-value of 0.05 to determine significance.

To determine the effects of seed provenance, watering treatment, and *B. tectorum* competition treatment on the performance of *P. secunda*, we ran a three-way ANOVA on mean establishment rates and mean total biomass followed by a post-hoc Tukey test using the TukeyHSD function in the "multcomp" package (Hothorn et al. 2018). To assess the drought tolerance of each population, we analyzed the effect of watering treatment on establishment rate of *P. secunda* with a linear mixed model with population and watering treatment as fixed effects and block as a random effect using the lme function in the "nlme" package (Pinheiro et al. 2019). We did this analysis separately for each *B. tectorum* competition treatment. We checked the assumptions for the linear mixed model that the residuals and random effect are approximately normally distributed by graphically comparing the residuals and random intercepts to the quantiles of the standard normal distribution.

To characterize the seedling phenotypic trait responses to water stress and *B. tectorum* competition, trait values were standardized to z-scores and summarized via Principal Component Analysis (PCA) using the "vegan" package (Oksanen et al. 2017). We tested for significant differences among watering and competition treatments with PERMANOVA using the adonis function in "vegan." We analyzed dispersion (distance around centroids) of traits with the multivariate_difference function in the "codyn" package (Avolio et al. 2019) to determine how individuals within populations varied in response to each treatment. The data met the only assumption for PERMANOVA, which is that objects in the data are exchangeable and independent.

To determine the relationships between seedling phenotypic traits and establishment rates depending on the presence or absence of *B. tectorum*, standardized trait values in z-scores and establishment rates were analyzed with linear regression models separately for each *B. tectorum* competition treatments. To quantify phenotypic trait plasticity, we calculated the relative change in mean trait values between wet and dry conditions for each population and competition treatment:

$$Trait\ plasticity = \frac{trait_{dry} - trait_{wet}}{trait_{wet}}$$

We analyzed the effect of trait plasticity on drought tolerance (the difference in mean establishment rates between wet and dry conditions) with linear regression models. $R^2$ values are used to assess relative model fit. Because some traits were collinear, we analyzed each trait separately. We checked for the linear relationships and homoscedasticity with scatter plots, and the normal assumption using quantile-quantile plots.

## Results

### *Seedling performance response*

Seed provenance had a significant effect on mean seedling establishment rate of *P. secunda* ($F_{4, 155} = 5.77$, $p < 0.001$). In particular, seeds from Butte Valley, CA the wettest site, had the highest mean establishment rate compared to other populations (Figure 4.2a). Seed weights of *P. secunda* differed among populations ($F_{9, 20} = 23.01$, $p < 0.001$). Seed mass was significantly positively correlated with seedling establishment rate ($R^2 = 6.02e\text{-}5$, $p < 0.001$) but not biomass ($R^2 = 0.32$, $p = 0.32$; Figure S4.1). Seeds from Butte Valley were significantly larger than those from other sites. Reduced watering treatment significantly decreased mean seedling establishment rate ($F_{1, 158} = 34.95$, $p < 0.001$). However, *B. tectorum* competition treatment ($F_{1, 158} = 0.11$, $p = 0.73$), the two-way interaction of water and competition ($F_{1, 158} = 2.82$, $p = 0.09$), or the three-way interaction of water, competition, and provenance ($F_{4, 155} = 0.744$, $p = 0.56$) did not affect mean seedling establishment rate. Without *B. tectorum* competition, *P. secunda* from Butte Valley, CA and EOARC (Eastern Oregon Agricultural Research Center in Burns, OR) tolerated reduced water availability, with no difference in response between the dry and wet treatments (Figure 4.2b). With *B. tectorum* competition, all populations except for Reno tolerated reduced water availability (Figure 4.2c).

Seed provenance did not have a significant effect on mean seedling growth, or total biomass, of *P. secunda* ($F_{4, 155} = 0.434$, $p = 0.78$; Figure 4.2d). Both the reduced watering treatment ($F_{1, 158} = 74.93$, $p < 0.001$) and the *B. tectorum* competition treatment ($F_{1, 158} = 315.55$, $p < 0.001$) significantly decreased seedling growth, and the two-way interaction of water and competition had a significant interactive effect on seedling growth ($F_{1, 158} = 63.97$, $p < 0.001$). The three-way interaction of water, competition, and provenance ($F_{4, 155} = 0.75$, $p = 0.55$) did not affect mean seedling establishment rate. Without *B. tectorum* competition, all populations decreased in seedling growth under water stress (Figure 4.2e). The overall seedling growth dramatically decreased to less than 5% of its biomass in response to *B. tectorum* competition. Only seeds from Reno had a significant difference in seedling growth between wet and dry treatment within the *B. tectorum* competition treatment (Figure 4.2f).

### *Phenotypic trait response*

Watering and *B. tectorum* treatments had a significant effect ($p = 0.001$) on *P. secunda* seedling traits. In particular, the multi-dimensional trait response between the no competition-wet treatment and the competition-dry treatment was significantly different from each other ($p = 0.001$). The first axis of PCA, which explains 43% of the variation in seedling traits, represented shifts in phenology, height, and root growth. The second axis of PCA, which explains 14% of the variation in seedling traits, represented shifts in leaf economics and proportion of fine roots. Seedling traits shifted along the first axis of PCA in response to *B. tectorum* competition (Figure 4.3a). Specifically, *P. secunda* seedlings emerged later, grew shorter in height, shorter roots, and fewer root tips in response to *B. tectorum* competition (Figure S4.2). In contrast, seedling traits shifted along the second axis of PCA in response to watering treatment (Figure 4.3a). The directionality of these trait shifts depended on the presence of *B. tectorum* (Figure 4.3b). Specifically, the relationship between proportion of fine roots and fitness (establishment rate/biomass) was positive with cheatgrass competition, but negative without cheatgrass competition (Figure 4.4; Figure S4.3). Dispersion of traits around the centroid of each treatment was greatest in the competition-dry treatment and lowest in the no competition-wet treatment (Figure 4.3c).

The following traits were predictive of greater seedling establishment rate of *P. secunda* regardless of *B. tectorum* competition: early emergence, longer root length, higher root mass ratio, lower specific root length, and more root tips (Figure 4.4). Without *B. tectorum* competition, lower proportion of fine roots was associated with higher seedling establishment rate (Figure 4.4). With *B. tectorum* competition, in contrast, a higher proportion of fine roots and lower LDMC were associated with higher seedling establishment rate (Figure 4.4). The plasticity of seedling emergence (t = -2.48, p = 0.03) and root length (t = -2.50, p = 0.03) were negatively correlated with tolerance to reduced water availability (Figure 4.5).

## Discussion

Determining how trait-fitness relationships shift with environmental and competitive conditions is a fundamental ecology question that is increasingly relevant for restoration in face of climate change (Laughlin et al. 2017), invasion (Leger 2008), and altered disturbance regimes (Gómez-

González et al. 2011). When selecting the appropriate seeds for native plant restoration in semiarid systems, understanding how seed provenance and traits predict seedling performance in face of environmental change - such as drought and annual grass invasion - can inform restoration decisions (Funk et al. 2008; Laughlin 2014). We expected that seeds from drier sites would tolerate stress better than those from wetter sites, but we observed that seeds from wetter sites tolerated water stress better, especially under cheatgrass competition, compared to seeds from drier sites. Without cheatgrass competition, *P. secunda* seedling traits under water stress expressed resource-conservative traits, such as lower specific leaf area (SLA) and higher proportion of fine roots, whereas the relationship shifted such that with cheatgrass competition *P. secunda* exhibited more resource-acquisitive traits under drought. In return, the predictive relationship between proportion of fine roots and seedling performance depended on the context of cheatgrass competition. As trait-based approaches are increasingly used for restoration (Leger et al. 2021; Funk 2021), our results highlight the importance of understanding the effect of interacting environmental filters on intraspecific variability in traits and seedling performance.

Selecting seed provenance that yields high plant performance is one of the first steps in improving native plant restoration success (Baughman et al. 2022). Contrary to our expectation, *P. secunda* seeds from Butte Valley (the wettest site) had the highest mean establishment rate and total biomass (i.e., shoot and root growth) across watering and cheatgrass competition treatments. Furthermore, seedling drought tolerance, calculated by how much seedlings resisted change in establishment rate and biomass in response to low water availability, was lowest in Reno (the driest site). We were struck by this result because there is strong evidence of local adaptation in native plants across the Great Basin (Baughman et al. 2019). One possible explanation for our observation is that it reflected intraspecific variation in drought strategies:

seeds from mesic sites have higher emergence rate and greater biomass, but risk higher mortality in the dry season, while seeds from xeric sites have lower emergence rate and grow smaller, but survive better through the dry season. Alternatively, the "artificial" drought effect in the greenhouse deviated from the actual drought effect observed in the field (Marchin et al. 2020). In other words, we experimentally imposed water deficits by varying the frequency of water applied to potted plants in the greenhouse, but low evapotranspiration in a highly controlled environment in the greenhouse may have preferred germination and growth of seeds from wetter sites.

Interspecific competition with cheatgrass mediated the seedling trait response of *P. secunda* to water stress. *P. secunda* seedlings with cheatgrass competition grew more resource-conservative leaf traits - lower specific leaf area (SLA) and higher leaf dry matter content (LDMC) - compared to *P. secunda* seedlings without cheatgrass competition. These leaf trait responses suggest that *P. secunda* seedlings in competition with cheatgrass were increasing water use efficiency by growing thicker leaves, which are also beneficial for drought tolerance (e.g., Kursar et al. 2009; Vitra et al 2019; Wilcox et al 2021). On the other hand, *P. secunda* seedlings growing without cheatgrass competition had more resource-acquisitive root traits. Specifically, the proportion of fine roots in *P. secunda* without cheatgrass competition increased in response to dry conditions, but decreased with cheatgrass competition. This result was unexpected because studies on *P. secunda* and other native perennial grasses have shown that cheatgrass competition induces greater allocation to root mass and fine roots (Phillips & Leger 2015; Foxx 2021). The trade-off in seedling leaf and root traits makes seed selection for native grass restoration in cheatgrass dominated areas complicated as traits selected for drought tolerance may conflict with traits that resist cheatgrass.

As a consequence of the trade-off in traits, the trait-fitness relationships varied by water availability and cheatgrass competition. In particular, the directionality of the relationship between proportion of fine roots and fitness (establishment rate/biomass) depended whether *P. secunda* seedlings were grown with cheatgrass. Proportion of fine roots, which is important for nutrient and water uptake, is a sensitive predictor of changing environments in trees (Ostonen et al. 2007; McCormack & Guo 2014), forbs (Bowsher et al. 2016), and grasses (Larson et al. 2020). Meanwhile, other traits such as early emergence, longer root length, higher root mass ratio, and more root tips were consistent predictors of increasing establishment rate and biomass regardless of water stress or cheatgrass presence. Our results were consistent with the previous work on seedling traits of native grasses in the Great Basin (e.g., Larson et al. 2015; Leger et al. 2019; Leger 2021). Seed mass is a trait that is known to be influenced by maternal growing environment (Roach & Wulff 1987). Larger-seed sources are linked with higher seedling survival and biomass (Leger et al. 2019; Leger et al. 2021). In our study, seed mass had a significant positive effect on emergence rate but not on biomass. The effects of seed mass on seedling performance could be due to more indirect effects on other phenotypic traits, such as emergence timing, than direct effects on plant fitness (Atwater et al. 2015). While links between traits and seedling establishment have been developed, efforts to understand the predictability of seedling establishment success from traits have been limited to plastic responses to one variable environmental factor: drought (Comas et al. 2013; Harrison & LaForgia 2019; Larson et al. 2020) or cheatgrass (Phillips & Leger 2015; Leger Goergen 2017), but not both. Our work demonstrates that abiotic and biotic filters interact to influence trait-fitness relationships, which should be accounted for in the trait screening process for restoration. As a recent review on trait-based framework has highlighted (Funk 2021), incorporating interacting filters into restoration design is important to establish restored communities that are resilient to climate change, invasive species, and altered disturbance regimes.

While trait plasticity could be an adaptive response to environmental change, we found no evidence that the phenotypic trait shifts in *P. secunda* in response to water stress and cheatgrass competition represent adaptive plasticity. We expected a positive correlation between the magnitude of trait shifts and drought tolerance of *P. secunda*, such that more plastic populations are able to maintain or resist change in establishment rates despite fluctuating water availability. For example, in the central Chilean Desert, higher levels of trait plasticity, especially root:shoot ratios, of desert shrubs positively correlated with plant performance, suggesting adaptive plasticity (Lazaro-Nogal et al. 2015; Carvajal et al. 2017). However, we found that emergence and root length trait plasticity negatively correlated with drought tolerance, and the rest of the traits' plasticity did not correlate with drought tolerance. Our results support growing evidence that much phenotypic plasticity might be neutral or non-adaptive (Caruso et al. 2006; Ghalambor et al. 2015; Acasuso-Rivero et al. 2019), despite the widespread expectation that phenotypic plasticity should be adaptive (Schlichting 1986; Chevin et al. 2010; Valladares et al. 2014). Seeds from EOARC showed the least trait plasticity and the highest drought tolerance regardless of cheatgrass competition compared to other populations. This suggests that seeds with less non-adaptive plasticity are perhaps less vulnerable to environmental fluctuations. More studies are needed that test the adaptive nature of phenotypic plasticity that could be directly applied to seed selection for native plant restoration.

We used wild-collected *P. secunda* seeds from areas with low density of cheatgrass for this study, but the natural selection of native perennial grasses by cheatgrass is evident in other studies (Phillips & Leger 2015; Leger & Goergen 2017). *P. secunda* would likely express more resource-acquisitive traits and less trait plasticity if we had used seeds in sites heavily invaded by cheatgrass. Furthermore, we focused on the seedling establishment stage, due to its importance on plant regeneration (Larson et al. 2015), but survival and seed production are clearly important for restoration success. An experiment that combines seeding and transplanting juvenile and adult plants from the same populations could allow for identifying traits that trade-off with fitness at different life-stages and identifying populations that perform optimally across life-stages.

Successful seedling establishment of native plants is increasingly desired in global grasslands, shrublands, and savannahs. In post-fire restoration in the Great Basin, a careful selection of seeds is detrimental to the success of seedling establishment (Leger et al. 2019; Leger et al. 2021). To meet the dual challenges of water limitation and invasive species competition, we should consider not only the suitable seed provenance and traits but the direction and magnitude of trait shifts in response to environmental change. Understanding the interactive effects of environmental filters (Funk 2021) on plant performance, traits, and their plasticity advances the trait-based approach to seed selection for native plant restoration.

## Figures

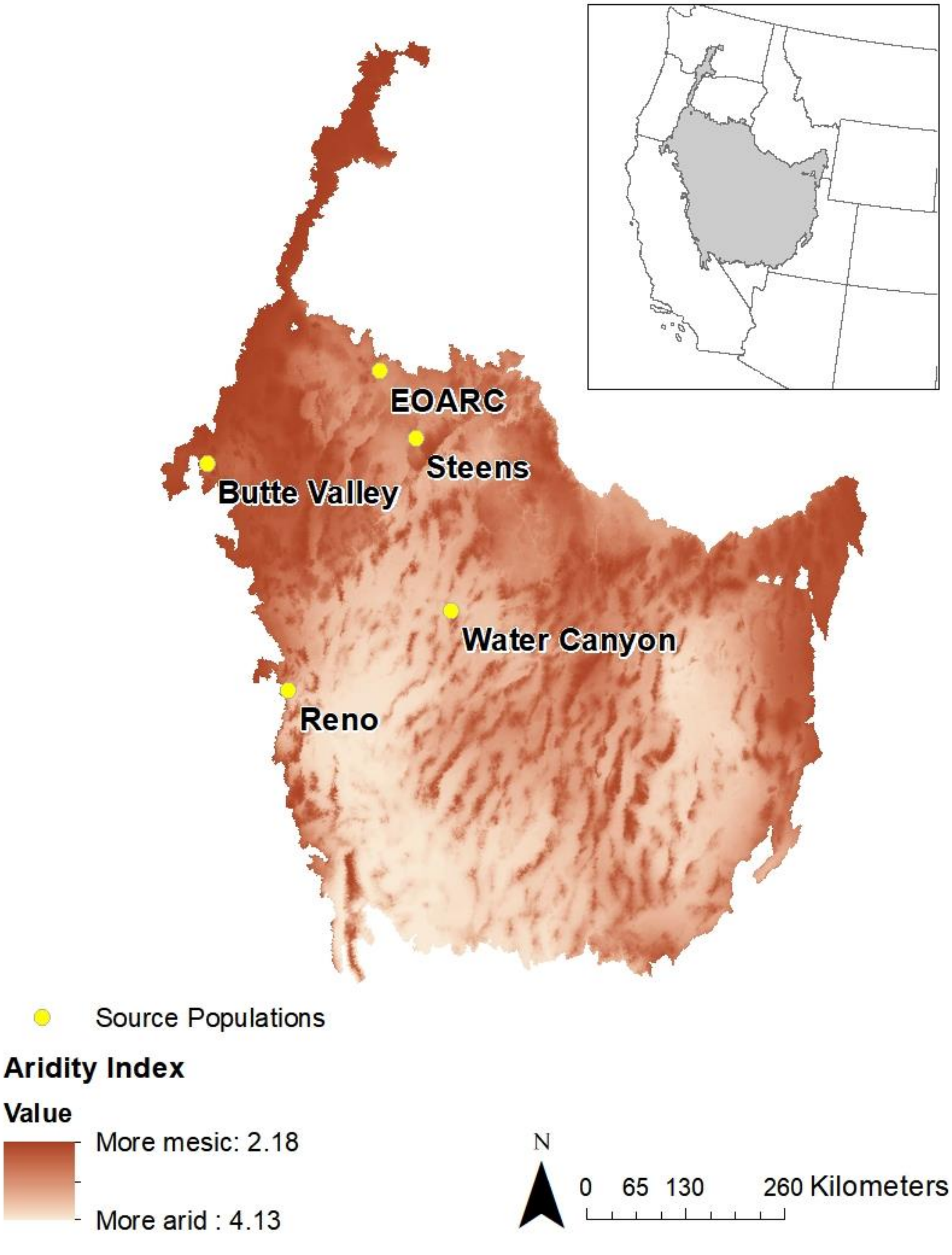

**Figure 4.1.** Source populations of wild-collected *Poa secunda* seeds across the Great Basin. An Aridity Index, calculated as mean annual precipitation over mean annual potential evapo-transpiration, is an indicator of dryness at a given location. Higher values of the Aridity Index indicate less aridity (more mesic). Wettest to driest populations: Butte Valley (0.23), EOARC (0.18), Steens (0.16), Water Canyon (0.14), Reno (0.13). Source Data: Global mean Aridity Index of 1950-2000 extracted from CGIAR-CSI (https://cgiarcsi.community/data/global-aridity-and-pet-database/?amp).

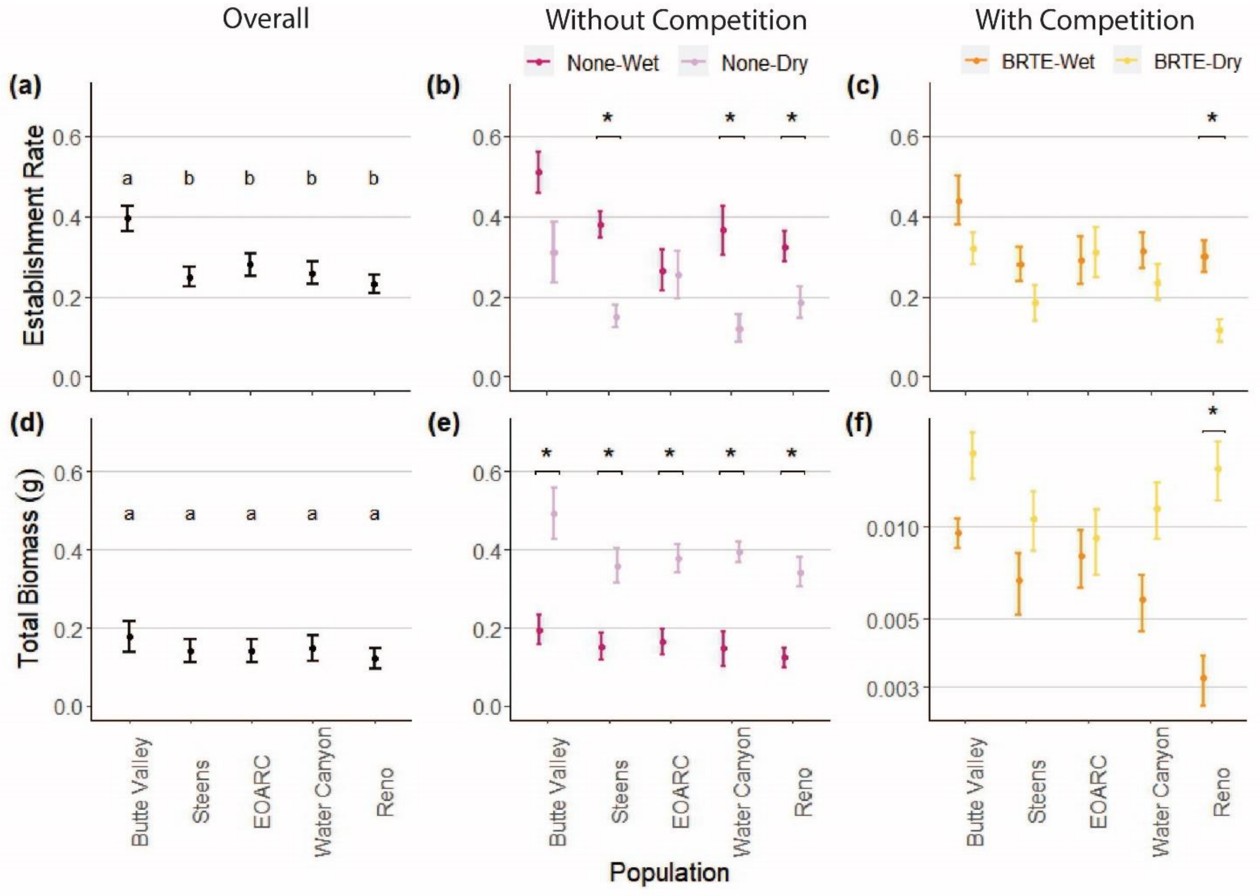

**Figure 4.2.** Intraspecific variation in *Poa secunda* seedling establishment rate and total biomass (grams) response to water stress and *Bromus tectorum* competition. Overall mean establishment rates (a) and total biomass (d) of *P. secunda* at week 8 since germination by populations. Letters above the box plots denote significant differences ($p < 0.05$) among populations. Fitness response to water stress (b, e) without *B. tectorum* competition and (c, f) with *B. tectorum* competition. Asterisks in these plots denote significant differences ($p < 0.05$) between wet and dry conditions. Points represent means, and error bars represent ± 1 SE.

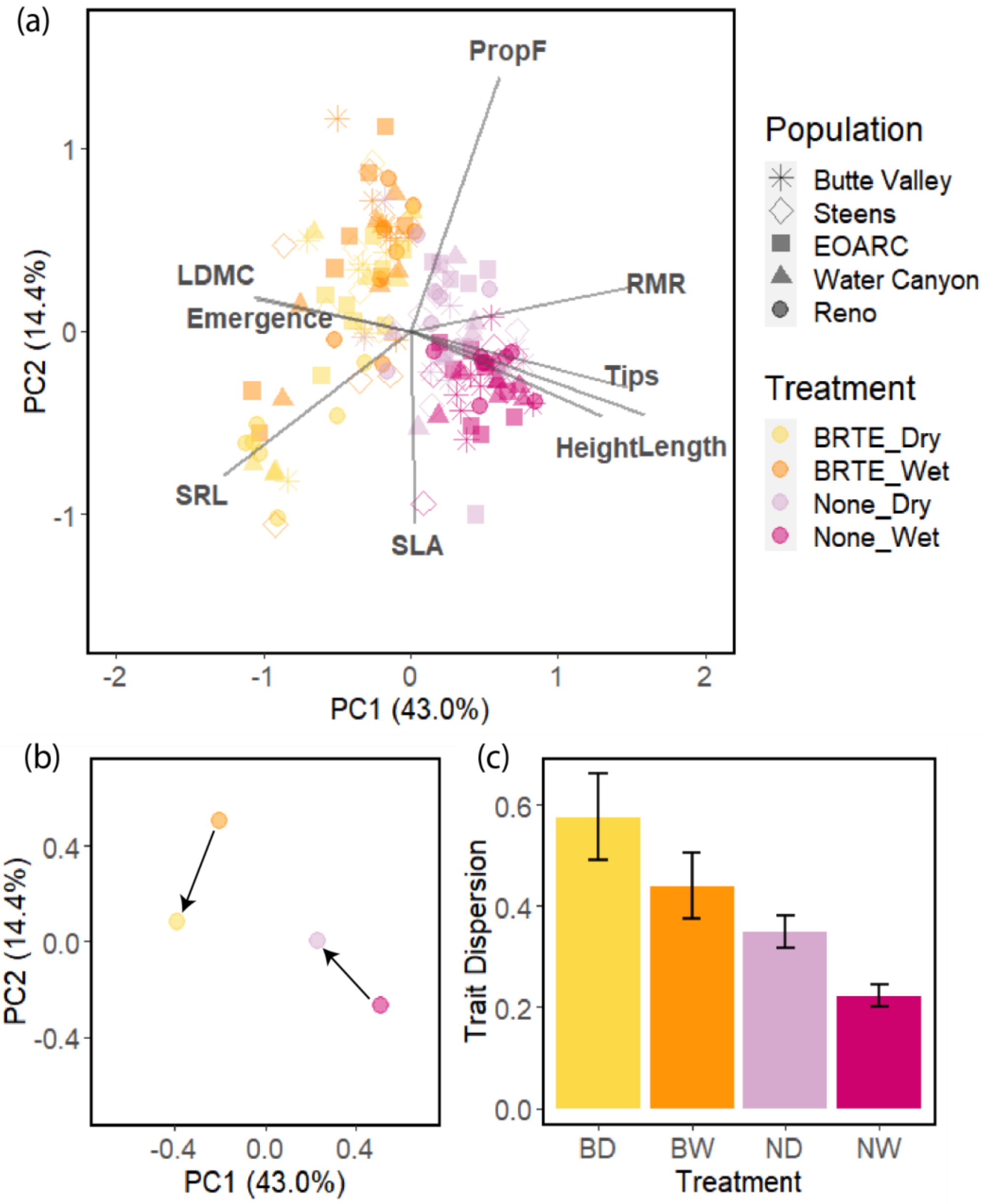

**Figure 4.3.** *Poa secunda* seedling traits shifted with water stress and *Bromus tectorum* competition. a) Principal Component Analysis (PCA) of *P. secunda* seedling traits colored by treatment and shaped by population. Percentages inside the parentheses are the proportion of variation explained by the principal component axis. Abbreviations of traits: Proportion of fine roots (PropF), root mass ratio (RMR), number of root tips (Tips), total root length (Length), tiller height (Height), specific leaf area (SLA), specific root length (SRL), timing of emergence (Emergence), and leaf dry matter content (LDMC). b) Centroid of each treatment. Arrows indicate the direction in which the mean traits shifted in response to wet and try treatments. c) Dispersion around the centroid for each treatment: with cheatgrass - dry (BD), with cheatgrass - wet (BW), no cheatgrass - dry (ND), and no cheatgrass - wet (NW). Boxes represent means, and error bars represent 1 ± SE.

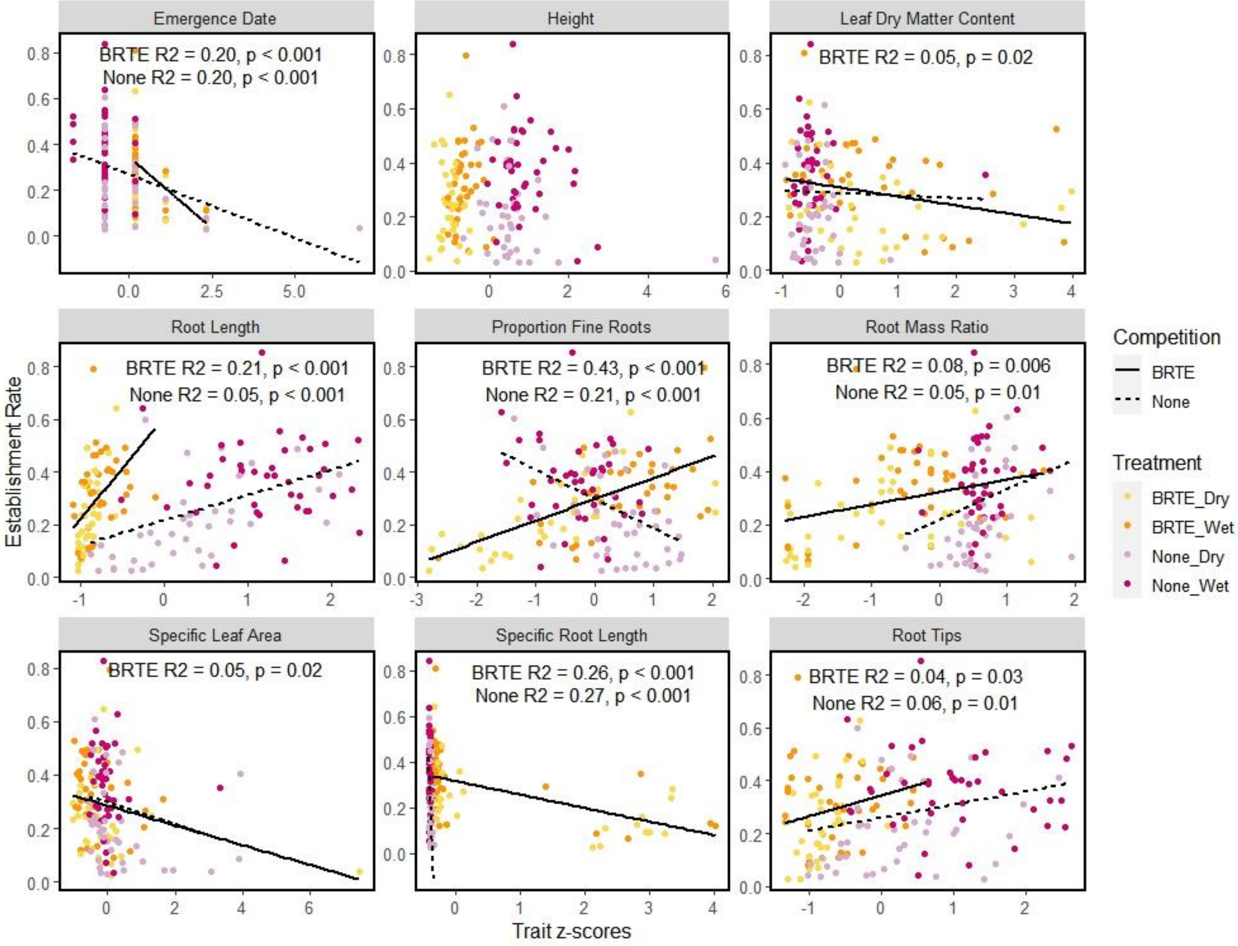

**Figure 4.4.** *Poa secunda*'s seedling establishment rate depends jointly on water availability and *Bromus tectorum* competition. Standardized trait values (z-scores) represented are the following: emergence date, height, leaf dry matter content, root length, proportion of fine roots, root mass ratio, specific leaf area, specific root length, and number of root tips. Colors represent watering and competition treatments: No cheatgrass - wet (dark pink), No cheatgrass - dry (light pink), With cheatgrass - wet (dark orange), and With cheatgrass - dry (light orange). Solid lines represent the regression lines in the competition treatment and dashed lines represent the regression lines in the no-competition treatment.

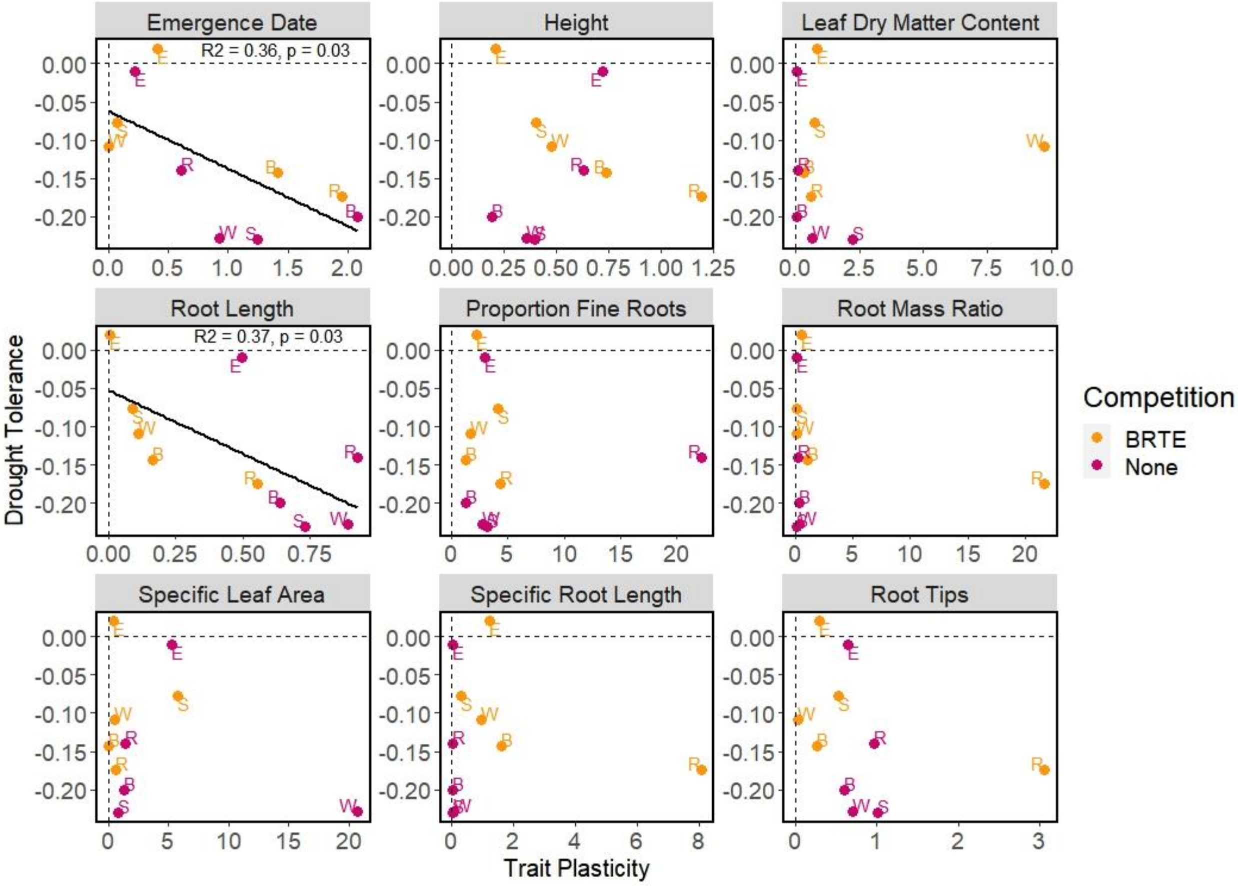

**Figure 4.5.** *Poa secunda*'s plasticity of seedling emergence and root length negatively correlated with drought tolerance. Relative change in trait z-scores from wet to dry conditions represents phenotypic trait plasticity. The difference in mean establishment rates from wet to dry conditions represents drought tolerance (below zero values mean less tolerant). Each point is colored by competition treatments (orange = with cheatgrass; pink = no cheatgrass), and labeled by population (B = Butte Valley, E = EOARC, R = Reno, S = Steens, W = Water Canyon).

## Table

**Table 4.1.** Seed source of *Poa secunda* and site characteristics including elevation, soil type, mean annual precipitation, mean max air temperature, mean minimum air temperature, mean maximum vapor pressure deficit, and mean minimum vapor pressure deficit. Soil data is obtained from USDA Web of Soil (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx). Climate data is the 30 year normals (1981-2010) obtained from PRISM (https://prism.oregonstate.edu/).

| **Site** | **Region** | **Coordinates (Lat, Long)** | **Elevation (m)** | **Soil type** | **Mean Annual PPT (mm)** | **Mean Max Temp (ºC)** | **Mean Min Temp (ºC)** | **Mean Max VPD (kPa)** | **Mean Min VPD (kPa)** |
|---|---|---|---|---|---|---|---|---|---|
| Butte Valley National Grassland | Siskiyou Co., CA | 41.897372785,<br>-121.996198989 | 1293 | Inlow-Ocho-Modoc complex, silt loam | 340 | 28.79 | -5.56 | 31.38 | 0.20 |
| Steens Mountains | Harney Co., OR | 42.811151722,<br>-118.865629523 | 1313 | Robson-Fourwheel complex, very cobbly clay loam | 380 | 28.80 | -5.66 | 28.80 | 0.73 |
| Eastern Oregon Agricultural Research Center | Harney Co., OR | 43.472769957,<br>-119.693315061 | 1395 | Gradon gravelly fine sandy loam | 263 | 29.08 | -7.52 | 35.04 | 0.36 |
| Water Canyon Recreation Area | Humboldt Co., NV | 40.928466666,<br>-117.671302816 | 1672 | Bliss-Chiara association, very fine sandy loam | 286 | 32.08 | -6.05 | 42.74 | 0.82 |
| Reno | Washoe Co., NV | 39.572570923,<br>-119.850898985 | 1622 | Zephan-Rock outcrop-Smallcone complex, very gravelly sandy loam | 285 | 31.13 | -4.61 | 39.88 | 1.11 |

# CHAPTER V

# FUNCTIONAL DIVERSITY BUFFERS BIOMASS PRODUCTION ACROSS VARIABLE RAINFALL CONDITIONS THROUGH DIFFERENT PROCESSES ABOVE- VERSUS BELOWGROUND

From Aoyama, L., E. A. Shaw, C. T. White, K. N. Suding, and L. M. Hallett. 2023. Functional diversity buffers biomass production across variable rainfall conditions through different processes above- vs belowground. *Functional Ecology* 37(9):2371-2385.

## Contributions

KNS & LMH conceived the ideas and designed the experiment; LA, EAS, CTW, & LMH collected the data; LA & EAS analyzed the data; LA & EAS led the writing of the manuscript with contributions from KNS and LMH.

## Introduction

Understanding plant community responses to changing environmental conditions is key to predicting ecosystem function provisions in the face of global change (Lavorel & Garnier, 2002). Trait-based approach is a tractable way to predict ecosystem functions from community-wide functional trait values that is generalizable across systems (Díaz et al., 2013; Funk et al., 2017). A developing consensus is that functional diversity, defined as the diversity of functional trait values within a community, promotes ecosystem functioning (Gagic et al., 2015; Tilman et al., 1997). While aboveground plant trait research has been successful in predicting plant functions (Lavorel & Garnier, 2002) and identifying patterns of aboveground diversity (Shipley et al., 2006), belowground trait research lags behind (but see Klumpp and Soussana, 2009; Picon-Cochard et al., 2021). The key challenge is applying this trait-based approach to understand how plant community change influences ecosystem functions, such as above-and belowground biomass production, under changing environmental conditions.

Water availability is a strong driver of plant productivity in a number of systems (e.g., Huxman et al., 2004; Wilcox et al., 2017), and responses of plant communities to changing rainfall patterns are mediated by species composition and functional diversity (Cleland et al., 2013). For example, in semi-arid grasslands, drought years can negatively affect dominant grasses, while less productive subordinate groups, particularly forbs, do relatively well due to decreased competition (Dudney et al., 2017; Kardol et al., 2010; Mariotte et al., 2013). These rainfall-driven fluctuations are particularly pronounced in annual grasslands, where species turnover happens on an annual basis. In California's annual grasslands, the intra-annual rainfall is characterized by the Mediterranean climate, where the growing season commences with a cool rain in the fall, and ends as it gets warmer and drier into the summer. In such systems, plant community dynamics are mediated by plant germination (Keeler-wolf et al., 2007; Pitt & Heady, 1979). For example, in fall drought, germinated grasses likely die, while forbs and legumes dominate from surviving the drought or germinating late (Bartolome et al., 2007; Keeler-wolf et al., 2007; Young & Evans, 1989). By contrast, wet fall and spring drought would favor annual grasses that grow tall earlier in the season and outcompete forbs for light (Pitt & Heady, 1979). These drought-related shifts in species composition affect functional trait diversity, which indirectly affects the plant communities' biomass production (Hallett et al., 2017; Shaw et al., 2022).

Two main mechanisms explain the relationship between functional diversity and ecosystem functioning: the mass ratio hypothesis and niche complementarity. The mass ratio hypothesis reflects that dominant species with particular trait values drive biomass production of the community (Grime, 1998), while niche complementarity emphasizes that greater functional diversity creates the potential for resource partitioning among species and therefore more complete resource utilization at the community level (Griffin et al., 2009; Tilman et al., 1997). These two hypotheses do not contradict each other as they address different types of functional diversity: the mass-ratio hypothesis is about the central tendency of a trait distribution, and the niche complementarity is about its breath. A correlation between biomass and the average trait value in a community suggests that dominant species drive ecosystem function (mass-ratio hypothesis; Garnier et al., 2004). Alternatively, if biomass increases with greater trait evenness or dispersion in a community, this suggests that niche differences (i.e., interspecific resource use) lead to more complete resource utilization at the community level (niche complementarity; Rao, 1982). Which of these mechanisms operate above- and belowground in variable rainfall conditions remains unknown.

Biomass production is a product of an ecosystem's functional diversity and the resource capturing strategies of all species in the plant community. Differences in functional traits between dominant and subordinate groups allow for divergent resource capturing strategies. While the traits of dominant species often reflect a strategy of rapid resource acquisition, those of subordinate species are associated with resource conservation (Díaz et al., 2013; Grime, 1998; Mariotte et al., 2013). For example, dominant species are generally taller and more efficient at light and carbon capture (i.e., high specific leaf area, SLA) than subordinate species (Mariotte et al., 2013). As such, these aboveground traits that reflect resource acquisition strategies are consistent predictors of aboveground biomass (e.g., Butterfield & Suding, 2013; Cheng et al., 2021; Finegan et al., 2015). Drought can either shift the community-level trait means to more resource conservative spectrum (e.g., lower SLA) by selection (Miller et al., 2019) or have no effect on functional diversity (Copeland et al., 2016). Furthermore, the presence of unique species, such as invasive species with strong competitive effects, could also disproportionately affect aboveground biomass.

A stronger emphasis on belowground traits is needed in trait-based ecology to better predict future changes in ecosystem functioning (Bardgett et al., 2014; Laliberté, 2017). This is particularly important in grasslands where plant species allocate significant fractions of biomass belowground (Poorter et al., 2012). Roots play a key role in reducing soil erosion by physically and chemically stabilizing the soil (Gyssels et al., 2005), contributing to nutrient cycling in the rhizosphere (Rengel & Marschner, 2005), and providing carbon sequestration in the soil (Balesdent & Balabane, 1996; Miller & Jastrow, 1990). As a result, belowground traits reflect a wide range of strategies that plants have evolved to capture resources and to respond to changes in their availability. For example, plant species from the resource-acquisitive spectrum are hypothesized to exhibit low root tissue density (RTD), high specific root length (SRL), high nitrogen content, and high root respiration, and *vice versa* for the resource-conservative spectrum (Roumet et al., 2006). Because root traits are multidimensional, some root traits respond to resource variation independently (Kramer-Walter et al., 2016). For example, RTD shifts toward more resource-conservative spectrum (higher RTD) in response to aridity, while SRL responds to aridity idiosyncratically (Butterfield et al., 2017). The existence of contrasting resource strategies among co-occurring species suggest that greater functional diversity could potentially buffer the effect of resource variation, such as seasonal droughts.

Here, we asked the following questions: 1) Does functional diversity beget biomass?; and 2) Does functional diversity buffer biomass under seasonal drought? We empirically explored these questions using a fully crossed experiment that imposed drought timing (fall dry, spring dry, consistent dry, and ambient rainfall) treatments across community composition treatments (grass-dominated, forb-dominated, and mixed grass-forb) over one growing season in a California annual grassland. First, we hypothesized that functionally diverse communities (i.e., mixed grass-forb) have higher biomass than grass- or forb-dominated communities across rainfall manipulations. We expected grass-dominated communities to be negatively affected by fall drought due to growth inhibition, and forb-dominated communities to be negatively affected by spring drought due to competition from grasses. Second, we hypothesized that biomass correlates with the average trait value of a community (mass-ratio hypothesis) but also increases with the breath of functional diversity (niche complementarity). Lastly, we hypothesized that functionally diverse communities are more robust to seasonal drought. We expected the slope of the functional diversity-biomass relationship to increase and the intercept to decrease in drought compared to control (ambient) rainfall condition. We tested these hypotheses to better predict the total biomass change under variable rainfall conditions.

## Methods

### *Study Site*

This study was conducted in the University of California Sierra Foothills Research and Extension Center (SFREC), which is located north of Sacramento in Browns Valley, California (39º15’ N, 121º17’ W). The site is characterized by a Mediterranean climate with cool, wet winters and hot, dry summers. Average annual rainfall is 1036 mm (PRISM Climate Group). The first germinating rain in this region usually falls in October, rapid spring growth commences with warming conditions in late winter or early spring, and biomass peaks in May (George et al., 2001). The elevation of the site ranges from less than 100 m to 600 m. Soils at the field site are derived from Mesozoic and Franciscan volcanic rock and classified as xeric inceptisols and alfisols in the Auburn-Sobrante complex (Beaudette & O'Geen, 2009). Soils are silty loams, are well drained, and are relatively shallow (~50 cm; Ryals et al., 2014). Vegetation of the site is valley grassland, dominated by slender ripgut brome (*Bromus diandrus*), wild oats (*Avena barbata*), soft chess (*Bromus hordeaceus*), broadleaf filaree (*Erodium botrys*), burclover (*Medicago polymorpha*) and clovers (*Trifolium* spp.).

### *Experimental Design*

In October 2014, we set up an experiment that manipulated the quantity and timing of rain and the plant community composition (Hallett et al., 2019; Shaw et al., 2022). We did not need permits for fieldwork. Water year 2015 (October 2014 to May 2015) was the final year of a 6-year drought, among the worst on state record since record keeping began in 1895 in California (California Department of Water Resources 2017). In a random-block design, plant community composition treatments were nested within rainfall treatment plots in 4 blocks (Fig. S5.1). Rainfall treatments consisted of control (ambient rainfall), consistent dry (50% of rain blocked from October – May), fall dry (50% of rain blocked from October - January), and spring dry (50% of rain blocked from February-May). A 50% rain reduction represents roughly a one in ten year drought. Rainfall treatments were effective in their respective windows (e.g., fall dry and consistent dry lowered volumetric soil moisture in the fall), although duration of the drought effect in fall was shorter compared to that in spring due to a late start to the season (Fig. S5.2). Within rainfall treatments, three 1 x 2 m community composition subplots were established for a total of 48 subplots: 4 rainfall plots x 3 composition subplots x 4 blocks. Community composition treatments were two single functional group treatments (only annual grasses or only forbs) and a mixture of both functional groups. Prior to seeding the composition treatments, we removed litter, and applied post-emergence herbicide during a sunny period when seedlings were around 1 inch tall. We used Poast herbicide (BASF Ag Products) to remove grass seedlings in the only forb plots, and 2,4D herbicide (Dow Chemical) to remove forb seedlings in the only annual grass plots. No herbicide was applied in the mixed plots. We followed the herbicide application with hand weeding of legumes in all three composition treatments. We seeded 4 g/m$^2$ of *Erodium botrys* in the forb only plots, 4 g/m$^2$ of *Bromus hordeaceus*, *Lolium multiflorum*, and *Avena barbata* in the grass only plots, and nothing in the mixed plots. We seeded these species because they are the most dominant forb and grass species at the field site. We did not think the difference in number of species sown would disproportionately increase functional trait diversity in the grass plots, because forbs from the seedbank emerged after seeding, and functional diversity in this system is largely influenced by forb abundance (Hallett et al. 2017). Because *E. botrys* densities were variable across blocks, we transplanted individuals into the plots to reach a density of at least 10 individuals/m$^2$ in the forb and mixture plots. Apart from *E. botrys* transplants, the mixed functional group treatment was simply what emerged from the existing seed bank.

### *Species composition and biomass*

Following one growing season, peak species composition and biomass were collected in May 2015. A 1 m$^2$ quadrat was laid out within each subplot, and all plants present in the quadrat were identified to species and visual estimates of their percent cover were recorded. Additionally, a visual estimate of the percent cover of grass, forb, bare ground, and litter cover was also recorded. Aboveground net primary productivity (ANPP) was harvested by clipping plant biomass down to the soil surface from a 0.25 m x 0.25 m quadrat. Fresh biomass was placed in a drying oven at 60°C for 48 h. Samples were weighed after drying. Belowground net primary productivity (BNPP) was harvested by separating roots from a 5 cm diameter x 30 cm deep soil core in the same location as the ANPP clipping. Briefly, the core was divided into three 10 cm segments and roots were picked out of each segment with forceps in 10 minute intervals, for a total of 40 minutes per segment (Metcalfe et al., 2007). Roots were gently washed with tap water over a 2 mm sieve to remove any soil particles (Fisher Scientific No. 10), dried in a 60 °C oven for 48 h and then weighed. ANPP and BNPP data are presented as grams of dry biomass per $m^2$.

### *Plant traits*

For 16 out of 37 species present at our site, we used a trait database available from Butterfield & Suding (2013). We replicated their methods to collect traits on the remaining 21 species, with 5 species not included because they were rare members of the community or did not germinate (Table S5.1). Specifically, we collected plant traits from individuals grown in a greenhouse for one season (6 weeks after germination). We used the mean trait value of six individuals as the trait value for each species. The following aboveground traits were measured: plant height, specific leaf area (SLA), and leaf dry matter content (LDMC). Height was measured from the tip of the newest tiller to the bottom of the oldest tiller using a ruler. One leaf (second newest, mature leaf) per individual was cut, scanned, and weighed fresh for fresh leaf area and weight. Then, these leaves were dried in a 60 °C oven for 48 h and weighed to obtain dry leaf weight. Resource-acquisitive species are generally taller and have larger and fleshier leaves (i.e., high SLA; low LDMC) than resource-conservative species. These traits are consistent predictors of aboveground biomass (e.g., Butterfield & Suding, 2013; Cheng et al., 2021; Finegan et al., 2015).

The following belowground traits were measured: root tissue density, specific root length of coarse (> 2 mm diameter) and fine roots (≤ 2 mm diameter) separately, coarse root diameter, and proportion of fine roots. Roots were washed with tap water over 2 mm sieve, stored in 50% ethanol in a 4 °C refrigerator, then scanned and analyzed using WinRhizo (Regent Instruments, Siante-Foy, Quebec, Canada) to measure belowground traits. Resource-acquisitive species have finer roots with low root tissue density and high specific root length compared to resource-conservative species (Reich, 2014; Tjoelker et al., 2005; Weemstra et al., 2016). Specific root length is a trait that has been related to root's efficiency to water and nutrient acquisition, since it indicates the amount of root length achieved per unit root mass invested (Lambers et al., 2006; Ostonen et al., 2007). Root tissue density has been linked to drought tolerance in arid environments (Butterfield et al., 2017).

### *Functional diversity*

With species composition and trait data, we calculated three indices of functional diversity for each quadrat (n= 48): community-level weighted trait means (CWM), functional evenness, and functional dispersion. The CWM was calculated following Garnier et al. (2004):

$$CWM = \sum_{i=1}^{s} p_i t_i$$

where S is the number of species in a community, p is the relative abundance of species i multiplied by its functional group's biomass within the subplot, t is the species-specific trait value. To reduce the dimensionality of functional trait spaces, we performed separate principal component analyses (PCAs) of above- and belowground trait values for all species. We then treated the first two PCA axes of each as “trait axes” and extracted species’ PCA scores. Using these PCA scores as species “trait values,” we calculated CWMs of PC1 and PC2 scores as means of functional diversity.

We chose functional evenness and Rao’s Q to capture the breath of functional diversity. Functional evenness (FEve) was calculated following Villéger et al. (2008):

$$FEve = \frac{\sum_{l=1}^{S-1} \min\left(PEW_l, \frac{1}{S-1}\right) - \frac{1}{S-1}}{1 - \frac{1}{S-1}}$$

where S is the number of species in the community, *l* is the minimum number of links between species in a functional trait space, and $\mathrm{PEW}_l$ is the partial weighted evenness. $\mathrm{PEW}_l$ is the proportion of weighted evenness (Euclidean distance between species i and j divided by the sum of relative abundance of species i and j) to the sum of weighted evenness across all species in the community. FEve measures both the regularity of spacing between species along a functional trait gradient and evenness in the distribution of abundance across species.

Rao’s Q, or quadratic entropy ($\mathrm{FD_Q}$) was calculated as a measure of functional dispersion, following Botta-Dukát (2005):

$$FD_Q = \sum_{i=1}^{s-1} \sum_{j=i+1}^{s} d_{ij} p_i p_j$$

where dij is the difference between the i-th and j-th species (dij = dji and dii = 0), p is the relative abundance for each species, and $\mathrm{FD_Q}$ expresses the average difference between two randomly selected individuals with replacements. We calculated FEve and Rao’s Q using the *FD* package (Laliberté et al., 2014).

*Statistical analysis*

All statistical analyses were performed in R version 4.0.2 (R Development Core Team, 2020). To test community composition and rainfall treatment effects on biomass production, we built linear mixed effect models for ANPP and BNPP where composition and rainfall treatments and their interactions were fixed effects, and block was included as a random effect to control for site-specific effects. Further, to understand the effects of composition and rainfall treatments on functional diversity, we built linear mixed effect models for each functional diversity metric (CWMs, FEve, and Rao's Q) where we included composition and rainfall treatments as fixed effects and block as a random effect. We built all mixed effect models using the "lme" function in the *nlme* package (Pinheiro et al., 2020). We followed this with post-hoc pairwise comparisons of treatments using least-square means tests in the *emmeans* package with Tukey adjustments for multiple comparisons (Lenth, 2016). We accepted significance at p values less than 0.05.

To understand the mechanisms underlying the functional diversity-biomass relationships, we built generalized linear regression models of ANPP and BNPP following the procedure adapted from Díaz et al. (2007)(Díaz et al., 2007). Trait data was transformed to z-scores prior to analysis to meet the assumptions of normality. Step 1. We tested the effects of CWMs of PC1 and PC2 of above- and belowground traits on ANPP and BNPP, separately. The mass ratio hypothesis is supported if there is a significant (positive or negative) relationship between CWMs and biomass. Step 2. We then tested the effects of FEve and Rao's Q on ANPP and BNPP, separately. Niche complementarity is supported if there is a significant positive relationship between either FEve or Rao's Q and biomass. Step 3. We included the abundance of two unique invasive species, *Centaurea solstitialis* and *Taeniatherum caput-medusae*, as additional predictors because they have strong competitive effects on community composition

(Fig. S3). Steps 1-3 were completed sequentially, then in step 4 all significant terms from steps 1-3 were combined in a step-wise ascending procedure. The most parsimonious model was selected by using the Akaike criterion (Burnham & Anderson, 2004).

To assess how seasonal drought affects the functional diversity-biomass relationships, we fitted mixed effects models of total biomass (ANPP plus BNPP) with functional trait diversity (Rao’s Q and FEve), rainfall treatments, and their interactions as main effects and block as a random effect. We compared the difference in slope and intercept of these models against control (ambient) rainfall condition.

## Results

### *Biomass responses to composition and rainfall treatments*

Overall, there was a significant effect of community composition treatment on both above- and belowground biomass (Table S5.2). Specifically, ANPP was greater in mixed plots compared to forb plots ($p < 0.001$), but not grass plots ($p = 0.11$; Fig. 5.1a, Table S5.3). Additionally, BNPP was significantly greater in mixed plots compared to both forb ($p = 0.001$) and grass plots ($p = 0.02$; Fig. 5.1b, Table S5.3). Total biomass was significantly greater in mixed plots compared to both forb ($p < 0.001$; Fig. 5.1c) and grass plots ($p = 0.004$; Fig. 5.1c, Table S5.3). There was no main effect of rainfall treatment on either above- or belowground biomass and no interaction effect of community and rainfall treatments (Fig. 1d, e, f, Table S5.2).

### *Functional trait space*

For the PCA of aboveground functional traits, the cumulative variance explained by the first two axes was 88.0% (Fig. 5.2a). The first axis, which explained 51.4% of the variance, was associated with resource-conservative to acquisitive traits: high LDMC to high SLA, irrespective of functional group. The second axis, which explained 36.0% of the variance, was associated with taller grasses to shorter forbs. For the PCA of belowground functional traits, the cumulative variance explained by the first two axes was 79.2% (Fig. 5.2b). The first axis, which explained 43.1% of the variance, grouped forbs with coarser roots versus grasses with denser roots with greater allocation to fine roots. The second axis, which explained 26.1% of the variance, was associated with SRL: longer to shorter roots. Root tissue density was orthogonal to SRL; grasses tended to have higher root tissue density, while SRL was largely independent of functional group.

### *Functional diversity – Biomass relationships*

ANPP significantly decreased with the CWM of aboveground PC1 scores (higher SLA; $R^2 = 0.12$, $p = 0.01$, Fig. 5.3a) and the CWM of aboveground PC2 scores (shorter; $R^2 = 0.07$, $p = 0.06$, Fig. 5.3c). There was no relationship between BNPP and CWM of belowground PC1 scores (Fig. 5.3b, $p = 0.84$) or PC 2 scores (Fig. 5.3d, $p = 0.87$).

ANPP significantly decreased with functional trait dispersion ($R^2 = 0.16$, $p = 0.004$, Fig. 5.4c) but not with functional trait evenness ($R^2 = 0.42$, $p = 0.42$, Fig. 5.4a). BNPP slightly decreased with functional trait dispersion ($R^2 = 0.045$, $p = 0.15$, Fig. 4d) but not with functional trait evenness ($R^2 = 0.0005$, $p = 0.87$, Fig. 5.4b).

The predictive models showed that ANPP was driven by the CWM of aboveground PC1 scores, whereas BNPP was not predicted by any of the functional diversity metrics in this study (Table 5.1).

### *Functional diversity responses to composition and rainfall treatments*

Forb-dominated plots had more genera present and more evenness of forbs and grasses than mixed or grass plots (Fig. S5.3). In forb-dominated plots, cover of forbs increased in spring dry and grasses increased in consistent dry, but rainfall treatment did not affect the cover of functional groups in mixed or grass plots (Fig. S5.4). Forb-dominated plots had higher SLA and shorter plants compared to mixed plots (Fig. 5.5a and c). The composition treatment did not affect the central tendency of belowground traits (Fig. 5.5b and d). In response to rainfall treatments, aboveground traits shifted to higher SLA in consistent dry compared to control (Fig. 5.5e). The CWM of aboveground PC2 scores did not differ across rainfall treatments (Fig. 5.5g). Belowground traits shifted to longer, finer and denser roots in fall and consistent dry plots (Fig. 5.5f and h) and shorter and coarser roots in spring dry plots (Fig. 5.5f and h). There was no interaction effect of composition and rainfall treatments on CWMs of above- and belowground traits (Table S5.4).

Forb-dominated plots had higher above- and belowground functional trait evenness compared to grass plots but not mixed plots (Fig. S5.5a and b). Forb-dominated plots also had higher above- and belowground functional trait dispersion compared to mixed and grass plots (Fig. S5.5c and d). Rainfall treatment did not affect the above- or belowground functional trait evenness or dispersion (Fig. S5.5 e-h). There was a significant interaction effect of rainfall and composition treatments on belowground functional trait dispersion (Table S5.5). Specifically, in grass plots, Rao’s Q significantly decreased in drought conditions, whereas in forb and mixed plots, rainfall treatments had no significant effect on Rao’s Q.

### *Functional diversity - Biomass relationships in response to rainfall treatments*

The relationship between Rao’s Q and total biomass (ANPP and BNPP) was significantly negatively correlated ($F_{1, 37} = 11.33$, $p = 0.001$; Fig. 5.6). There was a weak interaction effect of

Rao's Q and rainfall treatment on total biomass ($F_{1, 37} = 1.70$, $p = 0.18$). The Rao's Q vs. total biomass relationship in control (ambient) rainfall condition had the steepest slope and highest intercept compared to other rainfall conditions (Fig. 5.6; Table 5.2). Though not statistically significant, the largest differences in slope and intercept compared to control were in spring dry and consistent dry, respectively (Table 5.2).

The relationship between functional evenness and total biomass was not significantly correlated ($F_{1, 37} = 0.05$, $p = 0.80$; Fig. S5.6). There was no interaction effect of functional evenness and rainfall treatment on total biomass ($F_{1, 37} = 0.82$, $p = 0.48$).

## Discussion

Understanding precipitation controls on community composition and functional diversity, and subsequent links between composition and production, is important in predicting how variable rainfall will alter plant productivity in the future. Here, we took a functional trait-based approach to predicting above- and belowground biomass under changing rainfall conditions. We provide evidence that community composition significantly affects biomass production, where plant communities containing mixed functional groups (i.e., grasses, forbs, and legumes) have higher biomass than either the grass- or forb-dominant communities. We found divergent relationships between functional diversity and biomass above- and belowground. The inverse relationship between CWM of functional traits (i.e., SLA and height) and aboveground biomass suggested support for the mass ratio hypothesis, where aboveground biomass is driven by specific traits from a few dominant species. On the other hand, no clear relationships between functional diversity and belowground biomass emerged that provided evidence of either the mass ratio hypothesis or the niche complementarity. While biomass was largely unaffected by the timing of drought in one season, above and below ground traits shifted due to species shuffling in response to seasonal drought. When we compared functional diversity-biomass relationships across variable rainfall conditions, we found that functionally diverse communities based on Rao's Q are overall less productive but more robust (no difference in total biomass) to seasonal drought.

Given the coexistence of resource-acquisitive and resource-conservative species in California grasslands, we predicted that plant communities with more functional groups (i.e., mixed grasses, forbs, and legumes) would have higher biomass than grass- or forb-dominated communities. Indeed, we found higher biomass in mixed plant communities (Fig. 5.1), supporting our first hypothesis. Yet, we did not observe a positive relationship between biomass and breath of functional trait diversity. This paradox is perhaps explained by the disconnect in the timing of when the benefits of growing in mixed communities manifested and when plant community composition was measured. For example, over 90% of annual plants in California germinate at the start of the growing season, but up to 50% of germinated seedlings can die within the first few weeks of the growing season (Eviner 2016). A self-thinning of legumes or forbs with high nitrogen content early in the growing season could help the growth of resource-acquisitive grasses via slow release of nitrogen (Eviner and Firestone 2007) and reduction in interspecific competition. But this signature of functional diversity in mixed communities (i.e., facilitation) may have disappeared by the time plant composition is measured at the end of the growing season. Though sampling species composition early in the growing season, as well as the peak, could have captured the full suite of functional diversity in mixed communities, identifying germinates or seedlings to species with high accuracy is non-trivial.

Our second hypothesis was partially supported in aboveground biomass. Aboveground biomass correlated with the average trait value of a community (Fig. 5.3), which supports the mass ratio hypothesis. However, aboveground biomass decreased with functional trait dispersion

(Fig. 4), which does not support niche complementarity. According to the mass ratio hypothesis, plant biomass should be predictable from the CWMs of a few traits within the community (Díaz et al., 2013). In our study, plant communities with lower SLA and taller plants positively correlated with greater aboveground biomass across all composition and rainfall treatments. In particular, the dominance of *Avena barbata* and *Festuca perennis* (former name *Lolium multiflorum*) in high productivity communities suggested that there is a trait selection for taller grass species that can better compete for light compared to shorter forb species. This is consistent with previous studies which found that aboveground productivity is best predicted by CWM of SLA (LaForgia et al. 2018) and height (Butterfield & Suding 2013) in California annual grasslands.

Belowground, three-dimensional competition for multiple limiting resources such as water and nutrients allows for coexistence of plant species with different and complementary resource strategies. It has been suggested that resource complementarity, such that plant biomass increases with functional trait diversity, occurs mainly belowground (e.g., Bardgett et al., 2014; Oram et al., 2018; van Ruijven & Berendse, 2005). However, we found no direct evidence for niche complementarity; belowground biomass neither increased nor decreased with evenness or dispersion of functional traits (Fig. 5.4). Similarly, Bakker et al. (2018) did not find a relationship between root traits and complementarity in a grassland biodiversity experiment. Instead, we found shifts in community-level means of belowground traits from species reshuffling in response to drought: we observed longer, finer and denser roots in fall and consistent dry plots, and shorter and coarser roots in spring dry plots (Fig. 5.5). While the means shifted, the breaths of belowground trait diversity (Fig. S5.5) and biomass (Fig. 5.1) were not affected by seasonal drought treatments. Reduction of root elongation and sacrifice of fine roots are different strategies that may promote nutrient and water acquisition, depending on plant species identity. Heterogeneous root trait responses to drought reflect coexistence of species with different drought tolerance strategies (Lozano et al. 2020). Perhaps the benefit of functionally diverse plant communities is not necessarily the increase in biomass, but the ability to maintain belowground niche space, and therefore biomass, under fluctuating resource conditions.

While we expected the timing of drought to differentially affect biomass depending on plant community composition, we found no effects of seasonal drought on biomass production across composition treatments (Fig. 5.1). This result is initially surprising, but it also indicates the robustness of plant productivity in this system. It is important to note that our experiment was conducted in water year 2015, at the tail end of a multi-year drought experienced in California from fall 2011 to fall 2015. Shaw et al. (2022), who used unmanipulated vegetation plots within the same rainfall infrastructure as our study over three years, found that aboveground biomass increased in 2016 and 2017 compared to 2015. The lagged effects of drought are well established in California grasslands, where rainfall in one year alters composition in the next via litter accumulation and seed production (Dudney et al., 2017). Furthermore, community composition, along with associated trait representation, shifts to more drought tolerant species and traits in a multi-year drought. For example, there is evidence that seed banks of native forb species increased, while those of exotic grass species decreased, during this extended drought, which effectively lowered CWM of SLA (LaForgia et al., 2018). The multiple years of natural drought prior to our study thus could have already selected for communities that were more robust to further experimental drought treatments. Overall, our seasonal drought treatment may have been dampened by the effects of this multi-year drought, highlighting the considerable impact of longer-term rainfall variability dynamics on biomass production over time.

Despite no direct effect of precipitation on biomass, we found that functional diversity-biomass relationships were weakly responsive to variable rainfall conditions. Specifically, slopes of the functional diversity-biomass relationships flattened and the intercepts declined in drought conditions compared to control (Fig. 5.6; Table 5.2). In other words, functionally less diverse communities were highly productive in ambient conditions but more volatile to drought, while functionally diverse communities were overall less productive but more robust to drought. Similarly, another field experiment in California grassland has shown that maintaining functional diversity (via the presence of subordinate, stress-tolerant forb species) in wet years is key to stabilize biomass production over dry years (Hallett et al. 2017). These relationships support the theoretical expectation that functionally diverse communities are more stable in environments with fluctuating resources (Wang, Yu, & Wang, 2007). Here, we assessed a few scenarios of seasonal rainfall variation, but it is uncertain how the functional diversity-biomass relationships shift across extreme climate conditions. As rainfall patterns are expected to be more variable in the future, understanding the precipitation control on functional diversity-biomass, and its limits, is important in predicting grassland biomass production under different rainfall scenarios.

Changes in community-wide functional trait diversity could arise from shuffling of plant composition and phenotypic plasticity (e.g., Pigliucci 2001). In this study, we measured all functional traits in the greenhouse and averaged the trait values for each species, because 1) we wanted to capture the potential of each species to express each trait, and 2) the root traits are notoriously difficult to accurately measure in the field. Unfortunately, this approach limited our ability to capture the functional trait shifts from phenotypic plasticity. Recent studies have shown that root trait responses to drought are more heterogeneous than aboveground trait responses (Lozano et al. 2020), thus we may have observed more functional trait dispersion belowground if we had included phenotypic plasticity in our study. But plasticity patterns are species-specific (Weemstra et al. 2020) and community context dependent (Aoyama et al. 2022). For example, some species may exhibit large plasticity in multiple traits to change from an acquisitive to more conservative strategy as an adaptive mechanism for a more efficient use of resources, which has consequences to the competitive trade-offs between species. (Pérez-Ramos et al. 2019). We have yet to understand which species and functional trait are more or less plastic than others, and how that affects the functional diversity-biomass relationships in changing environmental conditions. Though quantifying intraspecific trait variation for each species across environmental gradient is data-intensive, future studies that include such variation could improve our predictions for plant community responses to changing rainfall patterns.

Trait-based approach to predict plant productivity and its mechanisms has wide applications to ecosystems such as in mesic grasslands (Bakker et al., 2018; Chanteloup & Bonis, 2013; Schumacher & Roscher, 2009) and in temperate forests (Jing et al., 2021). Shifts in trait-fitness relationships can give rise to turnover in plant phenotypes across environmental gradients (Kandlikar et al. 2022). We highlight the value of above- and belowground plant functional traits in predicting community responses to environmental variation, and emphasize the need for more widespread study of whole-plant functional diversity-biomass relationships to predict changes in plant communities from global changes.

## Figures

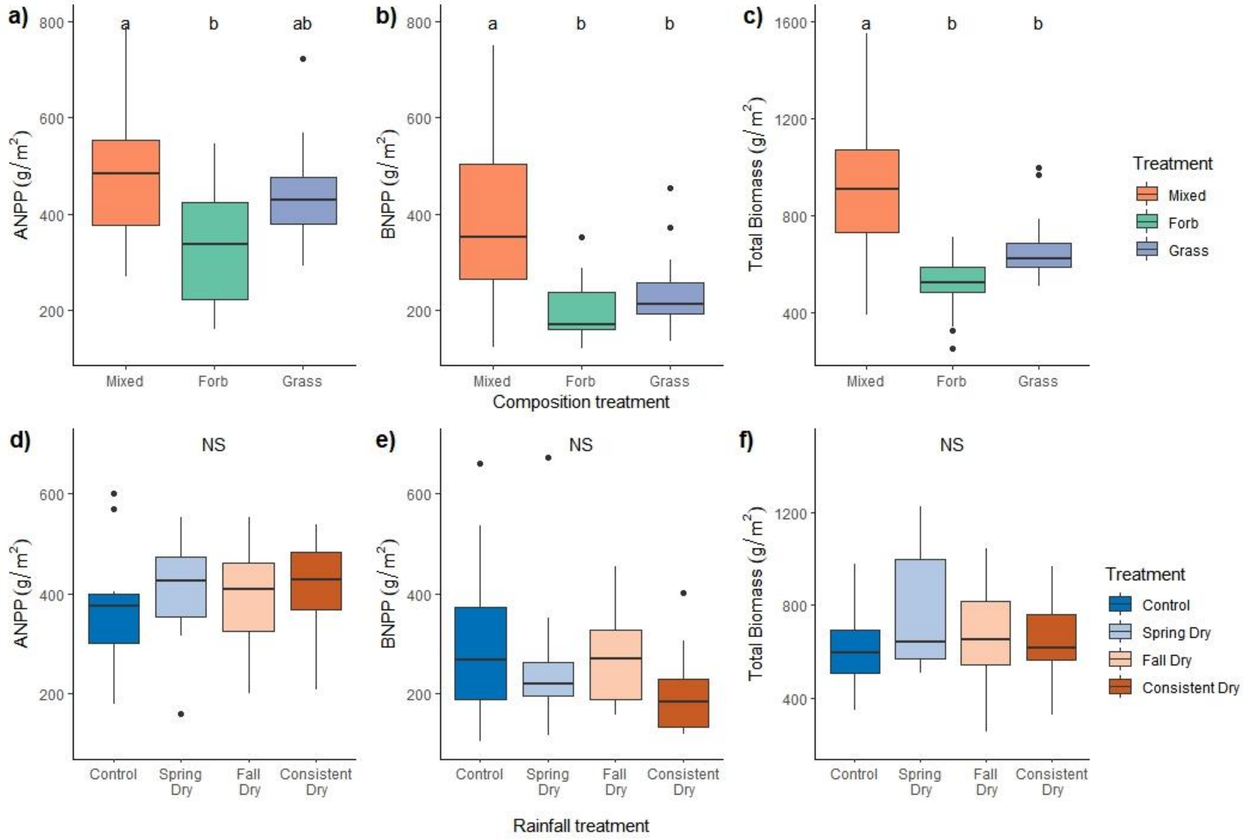

**Figure 5.1.** Biomass by composition treatment (a-c) and precipitation treatment (d-f) for (a,d) ANPP (b,e) BNPP, and (c,f) total biomass. Boxplots in a-c are colored by the composition treatments: mixed (orange), forb-only (green), grass-only (purple). Boxplots in d-f are colored by the rainfall treatments: control (dark blue), spring dry (light blue), fall dry (beige), and consistent dry (brown). Central values correspond to median; box limits correspond to the 25th and 75th percentiles; and error bars correspond to maximum and minimum values no further than 1.5 * IQR (inter-quantile range, or distance between the 25th and 75th percentiles). Points correspond to outliers. Overall significant difference ($p < 0.05$) between composition treatments is indicated by different letters above each box. There is neither a significant (NS = not significant) rainfall effect nor a significant rainfall x composition effect.

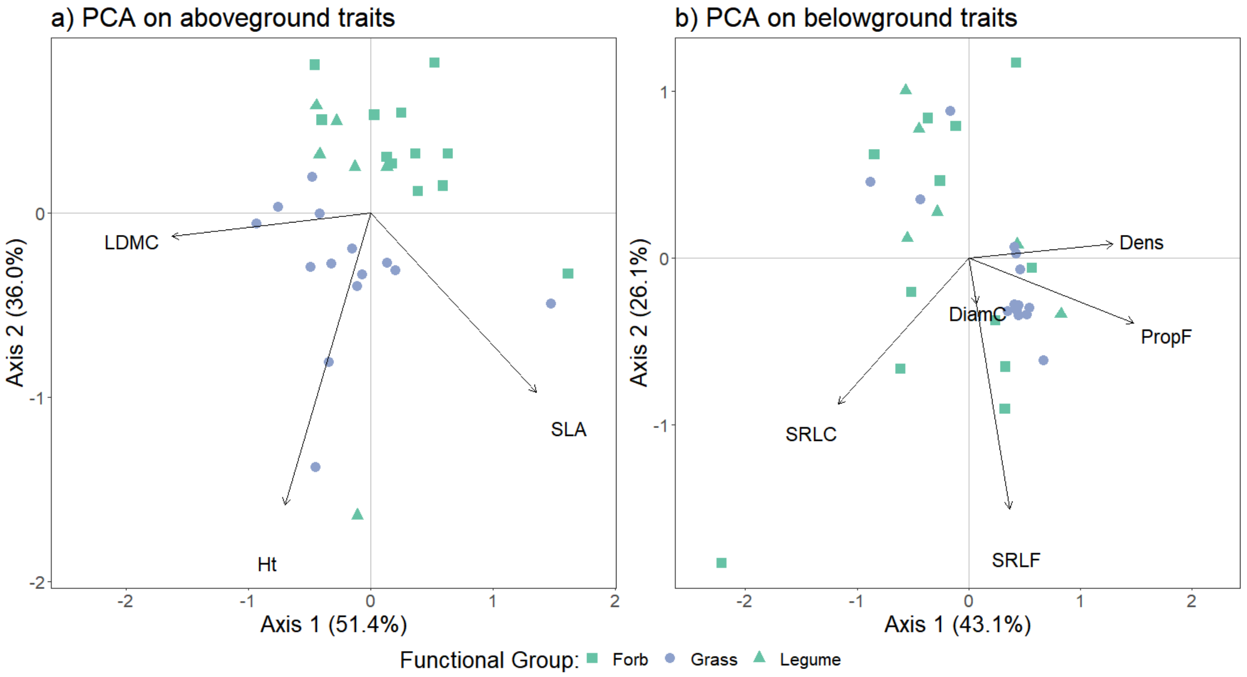

**Figure 5.2.** Principal component analysis of a) three aboveground and b) five belowground functional traits derived from plant composition present in our field experiment and traits collected in the greenhouse. Trait abbreviations are: Ht height, SLA specific leaf area, LDMC leaf dry matter content, Dens root density, DiamC coarse root diameter, SRLC specific root length of coarse roots (> 0.2 mm diameter), and SRLF specific root length of fine roots (> 0.2 mm diameter). Functional groups are: grasses (purple circles), legumes (green triangles), and forbs (green squares). See Table S6 for PC loadings by species.

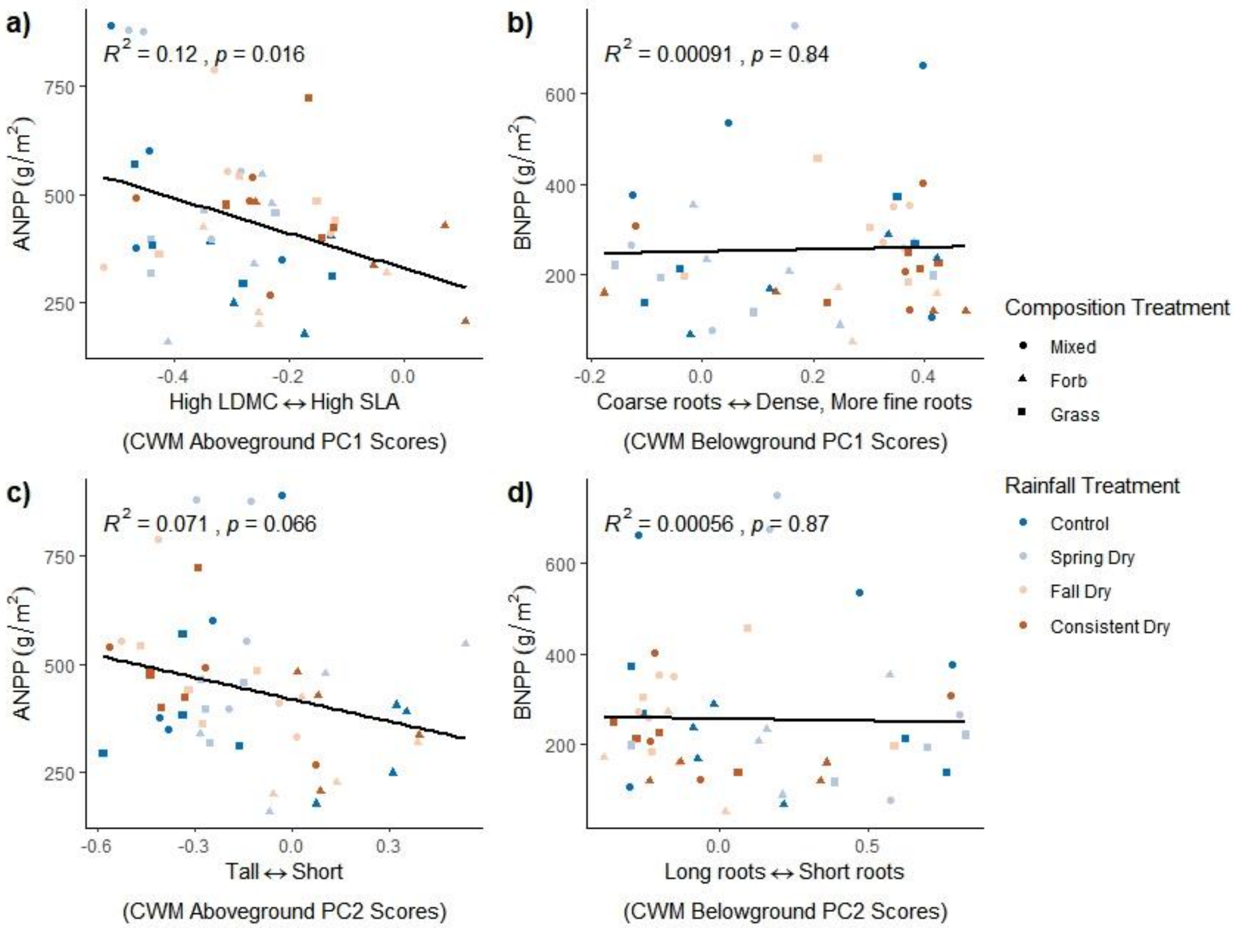

**Figure 5.3.** Linear regressions between biomass productivity and community weighted mean (CWM) of functional traits. Panel a) and c) show regressions of aboveground net primary productivity (ANPP) on the CWM of PC1 and PC2 from PCA of aboveground traits (see Figure 2a). Panel b) and d) show regressions of belowground net primary productivity (BNPP) on the CWM of PC1 and PC2 axis scores from PCA of belowground traits (see Figure 2b). Composition treatments are differentiated by shapes, and rainfall treatments are differentiated by colors.

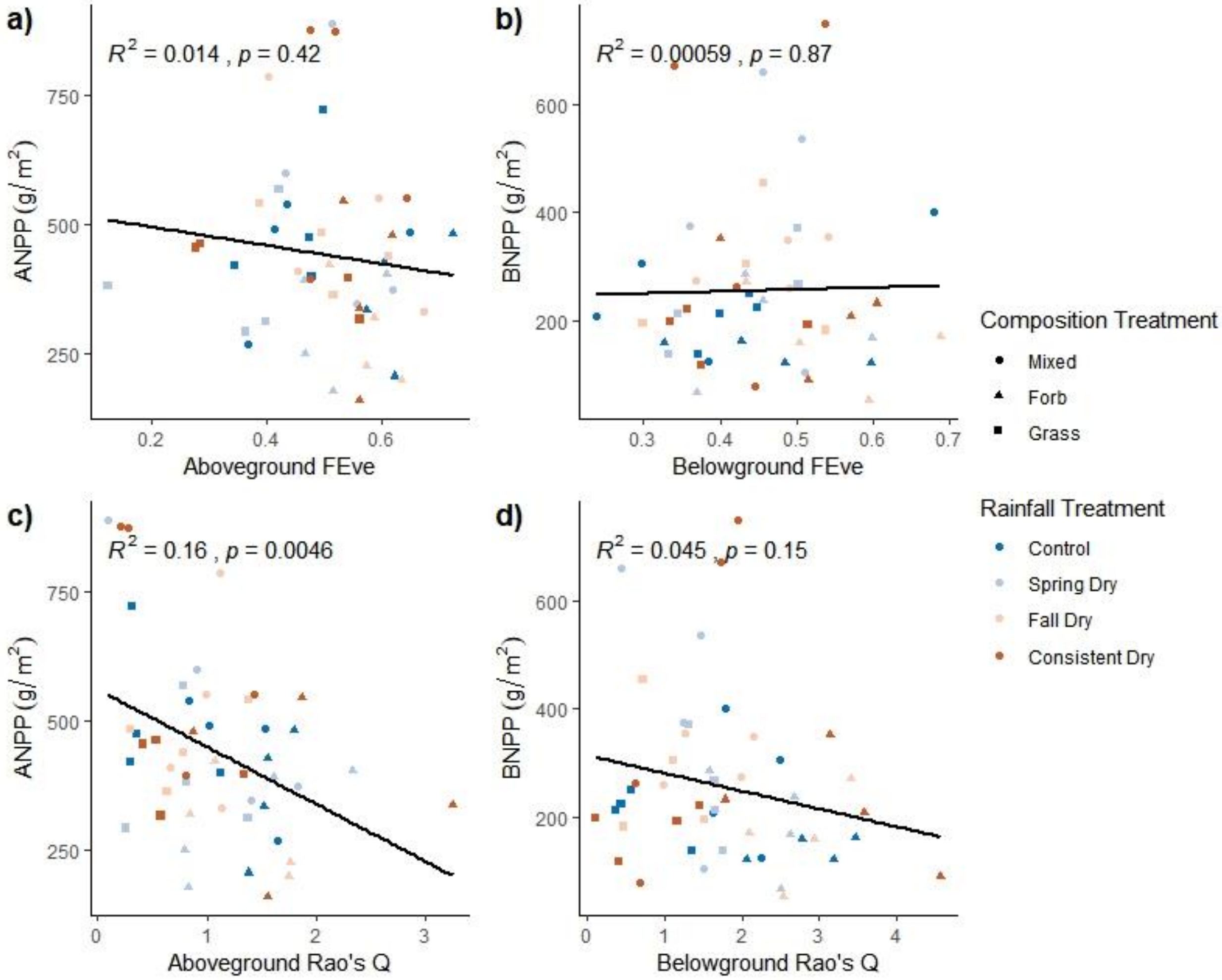

**Figure 5.4.** Linear regressions between biomass productivity and functional diversity of community traits. Panels a) and c) show relationships of aboveground net primary productivity (ANPP) with functional evenness (FEve) and functional dispersion (Rao’s Q) of aboveground traits. Panels b) and d) show belowground net primary productivity (BNPP) with functional evenness and functional dispersion of belowground traits. Composition treatments are differentiated by shapes, and rainfall treatments are differentiated by colors.

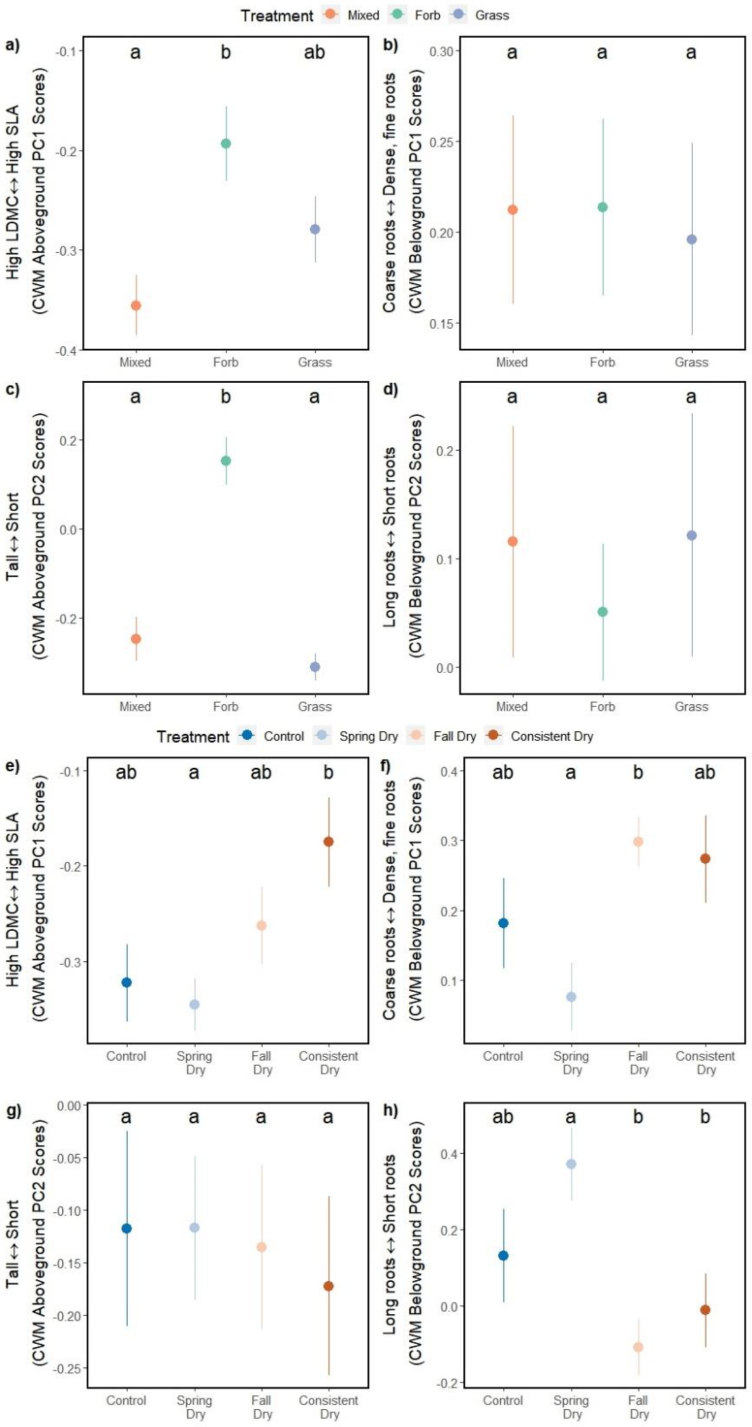

**Figure 5.5.** Shifts in community weighted mean (CWM) of functional traits by composition and rainfall treatments. Left panels show the CWM of PC1 and PC2 from PCA of aboveground traits (see Figure 2a) across composition treatments (a and c) and rainfall treatments (e and g). Right panels show the CWM of PC1 and PC2 from PCA of belowground traits (see Figure 2b) across composition treatments (b and d) and rainfall treatments (f and h). Overall significant difference between each treatment is indicated by different letters above each bar.

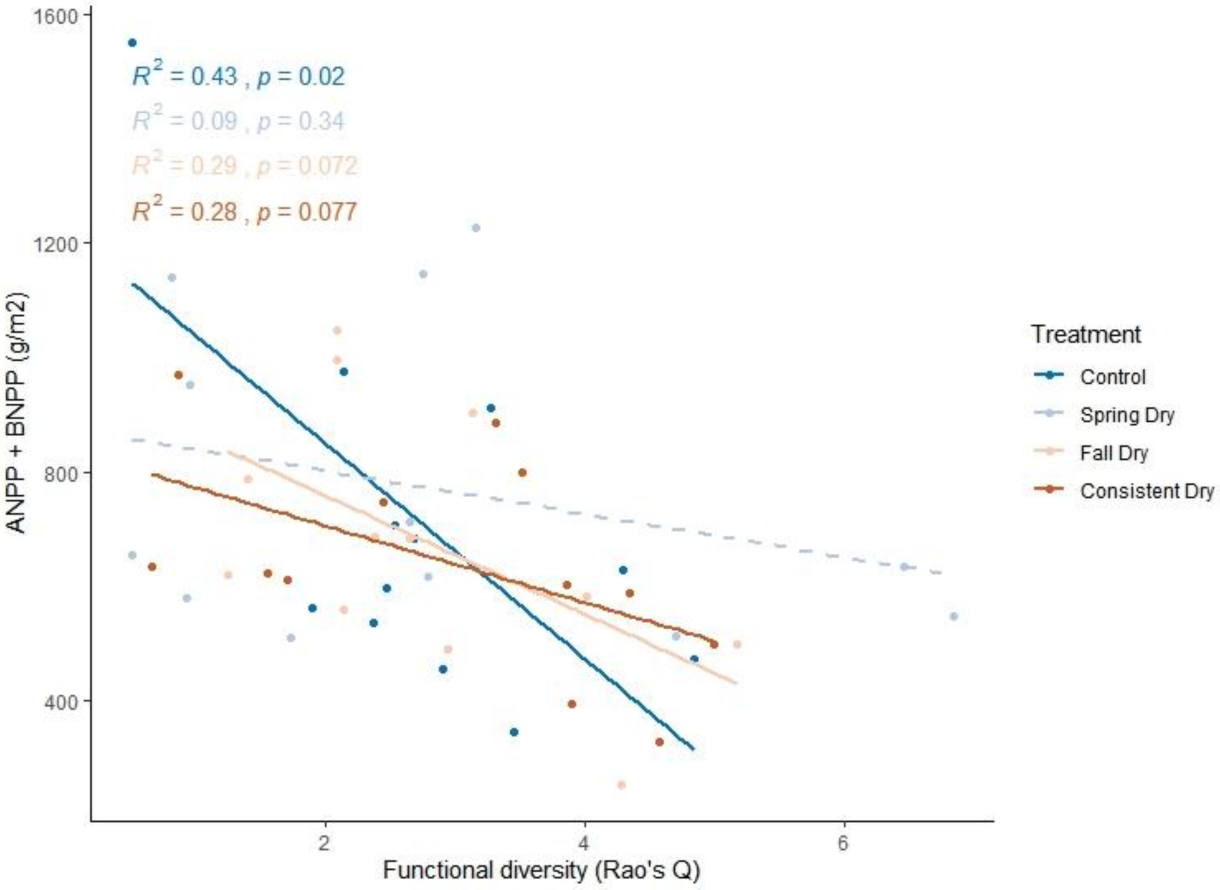

**Figure 5.6.** Total biomass (ANPP and BNPP) and functional trait dispersion (Rao's Q) relationships compared across rainfall treatments. Points and linear regression lines colored by rainfall treatments: control (dark blue), spring dry (light blue), fall dry (beige), and consistent dry (brown). There was a weak interaction effect of Rao's Q and rainfall treatment on total biomass ($F_{1, 37}$ = 1.70, p = 0.18).

## Tables

**Table 5.1.** Predictive models of above- and belowground biomass on the basis of functional trait diversity. Models were developed for primary productivity (ANPP) and belowground net primary productivity (BNPP) separately following steps 1-4 outlined in the methods.

| | Stage 1: Individual effects of functional diversity on biomass | | | | | | Stage 2: Combining significant effects into the best predictive model | |
|---|---|---|---|---|---|---|---|---|
| | Step 1: CWM traits | | Step 2: Trait distribution | | Step 3: Unique species | | Step 4: Final model | |
| | Variable | p value | Variable | p value | Variable | p value | Model | % var |
| ANPP | CWM PC1 | 0.016 | FEve | 0.371 | Centaurea solstitialis | 0.783 | ANPP ~ 329.72 - 404.37 CWM.PC1 | 12.01 |
| | CWM PC2 | 0.066 | RaoQ | 0.127 | Taeniatherum caput-medusae | 0.893 | | |
| BNPP | CWM PC1 | 0.839 | FEve | 0.881 | Centaurea solstitialis | 0.315 | No suitable model | |
| | CWM PC2 | 0.874 | RaoQ | 0.832 | Taeniatherum caput-medusae | 0.436 | | |

**Table 5.2.** Linear regressions of total biomass (ANPP + BNPP) and functional trait dispersion (Rao's Q) across variable rainfall treatments. Slope, intercept, model statistics ($R^2$), and p-value of each linear regressions. Difference in slope and intercept compared to control.

| Rainfall treatment | Slope | Intercept | $R^2$ | p-value | Δ Slope compared to control | Δ Intercept compared to control |
|---|---|---|---|---|---|---|
| Control | -189.19 | 1228.47 | 0.4323 | 0.02014 | 0 | 0 |
| Spring Dry | -37.78 | 876.99 | 0.0896 | 0.3444 | 151.41 | -351.48 |
| Fall Dry | -104.04 | 966.37 | 0.2885 | 0.07171 | 85.15 | -262.1 |
| Consistent Dry | -67.22 | 839.93 | 0.2797 | 0.07709 | 121.97 | -388.54 |

# CHAPTER VI

# APPLICATION OF MODERN COEXISTENCE THEORY TO RARE PLANT RESTORATION PROVIDES EARLY INDICATION OF RESTORATION TRAJECTORIES AND MANAGEMENT ACTIONS

From Aoyama, L., L. G. Shoemaker, B. Gilbert, S. K. Collinge, A. M. Faist, N. A. Shackelford, V. Temperton, G. Barabás, L. Larios, E. R. Ladouceur, O. Godoy, C. Bowler, and L. M. Hallett. 2022. Application of modern coexistence theory to rare plant restoration provides early indication of restoration trajectories and management actions. *Ecological Applications* 32(7):e2649.

## Contributions

All authors contributed to the ideas and concepts in this research. SKC designed the experiment and SKC and AMF collected data; LA led the analysis with input from LGS, BG, and LMH; LA, LGS, LMH, BG, SKC, AMF, NS, and VMT wrote the first draft and all authors contributed to manuscript edits.

## Introduction

Ecological restoration is an increasingly important tool to reverse the effects of land degradation, but the success of restoration efforts is often uncertain. Success is often measured by comparing the abundance of target species in the restored community to a natural "reference" community, where similar species abundances between restored and reference are presumed to indicate success (Ruiz-Jaen and Aide, 2005; Society of Ecological Restoration, 2004). This approach is best suited when ecosystems have a monotonic recovery trajectory, which is predicted by the most common ecological theories used to inform restoration, such as succession and community assembly (Wainwright et al., 2017). At the same time, more recent developments in ecological theory, such as Modern Coexistence Theory (MCT), have highlighted that environmental variation and associated fluctuations in species abundances are not only common, but at times essential for maintaining species populations (Chesson, 2000; Adler et al., 2007; HilleRisLambers et al., 2012), complicating efforts to assess success on abundance alone. Applying MCT to ecological restoration may provide a clearer picture of restoration trajectories and illuminate pathways to improve restoration outcomes, particularly in variable environments.

Modern coexistence theory has gained prominence as a tool to understand how the environment and species interactions jointly structure species diversity (HilleRisLambers et al., 2012; Letten et al., 2018; Grainger et al., 2019). A cornerstone of MCT is that species can coexist if they can increase when rare (Baraba´s et al., 2018). Critically, the ability to increase when rare is assessed by average low-density growth rates; many persistent species still experience periods of negative growth and low abundance (Letten et al., 2018; Hallett et al., 2019). Moreover, this variability can in fact be key to maintaining coexistence among species (Chesson, 2000). For example, the storage effect - in which species capitalize on good years while “storing” through bad years, such as in the seed bank - is a classic fluctuation-dependent mechanism of coexistence (Chesson, 2018). While the storage effect is based on species-specific responses to the environment, relative nonlinearity in species’ responses to the environment or competition can also enhance coexistence if species experience a greater magnitude of benefit under favorable conditions compared to their magnitude of decline 56 under unfavorable conditions (Letten et al., 2018; Hallett et al., 2019).

Integrating methods from MCT into restoration ecology may improve our ability to diagnose restoration trajectories. A key lesson from fluctuation-dependent coexistence is that a low abundance of target species may not necessarily indicate failure; rather, abundance fluctuations can be essential to coexistence (Warner and Chesson, 1985; Gravel et al., 2011). Conversely, high abundances of target species may not indicate success, because restoration activities such as seed addition may yield abundances that belie an overall negative low-density growth rate (Martin and Wilsey, 2014). Gauging success by annual low-density growth rate rather than abundance may therefore better provide an early indication of restoration trajectories. Furthermore, when restoration projects are monitored over time, average low-density growth rate may indicate whether or not the target species will persist 67 in the long-term, which is critical to restoration success.

Assessing restoration outcomes through the lens of MCT may also improve our ability to identify the factors governing success or failure, ultimately improving the adaptive management process. Classic models of ecological restoration (e.g. ecological filters framework) have highlighted that both altered environmental conditions and novel competitive interactions can act as barriers to restoration success (Temperton et al., 2004). Management efforts in restoration regularly tailor both barriers. For example, land-moving to reshape hydrology or amendments to alter soil conditions are common tools in riparian and grassland restoration (Nilsson et al., 2015; Havrilla et al., 2020), respectively, and mowing or grazing to reduce exotic grasses are common tools to promote native forbs in annual grasslands (Weiss, 1999; Hernandez et al., 2021) and vernal pools (shallow, ephemeral wetlands in the Mediterranean climates) (Marty, 2015). A strength of MCT is that it decomposes the effects of the environment, competitive interactions, and their responses to varying environments on species growth rates (Barabás et al., 2018). Typically, an MCT invasion analysis is applied to understand species coexistence with the resident community (i.e., a pairwise, multispecies approach), but it can also be applied to examine persistence of a single species (Godoy, 2019). Applying this partitioning to restoration monitoring data may help isolate the effect of the restoration actions on abiotic versus biotic barriers. For example, a strong negative effect of environmental variability on species' intrinsic growth rate would indicate that an intervention is needed to ameliorate the abiotic environment. Similarly, MCT can indicate periods in which competitor removal is most likely to benefit a target species (Godoy and Levine, 2014), even when these dynamics would be hard to discern from abundance patterns due to covariance of competition and environment.

Here, we demonstrate how principles and methods of MCT can be used to improve the assessment of restoration trajectories and guide restoration actions. We leverage long-term monitoring data of an endangered annual forb species, *Lasthenia conjugens* Greene (Contra Costa goldfields, Asteraceae: Heliantheae), from a restoration project in California vernal pools. Vernal pools are critical habitats for rare plant diversity. While native vernal pool forbs are better adapted to the highly variable ephemeral ponding (Emery et al., 2009; Faist and Collinge, 2015; Tittes et al., 2019), exotic grasses historically are not (Gerhardt and Collinge, 2007). In particular, *L. conjugens* thrives in wet early-season rain years (Gerhardt and Collinge, 2007), while exotic grasses are competitive in wet late-season rain years (Javornik and Collinge, 2016). Because of these environmental fluctuations, determining restoration success solely from the abundance of *L. conjugens* is difficult (Collinge et al., 2013; Schlatter et al., 2016). We hypothesized that i) model-derived growth rates are more reliable metrics to assess long-term trends than observed abundance in early years; ii) *L. conjugens* can persist (as indicated by a positive average low-density growth rate) because environmental variability has a positive effect on the intrinsic growth rate and relative nonlinearity in competition; and iii) the average low-density growth rate of *L. conjugens* increases with greater amount of exotic grass removal. To test these hypotheses, we parameterized stochastic population models with monitoring data, partitioned growth rates with MCT invasion analysis (Ellner et al., 2019), and experimentally simulated the effects of active management on restored populations. This approach has a wide application potential for restoration of rare species and species of conservation concern.

## Methods

### *Study site*

We conducted this study in a vernal pool system at Travis Air Force Base (AFB) near Fairfield, California, USA (38◦15’00” N, 122◦00’00” W, 6 m elevation). Approximately 100 naturally occurring vernal pools exist in the 15-ha study area. The site experiences cool, wet winters and hot, dry summers. The pools fill with water and the growing season begins with winter rains, usually starting in October. The pools dry out and the growing season ends in April or May, when the rain stops and temperature increases (Keeley and Zedler, 1998). The amount of water in the pools varies each year due to high seasonal and annual rainfall variability [growing season rainfall averages 455.4 mm but has ranged 120 from 159.8 mm to 869.2 mm over the past 50 years (PRISM Climate Group, 2020)].

While the pools support a diverse native plant community, restoration efforts often center on the annual forb *L. conjugens*, because it is a protected endangered species (Federal Register, 1997). Previous work has shown that *L. conjugens* populations are weakly positively correlated with higher early-season rainfall in October to December, deeper pools, and longer inundation

(Javornik and Collinge, 2016). Additional focal species native to vernal pools at this site include *Eryngium vaseyi* (button celery), *Downingia concolor* (spotted throat downingia), and *Plagiobothrys stipitatus* (popcorn flower) (Collinge and Ray 2009). Exotic annual grasses dominant in this system are *Bromus hordeaceus, Hordeum marinum*, and *Festuca perennis* (previously called *Lolium multiflorum*).

*Restoration design*

To restore *L. conjugens*, 255 artificial pools were constructed in December 1999 in the vicinity of reference pools as described in Collinge and Ray (2009). Pool sizes were randomly assigned as either small (5 x 5 m), medium (5 x 10 m), or large (5 x 20 m), n = 85 each, to reflect the size variation in the reference pools. Constructed pools were also similar to references in maximum depth (0-10 cm), elevation, topography (0-2% slopes), and soil type (Antioch San Ysidro complex and San Ysidro sandy loam). To establish populations of *L. conjugens* (LACO) in constructed pools, SKC collected seeds from reference pools during May and June of 1999, 2000, and 2001. In early December of 1999, 2000, and 2001, constructed pools were sown with seeds within permanently marked 0.5 x 0.5 m plots (one plot per pool). Five seed addition treatments were randomly assigned to constructed pools: control (no seeding); LACO 1x (100 *L. conjugens* seeds in 1999); LACO 3x (100 *L. conjugens* seeds in 1999, 2000, and 2001); Group A-Group B (100 seeds of group A species in 1999, 100 seeds of group B species in 2000); Group B-Group A (100 seeds of group B species in 1999, 100 seeds of group A species in 2000). Group A consisted of *L. conjugens, Deschampsia danthonioides,* and *E. vaseyi*. Group B consisted of *L. conjugens, P. stipitatus*, and *Layia chrysanthemoides*. Plots were initially raked prior to seeding in 1999 but not in 146 2000 and 2001 as to not disturb seeds from previous years.

In April, during approximate peak biomass, each year from 2000 to 2017, plant occurrence was monitored (Collinge and Faist, 2020). A 0.5 x 0.5 m frame divided into 100 subquadrats (5 x 5 cm) was placed in a permanently marked plot in each pool, and stem counts of five focal species and frequency (number of subquadrats out of 100 in which the species occurred) of all species present were measured within each plot. At weekly intervals during the wet seasons of 1999-2000, 2001-2002, and 2008-2012, water depths at the center of sampling quadrats in each pool were measured.

### *Dynamical model*

To project the population dynamics of L. conjugens, we took into account the effect of environmental conditions as well as the competition experienced from both conspecific and heterospecific individuals. We used the Beverton-Holt model, which is well-suited as an annual plant model (Levine and HilleRisLambers, 2009; Larios et al., 2017). This model tracks the number of seeds (*X*) at the end of each year, as the entire population of the annual plant is captured in its seeds just prior to germination cues (Figure 6.1). The 160 number of seeds in year *t* of group 1 (i.e. *L. conjugens*) in vernal pool p denoted by $X_{t,1,p}$

is:

$$X_{t+1,1,p} = s(1 - g_t)X_{t,1,p} + \frac{\lambda_t}{C_{t,p}} g_t X_{t,1,p} \tag{1}$$

where *s* is the (time- and pool-independent) annual survival probability of each *L. conjugens* seed, and $g_t$ is its germination rate in year *t*. The term $s(1-g_t)X_{t,1,p}$ describes the carryover of seeds in the seed bank that contribute to future years' *L. conjugens* population size. In turn, $\lambda_t$ is the maximum, density independent number of *L. conjugens* seeds produced in 166 year *t* by a single plant, and

$$C_{t,p} = 1 + \sum_{k=1}^{4} \alpha_{t,k} Y_{t,k,p} \tag{2}$$

measures the degree of competition experienced by *L. conjugens* stems in year $t$ and vernal pool $p$, translating into reductions in the maximum annual fecundity $\lambda_t$. Here $\alpha_{t,k}$ is the competition experienced by *L. conjugens* from individuals of group $k$ in year $t$, and $Y_{t,k,p}$ is the number of stems of group $k$ in year $t$ and pool $p$. This model assumes no dispersal among the vernal pools because *L. conjugens* is a self-incompatible annual with gravity-dispersed seeds, and its dispersal range is unknown.

*Statistical model*

The statistical model was designed to estimate the parameters of Eq 1 and Eq 2 to allow us to infer the processes that promote or limit the persistence of *L. conjugens*. Importantly, model fitting proceeds annually: the data we use to obtain estimates in year $t + 1$ are only those of year $t$. In other words, while long-term monitoring data is necessary to explicitly look at long-term trends, the model can be parameterized for shorter time series. Unlike experimental tests of MCT that typically collect more response data and include fewer competitors than restoration efforts (Ellner et al., 2019), several challenges arose when fitting restoration monitoring data to these models. Here, we outline the decisions we made to best adapt monitoring data to model *L. conjugens* using a MCT framework.

The seed bank (the first half of the Eq 1) can maintain annual populations during unfavorable years while enabling them to take advantage of favorable environmental conditions (Faist et al., 2013). It is, however, difficult to measure the seed bank directly, especially when destructive soil disturbance would threaten the success of restoration projects. In such cases, as with our data, only stems ($Y_{t,1,p}$) were measured, and we needed to estimate the total number of seeds ($X_{t,1,p}$). We did this by using the expected number of seeds given the number of stems: $X_{t,1,p} = Y_{t,1,p}/g_t$. Although stem number was our best estimate of population size, there were some pools that recorded zero stems in one year, followed by stems present in the subsequent year. To account for the possibility of observing zero stems when seeds are present but at low abundance, we used a different estimate of population number for the first year in which stems were absent from a pool. Specifically, in the first year with no stems recorded for *L. conjugens* ($Y_{t,1,p} = 0$), we used the population estimate of the prior year and adjusted seed survivability, so that $X_{t+1,1,p} = s^2 X_{t-1,1,p}$.

To account for manual seed addition in the first three years, we modeled stem counts of *L. conjugens* in constructed pools as follows. For the initial year, we drew the individuals from a binomial distribution: $Y_{t=1,1,p} \sim \text{Binomial}(X_{1,1,p}, g_1)$, where X1,1,p is a matrix of seeds added at time $t = 1$ in pool $p$, and $g_1$ is the germination rate of *L. conjugens*. For the second and third years, we added the number of seeds manually added via the experimental treatment to our modeled population $X_{t,1,p}$.

The California vernal pool system is species-rich, but most species occur at low abundance. To reduce the dimensionality of this competitive environment (Eq 2), we selected six species that comprised the majority of the cover (53% of cover across 16 years and plots) apart from *L. conjugens*, grouped them in three functional groups based on similar temporal fluctuations (Fig. S6.1). Specifically, we grouped *B. hordeaceus*, *H. marinum*, and *F. perennis* as an exotic grass group; and *P. stipitatus*, *D. concolor* as a native annual forb group. We kept *E. vaseyi* separate from the native forb group because it is a biennual plant, while other native forbs are annual plants. We labeled these aggregated groups as group 1 (*L. conjugens*), group 2 (exotic annual grass), group 3 (native annual forb), and group 4 (*E. vaseyi*). Of the four groups, we only created an explicit model for the population dynamics of our focal species *L. conjugens* (group 1), while estimating competition coefficients between *L. conjugens* and all other groups.

To include demographic stochasticity, which can increase extinction risk at small population sizes (Lande, 1993; Shoemaker et al., 2020), we drew the number of individuals at a given time and pool from a Poisson distribution with a mean given by the right-hand side of Eq 1. We checked for model-fit of the model by simulating *L. conjugens* population dynamics, and visualizing simulated vs. observed stem counts (Fig. S6.2).

We fitted our model separately for reference (no seed addition) and constructed pools (with seed addition). We only used data from pools with consecutive years of data. While the data for 2000-2017 was available, the data from 2016 and 2017 were dropped from our analysis because we did not have reference pools with consecutive years of data beyond 2015. For reference pools, we used frequency of *L. conjugens*, which we converted to abundance (Fig. S6.3), and frequency of other species from 7 pools in 2000-2015 and 2 additional pools in 2002-2015. For constructed pools, we used stem counts of *L. conjugens* and frequency of other species from 142 pools in 2000-2015. We compared the following seeding treatments: LACO 1x (n = 24), LACO 3x (n = 24), Group A-Group B (n = 43), and Group B-Group A (n = 51). We omitted the control plots because the stem counts of *L. conjugens* in control plots remained zero over time. Pool size classes included small (25 m$^2$; n = 52), medium (50 m$^2$; n = 52), and large (100 m$^2$; n = 38). Pool depth classes were determined by the range of annual maximum pool depth averaged across years: shallow (< 3.8 cm; < 25th percentile; n = 29), intermediate (3.8-8.0 cm; 25-75th percentile; n = 76), and deep (> 8 cm; > 75th percentile; n = 37).

We extracted the Bayesian posterior estimates of the seed survival probability s, annual fecundity estimates $\lambda_t$, and annual competition coefficient estimates $\alpha_{t,k}$. We assumed germination rates of *L. conjugens* were lower in years with a thick litter layer (Faist and Beals, 2018). As such, we set $g_t$ to 0.2 when previous year's total exotic grass cover was 100% or greater. Otherwise, the germination rate was set to 0.7 based on a seed germination trial of a related species, *L. californica* DC. ex Lindley (Gulmon, 1992). All models were fit using the Hamiltonian Monte Carlo (HMC) sampler Stan (Carpenter et al., 2017), using the rstan package in R (R Core Team, 2013). We specified that $\lambda_t$ has a partially informed prior (probability distribution of uncertainty based on a known range), $\lambda_t \sim$ Normal (60, 20), with a lower bound of 0 (Faist et al., 2015). Prior distributions on competition coefficients were half-normal distributions centered on 0 with standard deviations of 1. For survival, we used an uninformed Jeffreys prior, Beta (0.5, 0.5). We generated samples from posterior distributions using the Markov chain Monte Carlo (MCMC) sampling method with 4 chains and 1000 iterations. We checked for convergence using the Gelman-Rubin diagnostic (Rhat), and precision of parameter estimates using the effective sample size. We validated the models using a subsampling approach (i.e., leave-one-out sensitivity analysis), where we iteratively excluded one pool from the dataset and fit the model each time (Fig. S6.4).

### *Analyses*

#### *Restoration trajectory metrics:*

We tested whether model-derived mean per capita intrinsic growth rates ($\lambda_t$) or low-density growth rates ($r_t$) of L. conjugens in reference and constructed pools were better metrics for assessing restoration success compared to observed abundance, measured as mean density of *L. conjugens*. We directly assessed $\lambda_t$ at each year between 2000-2015 from our Bayesian model output. The low-density growth rate was measured as *L. conjugens*' growth rate from rarity into the rest of the community at equilibrium (Chesson, 2000). To do so, we first calculated the equilibrium distribution of the exotic annual grass group, native annual forb group, and *E. vaseyi* as average annual frequencies in control plots within constructed pools that did not include *L. conjugens*. We then modeled low-density growth rates of *L. conjugens*, introducing a single individual into the equilibrium resident community for each year (2000-2015), calculating $r_t = \ln(X_t+1/X_t)$ (Figure 1). The average low-density growth rate across environmental conditions ($r$) predicts whether L. conjugens can persist ($r > 0$) or goes locally extinct ($r < 0$). The average low-density growth rate of the invader is usually compared to the resident species to test for stable coexistence (Chesson, 2000; Barabás et al., 2018); however, we calculated it solely for *L. conjugens*, allowing us to focus on single-species persistence rather than multi-species coexistence.

*Partitioning of persistence mechanisms:*

To understand how environmentally-driven temporal variation in seed production ($\lambda_t$) and variation in competition coefficients ($\alpha_{t,k}$) are driving restoration trajectories, we decomposed the average low-density growth rates ($r$) of *L. conjugens* into the mechanisms that contribute to its persistence (Figure 6.1). Following the decomposition framework from Ellner et al. (2019), the average low-density growth rates ($r$) can be decomposed into four mechanisms:

$$\overline{r}_i = \epsilon_i^0 + \epsilon_i^\alpha + \epsilon_i^\lambda + \epsilon_i^{(\alpha\lambda)} \tag{3}$$

where the first decomposition term, $\epsilon_i^0$, is the growth rate under constant, averaged environmental conditions, where we use the weighted mean condition from 2000 to 2015. In other words, we set $\lambda$ and $\alpha_k$ terms to their average values, rather than letting them vary through time; we calculate the low-density growth rate given these averaged parameter values. The second decomposition term, $\epsilon_i^\alpha$, is the main effect of the environmental variation on competition coefficients, where we calculate the growth rate when $\alpha_{t,k}$ varies through time while $\lambda$ is held at its average value and subtract out the growth rate under averaged conditions ($\epsilon_i^0$). This is analogous to relative non-linearity in competition, which means the target species experiences different competitive effects from the neighboring species through time. Similarly, the third decomposition term, $\epsilon_i^\lambda$, is the main effect of the environmental variation on seed production (per capita intrinsic growth rates). This is calculated as the low-density growth rate of *L. conjugens* when $\lambda_t$ varies through time according to our statistical model output, but $\alpha_k$ are held at their averaged value minus $\epsilon_i^0$. This mechanism is analogous to relative non-linearity in seed production, which ecologically equates to favorable years having a stronger positive effect size on seed production than the magnitude of the negative effect in unfavorable years. Finally, the last decomposition term, $\epsilon_i^{(\alpha\lambda)}$, is the interaction effect between variability in α and λ and accounts for environmental fluctuations simultaneously affecting competition and fecundity that are not accounted for by each main effect (i.e., $\epsilon_i^{(\alpha\lambda)} = r_i - [\epsilon_i^0 + \epsilon_i^\alpha + \epsilon_i^\lambda]$). We calculate the above decomposition separately in the reference versus constructed pools to compare how average conditions versus temporally-dependent mechanisms alter the low density growth rate of *L. conjugens* in each pool type.

*Simulation of exotic grass removal:*

To test the effects of exotic grass removal on *L. conjugens*, we simulated an experimental manipulation of the percentage of exotic grass cover on a yearly basis. We simulated the population dynamics of *L. conjugens* with 0, 50, and 75% reduction of exotic grass cover each year from 2001 to 2015, using frequency and abundance data and parameter estimates from above, and estimated the expected abundances of *L. conjugens*. We then calculated the average low density growth rate of *L. conjugens* for each treatment given the simulated management reduction in exotic grasses.

## Results

### *Diagnosing population trajectories with growth rates:*

We compared the time series of observed annual density to model-derived mean per capita intrinsic growth rate ($\lambda_t$) and low density growth rate ($r_t$) of *L. conjugens* to assess restoration trajectories. The observed 306 mean density in constructed pools increased from 2002 to 2007 (158 [se = 36]/$m^2$ to 251[se = 55]/$m^2$), exceeding that of reference pools (103 [se = 57]/$m^2$ to 221 [se = 75]/$m^2$) (Figure 6.2a). However, as exotic grass cover increased since 2007 in both reference and constructed pools (Fig. S6.5a), and a multi-year drought hit from 2011 to 2015 (Fig. S6.5b), *L. conjugens* populations in constructed pools drastically declined from 2007 to 2015, while reference populations remained stable (Figure 6.2a). The mean per capita intrinsic growth rate in constructed pools was only 2.9% of that in reference pools in 2001, which signaled divergence of population trajectories from the start of the restoration effort (Figure 6.2b). The low-density growth rate in constructed pools was also lower than that in reference pools throughout the time series (Figure 6.2c). Overall, the average low-density growth rate ($r$) showed long-term persistence of *L. conjugens* in reference pools (0.47; 95% CI: 0.32 to 0.62) but eventual competitive exclusion in constructed pools (-0.42; 95% CI: -0.46 to -0.39).

### *Partitioning of persistence mechanisms:*

To determine if environmentally-driven effects in intrinsic seed production, competitive environments, or their interaction most strongly contribute to differences in persistence between reference and constructed pools, we decomposed the average low density growth rate of *L. conjugens*. The difference in population trajectories was primarily driven by a strong destabilizing effect of environmental variation on intrinsic growth rate ($\epsilon_i^{\lambda}$) in constructed pools. In reference pools, the positive effects of fluctuation-independent mechanisms (e.g., average fitness differences; $\epsilon_i^{0}$) and relative non-linearity in competition ($\epsilon_i^{\alpha}$) were large enough to off-set the negative effect of environmental variation on intrinsic growth rate ($\epsilon_i^{\lambda}$) for *L. conjugens*, allowing persistence in the community (Figure 3a). In constructed pools, the positive effect of relative nonlinearity in competition ($\epsilon_i^{\alpha}$) was less than the negative effect of environmental variation on intrinsic growth rate ($\epsilon_i^{\lambda}$), such that *L. conjugens* was excluded from the community in the simulations (Figure 6.3b). The interactive effect of the environment on competition and intrinsic growth rate ($\epsilon_i^{\alpha\lambda}$) was negligible in both reference and constructed pools. More generally, these results highlight how the response of *L. conjugens* to temporal environmental variation is altered in constructed pools versus their reference counterparts.

*Intermediate pool depth and exotic grass removal improve persistence:*

None of the seeding treatments improved long-term persistence of *L. conjugens*, even though LACO 3x seeding treatment initially increased density more than other treatments (Fig. S6.6). Average low-density growth rate was negative across all seeding treatments: LACO 1x (-0.58; 95% CI: -0.79 to -0.36), LACO 3x (-0.68; 95% CI: -0.84 to -0.52), Group A-Group 340 B (-0.90; 95% CI: -1.07 to -0.76), and Group B-Group A (-0.48; 95% CI: -0.64 to -0.32). Larger constructed pools showed higher average low-density growth rate of *L. conjugens* than smaller pools but they were all negative (Fig. S6.7): small (-0.57; 95% CI: -0.68 to -0.45), medium (-0.40; 95% CI: -0.49 to -0.32), and large (-0.20; 95% CI: -0.285 to -0.12). Meanwhile, pool depths, improved densities and growth rates of *L. conjugens* (Fig. S6.8). Specifically, L. conjugens in intermediate depth pools persisted – positive average low-density growth rate – (0.84; 95% CI: 0.82 to 0.87), while those in shallow (-0.68; 95% CI: -1.40 to -0.07) and deep (-0.57; 95% CI: -0.69 to -0.44) pools did not (Figure 6.4a, b).

Exotic grass cover in constructed pools increased over time regardless of the seeding treatment, pool size, or pool depth (Fig. S6.9). Mean annual exotic grass cover negatively affected the mean annual density of *L. conjugens*, but not alter the intrinsic growth rate or the low-density growth rate (Fig. S6.10). Given the stabilizing effect of variation in competition in constructed pools (Figure 6.3b), we simulated the effects of exotic grass removal on target species, and we found that on average, 50% exotic grass removal increased predicted mean abundance of *L. conjugens* by 2.1 fold compared to no removal, and 75% exotic grass removal increased mean abundance by 4.4 fold (Figure 6.4c). The effect of exotic grass removal was non-linear and diminishing over time; the effect sizes were higher in the first 5 years since the first seeding treatment in 1999 than in later years (after 2004) (Table S6.1). Moreover, the threshold at which the average low density growth rate of *L. conjugens* transitions from negative (local extinction) to positive (persistence) was around 50% exotic grass removal: -0.42 (95% CI: -0.46 to -0.39) in the no removal scenario, 0.01 (95% CI: -0.01 to 0.04) in the 50% removal scenario, and 0.38 (95% CI: 0.35 to 0.41) in the 75% removal scenario (Figure 6.4d).

## Discussion

Here we re-purposed analytical tools from modern coexistence theory (MCT) to diagnose restoration trajectories in a highly invaded landscape with large environmental fluctuations.

This approach enabled us to not only detect restoration failure quickly, but understand why the target species did not persist and identify interventions that would increase restoration success. Model-derived per capita intrinsic growth rates (i.e. density-independent seed production) and low-density growth rates (i.e. when accounting for interspecific interactions) were more reliable metrics of long-term trends than stem abundance, particularly in the initial years following seed addition. The average low-density growth rates indicate that the restored populations will eventually go extinct, while the reference populations will persist. The difference was explained by a stronger negative effect of environmental variability on per capita intrinsic growth rates than a positive effect of varying competitive environments in restored populations compared to reference populations. While neither seeding treatment nor pool size changed the restoration trajectories, pool depth, especially intermediate pool depth, supported the long-term persistence of target species. Furthermore, our experimental simulations showed that the effect of exotic grass removal is variable over time but can increase the average low-density growth rate of restored populations, even switching from predicted local extinction to persistence.

Assessing restoration success is complex when restoration activities like seed addition can overcome dispersal limitation and artificially increase the abundance of target species. In our study, we used the reference populations of *L. conjugens* to set expectations for restored populations. At a glance, restoration looked successful because the mean abundance in constructed pools was higher than that in reference pools in the first eight years since the seed addition in 1999 (Figure 6.2a). However, by using annual per capita intrinsic growth rates and annual low density growth rates as indicators, we found that restored populations lagged behind reference populations from the start. In particular, the annual per capita intrinsic growth rates in constructed pools diverged from those of reference pools as early as the first year (2000; Figure

6.2b). In other words, the initial surge in mean abundance in constructed pools was a temporary seed addition effect. As such, incorporating demographic growth rates as additional metrics of restoration success may aid in detecting problems much earlier than relying on abundances.

Modern coexistence theory has highlighted the importance of environmental variability for niche partitioning, which promotes species coexistence (Bimler et al., 2018; Mat´ıas et al., 2018). In California annual grassland systems, temporal rainfall variability is particularly important to maintain 398 forb species (Hallett et al., 2019). In our study, we expected *L. conjugens* to intrinsically favor environmental variability because it has adapted to fluctuating water levels of vernal pools already. However, by partitioning the contribution of environmental variability to average low density growth rates, we found that environmental variation had a negative effect on per capita intrinsic growth rate of *L. conjugens*. In other words, the bad years were worse than the good years were good for *L. conjugens*, and the abiotic environment of constructed pools was not as suitable as the reference pools (Faist and Beals, 2018). This highlights the need to consider redesigning the constructed pools for future vernal pool restoration projects. Specifically, we found that intermediate pool depths (3.8 to 8.0 cm) is an important feature to support long-term persistence of *L. conjugens*. This is likely because shallower pools favored exotic grasses, while the deepest pools favored other native forbs. A previous study has also highlighted that the pool depth effect was stronger than the pool size effect, and that there was no strong correlation between pool size and depth, even though that was the intention in the experimental design, due to heterogeneity across the site Javornik and Collinge (2016).

Variable competition with neighboring individuals may, however, maintain species coexistence (Chesson, 2000). We found that relative non-linearity in competition was the dominant stabilizing mechanism, and it was stronger in constructed pools than in reference pools (Figure 6.3). This result parallels empirical evidence that *L. conjugens* has a competitive release from the exotic grasses in wet early-season rain years, because it can maintain an immature state in inundated pools, while exotic grasses cannot (Javornik and Collinge, 2016). Since the biological reason for *L. conjugens* persistence is a competitive release under some environmental conditions as opposed to intrinsically favoring those conditions, reducing the competitive effect from competitors should promote persistence of *L. conjugens*. In practice, we may not be able to change the pools once they are constructed, but we can manipulate the competitive neighborhoods. Our results underscore the importance of partitioning multiple mechanisms of persistence to identify key targets for management interventions.

In adaptive management of restoration sites, one of the challenges is knowing how much and when active management is needed given limited time and resources (Williams, 2011). Building on our partitioning of low density growth rates showing a positive effect of competitive variability, our experimental simulation results showed a non-linear effect of exotic grass removal. Specifically, exotic grass removal was more effective when *L. conjugens* populations were increasing in early years than later years (Figure 6.4a). This suggests that management action should be taken proactively in the target species' favorable years to further reduce the competition they experience, which is contrary to the common practice of managing in reaction to declining restored populations (Williams, 2011). More importantly, positive average low density growth rates with exotic grasses removal indicated that the long-term persistence of restored populations is possible, even if the restored pools were not an optimal depth (Figure 6.4b). From a restoration perspective, these data-driven simulations can be helpful to inform adaptive management and set expectations for how active management will alter restoration outcomes.

Our study takes a novel approach to integrate methods from MCT and long-term monitoring data from a restoration project to assess restoration success. By leveraging the long-term data set, we were able to determine whether the focal species can persist over time and why. While long-term data is beneficial for assessing long-term persistence, it is not critical for discerning initial trajectories. The parameterization of the demographic model can proceed one time step at a time (in our case, the model was fit each year); this does not require a long-term data set. Even with few years of monitoring data, it is possible to fit the demographic model and assess initial restoration trajectories using annual low density growth rates. Moreover, lessons learned from this approach can be applied to other restoration goals across ecosystems. For instance, we focus here on conservation of a single species, but some restoration efforts may focus on removing a key invasive species or restoring a diverse community with multiple focal species (Perring et al., 2015). Although data collection requirements may increase and the demographic models may get more complicated in more specious communities, this should become more feasible as new methods for analyzing multi-species coexistence emerge (Saavedra et al., 2017).

Restoration in a time of rapid global change faces challenges where the baseline environmental conditions shift and invasive species can drastically disrupt the community's stability (Hobbs and Cramer, 2008). Using the framework of MCT in diagnosing restoration trajectories is particularly helpful when restoring species in a variable and competitive environment because we can understand what mechanisms are driving species persistence, which can point us to what we can do to change the restoration trajectories. Broader application of

MCT in ecological restoration has the potential to inform and update theoretical predictions, explain empirical dynamics, and identify management actions for desirable restoration outcomes.

## Figures

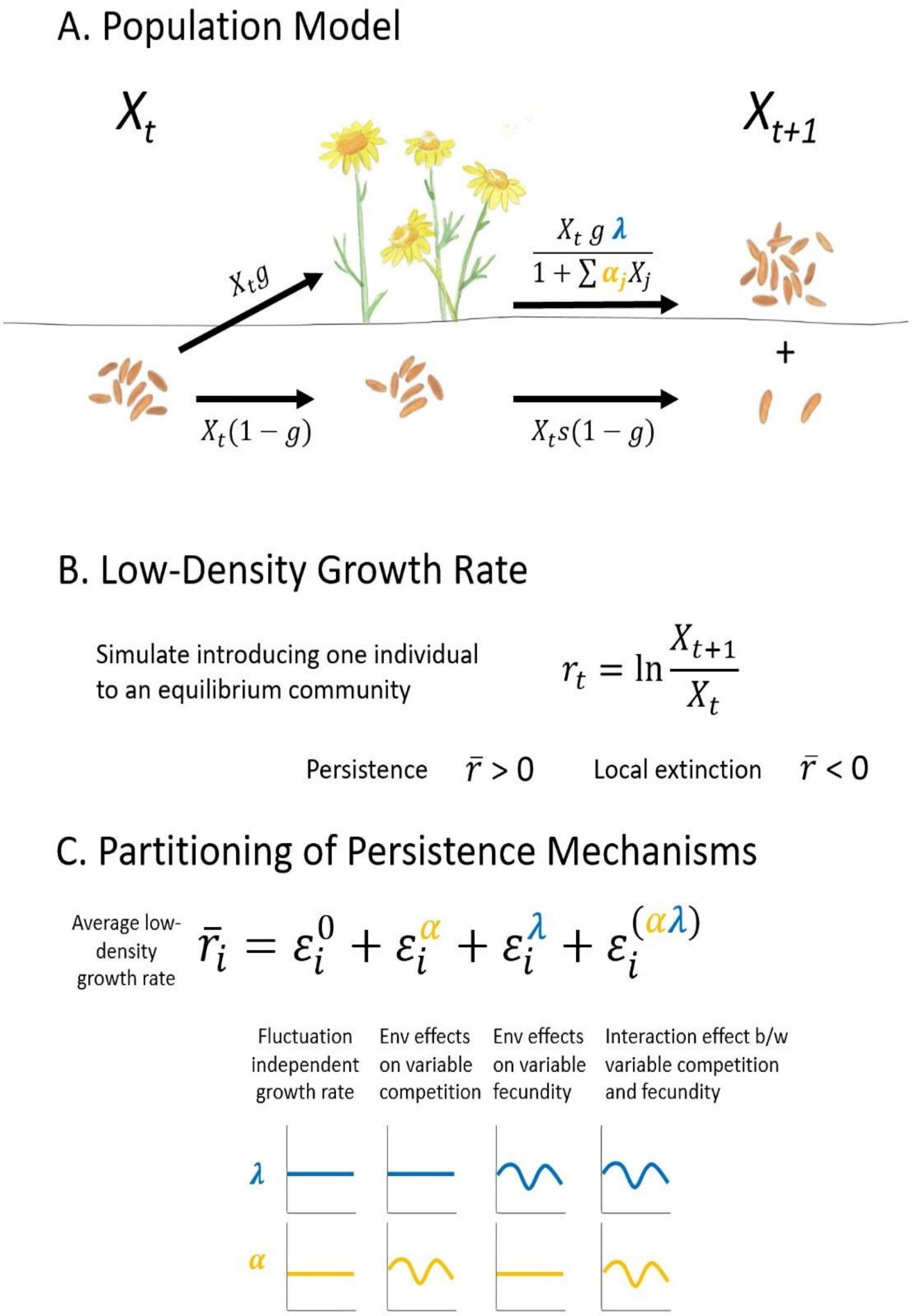
A. Population Model
$X_t$
$X_{t+1}$
$X_t g$
$\frac{X_t g \lambda}{1 + \sum \alpha_j X_j}$
+
$X_t(1-g)$
$X_t s(1-g)$
B. Low-Density Growth Rate
Simulate introducing one individual to an equilibrium community
$r_t = \ln \frac{X_{t+1}}{X_t}$
Persistence $\bar{r} > 0$
Local extinction $\bar{r} < 0$
C. Partitioning of Persistence Mechanisms
Average low-density growth rate
$\bar{r}_i = \varepsilon_i^0 + \varepsilon_i^\alpha + \varepsilon_i^\lambda + \varepsilon_i^{(\alpha\lambda)}$
Fluctuation independent growth rate
Env effects on variable competition
Env effects on variable fecundity
Interaction effect b/w variable competition and fecundity
$\lambda$
$\alpha$

**Figure 6.1.** Visual representation of the (A) population model, (B) low-density growth rate, and (C) partitioning of persistence mechanisms. (A) Beverton-Holt equation was used to model the life-cycle of the focal species, *Lasthenia conjugens*. The population prior to winter rainfall ($X_t$) is captured in the number of seeds in a pool. Following sufficient rain, a fraction of the seeds germinate ($g$) while the remainder do not (1-$g$). Those seeds that germinate grow to produce new seeds that depend on the maximum fecundity in the environmental conditions encountered that year ($\lambda$), which is reduced competition (denominator of the equation at top right, with the sum over all species in the community). The population at the outset of the next year ($X_{t+1}$) is the sum of the new seeds produced ($X_t g\lambda/1+\sum(\alpha_j X_j)$) plus the ungerminated seeds that survive ($X_t s(1 - g)$). Although the total population at time $t$ is given by the total number of seeds prior to germination, population size is estimated from the aboveground community (germinated plants). When germination rates are known, the ungerminated number of seeds is calculated as: (1-$g$)plants/$g$, where plants is the observed number of stems in a vernal pool. This calculation follows from our estimate of the population size (plants/$g$), and is necessary because ungerminated seeds cannot be sampled directly without disturbing the vernal pools. (B) To calculate the low-density growth rate ($r_t = \ln(N_{t+1}/N_t)$), introduction of a single individual into the equilibrium resident community is simulated for each year. The average low-density growth rate across years ($\bar{r}$) predicts persistence ($\bar{r} > 0$) or local extinction ($\bar{r} < 0$) of the focal species. (C) The average low-density growth rate is partitioned into four persistence mechanisms following the decomposition framework from Ellner et al. (2019). Fluctuation independent growth rate ($\epsilon_i^0$) is calculated by removing all variability in lambda (density-independent growth rates) and alpha (competition). Environmental effects on variable competition ($\epsilon_i^\alpha$) is calculated by subtracting $\epsilon_i^0$ from growth rate with varying alpha but constant lambda. Environmental effects on variable fecundity ($\epsilon_i^\lambda$) is calculated by subtracting $\epsilon_i^0$ from growth rate with varying lambda but constant alpha. The interaction effect ($\epsilon_i^{\alpha\lambda}$) is calculated by subtracting $\epsilon_i^0$ from growth rate with varying alpha but constant $\epsilon_i^0$, $\epsilon_i^\alpha$, and$\epsilon_i^\lambda$ from growth rate with varying lambda and alpha.

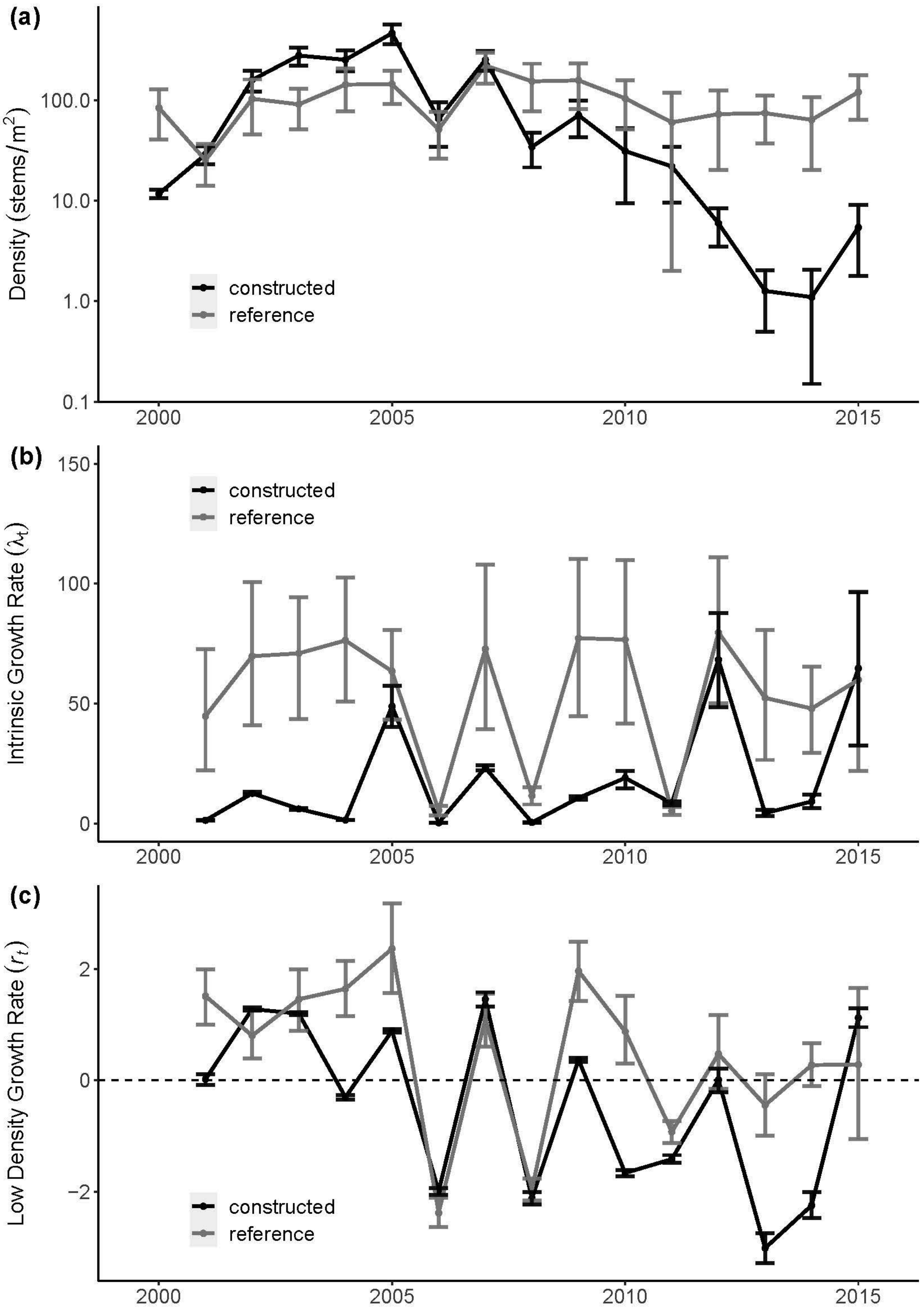
(a)
Density (stems/m$^2$)
100.0
10.0
1.0
0.1
constructed
reference
2000
2005
2010
2015
(b)
Intrinsic Growth Rate ($\lambda_t$)
150
100
50
0
constructed
reference
2000
2005
2010
2015
(c)
Low Density Growth Rate ($r_t$)
2
0
−2
constructed
reference
2000
2005
2010
2015

**Figure 6.2.** Restoration trajectories of *Lasthenia conjugens* are dynamic over time. Restoration success can be assessed by three metrics: (a) *Lasthenia conjugens* density (stems/m$^2$; note logarithmic y-axis), (b) posterior estimates of per capita intrinsic growth rate ($\lambda_t$, average number of seeds produced in the absence of competition), and (c) low-density growth rate ($r_t = \ln(N_{t+1}/N_t)$) in constructed (black) and reference (grey) pools. The error bars represent 95% credible intervals. A positive low-density growth rate predicts persistence, while a negative one indicates eventual competitive exclusion, and thus loss from the pools, even though $\lambda_t$ may be positive. For all three panels, we used data from 142 constructed pools from 2000-2015, 7 reference pools from 2000-2015, and 2 additional reference pools from 2002-2015 to maximize the use of data.

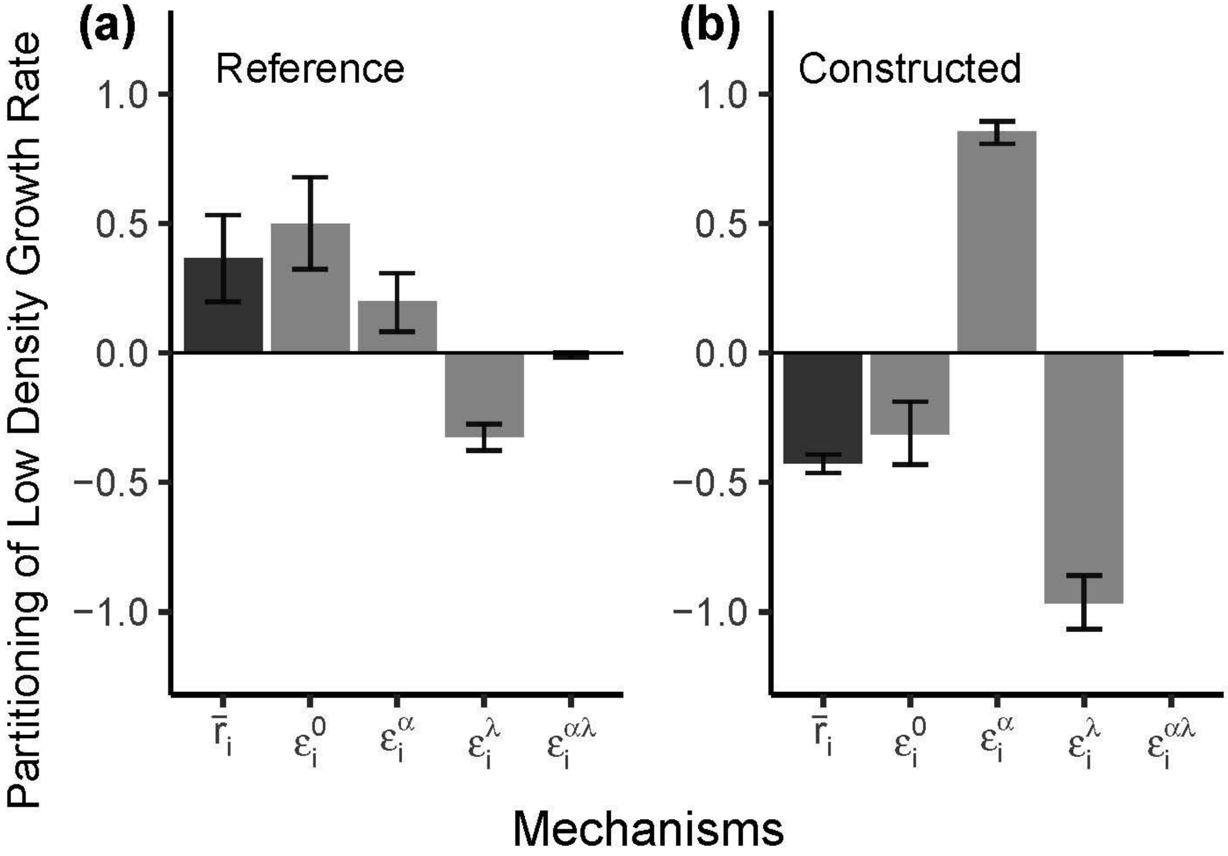

**Figure 6.3.** *Lasthenia conjugens* is predicted to persist in reference pools (a), but not in constructed pools (b), due to the effect of variation in $\lambda_t$. Partitioning of average low density growth rate ($\bar{r}$; dark grey) into contributions from different aspects of the species' environment on competition ($\epsilon_i^{\alpha}$), seed production ($\epsilon_i^{\lambda}$), and their combined interactive effect ($\epsilon_i^{\alpha\lambda}$) shown in light grey. The error bars represent standard errors from means. The interaction effects are present but too small to be visible.

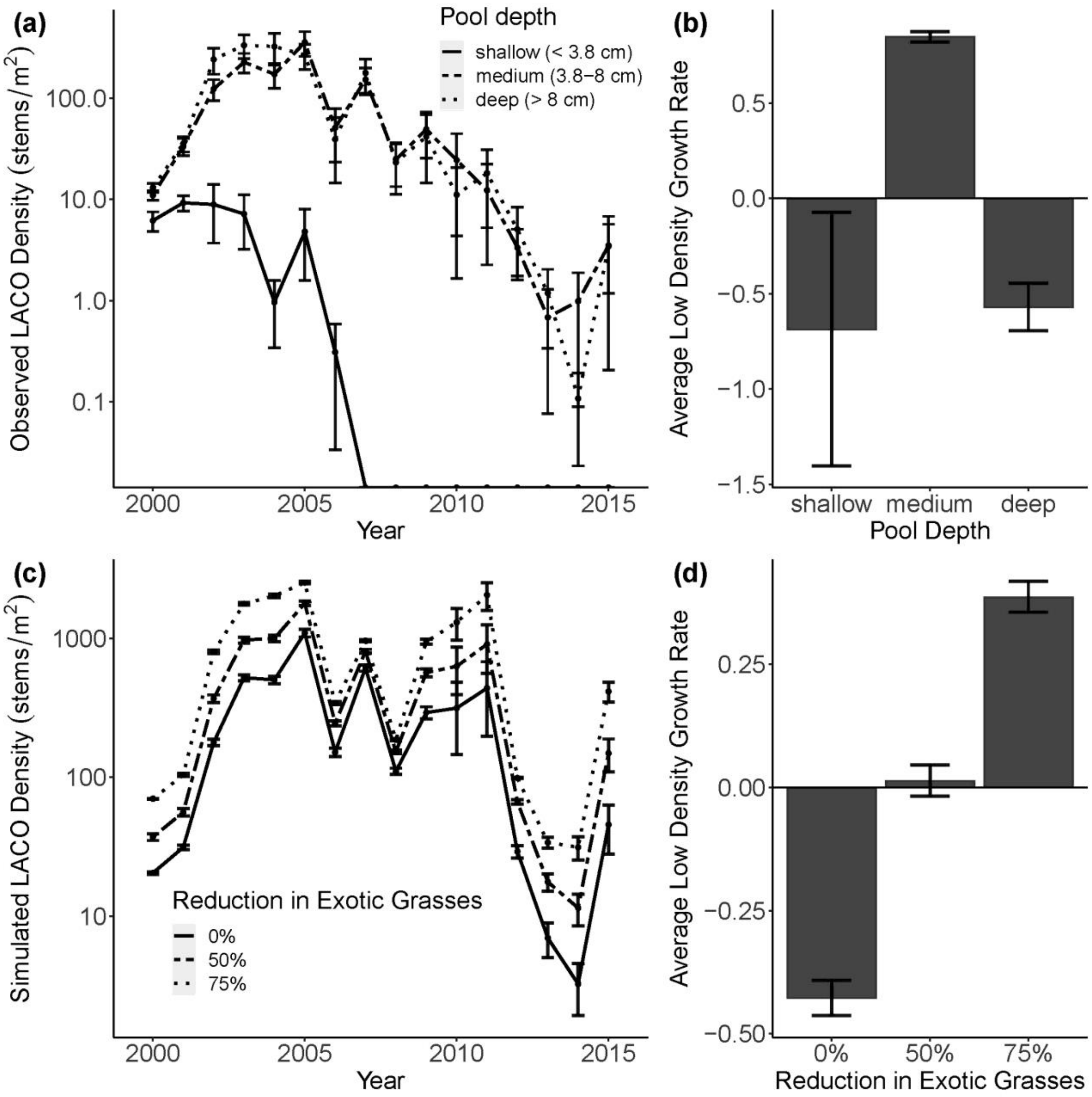

**Figure 6.4.** Pool depth and exotic grass reduction influence *Lasthenia conjugens*'s persistence in constructed pools. Top two panels are observed effects of pool depth on (a) density and (b) average low-density growth rate. Depth classes are determined by 25th and 75th percentiles of annual maximum pool depth averaged across years: shallow (< 3.8 cm; < 25th percentile; dotted), medium (3.8 - 8.0 cm; 25-75th percentile; two-dashed), deep (> 8.0 cm; > 75$^{\text{th}}$ percentile; solid). Bottom two panels are simulated effects of exotic grass reduction on (c) density and (d) average low-density growth rate. Simulated treatments include 0% (solid), 5% (two-dashed), and 75% (dotted) exotic grass reduction. Error bars in panel a and c represent standard error, those in panel b and d represent 95% credible intervals. The 95% credible interval for shallow pools was large because so few pools had viable populations in 2006 and none since 2007. A positive average low-density growth rate predicts long-term persistence.

# CHAPTER VII

## CONCLUSIONS

Ecological restoration is an important tool for biodiversity conservation and sustainability of working landscapes. On rangelands across the western United States, successful implementation of restoration efforts are necessary to tackle the vicious annual grass-fire cycle and halt species extinctions from habitat loss and fragmentation. The adaptive capacity of restored populations can increase by harnessing the diversity in genetics – thereby functional traits – of seeds used in restoration. While restoration outcomes vary due to inherent environmental variability, climate change, and invasive species, understanding these relationships to species persistence can help ensure effective restoration outcomes in a rapidly changing world.

Over the course of my dissertation, I considered how current seeding practice affects the genetic diversity of native grass populations across a burned landscape (Chapter II), how an emerging seed sourcing strategy called climate-adjusted provenancing affects seedling recruitment under drought (Chapter III), how seedling traits can be plastic under variable environmental and competitive conditions (Chapter IV), how community-level functional diversity can buffer biomass productions against seasonal drought (Chapter V), and how restoration success can be assessed from nonlinear population trajectories.

In Chapter II, I surveyed the genetic structure and diversity of *Pseudoroegneria spicata* populations in the fire perimeter of Soda Fire. I found genetic differentiation of *P. spicata* populations based on geography, not post-fire seeding treatment, and found no homogenizing effect of seeding on the genetic diversity of *P. spicata* populations even accounting for distance from fire edge. These results provide confidence to land managers that the native cultivar seeds they chose to sow at large scale were not impacting the genetic diversity of wildland populations.

In Chapter III, I tested the effects of climate-adjusted provenancing strategy on seedling recruitment under variable rainfall conditions using a field common garden experiment. I found strong interannual variation in seedling recruitment: in one year, some provenances from warmer/drier sites had high emergence and subsequent seedling survival under moderate drought, but in another year, emergence was low across all provenances and rainfall conditions. Intraspecific variation in seedling traits such as thermal performance and water use efficiency explained the variation in strategies to cope with frost and drought. This research provides a proof-of-concept to climate-adjusted provenancing that has potential to improve restoration outcomes in future climatic conditions.

In Chapter IV, I examined the seedling trait plasticity of *Poa secunda* under water stress and cheatgrass competition by conducting a greenhouse experiment. I found that *P. secunda*'s seedling trait expressions and establishment rates under drought depended on the neighboring cheatgrass competition. Traits selected for drought tolerance such as high proportion of fine roots may trade-off with traits selected for cheatgrass resistance, which complicates the seed selection process in the Great Basin. Furthermore, there was no evidence of adaptive plasticity at least for drought tolerance. As trait-based approaches are increasing used for seed selection in the Great Basin, these results highlight that traits can be plastic and that plasticity should be incorporated when interpreting the predictive power of traits for multiple stressors.

In Chapter V, I explored the effects of seasonal drought on above- and belowground functional diversity and biomass in California grasslands. Plant communities with mixed functional groups resulted in greater biomass than either the grass- or forb-dominant communities. Functional diversity buffered biomass production by enabling shifts in above- and belowground functional traits across variable drought scenarios. Linking functional trait diversity to both above- and largely neglected belowground biomass improves predictions of plant community responses to more variable rainfall patterns in the future.

In Chapter VI, I demonstrated how to apply principles and methods of modern coexistence theory, a largely theoretical framework, to improve the assessment of restoration trajectories and guide restoration actions. I showed that initial, short-term appearances of restoration success from population abundances is misleading, and model-derived low-density growth rates are more reliable metrics to assess long-term trends than abundances. By partitioning the low-density growth rate, I identified that the bad years were worse than the good years were good for the target species, and the abiotic environment of restoration sites were not as suitable as the reference sites. Simulation of annual grass removal showed that long-term persistence of restored populations is possible even if the restored sites were not an optimal condition. From a restoration perspective, these data-driven approaches to restoration assessment can be helpful for informing adaptive management and setting expectations for how active management will alter restoration outcomes.

In conclusion, these results highlight the importance of considering genetic diversity, trait plasticity, and community-wide functional diversity and their complex interactions with environmental variability when planning, implementing, and assessing restoration. These results provide actionable recommendations for land managers and demonstrate forward-looking approaches to restoration on semi-arid rangelands. At the same time, they revealed many more research questions to be answered. For example, are the seeded populations connecting gene flow with fragmented remnant populations across burned landscapes? Besides climate of origin, what are the environmental predictors of seedling survival in warmer and drier conditions? What are the cascading effects of introducing nonlocal seeds to community and ecosystem level? How do land managers perceive seed sourcing strategies that take into account of climate change, and what are the barriers to adoption? How do projected increase in rainfall variability and warming in the future affect the predictability of long-term species persistence in a community? Future research that boldly address these questions is urgently needed to advance the science and practice of ecological restoration in a variable world.

## APPENDICES

## A. SUPPLEMENTAL MATERIALS FOR CHAPTER II

**Table S2.1.** ANOVA of genetic diversity metrics against area, treatments, and the interaction effect of area and treatment. Treatments: Burned-seeded, burned-unseeded, unburned-unseeded. Area: Rockville, Salmon, and West (see Fig. 1). Ae = effective allelic richness, Ho = observed heterozygosity, He = expected heterozygosity. Significant p-values ($p < 0.01$) are bolded.

| Variable | Tukey pairwise comparisons | Ae | | Ho | | He | |
|---|---|---|---|---|---|---|---|
| | | F | p | F | p | F | p |
| Area | | F2, 266.03 = 65.79 | **<0.001** | F2, 10.08 = 104.95 | **<0.001** | F2, 5.74 = 51.73 | **<0.001** |
| | Rockville-Salmon | | **<0.001** | | **<0.001** | | **<0.001** |
| | Rockville-West | | **<0.001** | | **<0.001** | | **<0.001** |
| | Salmon-West | | 0.19 | | **0.003** | | **0.001** |
| Treatment | | F2, 6.11 = 1.51 | 0.22 | F2, 0.04 = 0.39 | 0.67 | F2, 0.08 = 0.79 | 0.45 |
| Treatment: Area | | F4, 1.58 = 0.19 | 0.94 | F4,0.09 = 0.50 | 0.73 | F4, 0.08 = 0.36 | 0.83 |

**Table S2.2.** General linear regression models of genetic diversity metrics against distance from the nearest fire edge. Treatments: Burned-seeded, burned-unseeded, unburned-unseeded. Area: Rockville, Salmon, and West (see Fig. 1). Ae = effective allelic richness, Ho = observed heterozygosity, He = expected heterozygosity.

| Diversity metric | Area | Burned-seeded | | Burned-unseeded | |
|---|---|---|---|---|---|
| Ae | Rockville | y = 0.0002 x + 3.59 | R2 = 0.01, p = 0.50 | y = -0.0005 x + 5.26 | R2 = 0.07, p = 0.15 |
| | Salmon | y = -0.00002 x + 2.12 | R2 = 0.0005, p = 0.86 | y = -0.0005 x + 5.26 | R2 = 0.01, p = 0.34 |
| | West | y = -0.00006 x + 2.49 | R2 = 0.005, p = 0.59 | y = 0.0001 x + 1.96 | R2 = 0.03, p = 0.24 |
| Ho | Rockville | y = -0.000006 x + 0.52 | R2 = 0.0007, p = 0.86 | y = -0.00004 x + 0.48 | R2 = 0.04, p = 0.27 |
| | Salmon | y = -0.00005 x + 0.15 | R2 = 0.001, p = 0.74 | y = -0.000004 x + 0.13 | R2 = 0.002, p = 0.68 |
| | West | y = -0.00003 x + 0.30 | R2 = 0.05, p = 0.08 | y = -0.00001 x + 0.25 | R2 = 0.003, p = 0.71 |
| He | Rockville | y = 0.00003 x + 0.58 | R2 = 0.03, p = 0.23 | y = -0.00005 x + 0.80 | R2 = 0.09, p = 0.09 |
| | Salmon | y = -0.000003 x + 0.36 | R2 = 0.0005, p = 0.86 | y = -0.00001 x + 0.43 | R2 = 0.02, p = 0.19 |
| | West | y = -0.000004 x + 0.49 | R2 = 0.0006, p = 0.85 | y = 0.00004 x + 0.38 | R2 = 0.06, p = 0.09 |

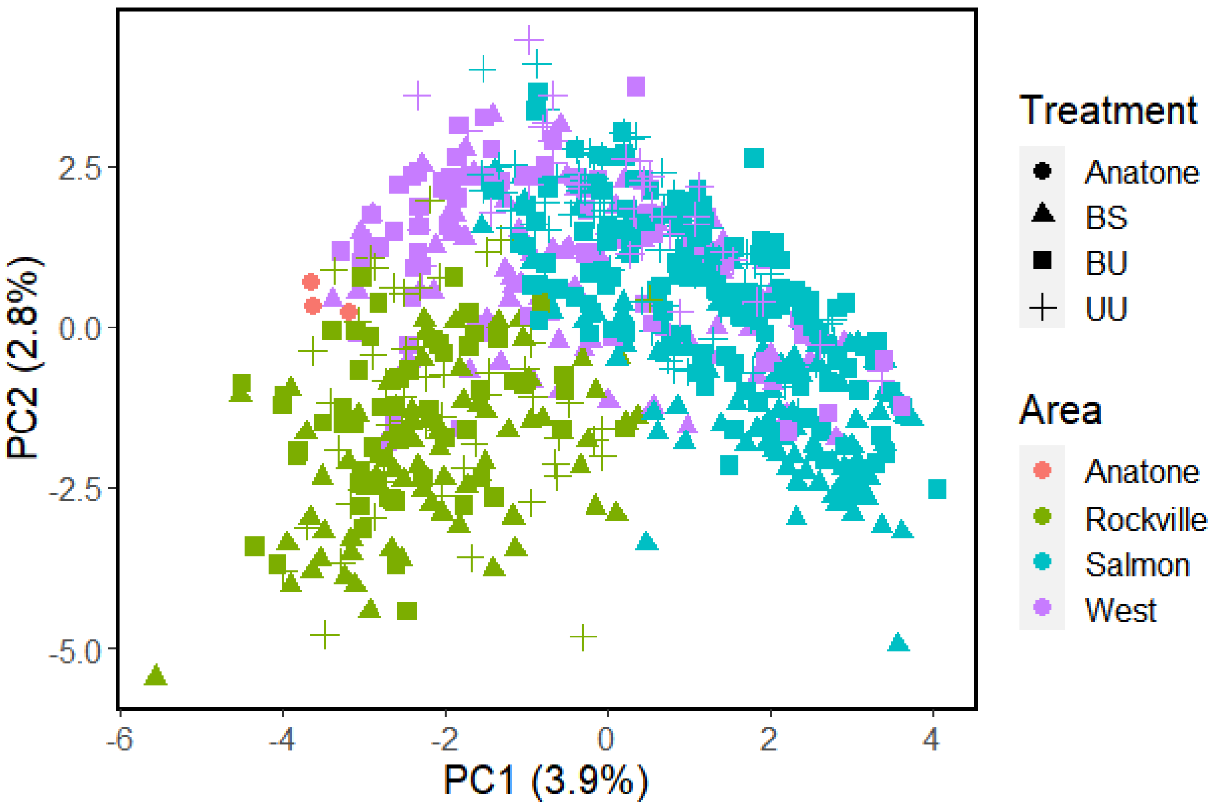

**Figure S2.1.** PCA of raw sequencing data with loci >50% no amplification (Locus 262, 307, and 396) removed.

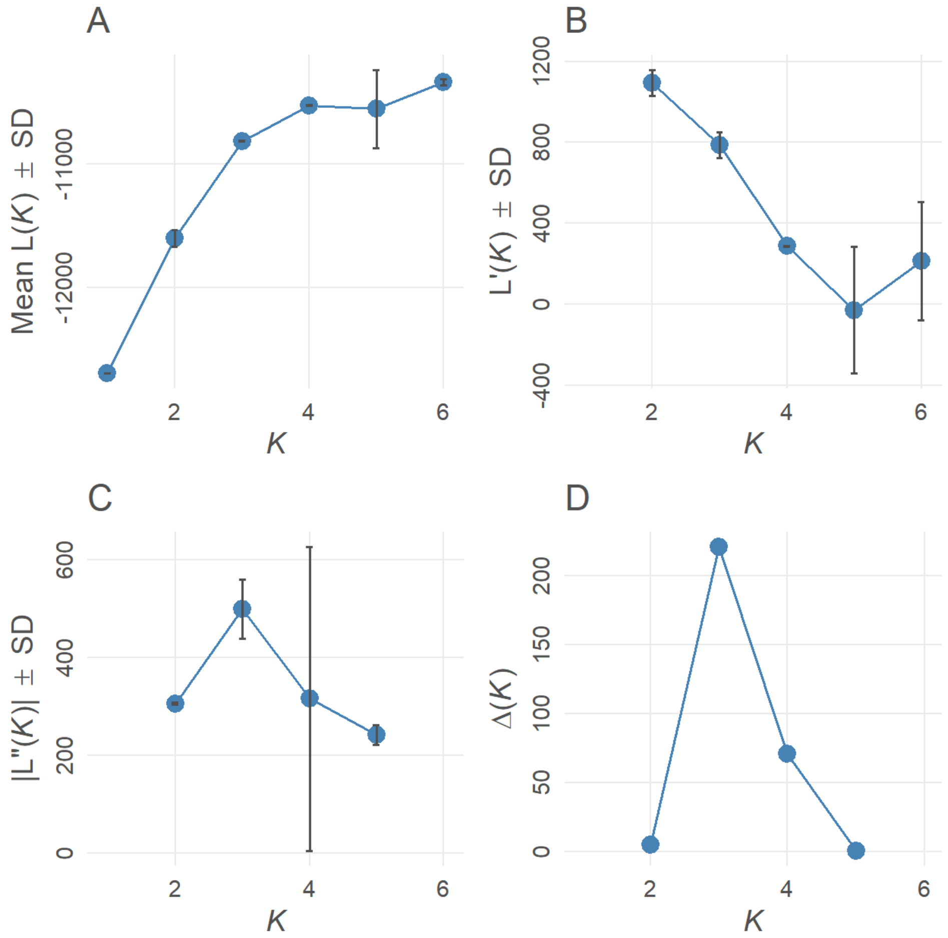

**Figure S2.2.** Evanno plots of STRUCTURE output for determining the optimal K value. A) Mean of estimated Ln prob of data, B) rate of change of the likelihood distribution (mean), C) absolute value of the 2nd order rate of change of the likelihood distribution (mean), D) delta K. Optimal number of clusters, K, identified based on where L(K) plateaus and delta K peaks, which is K = 3.

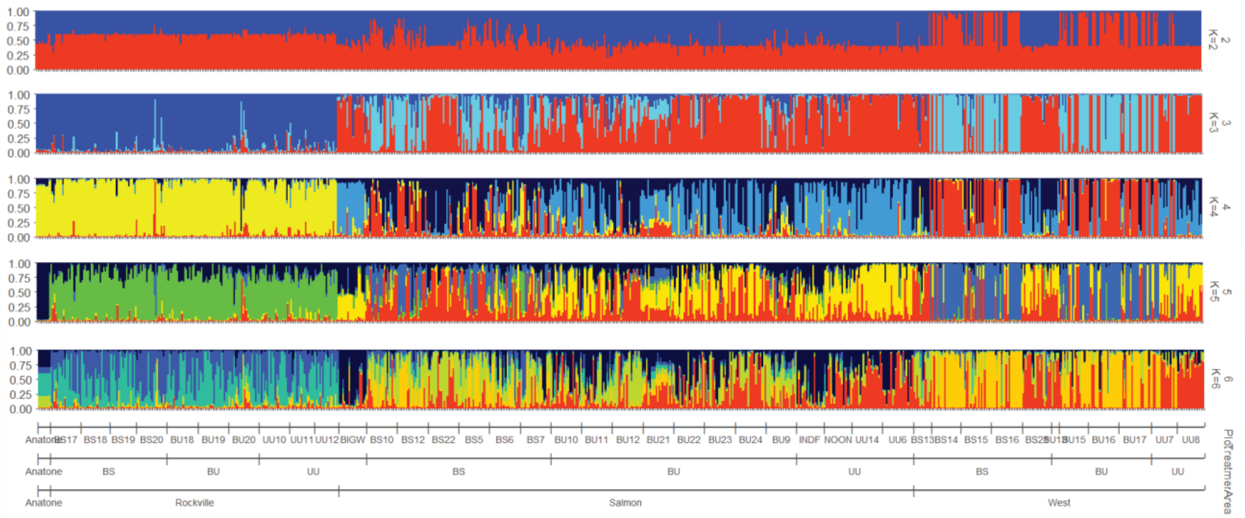

**Figure S2.3.** STRUCTURE bar plots of ancestry coefficients with K=2-6.

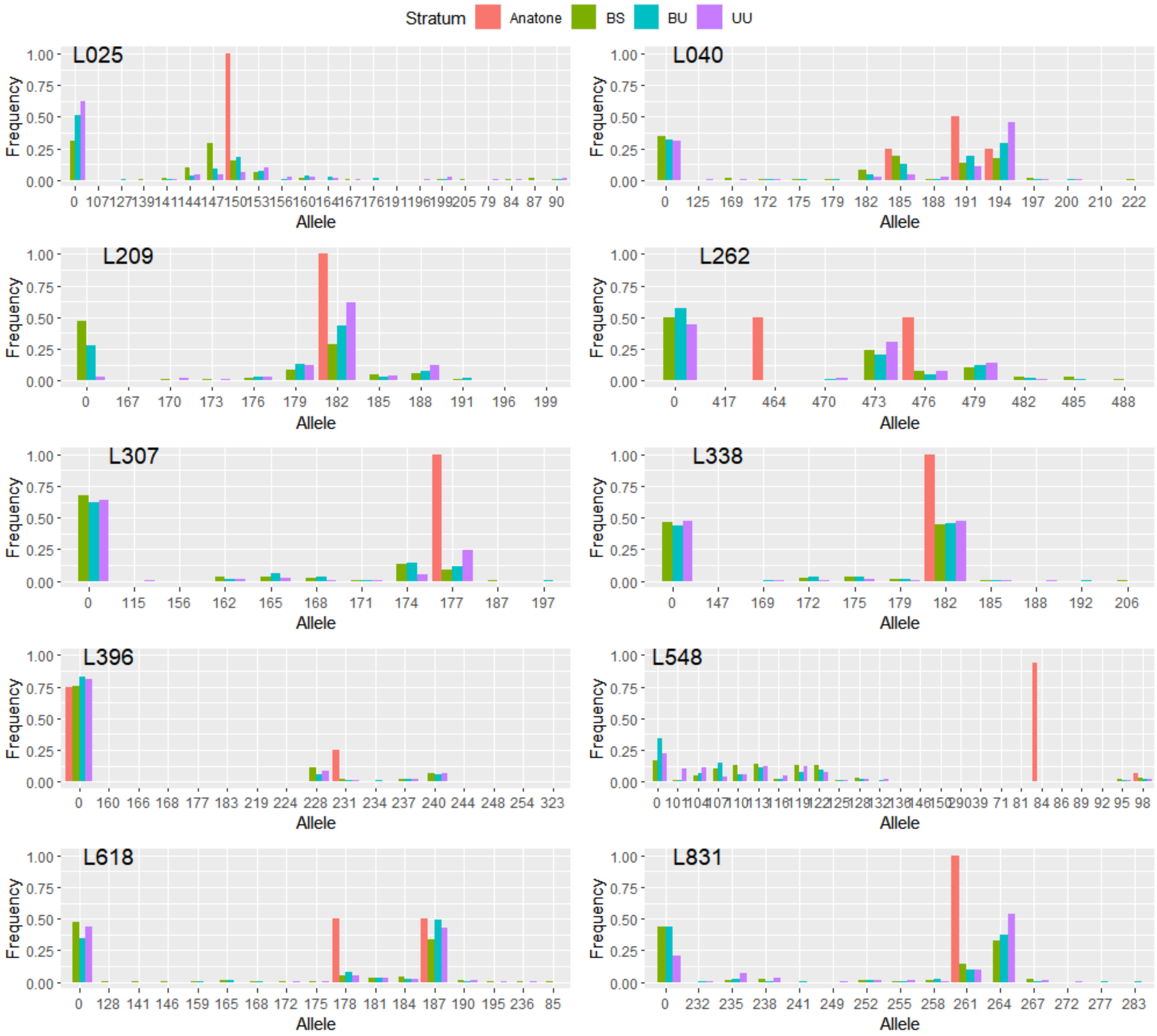

**Figure S2.4.** Allele frequencies for each locus. Anatone = red. Zero allele represents no peak/amplification.

# B. SUPPLEMENTAL MATERIALS FOR CHAPTER III

**Table S3.1.** Seed sources of *Elymus elymoides* seeds used in this study.

| Population | Collection | Location | Elevation | Weather station |
|---|---|---|---|---|
| Vale | Wild-collected | Malheur County, OR (44.0984, -117.8537) | 1,257 m | Vale, OR, USC00358797 (43.9814, -117.2439) |
| Little Sahara | Farm-grown | Millard County, UT (39.7167, -112.3388) | 1,566 m | Little Sahara Recreation Area, UT, USC00425138 (39.7268, -112.3069) |
| Roaring Springs | Farm-grown | Harney County, OR (42.5973, -119.0743) | 1,388 m | P Ranch Refuge, OR, USC00356853 (42.8080, -118.8778) |
| Norcross | Farm-grown | Klamath County, OR (41.9135, -122.0226) | 1,294 m | Klamath Falls 2 SSW, OR, USC00354506 (42.2008, -121.7814) |
| Elko ("Toe Jam Creek") | Farm-grown | Elko County, NV (40.9592, -115.4681) | 1,613 m | Elko Regional Airport, NV, USW00024121 (40.8288, -115.7886) |
| Susanville | Wild-collected | Lassen County, CA (40.4893, -120.1525) | 1,448 m | Susanville 2 SW, CA, USC00048702 (40.4167, -120.6631) |

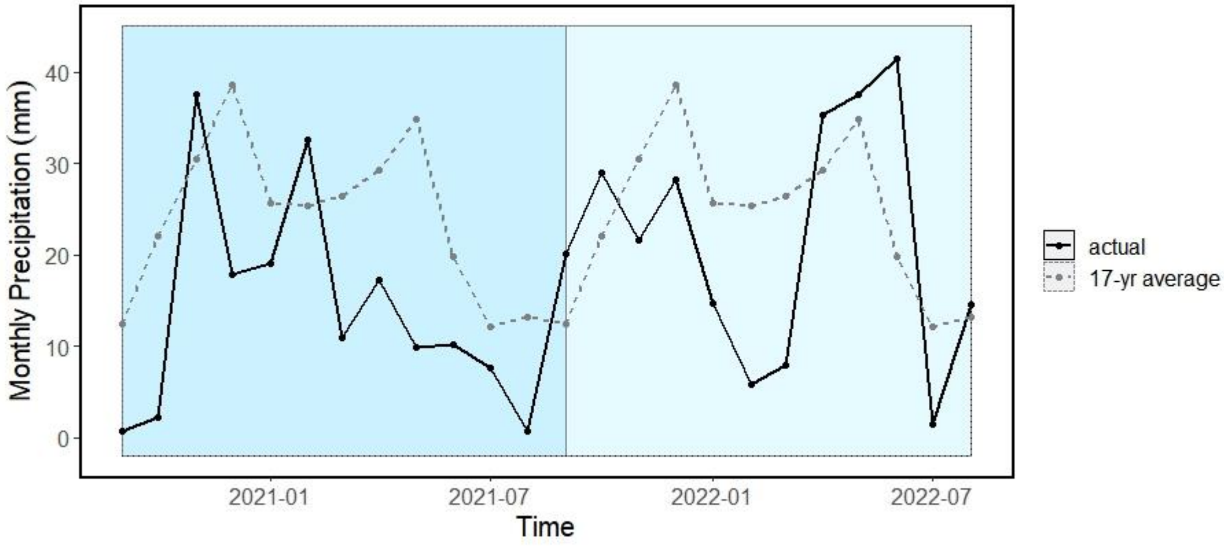

**Figure S3.1.** Monthly total precipitation (mm) at the Northern Great Basin Experimental Range in water year 2021-2022 (water year begins on October 1st and ends on September 30th of the following year). Dark blue and light blue boxes indicate water year 2021 and 2022, respectively. Solid line represents the actual rainfall and dashed line represents the average annual rainfall from water year 2004 to 2020. Data obtained from the NOAA weather station located at the experimental range (https://www.ncdc.noaa.gov/cdo-web/datasets/LCD/stations/WBAN:04128/detail).

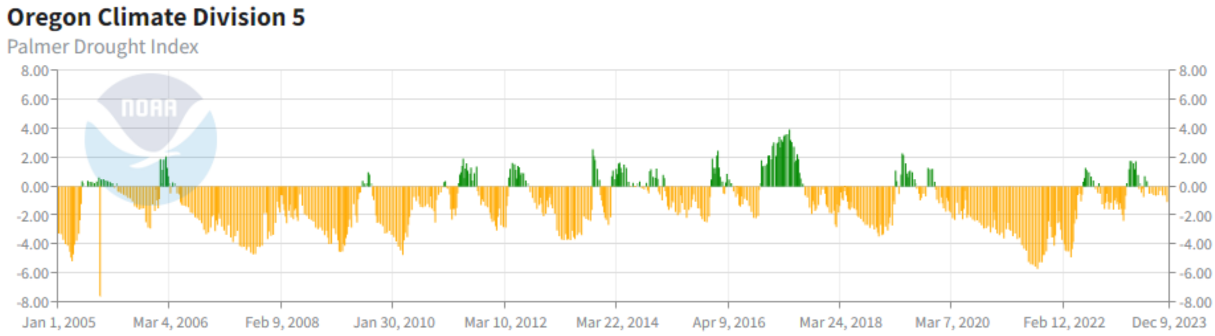

**Figure S3.2.** Historical timeseries of weekly Palmer Drought Severity Indices (PDSI) in Oregon High Plateau (Climate Division 5) from 2005 to 2023. The PDSI is a standardized index that ranges from -10 to 10, with negative values indicating drought conditions and positive values indicating wet conditions. A PDSI value between -2 to -4 represents moderate to severe drought, and less than -4 represents an extreme drought. Data source: NOAA National Centers for Environmental Information (https://www.ncei.noaa.gov/access/monitoring/weekly-palmers/time-series/3505).

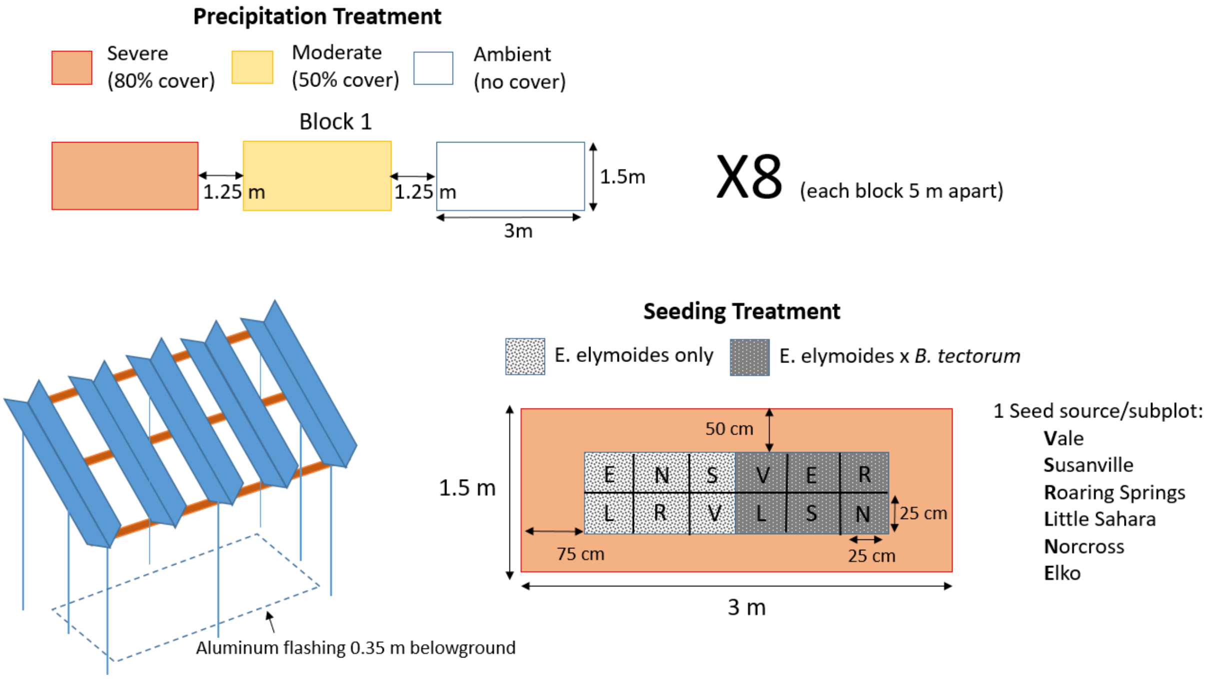

**Figure S3.3.** Experimental design of the common garden experiment at the Northern Great Basin Range, Oregon, USA. In a randomized block design, we installed 24 plots in eight blocks with seeding treatments nested within three rainfall treatments: severe drought, moderate drought, and ambient condition. Rainout shelters reduced rain by 80% for severe drought and 50% for moderate drought. No rainout shelters in the ambient plots. We divided each plot in twelve 25 x 25 cm subplots and sowed six sources of bottlebrush squirreltail (*Elymus elymoides*); half the plot (six subplots) was seeded with *E. elymoides* and cheatgrass (*Bromus tectorum*). Seeds were sown in two consecutive years, September 2020 and 2021.

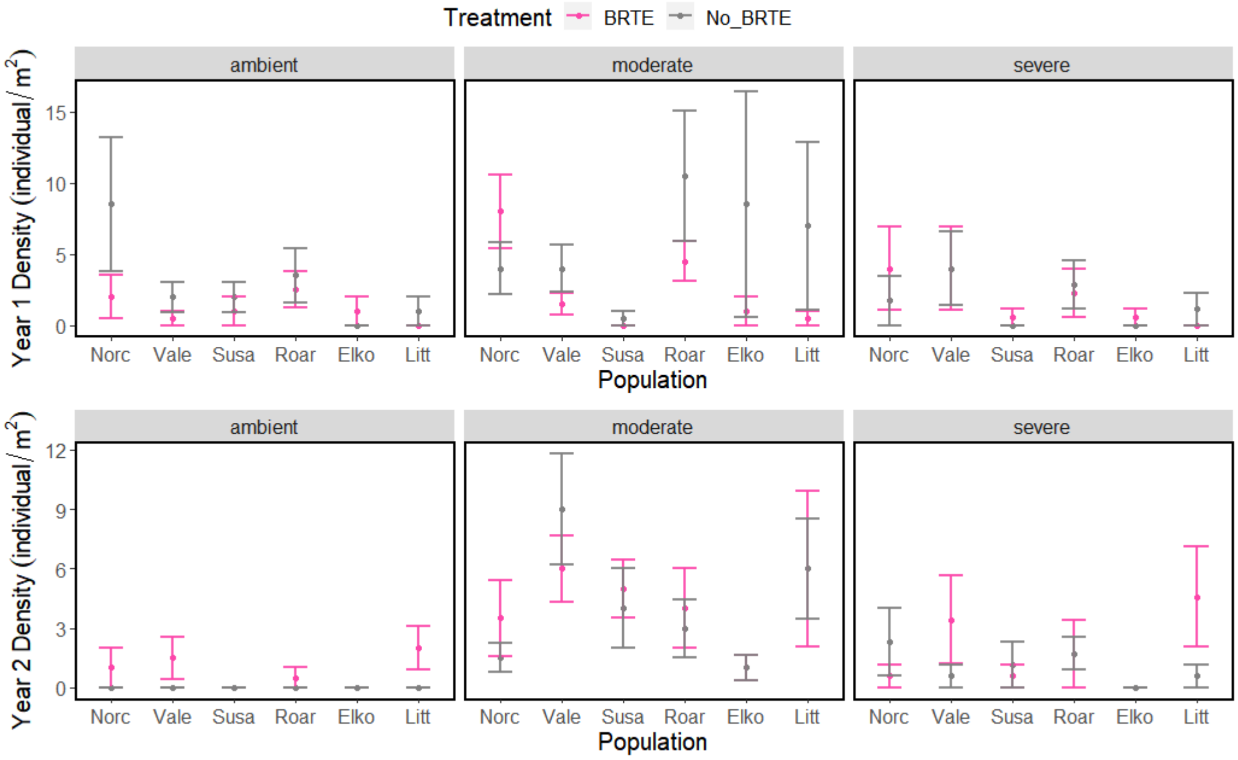

**Figure S3.4.** *Elymus elymoides* final densities of first-year and second-year seedlings of the 2020 cohort (mean ± SE). Panels from left to right show results from ambient, moderate drought, and severe drought rainfall treatments. Color of bars represent cheatgrass (*Bromus tectorum*) treatments: pink = with cheatgrass, grey = no cheatgrass.

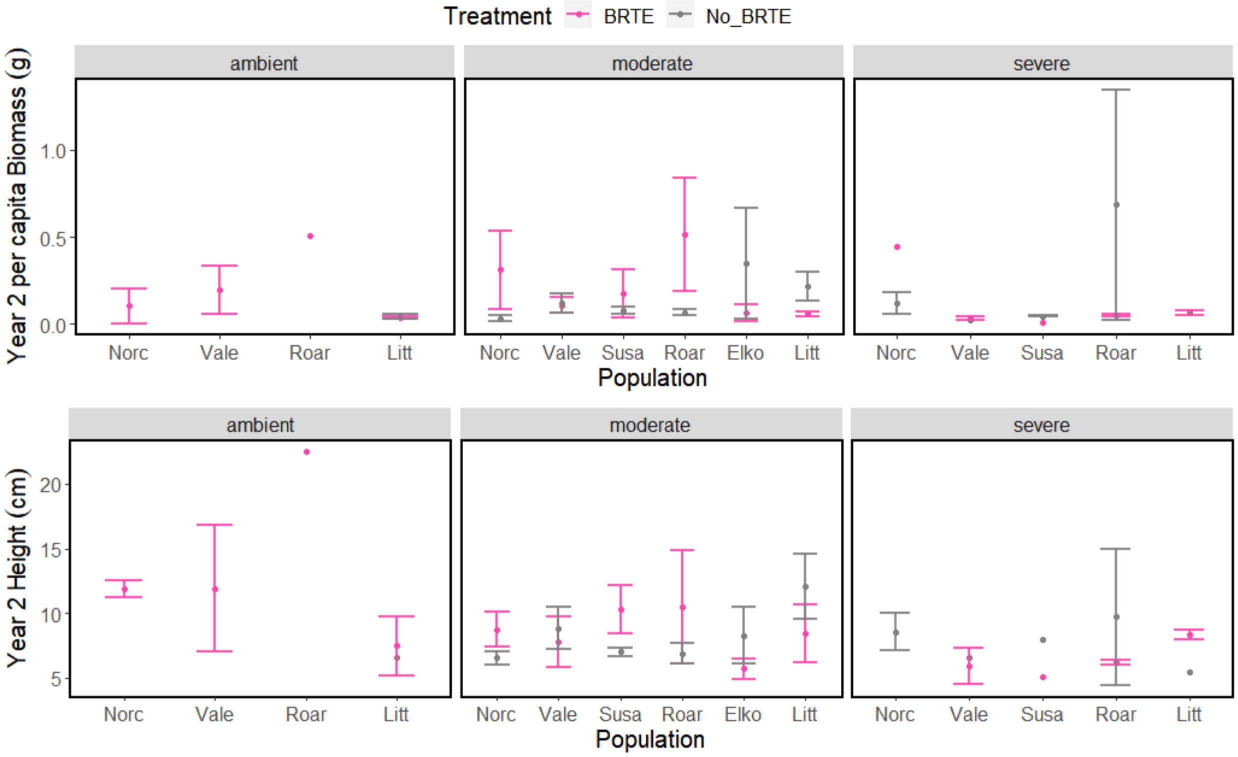

**Figure S3.5.** *Elymus elymoides* per capita biomass (g) and height (cm) of second-year seedlings of the 2020 cohort (mean ± SE). Panels from left to right show results from ambient, moderate drought, and severe drought rainfall treatments. Color of bars represent cheatgrass (*Bromus tectorum*) treatments: pink = with cheatgrass, grey = no cheatgrass.

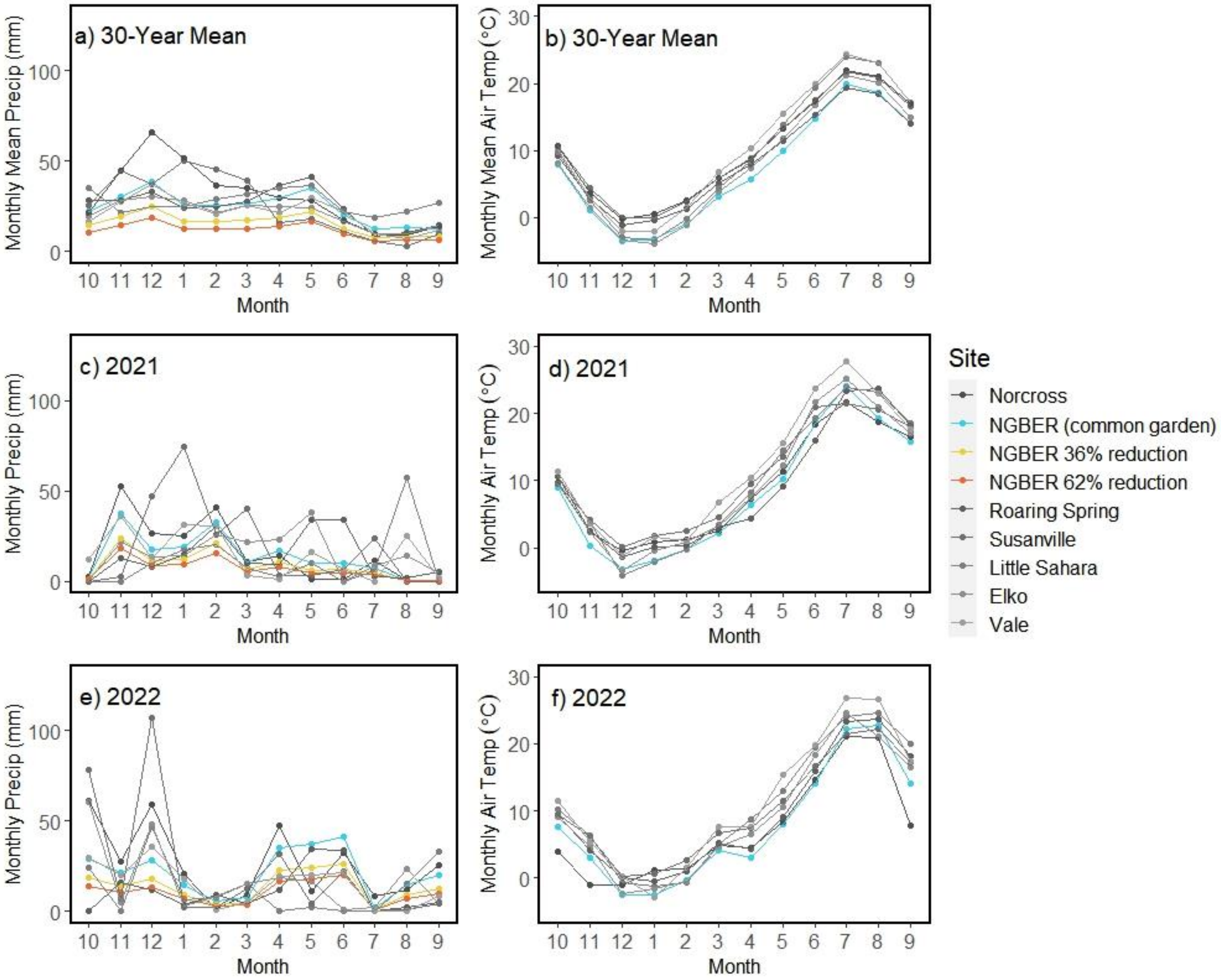

**Figure S3.6.** (a, b) 30-year mean monthly precipitation and air temperature of water year 1991 to 2020, (c, d) monthly precipitation (mm) and air temperature (˚C) in water year 2021, and (e, f) in water year 2022.

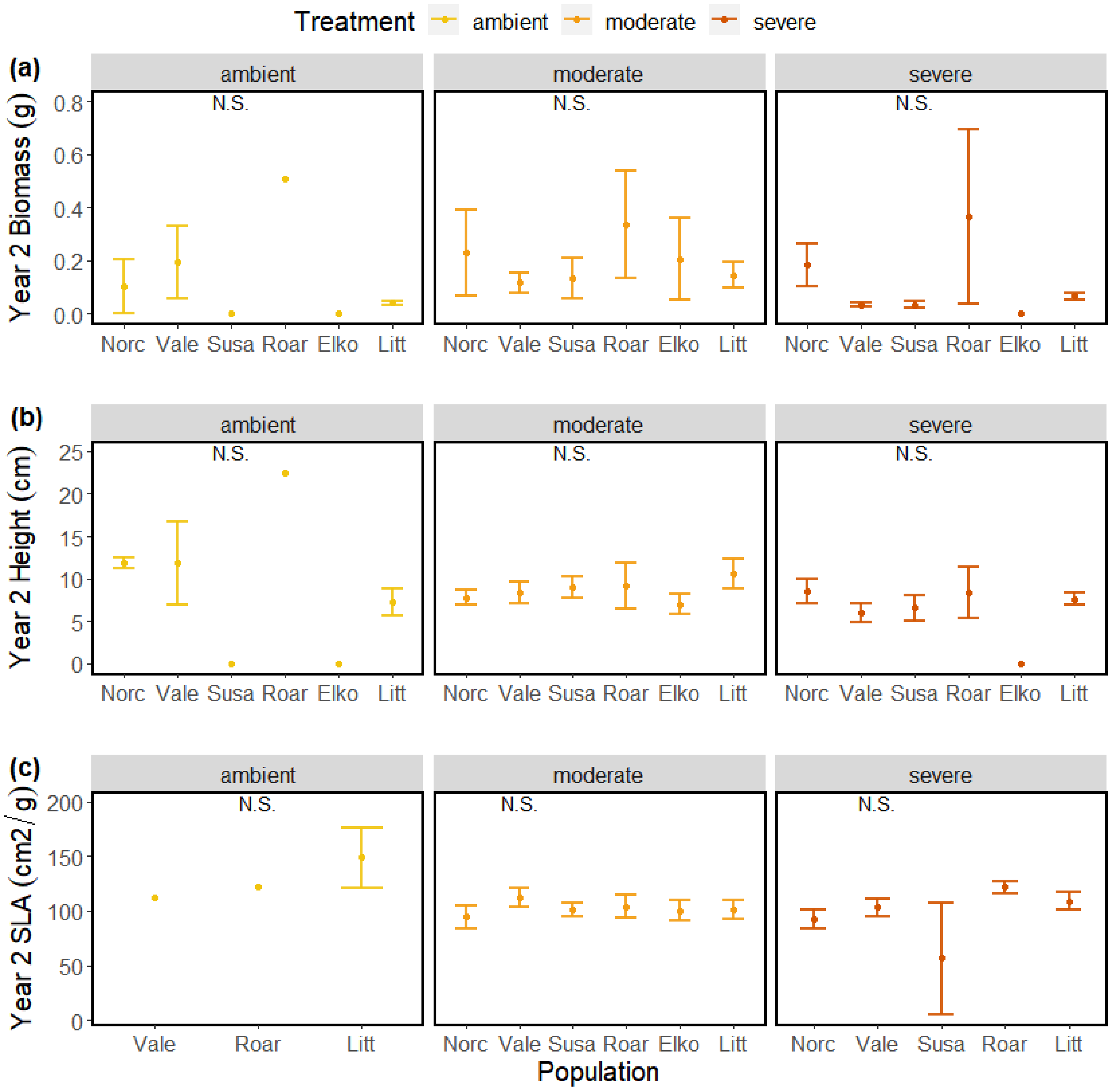

**Figure S3.7.** Per capita (a) biomass (g), (b) height (cm), and (c) specific leaf area (SLA, $cm^2/g$) of second-year seedlings (means ± SE) by rainfall treatments and seed sources. There was no significant difference ($p < 0.05$) between populations for each treatment.

# C. SUPPLEMENTAL MATERIALS FOR CHAPTER IV

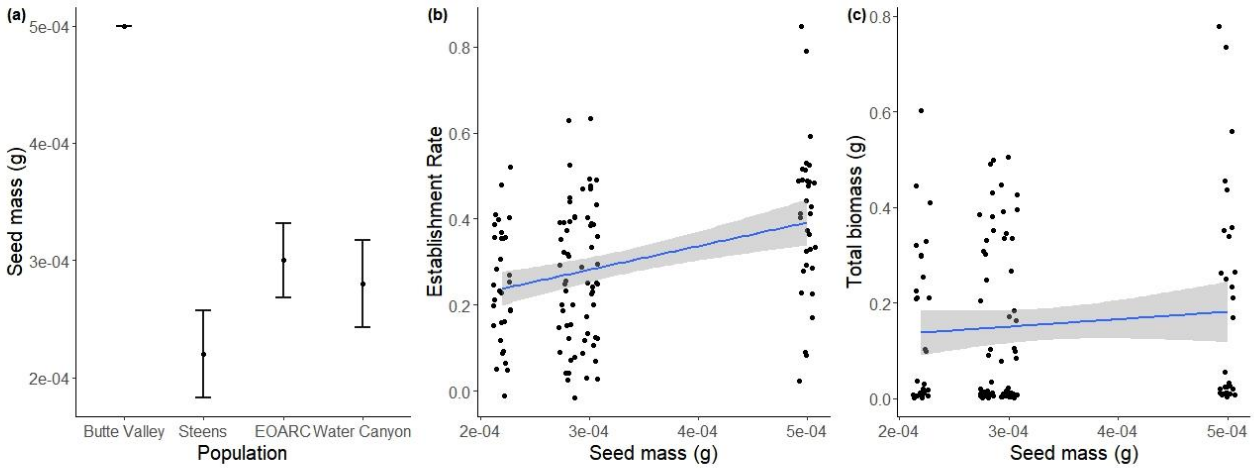

**Figure S4.1.** (a) *Poa secunda* seed mass (grams) by source population, (b) relationship between seed mass and seedling establishment rate, and (c) relationship between seed mass and total biomass. Points represent means, and error bars represent 1 ± SE. Grey areas in (b) and (c) represent the 95% CI for the linear regression models.

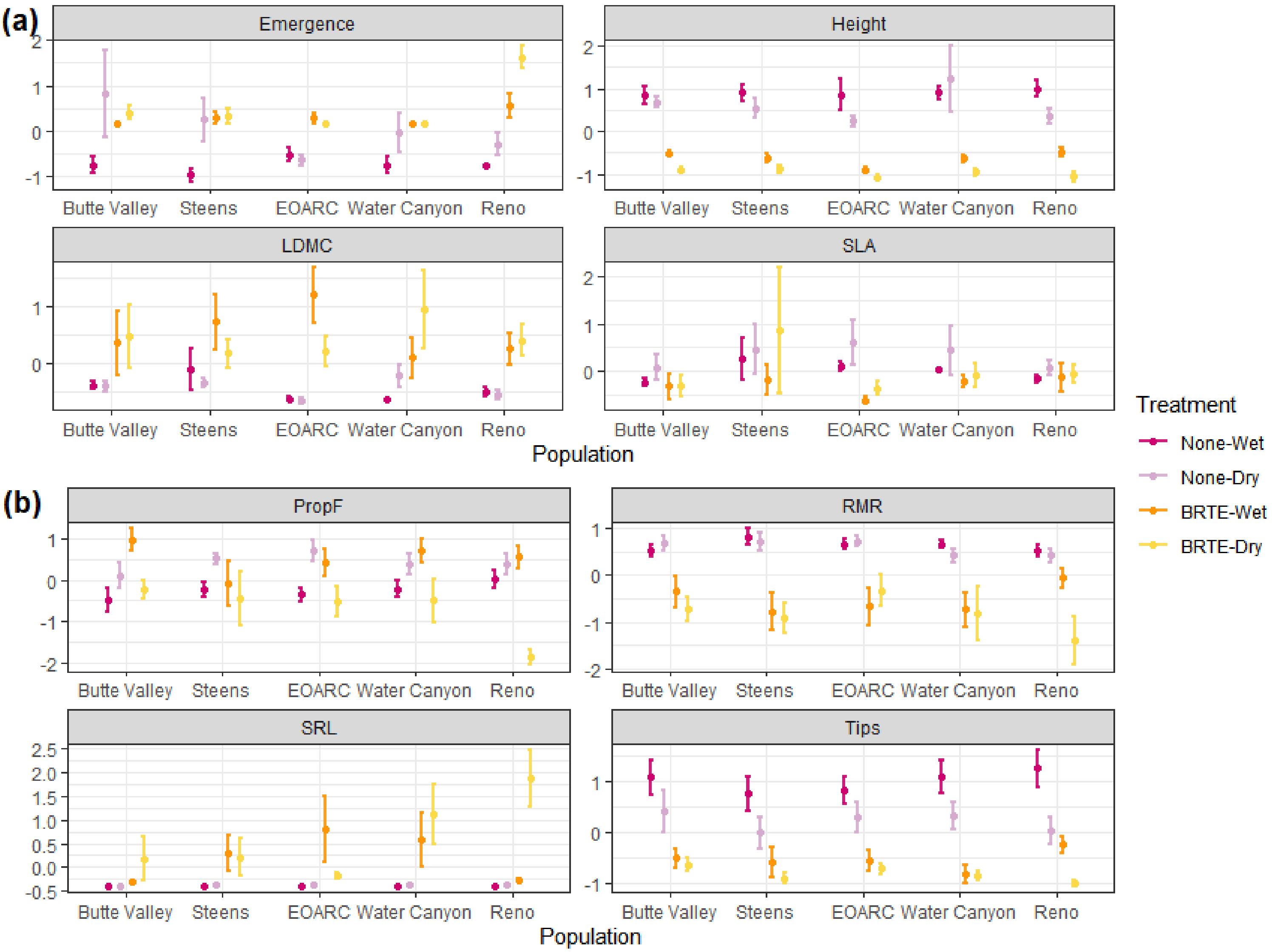

**Figure S4.2.** Standardized trait values (z-scores) by source population. (a) Aboveground traits include emergence date, height, leaf dry matter content (LDMC) and specific leaf area (SLA). (b) Belowground traits include proportion of fine roots (PropF), root matter ratio (RMR), specific root length (SRL), and root tips. Watering and competition treatments are differentiated by color: No cheatgrass - wet (dark pink), No cheatgrass - dry (light pink), With cheatgrass - wet (dark orange), and With cheatgrass - dry (light orange). Points represent means, and error bars represent 1 ± SE.

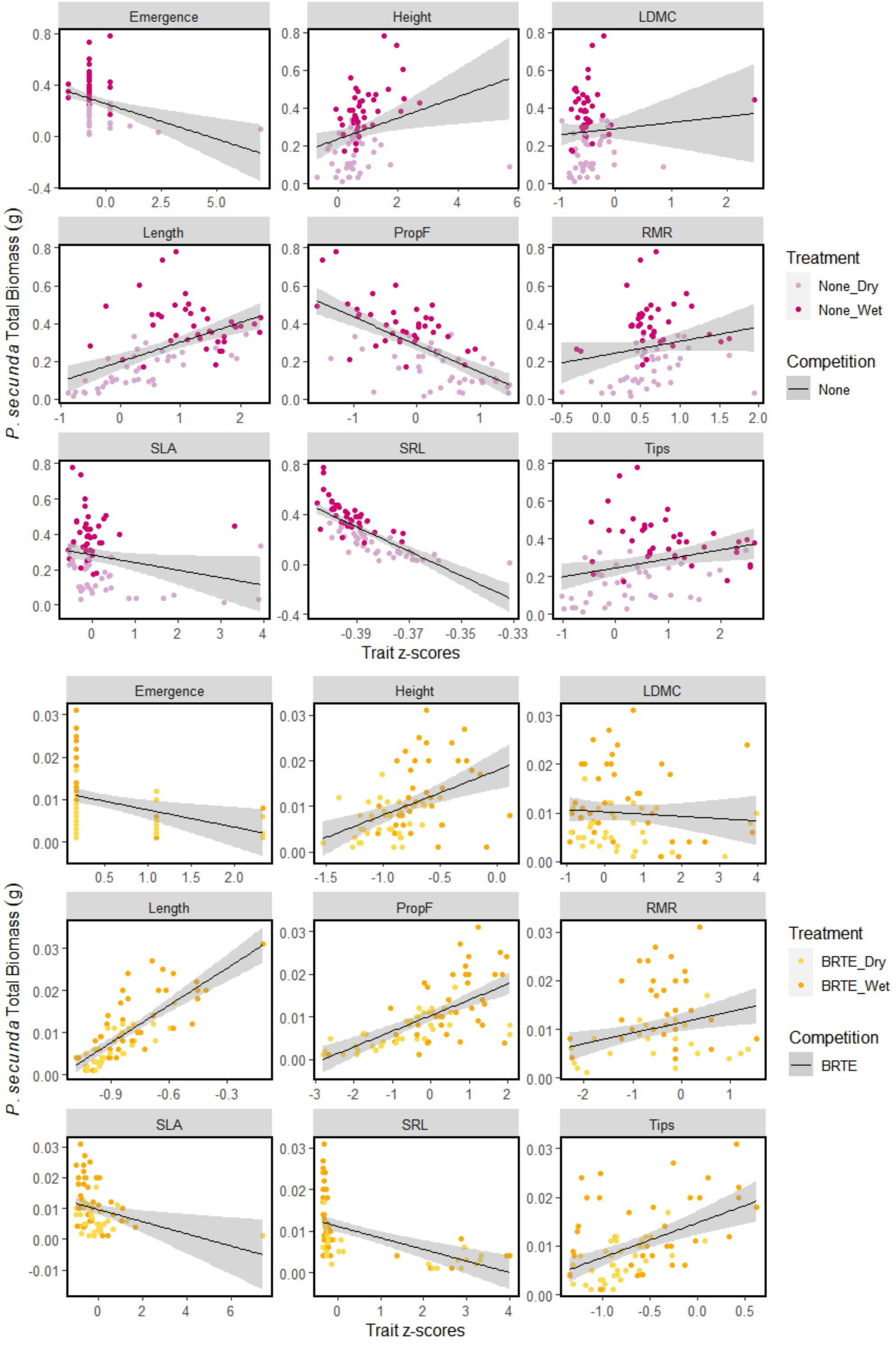

**Figure S4.3.** Relationships between standardized trait values (z-scores) and total biomass (g) by watering and competition treatments: No cheatgrass - wet (dark pink), No cheatgrass - dry (light pink), With cheatgrass - wet (dark orange), and With cheatgrass - dry (light orange). Grey areas represent the 95% CI for linear regression models.

# D. SUPPLEMENTAL MATERIALS FOR CHAPTER V

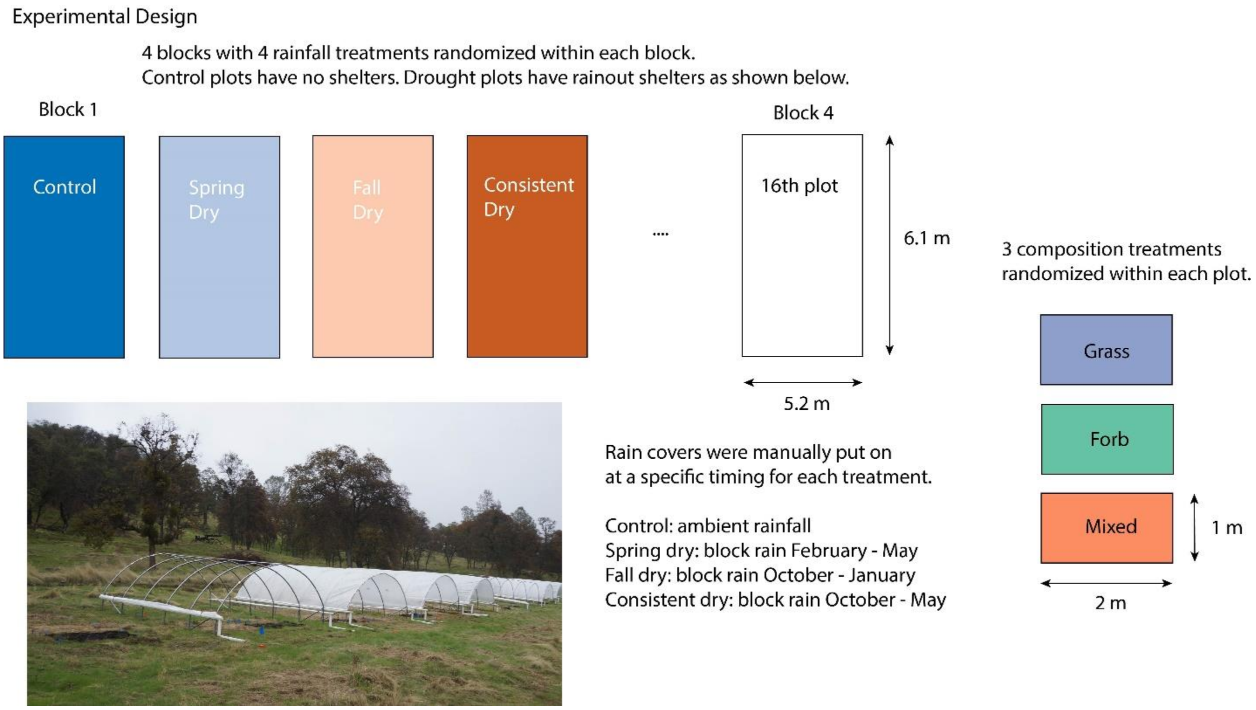

**Figure S5.1.** Schematic of the experiment at the University of California Sierra Foothills Research and Extension Center in Browns Valley, California. In a randomized-block design, plant community composition treatments were nested within rainfall treatment plots in 4 blocks. Rainout shelters were used to block the rain at a specific time of the growing season.

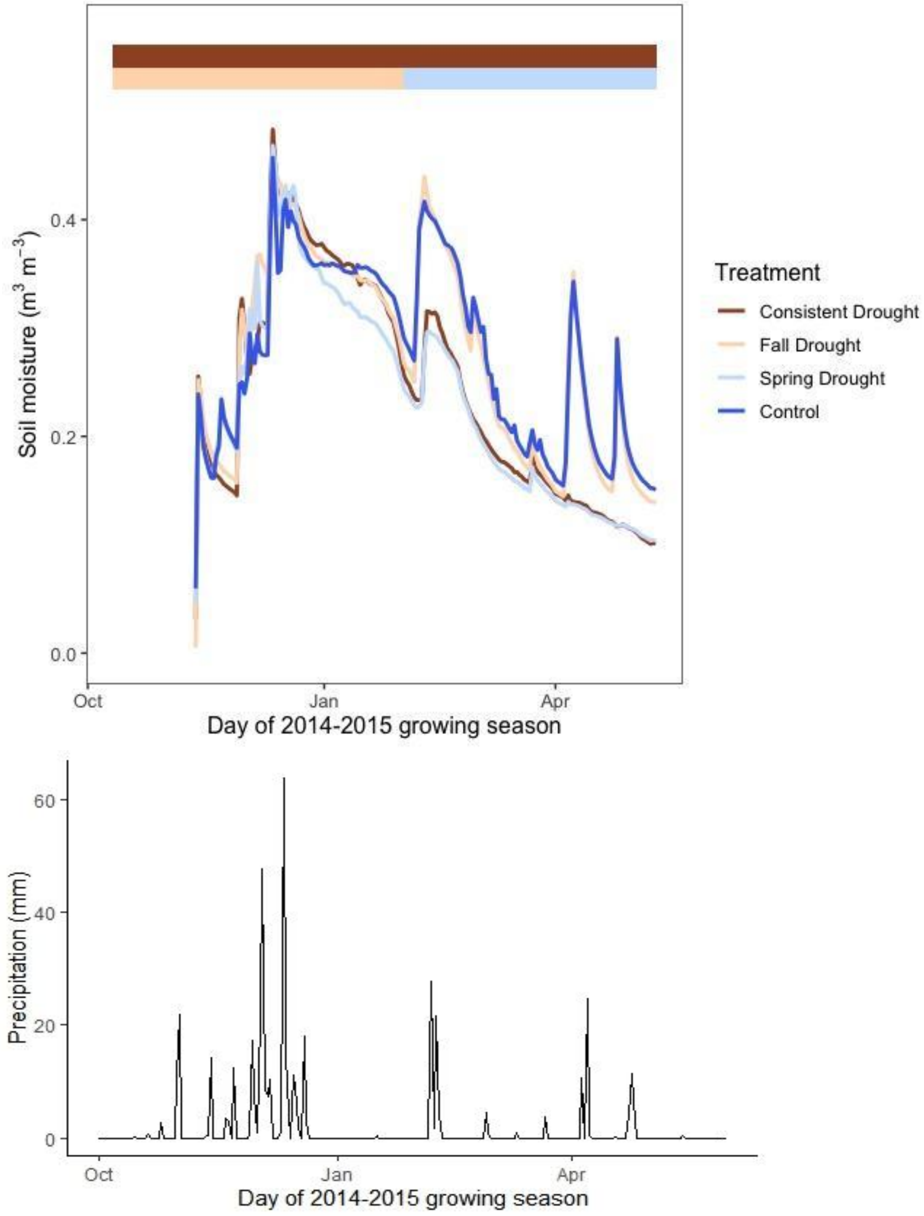

**Figure S5.2.** Volumetric soil moisture by treatment over time for water year 2015. Colored bars show time periods that consistent, fall, and spring drought treatments were applied, matching colors shown in the figure legend. Control received all ambient precipitation. Daily precipitation data in Browns Valley, CA, was sourced from California Irrigation Management Information System (https://cimis.water.ca.gov/).

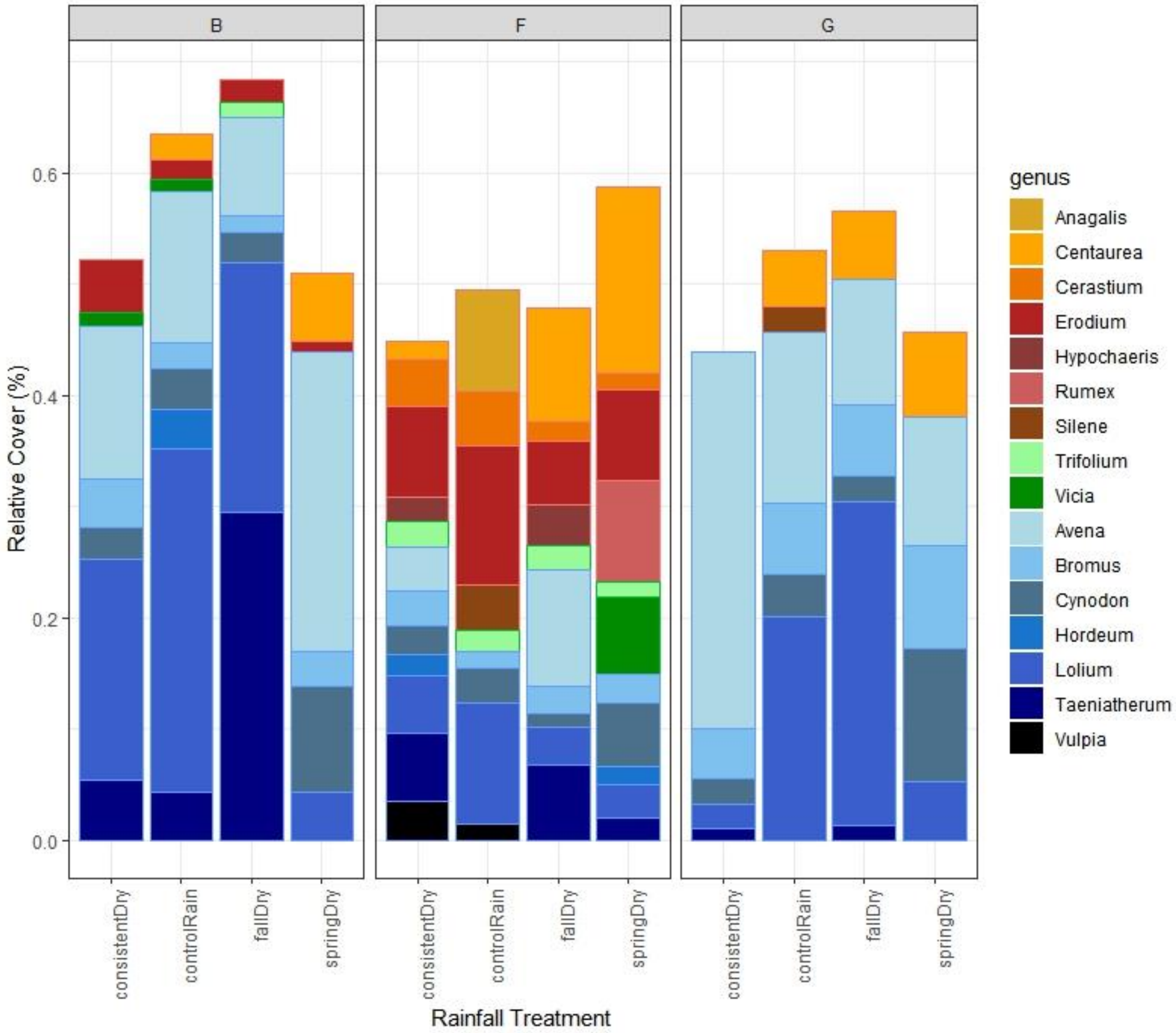

**Figure S5.3.** Mean relative cover of each genus by composition and rainfall treatments averaged across four blocks in May 2015. B = mixed plots, F = forb plots, G = grass plots. Genera are colored by functional groups: forb (orange/red), legume (green), and grass (blue).

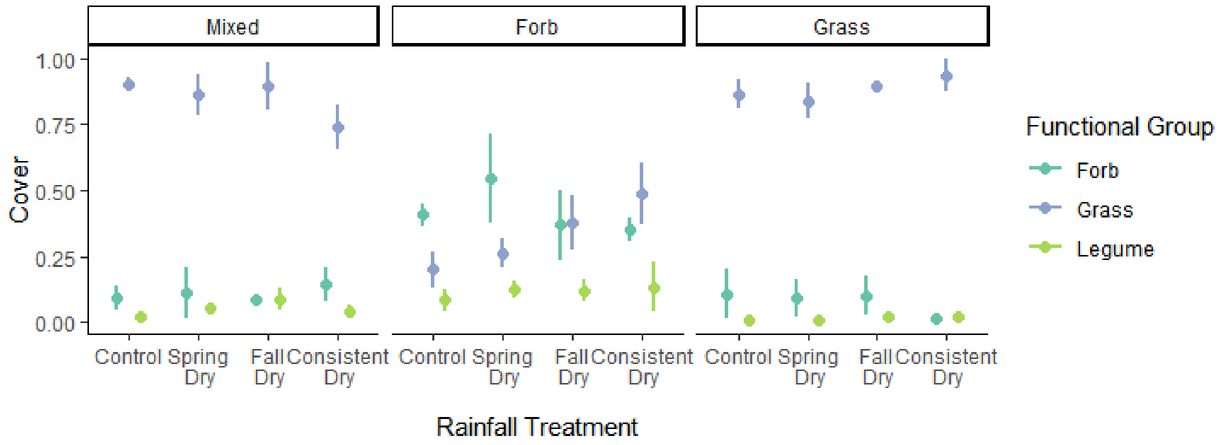

**Figure S5.4.** Rainfall treatment effects on plant composition. Mean percent cover by functional group across rainfall treatments and composition treatments. Bars colored by functional groups: forb (dark green), grass (blue), and legume (light green).

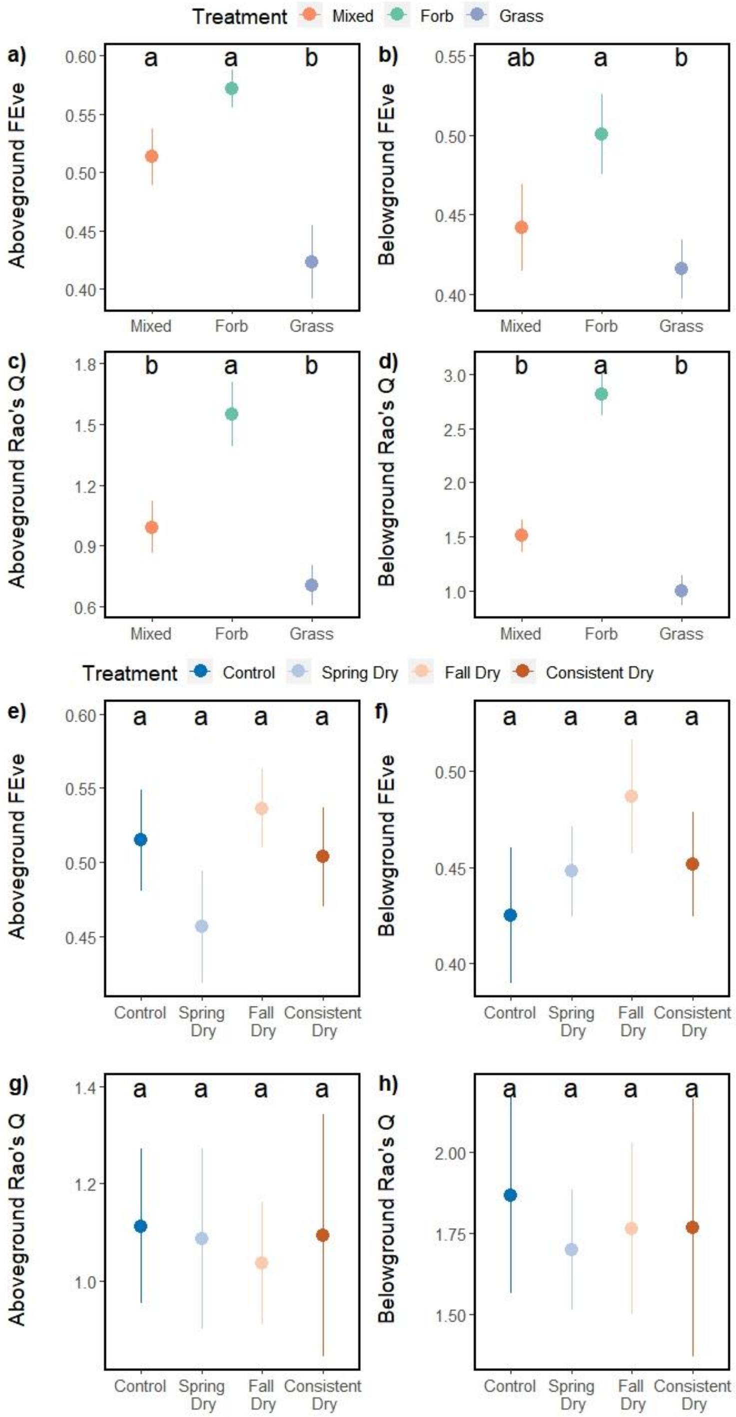

**Figure S5.5.** Treatment effects on functional diversity of community traits (mean ± SE). Top panels show the functional evenness (FEve) and functional dispersion (Rao's Q) of aboveground traits (a and c) and belowground traits (b and d) across composition treatments. Bottom panels show the FEve and Rao's Q of aboveground traits (e and g) and belowground traits (f and h) across rainfall treatments. Overall significant difference between each treatment is indicated by different letters above each bar.

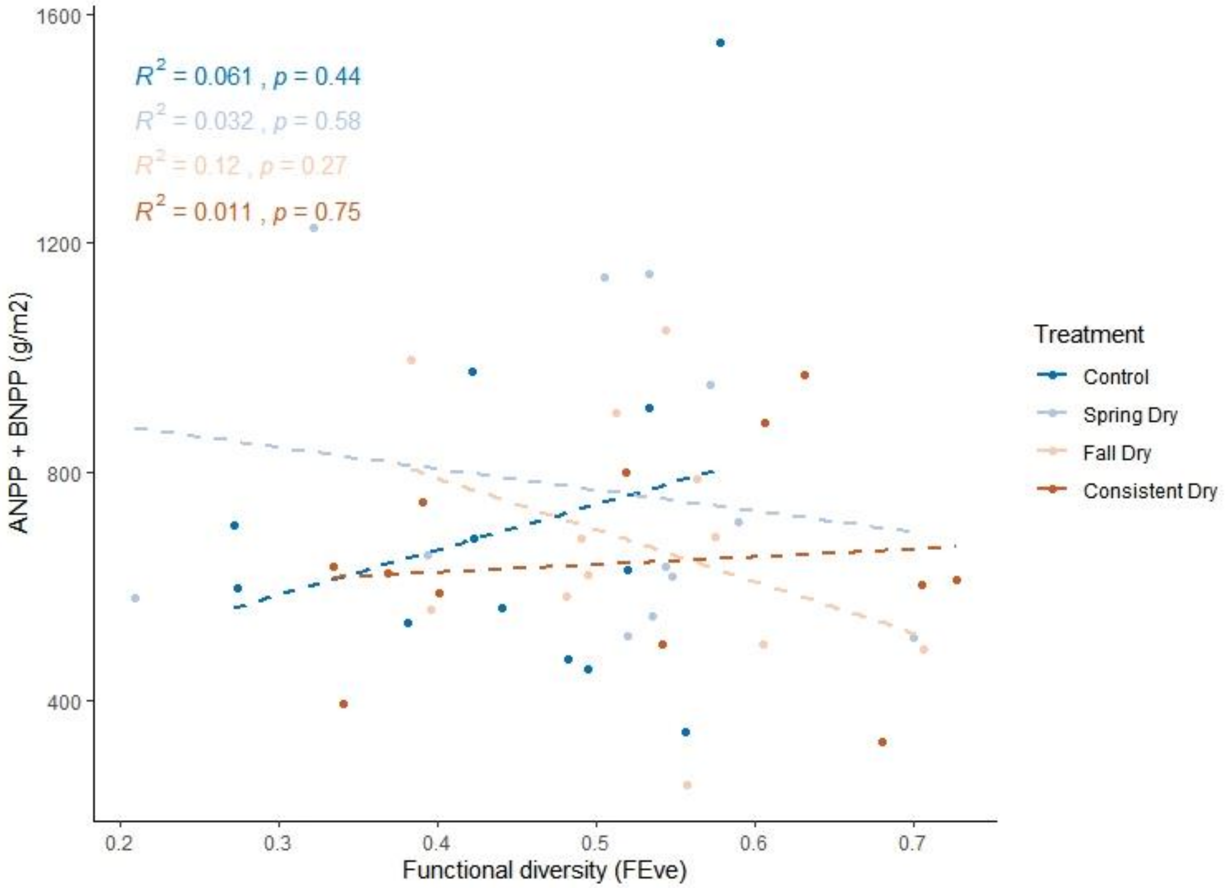

**Figure S5.6.** Total biomass (ANPP and BNPP) and functional trait evenness (FEve) relationships compared across rainfall treatments. Points and linear regression lines colored by rainfall treatments: control (dark blue), spring dry (light blue), fall dry (beige), and consistent dry (brown). There was no interaction effect of FEve and rainfall treatment on total biomass ($F_{1, 37}$ = 0.82, p = 0.48).

**Table S5.1.** List of plant species used in this study: name, functional group, whether traits were measured in the greenhouse, and maximum percent cover in any plot. Trait values of all species with “Yes” in the Traits Measured column were measured in the greenhouse. For species without asterisks (*), we extracted trait data from Butterfield & Suding (2013). For species with asterisks (*), we measured their traits directly in this study following the same trait collection protocol as Butterfield & Suding (2013) to be consistent across species.

| Species | Functional Group | Traits Measured | Maximum percent in any plot |
|---|---|---|---|
| *Achillea millefolium* | Forb | Yes | 1 |
| *Anagallis arvensis* | Forb | Yes* | 20 |
| *Avena barbata* | Grass | Yes* | 95 |
| *Avena fatua* | Grass | Yes | 10 |
| *Brachypodium distachyon* | Grass | Yes | 5 |
| *Briza minor* | Grass | Yes* | 1 |
| *Bromus diandrus* | Grass | Yes | 60 |
| *Bromus hordeaceus* | Grass | Yes | 70 |
| *Bromus madritensis* | Grass | Yes | 10 |
| *Carduus pycnocephalus* | Forb | Yes* | 2 |
| *Centaurea solstitialis* | Forb | Yes | 80 |
| *Cerastium glomeratum* | Forb | Yes* | 20 |
| *Clarkia amoena* | Forb | No | 0.5 |
| *Cynodon dactylon* | Grass | Yes | 30 |
| *Cynosaurus echinatus* | Grass | Yes | 0.5 |
| *Erodium botrys* | Forb | Yes | 40 |
| *Erodium moschatum* | Forb | Yes* | 10 |
| *Filago gallica* | Forb | Yes* | 0.5 |
| *Galium parisiense* | Forb | No | 1 |
| *Hordeum marinum* | Grass | No | 20 |
| *Hordeum murinum* | Grass | Yes | 10 |
| *Hypochaeris glabra* | Forb | Yes* | 10 |
| *Hypochaeris radicata* | Forb | Yes* | 5 |
| *Lactuca serriola* | Forb | Yes | 2 |
| *Lolium multiflorum* | Grass | Yes | 90 |
| *Rumex pulcher* | Forb | Yes* | 40 |

| | | | |
|---|---|---|---|
| *Silene gallica* | Forb | No | 10 |
| *Taeniatherum caput-medusae* | Grass | Yes | 70 |
| *Torilis arvensis* | Forb | No | 1 |
| *Trifolium dubium* | Legume | Yes* | 2 |
| *Trifolium glomeratum* | Legume | Yes* | 1 |
| *Trifolium hirtum* | Legume | Yes | 10 |
| *Trifolium sp.* | Legume | Yes | 5 |
| *Trifolium subterraneum* | Legume | Yes* | 40 |
| *Vicia sativa* | Legume | Yes* | 20 |
| *Vulpia bromoides* | Grass | Yes* | 20 |
| *Vulpia myuros* | Grass | Yes | 5 |

**Table S5.2.** Mixed model results of rainfall and composition treatment effects on ANPP, BNPP, and total biomass with block as a random effect. Statistically significant ($p < 0.05$) results are bolded.

| Response | Treatment | Degrees of freedom | F | p-value |
|---|---|---|---|---|
| ANPP | Rain | 3, 36 | 0.5263 | 0.6673 |
| | Composition | 2, 36 | 7.6192 | **0.0019** |
| | Rain:Composition | 6, 36 | 0.8813 | 0.5194 |
| BNPP | Rain | 3, 36 | 0.88807 | 0.4575 |
| | Composition | 2, 36 | 6.8685 | **0.0032** |
| | Rain:Composition | 6, 36 | 0.6462 | 0.6927 |
| Total | Rain | 3, 36 | 0.9994 | 0.4054 |
| | Composition | 2, 36 | 17.4264 | **<0.0001** |
| | Rain:Composition | 6, 36 | 1.7415 | 0.1423 |

**Table S5.3.** T-statistics and Tukey post-hoc pairwise comparisons of composition treatment on ANPP, BNPP, and total biomass. Statistically significant ($p < 0.05$) results are bolded.

| Response | Treatment | t-value | p-value | Tukey pairwise comparisons | | |
|---|---|---|---|---|---|---|
| | | | | Forb-mixed | Grass-Mixed | Grass-Forb |
| ANPP | Intercept (Mixed) | 14.503 | **<2e-16** | **0.0007091** | 0.1132203 | 0.1400661 |
| | Forb | -3.982 | **0.000247** | | | |
| | Grass | -2.045 | **0.046729** | | | |
| BNPP | Intercept (Mixed) | 10.438 | **1.33E-13** | **0.0016617** | **0.0297801** | 0.5446907 |
| | Forb | -3.701 | **0.000584** | | | |
| | Grass | -2.642 | **0.011283** | | | |
| Total | Intercept (Mixed) | 18.265 | **<2e-16** | **0.0000037** | **0.0041825** | 0.0807227 |
| | Forb | -5.591 | **1.26e-06** | | | |
| | Grass | -3.383 | **0.00149** | | | |

**Table S5.4.** Mixed model results of rainfall and composition treatment effects on CWM of above- and belowground traits with block as a random effect. Statistically significant ($p < 0.05$) results are bolded.

| Response | Treatment | Degrees of freedom | F | p-value |
|---|---|---|---|---|
| Aboveground CWM of PC1 | Rain | 3, 36 | 4.425 | **0.00952** |
| | Composition | 2, 36 | 6.770 | **0.00319** |
| | Rain:Composition | 6, 36 | 0.471 | 0.82546 |
| Aboveground CWM of PC2 | Rain | 3, 36 | 0.214 | 0.886 |
| | Composition | 2, 36 | 27.107 | **< 0.0001** |
| | Rain:Composition | 6, 36 | 0.561 | 0.758 |
| Belowground CWM of PC1 | Rain | 3, 36 | 3.045 | **0.0411** |
| | Composition | 2, 36 | 0.038 | 0.9630 |
| | Rain:Composition | 6, 36 | 0.402 | 0.8725 |
| Belowground CWM of PC2 | Rain | 3, 36 | 4.022 | **0.0145** |
| | Composition | 2, 36 | 0.189 | 0.8284 |
| | Rain:Composition | 6, 36 | 0.584 | 0.7404 |

**Table S5.5.** Mixed model results of rainfall and composition treatment effects on functional evenness and dispersion of above- and belowground traits with block as a random effect. Statistically significant ($p < 0.05$) results are bolded.

| Response | Treatment | Degrees of freedom | F | p-value |
|---|---|---|---|---|
| Aboveground FEve | Rain | 3, 36 | 1.451 | 0.244289 |
| | Composition | 2, 36 | 9.510 | **0.000483** |
| | Rain:Composition | 6, 36 | 1.140 | 0.359502 |
| Aboveground Rao's Q | Rain | 3, 36 | 0.043 | 0.987919 |
| | Composition | 2, 36 | 9.795 | **0.000401** |
| | Rain:Composition | 6, 36 | 0.855 | 0.536590 |
| Belowground FEve | Rain | 3, 36 | 0.758 | 0.5253 |
| | Composition | 2, 36 | 2.908 | 0.0675 |
| | Rain:Composition | 6, 36 | 0.277 | 0.9439 |
| Belowground Rao's Q | Rain | 3, 36 | 0.158 | 0.9236 |
| | Composition | 2, 36 | 38.416 | **< 0.0001** |
| | Rain:Composition | 6, 36 | 2.535 | **0.0377** |

**Table S5.6.** PC scores by species included PCAs on above and belowground traits. Taxon is the species name, FG is the functional group (F=forb, G=grass, L=legume), Origin indicates provenance (N=native, E=exotic).

| Taxon | GF | Origin | Above- PC1 | Above-PC2 | Below-PC1 | Below-PC2 |
|---|---|---|---|---|---|---|
| Achillea millefolium | F | N | 0.252385856 | 0.54322565 | 0.236797611 | -0.374072119 |
| Anagallis arvensis | F | E | 0.178212179 | 0.266787489 | -0.257148096 | 0.461794668 |
| Avena barbata | G | E | -0.484463193 | -0.293049048 | -0.166030937 | 0.877830087 |
| Avena fatua | G | E | -0.44670467 | -1.378541474 | 0.423950576 | 0.025021098 |
| Brachypodium distachyon | G | E | -0.927412073 | -0.058114943 | 0.540578651 | -0.299561895 |
| Briza minor | G | E | 1.476003613 | -0.493032741 | -0.875808551 | 0.454006205 |
| Bromus diandrus | G | E | -0.150460494 | -0.196376742 | 0.447365917 | -0.286609331 |
| Bromus hordeaceus | G | E | -0.063252043 | -0.332956473 | 0.429926008 | -0.312715488 |
| Bromus madritensis | G | E | 0.207728216 | -0.311082121 | 0.461880384 | -0.068905596 |
| Carduus pycnocephalus | F | E | 0.525948872 | 0.814049741 | -0.845940457 | 0.617105091 |
| Centaurea solstitialis | F | E | 0.033863599 | 0.531309829 | 0.330326589 | -0.651940459 |
| Cerastium glomeratum | F | E | 0.134980694 | 0.304395304 | -0.366842785 | 0.836021391 |
| Cynodon dactylon | G | E | -0.409516753 | -0.006751919 | 0.351287159 | -0.319199515 |
| Cynosurus echinatus | G | E | 0.13678546 | -0.271590227 | 0.52367323 | -0.338064688 |
| Erodium botrys | F | E | -0.454507952 | 0.803423257 | 0.566360631 | -0.061027778 |
| Erodium moschatum | F | E | -0.396304372 | 0.505009297 | -0.115398133 | 0.787653223 |
| Filago gallica | F | E | 1.616901442 | -0.329882061 | -2.21433686 | -1.825285411 |
| Hordeum murinum | G | E | -0.105516459 | -0.399113854 | 0.673396243 | -0.611893316 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| Hypochaeris glabra | F | E | 0.633590312 | 0.320861467 | -0.610537999 | -0.664221688 |
| Hypochaeris radicata | F | E | 0.370027436 | 0.322172778 | -0.516226726 | -0.205597708 |
| Lactuca serriola | F | E | 0.390998887 | 0.116259119 | 0.323046399 | -0.904577206 |
| Lolium multiflorum | G | E | -0.336599852 | -0.808300642 | 0.406838374 | -0.278357494 |
| Rumex pulcher | F | E | 0.592394836 | 0.146943738 | 0.424915502 | 1.166868312 |
| Taeniatherum caput-medusae | G | E | -0.750859016 | 0.032904555 | 0.444606212 | -0.344872507 |
| Trifolium dubium | L | E | -0.126222834 | 0.248783939 | -0.55082956 | 0.116562115 |
| Trifolium glomeratum | L | E | -0.441531149 | 0.581934572 | -0.28195549 | 0.27490396 |
| Trifolium hirtum | L | E | -0.275613422 | 0.497914215 | 0.435543541 | 0.080204734 |
| Trifolium repens | L | E | -0.414403022 | 0.317914369 | 0.82393173 | -0.336232851 |
| Trifolium subterraneum | L | E | 0.13308846 | 0.248990459 | -0.447733904 | 0.772705162 |
| Vicia sativa | L | E | -0.107465092 | -1.642707335 | -0.566986337 | 1.001845598 |
| Vulpia bromoides | G | E | -0.314969043 | -0.273918851 | -0.434968302 | 0.347379687 |
| Vulpia myuros | G | E | -0.477108421 | 0.192538652 | 0.406319381 | 0.06323372 |

# E. SUPPLEMENTAL MATERIALS FOR CHAPTER VI

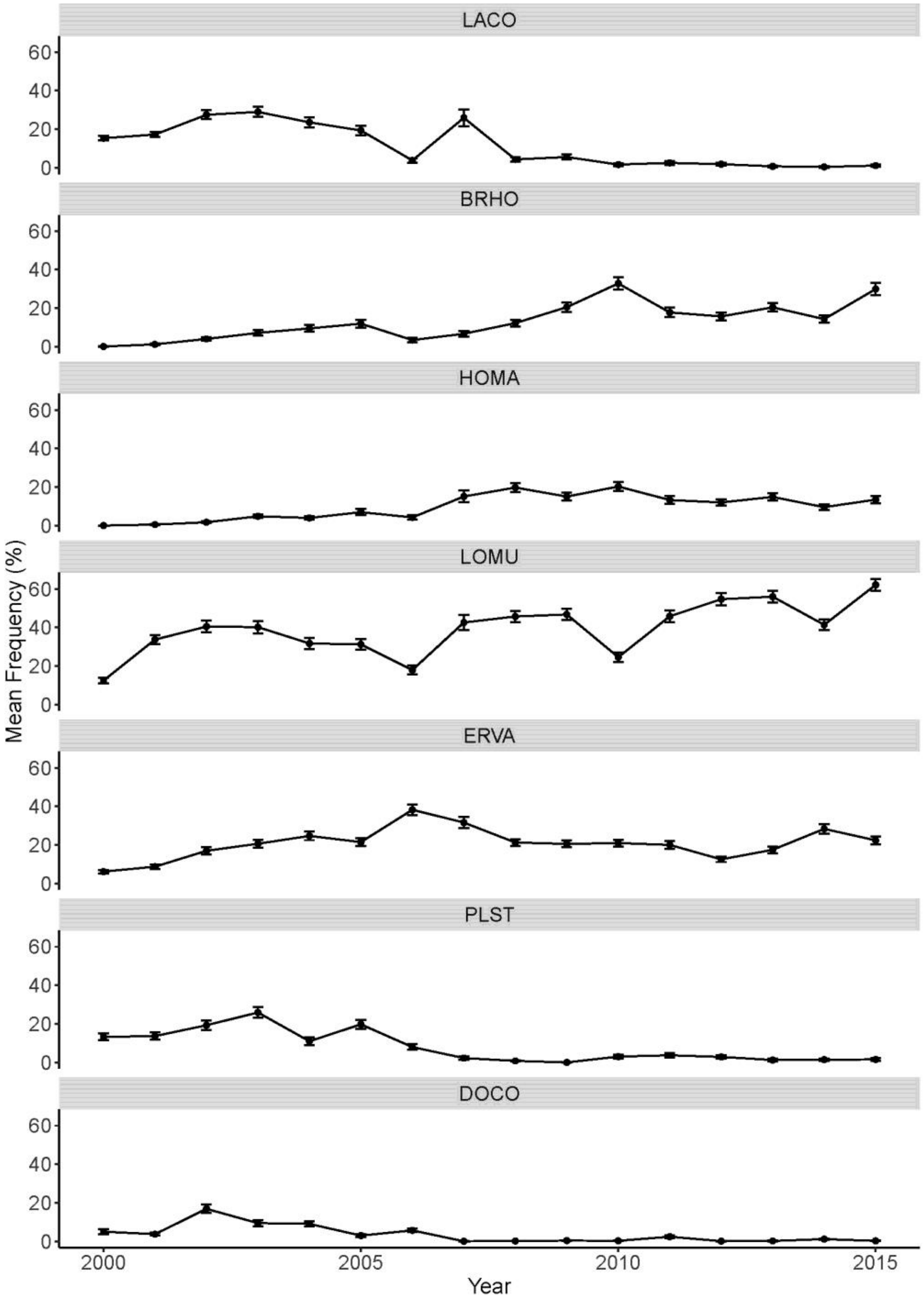

**Figure S6.1.** Trajectories of species in the constructed pools. Species included in the population models: *Lasthenia conjugens* (LACO; focal species); *Bromus hordeaceus* (BRHO), *Hordeum marinum* (HOMA), and *Festuca perennis* (previously called *Lolium multiflorum* (LOMU), exotic annual grasses); *Eryngium vaseyi* (ERVA; native biannual forb); *Plagiobothrys stipitatus var. micranthus* (PLST) and *Downingia concolor* (DOCO; native annual forb). Points and error bars represent and mean and standard error of frequency (%).

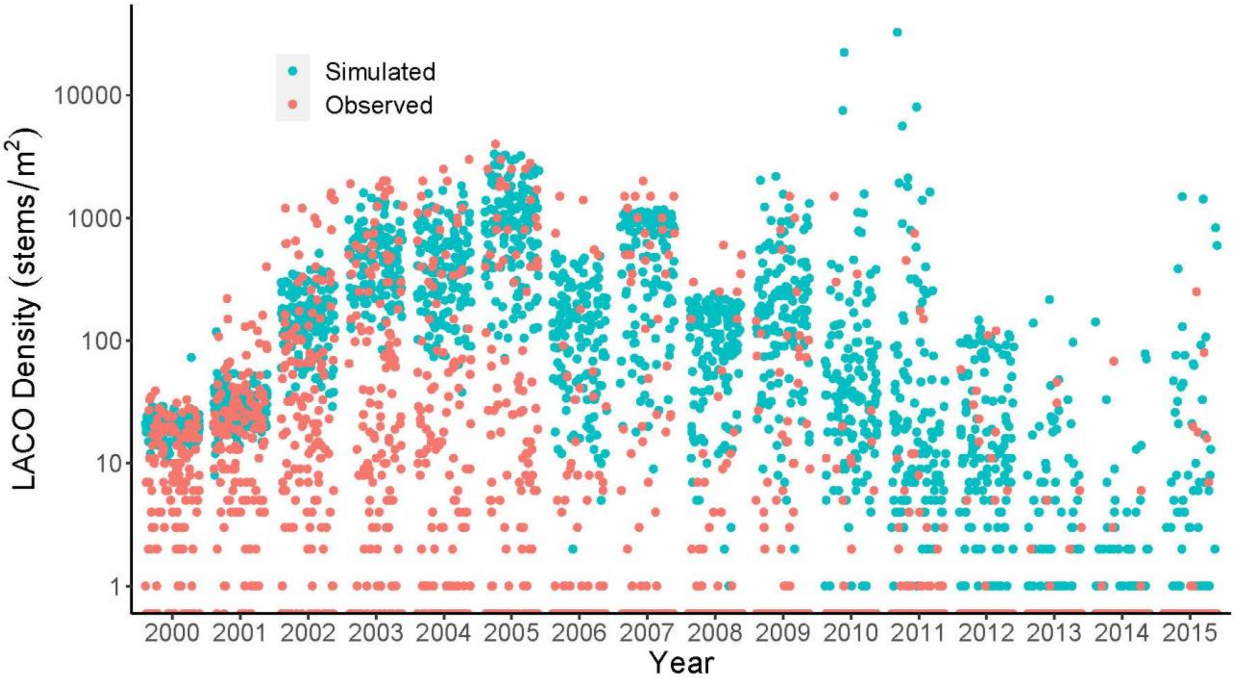

**Figure S6.2.** Time series of *Lasthenia conjugens*'s simulated vs. observed density in constructed pools.

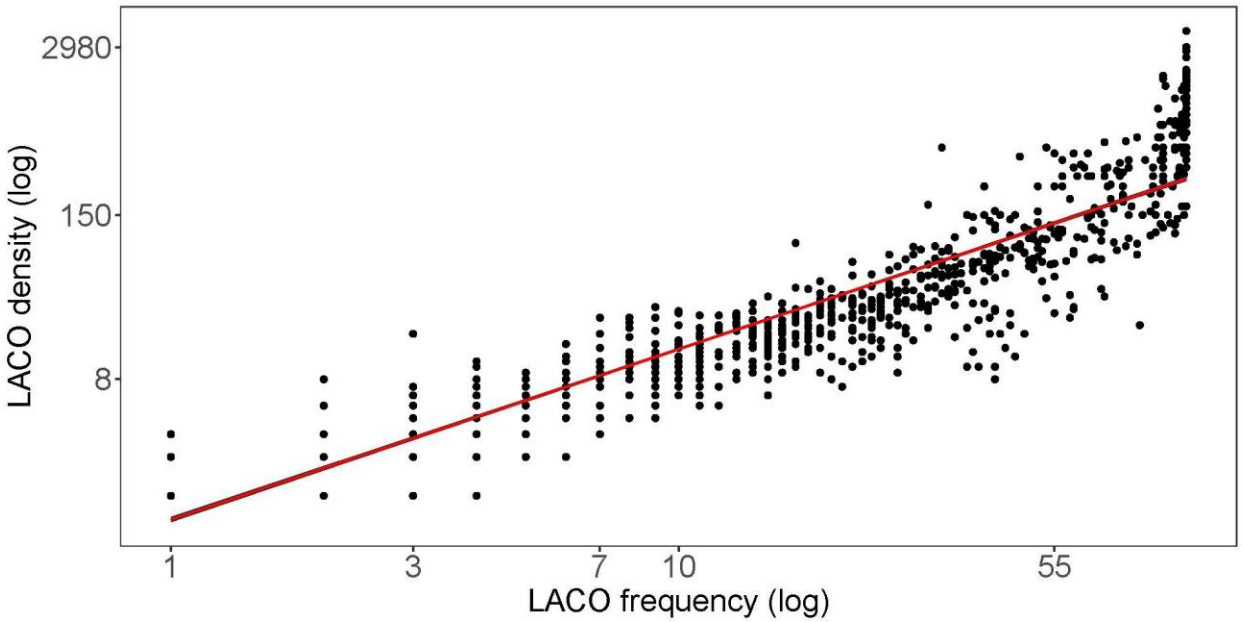

**Figure S6.3.** *Lasthenia conjugens*'s (LACO) density (natural log) and frequency (natural log) are correlated linearly. The points represent a pool in a given year where both density and frequency were measured.

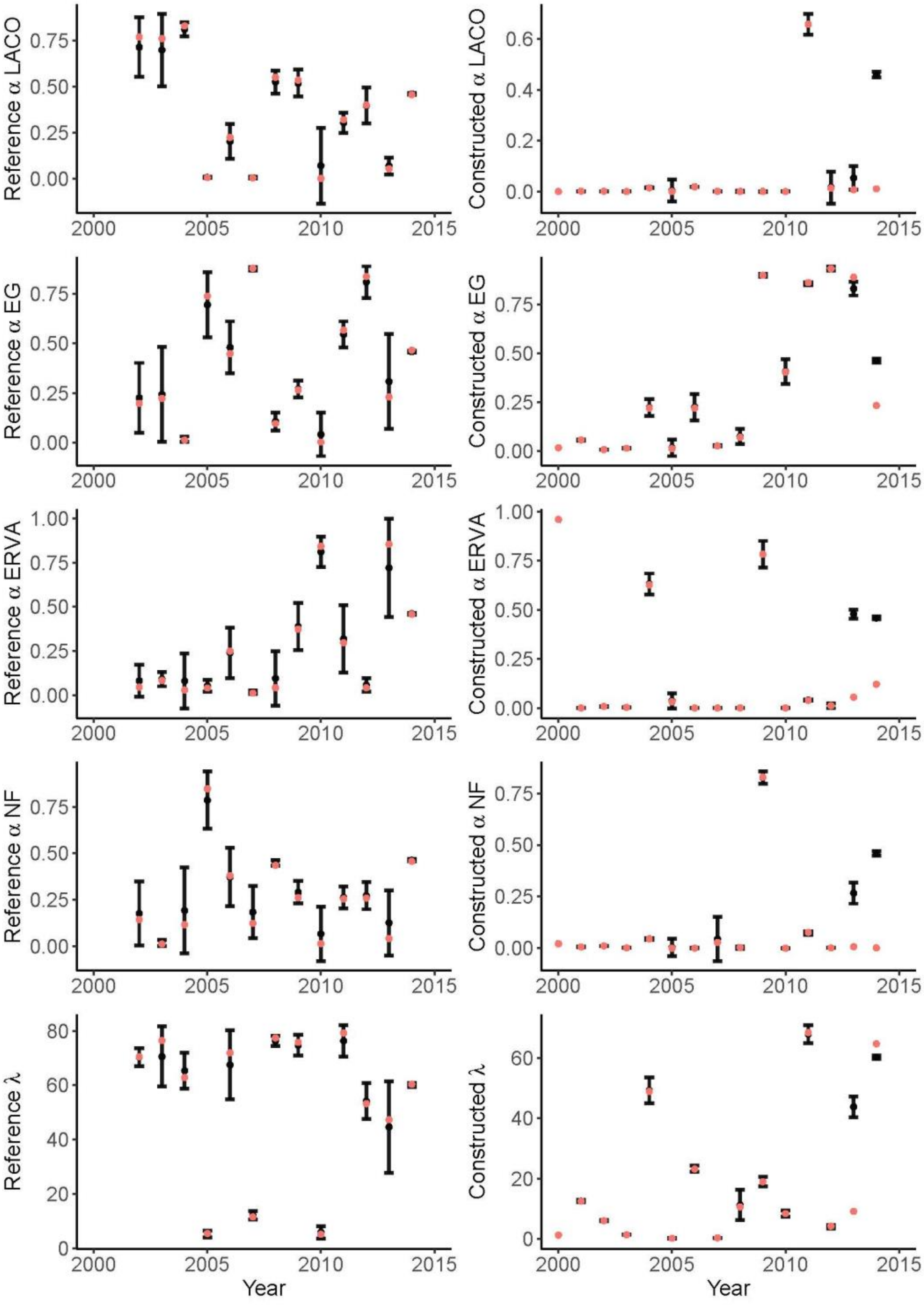

**Figure S6.4.** Time series of parameter estimates from the leave-one-out sensitivity analysis of constructed and reference pool models. Parameters estimated were the following: alpha of *Lasthenia conjugens*, alpha of exotic annual grasses, alpha of *Eryngium vaseyi*, alpha of native forbs, and lambda of *L. conjugens*. Red points represent the mean posterior estimates from the full dataset (141 pools in the constructed pool model and 9 pools in the reference pool model). Black points and error bars represent the mean and standard deviation of mean posterior estimates from models run on subset of the dataset by leaving out one pool each analysis.

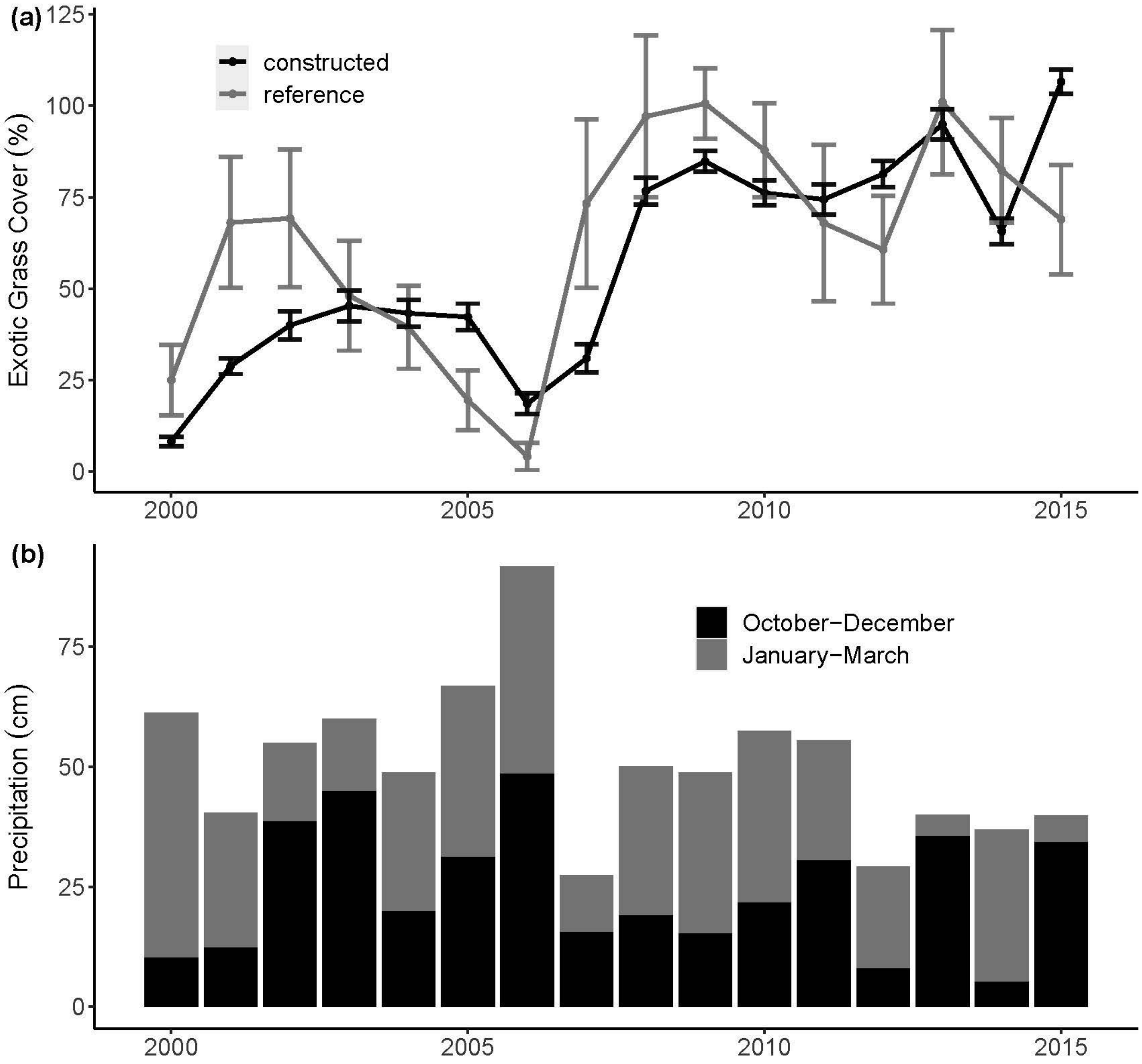

**Figure S6.5.** Timeseries of observed exotic grass frequency and precipitation. (a) Mean exotic grass frequency (%) increased over time in both reference (grey lines) and constructed pools (black lines). Mean exotic frequency was calculated by summing the frequency of three exotic grass species, *Bromus hordeaceus*, *Hordeum marinum*, and *Festuca perennis*, and averaging across pools each year. Points and error bars represent annual means and standard errors from means, respectively. (b) Growing season precipitation (cm) in Fairfield, CA varied across years. Black bars represent total rainfall in October to December of the previous year, and grey bars represent total rainfall in January to March of that year.

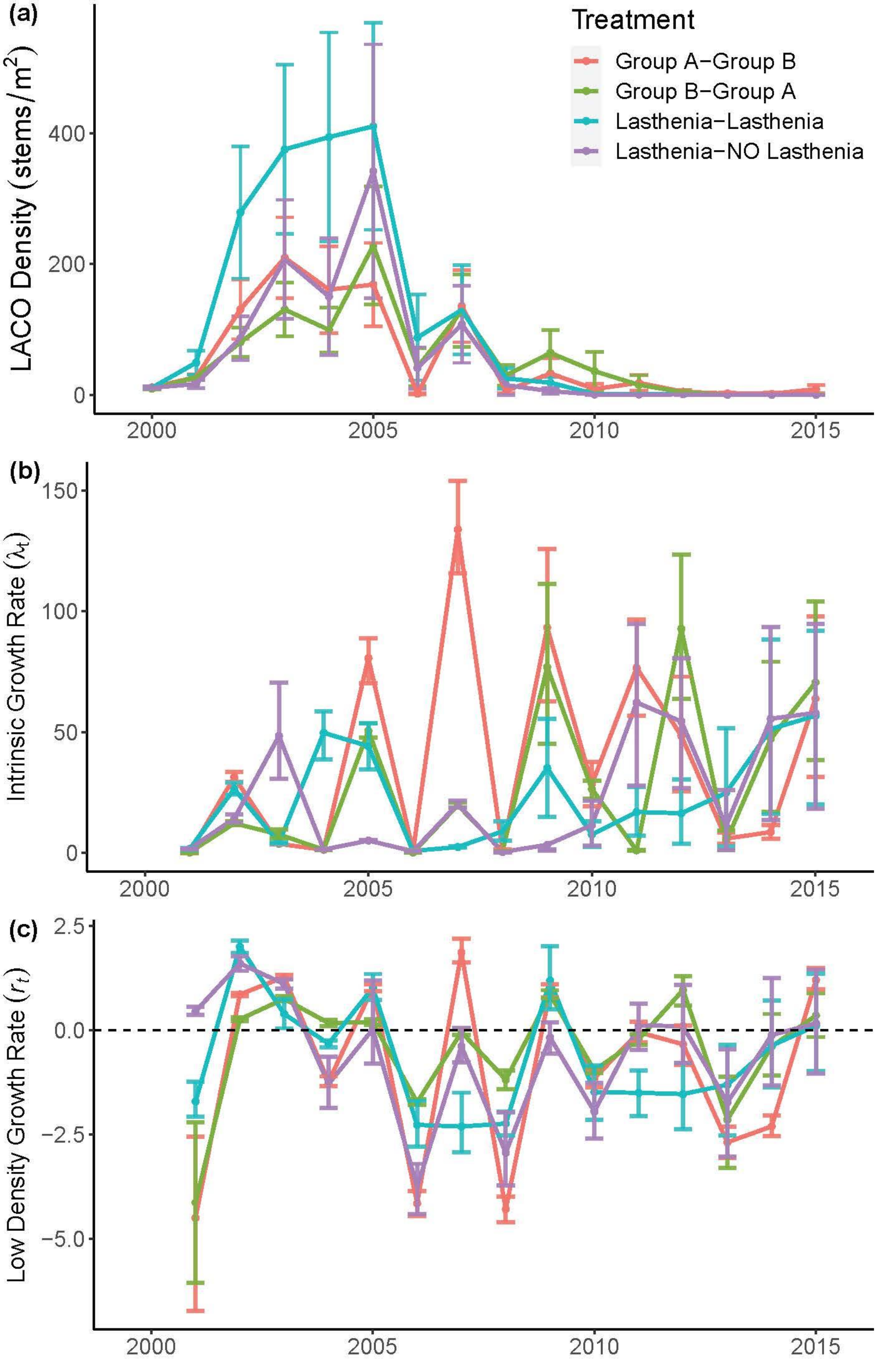
(a)
Treatment
Group A−Group B
Group B−Group A
Lasthenia−Lasthenia
Lasthenia−NO Lasthenia
LACO Density (stems/m$^2$)
0
200
400
2000
2005
2010
2015
(b)
Intrinsic Growth Rate ($\lambda_t$)
0
50
100
150
2000
2005
2010
2015
(c)
Low Density Growth Rate ($r_t$)
2.5
0.0
−2.5
−5.0
2000
2005
2010
2015

**Figure S6.6.** Effect of initial seeding treatment on *Lasthenia conjugens*'s (a) density, (b) intrinsic growth rate, and (c) low-density growth rate. Each experimental pool received one of five seeding treatments as detailed in Collinge and Ray (2009): (1) “Group A- Group B" pools received 300 seeds in 1999 (100 seeds each of Group A species *L. conjugens*, *Danthonia danthonioides*, *Eryngium vaseyi*), followed by 300 seeds in 2000 (100 seeds each of Group B species *L. conjugens*, *Layia chrysanthemoides*, *Plagiobothrys stipitatus*). (2) “Group B-Group A" pools received Group B species in 1999 followed by Group A species in 2000. (3) “Lasthenia-Lasthenia" pools received 100 seeds of *L. conjugens* in each year 1999-2001 (300 seeds total). (4) “Lasthenia-NO Lasthenia" pools received 100 seeds of *L. conjugens* in 1999.

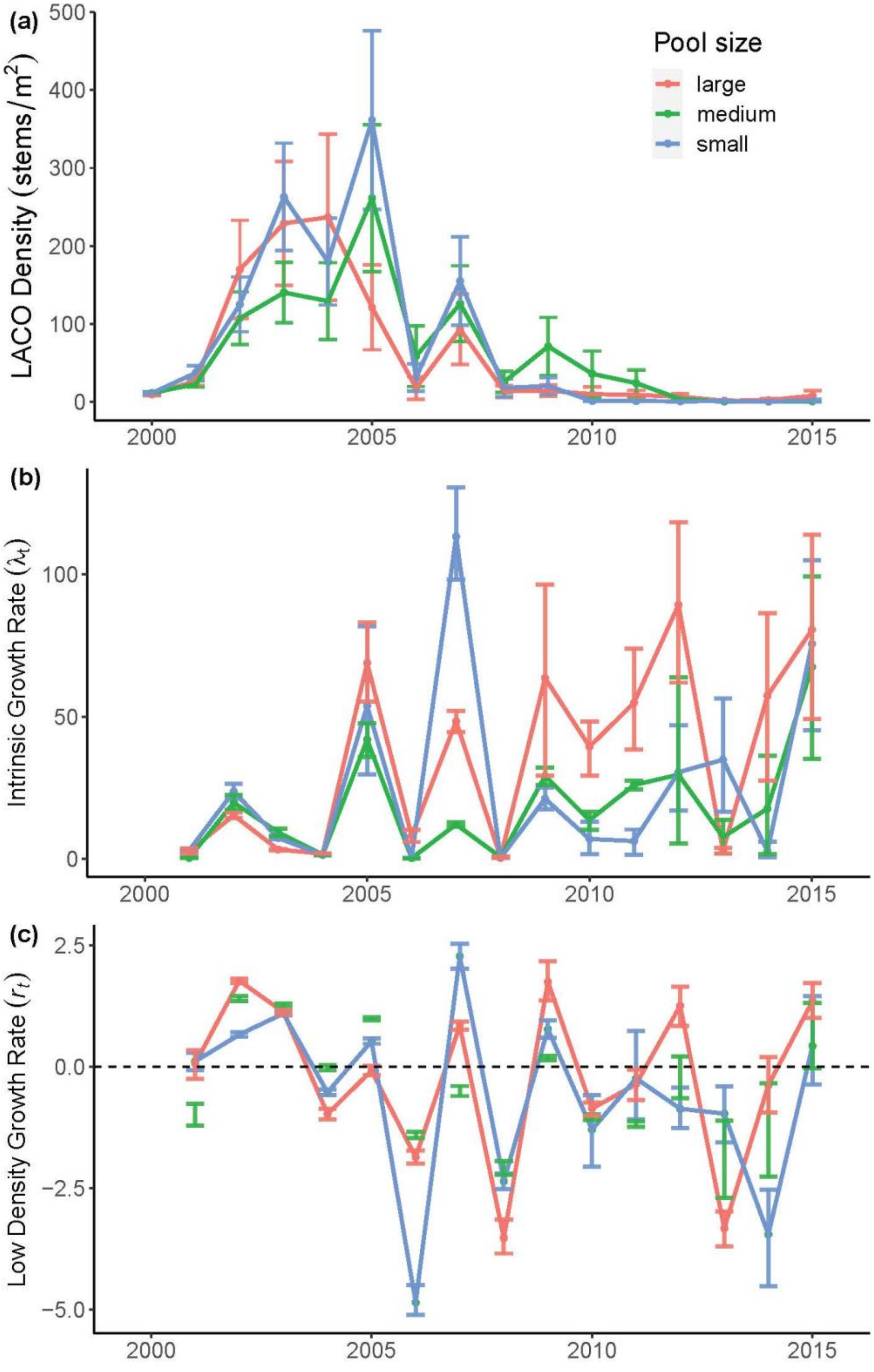

**Figure S6.7.** Effect of pool size on *Lasthenia conjugens*'s (a) density, (b) intrinsic growth rate, and (c) low-density growth rate. Pools were constructed in three size classes: small (25 m2), medium (50 m2), and large (100 m2), n = 85 each, to bracket the median area (78 m2) of natural pools within the study site.

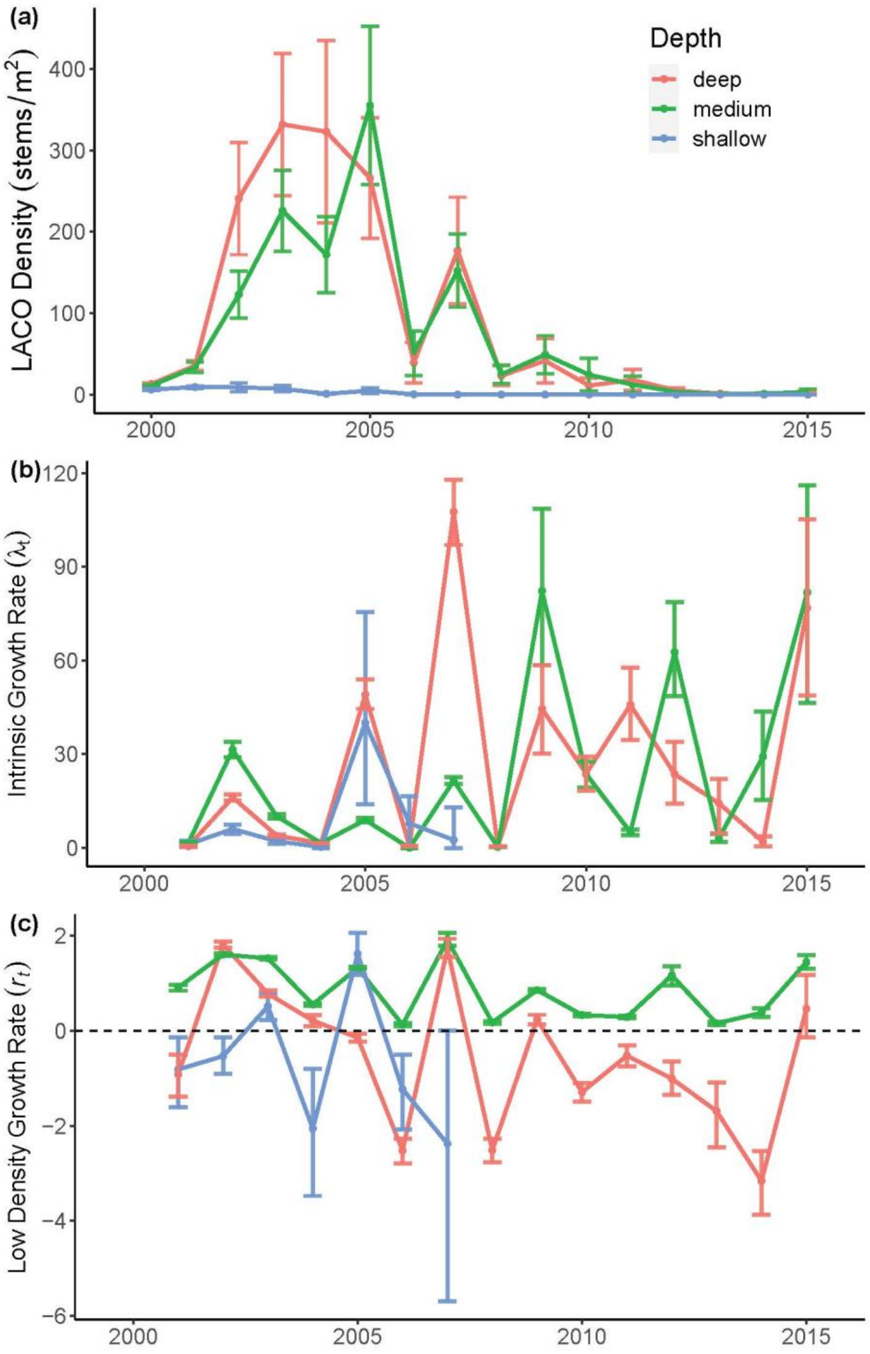

**Figure S6.8.** Effect of pool depth on *Lasthenia conjugens*'s (a) density, (b) intrinsic growth rate, and (c) low-density growth rate. Inundation or pool depth (cm) was measured from December to April in 1999-2000, 2001-2002, and 2008-2012. Depth classes were determined by 25th and 75th percentiles of annual maximum pool depth averaged across years: shallow (< 3.8 cm; < 25th percentile; n = 29), medium (3.8 - 8 cm; 25-75th percentile; n = 76), and deep (> 8 cm; >75th percentile; n = 37). *Lasthenia conjugens*'s density consistently declined to zero across all shallow pools since 2007, so we did not calculate intrinsic growth rate or low-density growth rate beyond 2007.

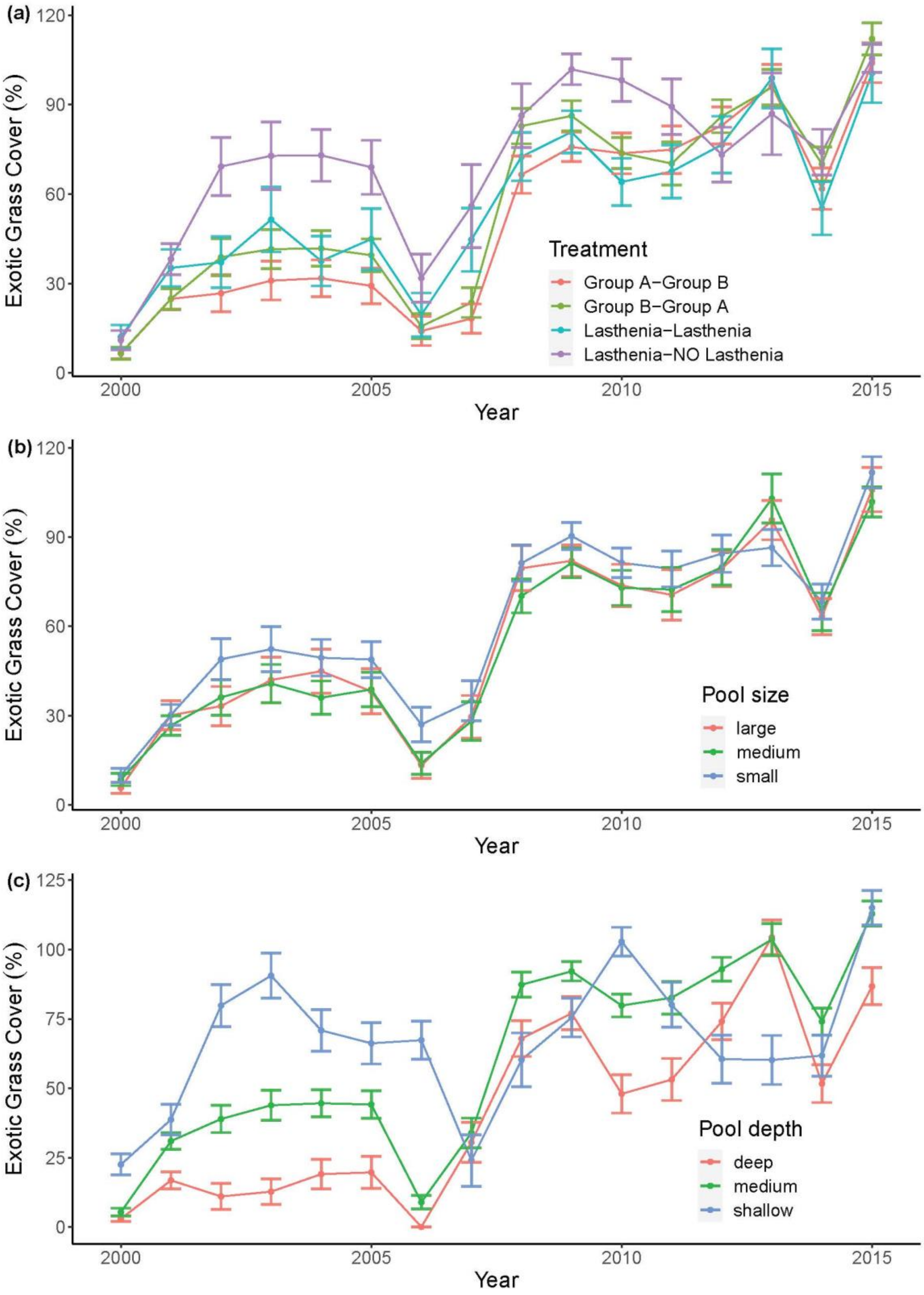

**Figure S6.9.** Frequency of exotic grasses (%) in constructed pools did not vary much by (a) pool size or (b) initial seeding treatment. See methods for details on the seeding treatment, pool size, and pool depth.

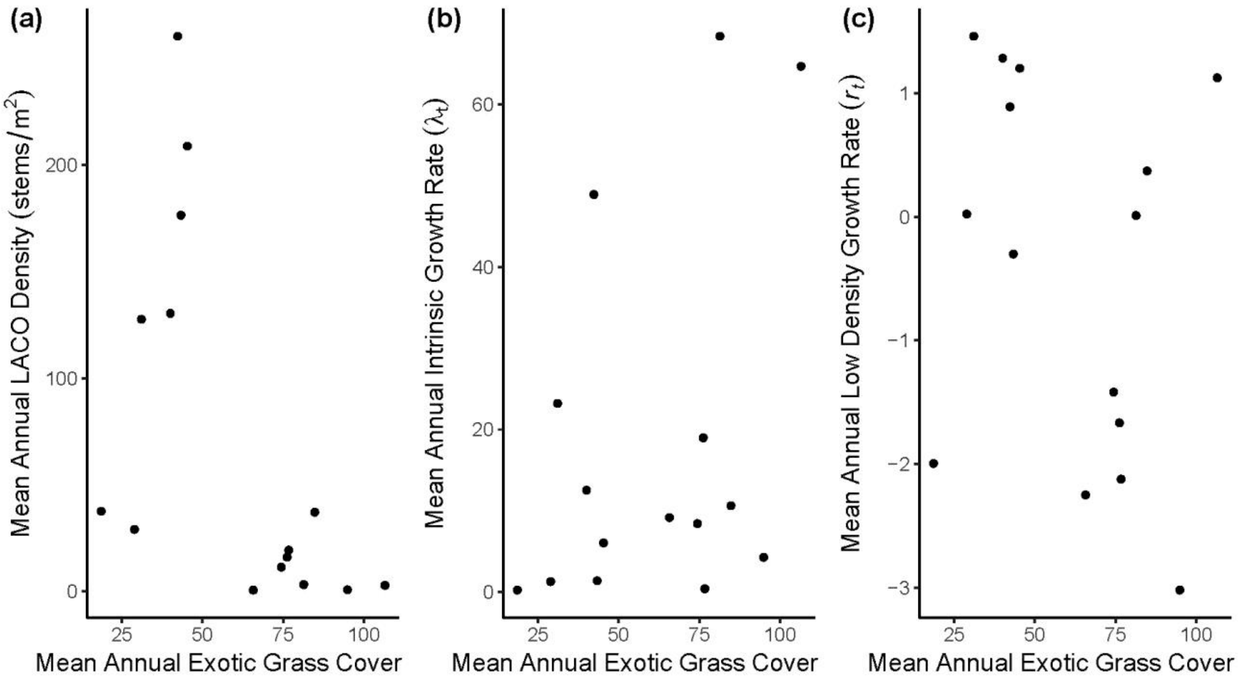

**Figure S6.10.** Mean annual grass cover (%) compared to Lasthenia conjugens's mean annual (a) density, (b) intrinsic growth rate, and (c) low-density growth rate in constructed pools. Each point represents a year.

**Table S6.1.** Effect sizes of simulated exotic grass removal on *Lasthenia conjugens* abundance by year. Two treatments include 50% grass removal and 75% grass removal. The effect sizes are calculated as the difference between mean *L. conjugens* abundance with and without treatment divided by the standard error of mean *L. conjugens* abundance without treatment.

| Year | 50% removal | 75% removal |
|---|---|---|
| 2000 | 2.970 | 8.469 |
| 2001 | 1.970 | 5.539 |
| 2002 | 1.925 | 6.016 |
| 2003 | 1.483 | 4.399 |
| 2004 | 1.194 | 3.985 |
| 2005 | 0.700 | 1.909 |
| 2006 | 0.665 | 1.761 |
| 2007 | 0.558 | 1.261 |
| 2008 | 0.797 | 1.633 |
| 2009 | 0.939 | 2.535 |
| 2010 | 0.392 | 1.222 |
| 2011 | 0.395 | 1.191 |
| 2012 | 0.686 | 2.185 |
| 2013 | 0.309 | 1.296 |
| 2014 | 0.574 | 2.785 |
| 2015 | 0.390 | 1.797 |

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