

POST-FIRE RESEEDING EFFECTS ON THE GENETIC DIVERSITY
OF NATIVE GRASS IN THE GREAT BASIN

by

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After large wildfires, land managers reseed burned areas with native perennial grass species such as blue bunch wheatgrass (*Pseudoroegneria spicata*) to support the regeneration of grasslands and prevent soil erosion and cheatgrass (*Bromus tectorum*) invasion. The seeds used for restoration often have lower genetic diversity than native populations, which would in turn lower the local genetic variation of the restored population. Low genetic variation is a concern for restoration because it lowers the population's adaptability and resilience to environmental changes. I want to learn the effects of reseeded on the genetic diversity of grasslands to enhance post-fire restoration. This study explores the effect of post-fire reseeded on the genetic diversity of the native bluebunch wheatgrass. I hypothesized that burned plots of grass that have not been reseeded will have a higher level of genetic diversity than that of the burned plots that were reseeded. After completing my analysis, I did not find any significance between the genetic diversity of the reseeded versus unseeded plots. These findings are likely due to the genetic makeup of the seed and how they established in the ecosystem. This research is important because post-fire restoration ecology is increasingly important to maintain the Northern Great Basin grasslands with the presence of invasive species.

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Introduction

Restoration is a critical tool for preventing the invasion of non-native plants into ecosystems after a wildfire. Although fire has always been present in the Great Basin, the recent change in land use because of colonization and livestock grazing has decreased the fire interval from decades or centuries to years (Boyte et al., 2016). Annual grasses, such as *Bromus tectorum*, better known as cheatgrass, spend more of their energy quickly growing dense above-ground biomass to set their seeds early in the season. Cheatgrass also dries earlier than perennial grasses, contributing to fire fuel before perennials can set their seeds, depleting their seed bank for future years (Fenesi et al., 2016). Cheatgrass has heavily invaded parts of the Great Basin and with the combination of these characteristics, quickly decreases fire intervals even more (Fenesi et al., 2016). Shortened fire intervals support the growth and spread of nonnative annual grasses such as cheatgrass because they establish quickly postfire while simultaneously decreasing the abundance of native plants that need more time to establish and set seeds after a fire, This positive feedback loop is why post-fire restoration is so important (Boyte et al., 2016). In the Great Basin between 2009-2018 13.5 million acres burned (BLM). Because of these large fires, restoration efforts are growing and roughly 223 million acres of sagebrush communities are being conserved and restored by the BLM (*The Bureau of Land Management Releases Decision to Reduce Fuels, Restore Sagebrush Areas in Great Basin | Bureau of Land Management, n.d.*). Perennial grasses resist the invasion of non-native annual grasses by establishing extensive root systems that the annuals cannot compete against when reestablishing yearly, which is why one of the most common ways of restoring grasslands is through reseeding with seed banks.

When restoring an ecosystem, biodiversity and genetic diversity are important components to focus on because they contribute to the resilience of the ecosystem. Resilience

can be understood as the likeliness of the individuals of a species to survive change in its environment (Oliver et al., 2015). Biodiversity and genetic diversity increase resilience through variation. Diversity and variation allow some individuals of a species to survive environmental changes that other individuals might not, allowing the species to survive the change. With low biodiversity, the loss of species in an ecosystem is more likely because one event or change could kill too many for the population to continue (Oliver et al., 2015). When restoring a grassland post-fire, nonnative seed, or seed with low genetic diversity from a high-volume nursery, can lead to continued damage to the restored ecosystem. Reseeding grasslands is the most beneficial when the species and genetic diversity of the seed are taken into consideration (McCormick et al., 2021). Native plants are adapted to their environment and therefore are the most efficient for restoration projects, especially native mixes with high species and genetic diversity (McCormick et al., 2021). However, these seed mixes are much more costly and labor-intensive than nursery ones made with lower diversity and are therefore not used as much (McCormick et al., 2021). The high price can have large effects on the stability and resilience of the restored grassland. In this study, we will be discussing genetic diversity in terms of allelic richness and heterozygosity. Allelic richness is the measurement of how many different alleles for a gene are present within a population while heterozygosity describes how those alleles are spread within individuals and the population.

Although ecologists know that biodiversity is important when restoring an ecosystem, producing seed with high genetic diversity, measured by allelic richness and heterozygosity, in big enough volumes for most restoration projects is very difficult. Commonly, agronomic seed mixes are selected toward specific traits such as high yield or fast growth with low diversity, but in restoration ecology, the goal is to have high genetic diversity across native species as well as

within one species such as bluebunch wheatgrass to increase biodiversity and resilience (Leger et al., 2021). But it is because of this unknown, and potentially low, genetic diversity of the commercially produced seeds that restoration sites can have variable success. Hypothetically, if the seed has comparable diversity to the native population, it is more likely to be successful and maintain the diversity of the ecosystem, and a lower-diversity seed will decrease the diversity or not establish. However, this narrative does not take adaptation and natural selection into consideration. Individuals that are more fit for their environment are more likely to survive and pass down their genes, and those that are not fit are less likely to. If one gene variation is significantly beneficial to individuals, over time, it will become more and more common in the population due to natural selection, decreasing the diversity of that specific gene. Therefore, introducing new variations via seeds in restoration efforts may increase genetic diversity.

The purpose of this research is to better understand how reseeded affects the genetic diversity of the restored bluebunch wheatgrass in the field. Bluebunch wheatgrass, *Pseudoroegneria spicata*, is a native perennial grass of the Great Basin. It is well adapted to the Great Basin and is drought and disturbance resistant. The restoration project studied in this research was done with a seed that contained bluebunch wheatgrass because of these characteristics. The research question is: How does post-fire reseeded affect the genetic diversity of the native bluebunch wheatgrass? I hypothesize that the burned plots of grass that have not been reseeded will have a higher level of genetic diversity than that of the burned plots that were reseeded. I also hypothesize that the population in the unburned plots will have higher genetic diversity than in both the burned plots.

Methods

Data collection

To test my hypotheses, I genotype sequenced microsattelites of bluebunch wheatgrass from a post-fire restoration site in the Northern Great Basin. I compared the genetic diversity of bluebunch wheatgrass across three different treatments: four plots of unburned/unseeded (UU), three plots of burned/unseeded (BU), and four plots of burned/reseeded (BS). The plots had a 50-meter radius and the average density of bluebunch wheatgrass UU was 2.00 individuals per m², the average density of BU was 2.29 individuals per m², and the average density of BS was 3.32 individuals per m². To do so, we collected fresh leaves of twenty individuals from all the plots (for a total of 220 individual samples) in the summer of 2020 and 2021 from the Soda Fire that burned in 2015 which was seeded with Anatone cultivated bluebunch wheatgrass in 2016 in Owyhee, Idaho on public land managed by the Bureau of Land Management (Figure 1).

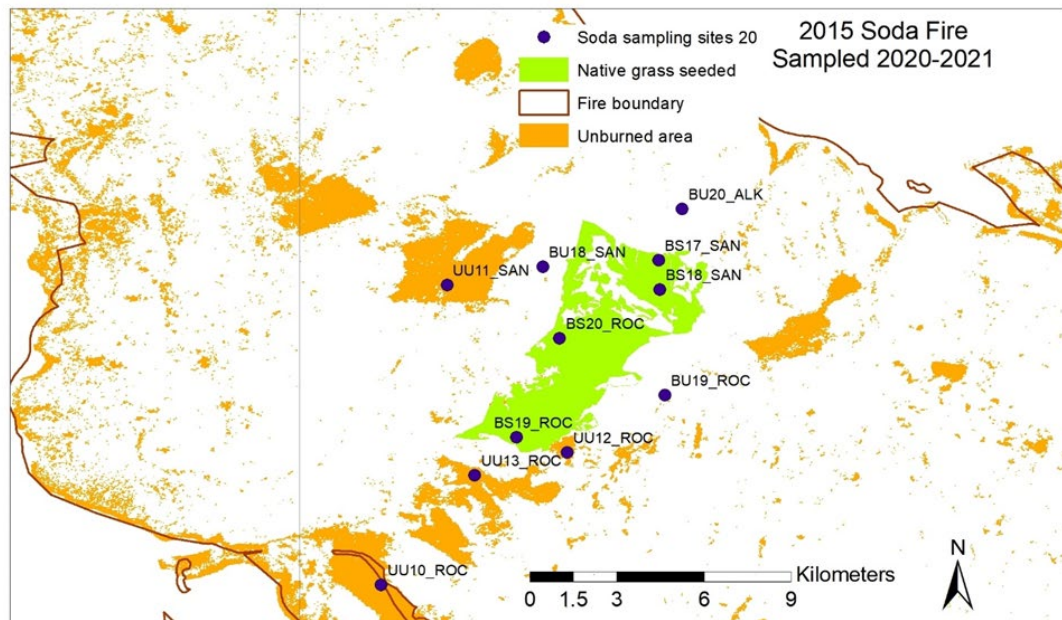


Figure 1: Map of the Soda Fire and sampling sites where individuals were collected.

Two batches of leaf samples were dried and stored in a -80° C freezer for 14 and 26 months. DNA extractions were completed using the cetyltrimethylammonium bromide (CTAB) method (Aboul-Ftooh Aboul-Maaty & Abdel-Sadek Oraby, n.d., 2019) Following DNA extractions, short fragments of DNA were copied via polymerase chain reaction (PCR) using nine primer sets. We randomly selected primers belonging to 20 genes and kept nine of them that had polymorphism which were the following: FF34758.1 HEX, FF343209.1 HEX, FF340831.1 FAM, FF340262.1 HEX, FF343025.1 FAM, FF344307.1 HEX, FF347040.1 HEX, FF344338.1 FAM, and FF344396.1 FAM. We do not know the function of these genes. The PCR samples were delivered to Oregon State University Genomic Core for genotype sequencing via an AB 3730 capillary DNA sequencer (Applied Biosystems).

Data Analysis

We analyzed the sequencing data using Geneious Prime software to count and measure the peaks of the present alleles (Geneious). To be conservative, the alleles were manually binned so that the peaks had to be at least three base pairs apart to be considered different alleles. The two genetic diversity metrics used in this experiment were heterozygosity and allelic richness. Heterozygosity was measured by the frequency of two peaks and allelic richness was measured by the number of unique alleles present after they had been binned. In this research, higher genetic variation is measured by higher heterozygosity and allelic richness. Analysis of Variance and Post Hoc Tukey statistical tests were used in R 4.2.1 (R Core Team 2022) programming language to measure the difference in heterozygosity and allelic richness between the treatments: Burned/Seeded, Burned/Unseeded, and Unburned/Unseeded.

Results and Figures

Heterozygosity

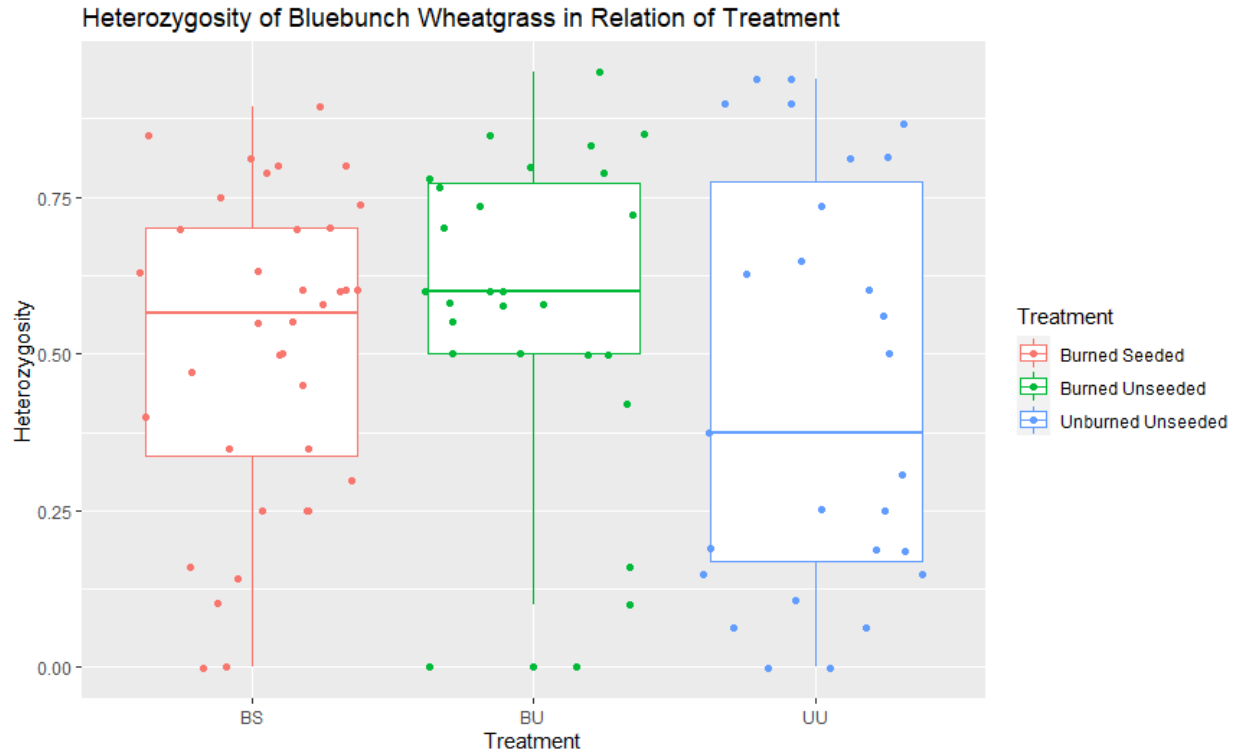


Figure 2: The heterozygosity of the group of individuals from each plot at the nine genes copied with PCR. BS- Burned Seeded, BU- Burned Unseeded, UU- Unburned Unseeded. Each point represents the heterozygosity of all the individuals from the plot at a gene copied by one of the primers used. The points are grouped by treatment. Box plots over the scatter plots show the five-number summary: the top of the superior vertical line is the maximum, the superior horizontal line is the upper quartile, the middle horizontal line is the median, the inferior horizontal line is the lower quartile, and the bottom of the inferior vertical line is the minimum.

Allelic Richness

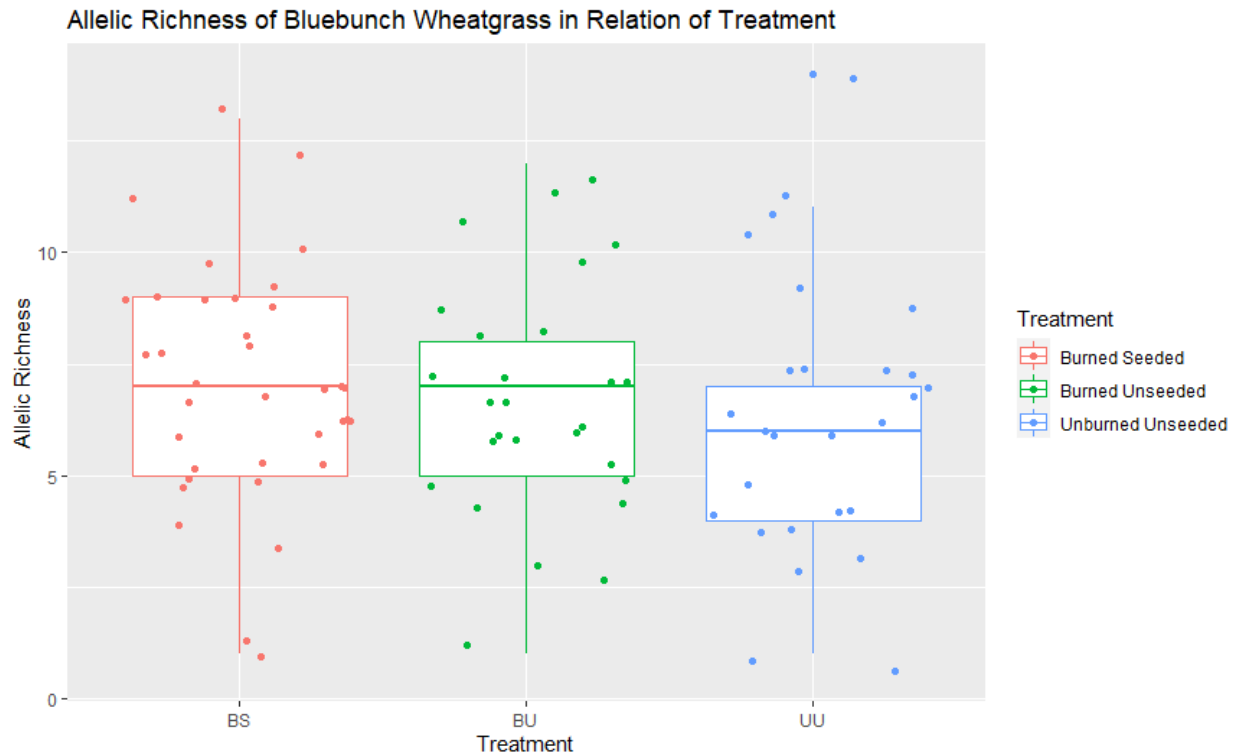


Figure 3: The allelic richness of the individuals from each plot at the nine genes copied with PCR. BS- Burned Seeded, BU- Burned Unseeded, UU- Unburned Unseeded. Each point represents the allelic richness of all the individuals from the plot at a gene copied by one of the primers used. Points are grouped by treatment. Boxplot shows the five-number summary: the top of the superior vertical line is the maximum, the superior horizontal line is the upper quartile, the middle horizontal line is the median, the inferior horizontal line is the lower quartile, and the bottom of the inferior vertical line is the minimum.

My results showed that the treatments did not create a significant difference in the genetic diversity of the individuals within the plots. An ANOVA statistical test ($\alpha=0.05$) on the heterozygosity data had 87 degrees of freedom, a p-value of 0.2486, and an F statistic value of 1.4143. This p-value is larger than the alpha level and fails to reject the null hypothesis. The mean heterozygosity of the BS treatment is 0.5096 with a standard error of 0.0413. The mean heterozygosity of the BU treatment is 0.5756 with a standard error of 0.0493. The mean heterozygosity of the UU treatment is 0.4488 with a standard error of 0.0634. An ANOVA statistical test ($\alpha=0.05$) on the allelic richness data had a p-value of 0.5565 and an F statistical

value of 0.5901. This p-value is larger than the alpha level and fails to reject the null hypothesis. The mean allelic richness of BS is 7.0278 with a standard error of 0.4445. The mean of BU is 6.7037 with a standard error of 0.5087. The mean of UU is 6.2593 with a standard error of 0.5861.

Discussion

The question of this research project was what effect does post-fire reseeding have on the genetic diversity of the native bluebunch wheatgrass? I hypothesized that the burned plots of grass that were not reseeded would have a higher level of genetic diversity than that of the burned plots that were reseeded and that the genetic diversity in the unburned plots will have higher genetic diversity than in both the burned plots. This is because the unseeded burned plots will have had some wind-dispersed cross-over from the nearby wild unburned grass while the reseeded burned plots were subject to seed mix that likely had lower genetic diversity. As seen in the results section, our results did not support the hypothesis. There was not any significant difference in allelic richness or heterozygosity between the plots based on treatment.

Because the genetic analysis of the plots did not support the hypothesis, we cannot say that reseeding restoration sites affects the heterozygosity of the population as we expected. The seeded plot's heterozygosity was not significantly different than the unburned plots, which likely means that the seed population's heterozygosity was nearly the same as the native wild population or that they did not establish and cross over enough to make a difference in the genetic diversity. Seeding also did not influence the allelic richness of the restored population. This too likely means that either the native bluebunch drill seed mix had comparable allelic richness or that we collected naturally occurring individuals as well as seeded individuals. 45% of the seed mix was made of the Anatone cultivar strain of bluebunch wheatgrass along with

23% Snake River Wheatgrass, 15% Thickspike Wheatgrass, and 17% Big Bluegrass (ESR, 2020). These results do not support the hypothesis which means that we cannot conclude that restoration with native seed mixes negatively affects the genetic diversity of restored grasslands in the Northern Great Basin.

These results were unexpected because we assumed that the seed mix had lower heterozygosity and allelic richness than the wild population. There are two explanations as to why these results might have happened. The first is, that because the mix was made of blue-bunch and other native perennial grasses it may have had relatively similar genetic diversity to the wild population and therefore did not affect it either way. This is possible because if done correctly, according to McCormick et al. seed mixes can be produced that will not negatively affect the restoration site's genetic diversity. By following the correct steps; seed collection, evaluation and development, field establishment, seed production, seed certification and procurement, seed storage, and restoration, producers can mitigate the harm of potentially lowering the wild population's genetic diversity and stability caused by the seed mixes they use. The harm they could do by using seed mix that was not produced following these steps could decrease the genetic diversity and resilience of the community, leading to an increased risk of annual grass invasion. Therefore, if the seed mix used for the Soda Fire restoration site was produced following these steps, it likely did not decrease genetic diversity because it was made of native perennials with diverse genetics. Another reason that could explain the unexpected results is that decades and centuries of natural selection and adaptation of the focus alleles decreased the variation of a certain gene in the wild grassland prior to collection, so the seed mix actually brought more variation to the restoration site and when mixed with the nearby unburned

wild population. It actually increased diversity of the focus genes by adding back alleles whose frequency had been decreased by natural selection.

A limitation of this study was the alleles were binned manually because there was not an available r package to use. Error due to this limitation would be random and likely not skew the data in one direction. Another limitation is that there might have been a crossover from the unburned wild grass to the burned plots which would cause an increase in diversity. To further this research, I would compare the effects on genetic diversity of restoration reseeded at varying distances from the burn edge. I think this research would be interesting because it would give insight into how crossover from unburned wild plants affects the genetic diversity of the restored sites. It could also help explain potential reasons why we found higher genetic diversity in the restored areas.

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