Leveraging Environmental Seed-Source Characteristics to Improve Resilience, Performance and Efficiency in Native Forage Grasses

Project Narrative

1. Response to previous review

This proposal is a new submission and has not been previously reviewed.

2. Progress Report

We have not received prior funding from this program.

3. Global Engagement

Not applicable to this proposal.

(a) Introduction

Objective:

We propose to address fundamental knowledge gaps linking seed-source environmental characteristics and simulated cultivation practices, specifically irrigation, to plant productivity in order to support the development of resistant and resilient native forage grass plant materials that will display high biomass productivity, seed production, and structural and physiological traits conducive to drought resistance including higher water-use efficiency. These materials will build rangeland resilience in a changing world, and support ranching and restoration activities to bolster the economic benefits of productive and functional rangeland ecosystems. **We propose a two-phase project (Fig. 1) that uses seed-collection environmental site characteristics (edaphic characteristics, temperature, precipitation amount and variability) to identify productive and drought resistant populations of two native dryland forage grasses (*Bouteloua curtipendula, Sporobolus cryptandrus)*. In addition, we will compare plant performance and drought resistance of wild-collected seeds from inside and outside the target ecoregions (Arizona/New Mexico Plateau and Colorado Plateau) with performance and resistance of widely available cultivars and commercially available seeds that are used in seed-increase and restoration activities. Finally, we will assess whether supplemental watering mimicking cultivation or ambient growing conditions induce transgenerational plasticity in ways that impact plant performance using a reciprocal transplant experiment across common gardens in Arizona and New Mexico.**

Background:

1. Magnitude and Relevance

The vast majority of grazed public lands in the United States are located in the arid southwest, and forage production is one of the primary ecosystem services generated on these lands. Recent studies of western US rangelands have predicted habitat losses

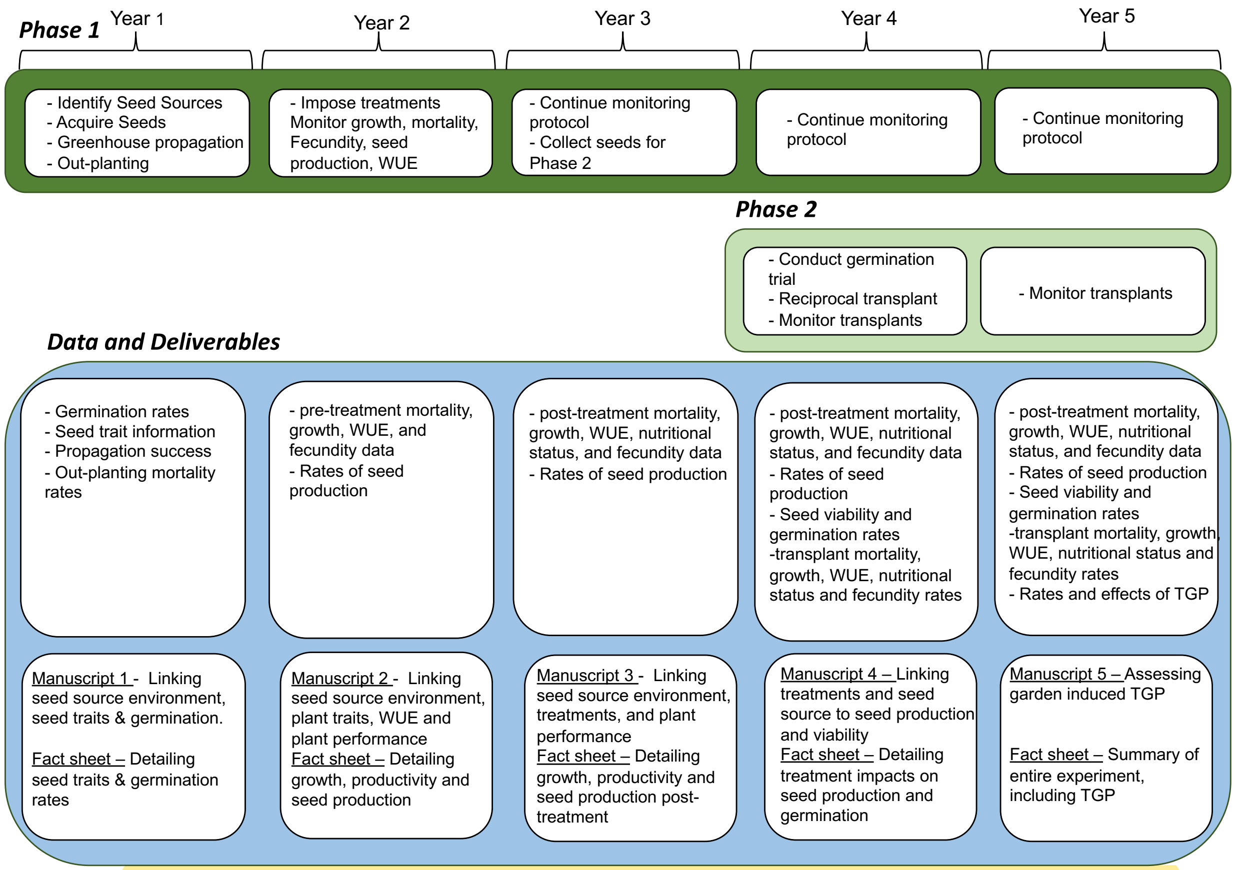

Figure 1. Our proposed, two-phase project will examine how seed source environmental characteristics impact performance, drought resistance, and productivity of two key native forage grass species across a temperature and aridity gradient representative of current and future climate conditions in the southwestern US. In Phase 1 (dark green rectangle), we will use seed from 12 populations of *Bouteloua curtipendula* and *Sporobolus cryptandrus* sourced from conditions ranging from hot and dry, to cool and wet, including three cultivars per species. Seeds will be propagated in the greenhouse and planted into one of three common gardens that span a climate gradient from hot/dry to cool/wet. In common gardens, plants will be subjected to either ambient conditions, or supplemental water mimicking cultivation. Plants will be monitored for mortality, growth, productivity, nutritional value, WUE, and fecundity. In Phase 2 (light green rectangle), seeds from the most productive populations at the hot/dry garden and cool/wet garden will undergo reciprocal transplant and will be monitored identically to plants in Phase 1. At the same time, seeds collected for Phase 2 will undergo germination trials. Key data products and deliverables (blue rectangle) that will enhance the success of seed increase, reseeding, and restoration activities will be provided to managers and growers in the form of fact sheets and scientific publications within the first year of the project.

of up to 37% as a result of land-use and environmental change (Byrd et al., 2015). Regional longitudinal climate analyses have found that over the past 50 years, amounts of precipitation have decreased while the duration between precipitation events has increased and temperatures have increased (Zhang et al., 2021). Models of interactions between climate and soil moisture indicate that in arid southwestern grasslands, altered climate will decrease soil moisture and increase the intensity and duration of aridity, leading to dramatic shifts in vegetation including loss of critical forage species (Schlaepfer et al., 2017) and reductions in livestock productivity (Craine et al., 2010; Havstad et al., 2018).

Such changes in climate, including the timing and intensity of rainfall, as well as changes to seasonal temperature, can impact arid rangeland vegetation in a number of ways. Recent work across multiple grassland biomes has indicated that dry grasslands are the least resilient (i.e., the ability to return to pre-drought levels of productivity) or resistant (i.e., able to withstand drought conditions without experiencing decreased growth or mortality) to extreme drought (Stuart-Haëntjens et al., 2018), and increasingly variable precipitation regimes reduce arid grassland net primary productivity (NPP; Ritter et al., 2020). In arid regions, rising atmospheric CO_2 and decreases in precipitation are expected to reduce productivity of forage grasses (Izaurralde et al., 2011) while increased heat stress is predicted to reduce the quality of forage grasses overall (Craine et al., 2010).

Economic losses in livestock production are directly linked to decreased forage production (Brownsey et al., 2013). Loss of livestock productivity in arid rangelands translates to a loss of economic productivity regionally and nationally. Sales of grazing permits to ranchers were estimated to total nearly $15 million in revenue in 2017, half of which was returned to managers to restore and improve arid rangeland ecosystems (USFS 2015, USDA 2016). In Arizona, approximately 76% of land area is owned by either the federal government or the state, and 72% of these lands are actively grazed (Conley et al., 2007) representing a key economic sector in the region. Grasses in arid rangelands are directly impacted by changes in temperature and precipitation (Izaurralde et al., 2011) and are particularly susceptible as they live near physiological limits for drought and heat-stress. Net primary productivity is projected to decline, and the presence of weedy annuals and grasses is expected to increase, as temperatures rise and precipitation becomes more variable (Archer & Predick, 2008) all of which are expected to negatively impact agricultural productivity in the region (Havstad et al., 2018). **In order to maintain agricultural sustainability and ecosystem services in the face of climate change, drought resistant populations of forage grasses must be identified, established, and their availability increased and leveraged for rangeland improvement and restoration.**

Because genotyping and development of novel germplasm is often expensive, many land managers rely on wild-collected and commercially available cultivated native seed stock (“cultivars”). Current seed-collection protocols often urge that locally adapted ecotypes are prioritized for increase and application in seeding and restoration activities. However, the importance and efficacy of using local ecotypes is not always clear (Baer et al., 2014; Bucharova et al., 2017) as these species may be maladapted to future climate conditions (Butterfield et al., 2017). One current method for selection of restoration and propagation materials is through collection and planting across seed transfer zones. These “zones” delineate areas where “plant material may be transferred with little risk of being poorly adapted” (Bower et al., 2014; Durka et al., 2017). However, these delineations are typically generated using climatic or geologic conditions that may not accurately reflect genetic variation and gene flow between populations (Listl et al., 2018). In addition, these zones are based on historical environmental conditions (Miller et al., 2011), and may not accurately reflect current or future environmental or disturbance conditions. A lack of suitable and resilient seed materials can lead to poor success during seeding and restoration (Banerjee et al., 2006; Gornish et al., 2019) and ultimately a loss of agricultural productivity.

In order to successfully maintain and increase native forage cover in the face of climate change, we must expand current seed collection and plant materials protocols beyond an emphasis on “locally adapted” populations, to focus instead on drought resistance to bolster rangeland resilience and productivity. Considering or directly manipulating the environmental conditions experienced by parent plants is a rarely explored pathway to increase the resilience of offspring. However, in a multi-garden study, populations of *Poa secunda* sourced from warmer, drier habitats tended to have greater plasticity in phenological traits (the timing of flower and seed production), considered critically important for coping with an unpredictable environment, than those from cooler, wetter climates (Espeland et al., 2018). Further, global analyses have indicated that xeric grassland ecosystems can rebound, and at times, overshoot productivity when drought stress is alleviated (Stuart-Haëntjens et al., 2018), suggesting that grass populations derived from more xeric locations may show greater resilience and productivity when used to restore less harsh environments where they are “released” from precipitation limitation. In addition, a rapidly growing body of literature suggests that stress experienced by parental plants can induce beneficial plastic change in offspring. Termed “transgenerational plasticity (TGP)”, this important mechanisms has been shown to enhance drought tolerance across a wide variety of plants (Donelan et al., 2020; Herman et al., 2012; Latzel et al., 2014; Münzbergová & Hadincová, 2017; Wadgymar et al., 2018), though with few studies in arid forage species. Because parental conditions can influence offspring resilience, cultivation conditions (e.g., supplemental watering) could have serious consequences for the tolerance and productivity of seed materials if garden conditions select for less drought hardy populations or genotypes. There is an increasing recognition that locally adapted genotypes may not be optimal under future conditions (Bucharova et al., 2017), and that seed should be sourced more broadly to enhance resistance and resilience by generating regional admixtures (Bucharova et al., 2019). **A more thoughtful and targeted approach to the regional admixture idea, one that incorporates environmental variability of seed source populations and considers cultivation conditions, may generate greater drought resistance and resilience in native forage grass plant materials.**

In order to address the pressing need for stable forage production in a warmer, drier future, we propose to leverage three common gardens spanning a temperature and aridity gradient to understand how seed source environmental characteristics influence germination, mortality, productivity, drought tolerance, and WUE in two native forage grass species.

2. *Stakeholder Roles*

This project has been developed in concert with the Institute for Applied Ecology Southwest Seed Partnership (SWSP), the New Mexico Land Conservancy, and New Mexico Bureau of Land Management (BLM-NM). We utilized the BLM-NM Plant

Conservation and Restoration priority species list to identify forage grasses that meet regional plant and seed material needs, and to build capacity to increase appropriate seed materials for restoration and reseeding initiatives. Letters of support included with this proposal reflect stakeholder interest in the project. Representatives from the BLM-NM, NMLC and SWSP will be updated annually and provided with management-oriented fact sheets and given the opportunity to provide feedback and guidance on project progress during an annual virtual stakeholder meeting. Culminating outcomes and results of both Phases of the proposed project will be formally presented to Stakeholders in Year 5 of the proposed project.

3. Reasons for Performing Work at Proposed Institution

Northern Arizona University (NAU) is ideally situated to support this project. The Southwest Experimental Garden Array (SEGA) provides ample protected experimental garden space to conduct the proposed research at low cost. PI Mitchell assisted in the foundation of a common garden on NMLC property in 2019. PIs Mitchell and Souther are well qualified to carry out the proposed research, with expertise in plant propagation, phenotypic and physiological monitoring, common garden studies, and plant maternal effects. PIs have access to necessary ecophysiology equipment (e.g., Portable photosynthesis system (LI-6800) and Vapor pressure osmometer (VAPRO 5520)), and facilities (NAU Stable Isotopes Lab) that will support the completion of this project. PIs Mitchell and Souther already have strong ties with the BLM-NM and NMLC.

Previous research conducted at the Santa Fe garden supported by the BLM-NM and NMLC by PI Mitchell has demonstrated that, for two native forage species *Bouteloua curtipendula* and *Sporobolus cryptandrus*, plant performance (survival, growth, and fecundity) did not necessarily decrease with increased geographic distance from a common garden (Fig. 2A), but rather matching environmental characteristics of seed sources to the environment of the garden better predicted performance (Doherty et al.,

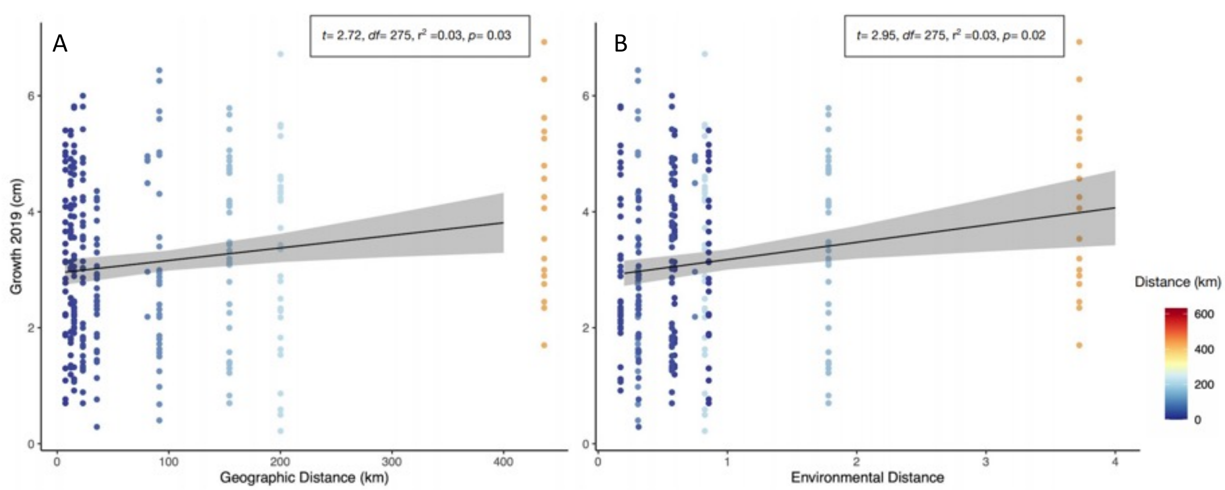

Figure 2. For eleven *Bouteloua curtipendula* populations, including one cultivar, growth in the Santa Fe common garden was better predicted by how closely seed source environmental conditions (environmental distance, panel B) matched garden conditions than by the how geographically distant a seed source was from the common garden (A).

2017; Fig. 2B). These results suggest that matching collection environments to future conditions may yield better results for establishment or seed increase operations than simply focusing on nearby “locally adapted” populations. However, future conditions across the southwestern US are expected to become hotter and drier, with more variable precipitation (Garfin et al., 2013). Thus, further research is required to understand how levels of precipitation and temperature, as well as variability in precipitation, impact forage grass drought resistance and performance. To best understand resistance, plants must not only be grown in a novel environment, but also subjected to stress mimicking future conditions. Therefore, we will leverage three common gardens spanning a temperature and aridity gradient in a provenance trial to examine the effects of seed-source environment, supplemental watering, and ambient temperature and precipitation on plant performance and drought resistance.

Research Questions and Hypotheses:

We propose a large, multi-garden provenance trial of two forage and restoration relevant perennial grass species. We will source seeds from sites that differ significantly in the amount and variability of temperature and precipitation experienced by the parent plants. We will collect seed from within and just outside of the target ecoregions, the Colorado and Arizona/New Mexico Plateaus. In addition, we will include commercially produced cultivar seed in our trials, to test water-use efficiency (WUE), plant performance, nutritional value, and drought resistance of widely available and commonly used seeds. This experiment will address three key questions: 1**) Does seed-source environment, particularly temperature, precipitation, and their variability, predict drought tolerance, WUE, and impact plant performance across a temperature and aridity gradient; 2) Do plants grown from seed sourced outside of the ecoregion necessarily display maladaptation in the common garden and in response to temperature and aridity? And 3) Can we detect a signal of transgenerational plasticity (TGP) in seed derived from common gardens and in response to temperature, aridity and supplemental watering?** That is, are plants derived from seed grown under ambient conditions or under supplemental watering conditions that simulate grower irrigation practices more similar to one another in terms of WUE and performance across common gardens? This last question provides critical information about whether the environments of commercial increase operations may alter the adaptive capacity of forage seed materials. This work will provide essential management and production relevant information about seed-sourcing and impacts of garden cultivation on the drought resistance and resilience of native drylands forage species.

H_1: Plants derived from seed-sources with lower and more variable precipitation regimes and higher mean annual temperatures will display higher WUE and better plant performance across all common gardens and in response to supplemental watering. We predict that plants from hotter, drier climates and with more variable precipitation regimes will have better survival, growth, and higher fecundity across all gardens and in response to watering. If correct, this would strongly support considering

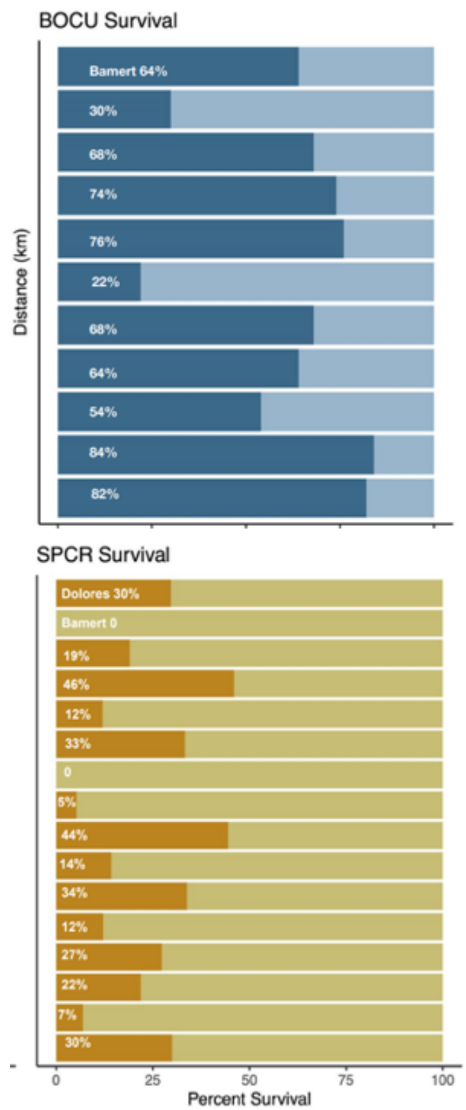

Figure 3. Survival data for populations of *B. curtipendula* (BOCU) and *S. cryptandrus* (SPCR) grown at the Santa Fe common garden indicate that commercial cultivars (Bamert and Dolores, respectively) can have significantly lower survival than some wild-collected populations in a common garden setting.

these site-level characteristics when collecting seed for restoration or seed-increase purposes.

H$_2$: Plants sourced from outside of the ecoregion, but from harsher (e.g., higher temperatures, more variable precipitation) climates will show higher WUE and enhanced performance in the garden. "General purpose" cultivars will have lower WUE and reduced performance in gardens which are hotter and drier. Although there is considerable research suggesting that locally adapted populations have the best performance in restoration settings (Baughman et al., 2019), local seed is often not available, or available in very limited quantities. If harsher, more variable climates convey adaptive capacity to future climate change, considering populations from *outside* of the ecoregion, but with high environmental matching to future conditions could boost seed availability as well as the resilience and success of seeding activities. Previous research with *B. curtipendula* and *S. cryptandrus* has demonstrated that commercially available cultivars performed poorly, exhibiting high or total mortality in the common garden compared to some wild-collected populations (Fig. 3). This result may be shaped by selective pressures imposed in commercial grow-out operations that limit the adaptive capacity and resilience of species.

H$_3$: Seeds derived from hotter, drier garden-grown plants will display positive effects of TGP on WUE and plant performance, but those grown with supplemental water will show strong negative effects of TGP on WUE and plant performance regardless of garden site. Transgenerational plasticity has been widely studied in annual plant species but is less understood for perennial plants and particularly for forage grasses. Plants grown under hotter and drier conditions may confer advantageous traits to offspring, resulting in higher drought tolerance, performance and resistance across all gardens, while those grown under supplemental water conditions, especially at lower temperatures and with higher precipitation, may not. If this hypothesis is correct, careful considerations of husbandry (e.g., geographic location of operation, temperature, and precipitation) in seed-increase operations and cultivar development must be considered.

In order to test these hypotheses, we will leverage three common gardens located across an aridity and temperature gradient. We will used two NAU SEGA (see Facilities document for description) sites located in Cameron, AZ (152mm precip, 15C; low precip/high temp) and at the Flagstaff Arboretum Meadow (556mm precip, 7.6C; high precip/low temp) and a third Bureau of Land Management/New Mexico Land Conservancy operated garden in Santa Fe, NM (328mm precip, 8.9C; intermediate precip and temp). All gardens are fenced to exclude herbivores and have water or water-delivery services available. We will select 12 populations representing a thermal and precipitation gradient (including cultivars) of two perennial forage species *(Bouteloua curtipendula* and *Sporobolus cryptandrus*) that have been identified as being of high forage, restoration, and seed-increase priority by the BLM-NM and SWSP.

Justification for Selected Species

Bouteloua curtipendula is a high quality, low fiber, high protein winter fodder, while *Sporobolus cryptandrus* is productive, palatable, and resilient to grazing (Humphrey, 1970). Both are considered generally drought tolerant and are widely distributed in the Southwest and across the United States, enhancing their utility as a seed crop for native forage grasses. Both are freely self-seeding, a strongly advantageous trait for establishment in range and restoration applications. In addition, both species are self-compatible, eliminating the need for elaborate experimental design to produce population specific seed for Phase 2 of the proposed project. Finally, both species have been identified as priority species for revegetation and restoration by the BLM-NM (see letters of support).

(b) Rationale and Significance

1. Rationale

The overarching objective of this project is to leverage seed-source environmental site characteristics to boost WUE, productivity, and resilience of native forage grasses. Presently, sourcing of plant materials typically relies on the assumption that locally adapted populations will perform better, in the short term, than materials sourced from more distant or environmentally dissimilar sites. This strategy ignores the rapidly warming and drying climate of the southwestern US (Garfin et al., 2013; Karl et al., 2009; Zhang et al., 2021) which threatens the productivity and viability of agriculture in arid rangelands (Craine et al., 2010; Havstad et al., 2018). At the same time, a growing demand for native grass seed is driving the selection of “general purpose” and readily available cultivars that may not be resilient to future conditions. Furthermore, garden conditions for commercial seed production may affect performance and WUE efficiency via TGP which may reduce the drought resistance of populations or cultivars and ultimately agricultural productivity. Research examining how seed site-selection, in combination with seed production protocols, build drought resistant, water-efficient, nutritious and productive plant materials is of pressing importance to rangeland sustainability, resilience, and restoration in arid regions experiencing increased drought frequency and severity.

This project will identify how seed-collection site characteristics influence resistance and resilience to hotter, drier conditions, as measured by carbon isotope discrimination (Δ), carbon-to-nitrogen ratios (C:N), fecundity, leaf gas exchange (CO_2 assimilation rate (A)); stomatal conductance (g_s); and instantaneous water-use efficiency (WUE, A/g_s), as well as quantifying whole-plant performance factors important for grower production, including seed size, seed production, germination, plant growth rates, biomass production, and mortality across a range of temperature and precipitation conditions, with and without supplemental watering. Furthermore, it will reveal whether garden conditions mimicking those for commercial seed production induce detrimental TGP in these important forage grasses, and whether TGP can be leveraged to enhance WUE, performance, and productivity of these grasses in an agricultural setting. If TGP strongly controls plant performance, protocols could be developed for growers to enhance WUE of rangeland seed products through adjustment to operation site selection and irrigation levels. This information can be leveraged to improve the "restoration economy", a rapidly growing sector of producers and practitioners increasingly relying on cultivated native seed materials (Bendor et al., 2015; Broadhurst et al., 2015; Tidwell & Brown, 2011). This information is especially relevant in this region as the Southwest Seed Partnership (SWSP, see letters of support), established in 2015 and in parallel with the National Seed Strategy, has begun assessing, prioritizing, and increasing production of native seeds through a network of small growers and farmers.

2. Relevance to Program Area Priorities

The proposed project addresses the following program priority areas:

1. We will use a combination of **whole-plant and ecophysiological approaches** to understand plant **response to abiotic stress to improve performance, productivity, and growth resilience to adverse environmental conditions** in two forage grasses of high economic, revegetation, and restoration value.
2. We will understand how seed source environment, common garden conditions, and supplemental water impact **"plant growth and developmental processes…"**
3. We will understand how seed source environment, common garden conditions, and irrigation shape "**mechanisms of plant response to abiotic stresses, including increased water use efficiency;"**

3. *Long-Range Improvements in Sustainability of U.S. Agriculture and Food Systems*

The proposed project addresses a pressing issue facing southwestern dryland agriculture: a loss of forage and livestock productivity in response to increasing aridity (Craine et al., 2010; Havstad et al., 2018). Our project leverages wild-collected and cultivar seed of two valuable, but understudied dryland forage grasses to identify the characteristics that lead to resilient and productive forage in a drier future. In addition, we will quantify how garden characteristics contribute to the adaptive capacity of garden produced seed, a key gap in our understanding of how to produce resilient seed at required scales. Results from our experiment will be directly communicated to the BLM-

NM, NMLC, and SWSP to inform current and planned seed material operations.
Our proposed work addresses the following AFRI priorities for sustainable agriculture and USDA strategic goals:

1) **Satisfy human food and fiber needs**

   The proposed work will provide useable information to improve the quality and amount of native forage available on public and private lands under increasing temperatures and aridity.

2) **Enhance environmental quality and the natural resource base upon which the agricultural economy depends**

   Information derived from the proposed work will directly contribute to seed-sourcing and seed-production decisions carried out by land management agencies and commercial growers. These seeds will be used in revegetation and restoration activities to improve environmental quality and natural resources on western rangelands.

3) **Make the most efficient use of nonrenewable resources**

   Our proposed project considers the impacts of irrigation on plant resilience and focuses on improving WUE in forage grasses that face increasing aridity and drought under climate change.

4) **Sustain the economic viability of agricultural operations.**

   Development of seed stock that leads to abundant, resilient, and diverse forage grasses in Western US rangelands will improve the economic viability of ranch operations in the US.

(c) Approach

1. Objectives

This project will provide practical information about how seed source and garden conditions impact WUE and plant performance in two native forage grass species. This information will be translated directly to stakeholders at the NMLC, BLM-NM, and SWSP, as well as disseminated more broadly via scientific publications and presentations to improve selection, production and cultivar development in native forage grasses.

The proposed project has two phases: 1) a multi-garden provenance trial that will test the impacts of seed-source environmental conditions on plant performance and WUE in two native forage grasses in ambient and supplemental water conditions; and 2) a reciprocal transplant experiment to detect effects of garden- and watering-induced TGP on WUE and plant performance. The primary objective of these experiments is **to identify seed collection site characteristics and garden environments that lead to drought resistant, high-yielding, nutritious and water use-efficient populations of native forage species.**

2. Methods

a) Stakeholder Involvement

Personnel at BLM-NM and the SWSP will inform plant population selection from available seed stock, or provide guidance on seed collection sites if necessary. Interested volunteers from the NMLC will be solicited for construction of common garden plots at the NMLC Santa Fe garden. Annually, stakeholders from BLM-NM, NMLC, and SWSP will be invited to a virtual stakeholder meeting, where project progress will be presented and stakeholder feedback and guidance will be solicited (see letters of support).

b) Project Activities

<u>Phase 1: Common Garden Experiment; Years 1-5</u>

Garden Sites and Population Selection

Leveraging existing partnerships with the BLM-NM, SWSP, and NMLC, we will establish common garden provenance and experimental trials at three sites along a temperature

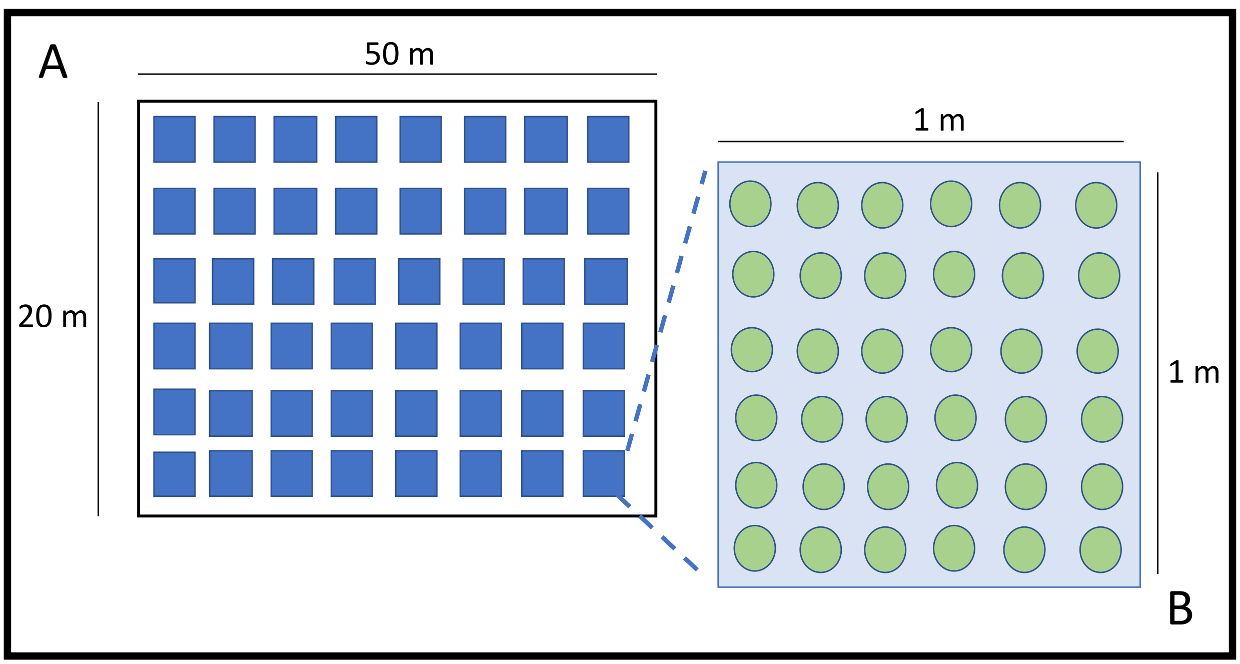

Figure 4. A schematic of common garden plot arrangement (A), with 48 1 m^2 research plots (B). Each research plot will have 36 individuals from each population randomly planted within it.

and precipitation gradient with gardens located in Cameron, AZ (152mm precip, 15C), at the Flagstaff Arboretum Meadow (556mm precip, 7.6C) and a Bureau of Land Management/New Mexico Land Conservancy operated garden in Santa Fe, NM (328mm precip, 8.9C). We will use collected and cultivated populations of *Boutaloua curtipendula* and *Sporobolus cryptandrus*. These species were selected in partnership with BLM-NM as they have high forage and restoration value, and are of high interest for seed increase. Populations will be selected to span a range of mean annual temperature and precipitation levels, as well as a range of precipitation variation, quantified by the coefficient of variation (CV) for precipitation. We will use the PRISM climate database (www.prismclimate.org) and WorldClim (www.worldclim.org) as well as Natural Resources Conservation Service (NRCS) soil series data to characterize seed collection site characteristics. Seeds will be obtained directly from NM-BLM and SWSP as well as from regional seed vendors for commercial cultivars. In the circumstance that suitable seed (e.g., seed from a hot location with highly variable precipitation) is not available, seed will be collected from a suitable site located within Arizona, Utah, Colorado, or New Mexico.

Experimental Design and Data Collection.

Each common garden will be planted with 72 individuals from each of 12 populations, including three commercial cultivar sources for each species, for a total of 864 individuals per species in each garden (n=2593 individuals per species across all gardens). Thirty-six individuals from each population will be assigned to either supplemental watering, or control treatments. Seeds will initially be planted in the NAU greenhouse to maximize replication in the common garden. Individuals from each population will be randomly planted into 1m^2 research plots in each garden to minimize edge effects in late spring after all damage of frost has passed (Fig. 4). Half of research plots will be randomly assigned to a supplemental watering treatment. However, supplemental watering treatments will not be imposed until Year 2 of the experiment, as all plots will receive supplemental water in Year 1 to ensure strong establishment of replicates.

On each plant, we will measure a suite of structural and physiological traits and parameters (Table 1). In the first year, we will sacrifice aboveground biomass of one individual from each population to calibrate visual cover estimates of biomass production. Visual estimation of aerial cover has been demonstrated to accurately reflect biomass production in dryland grasses and is a non-destructive way to quantify biomass production, aboveground net primary productivity, and forage availability while maintaining longer-term use of these grasses (Flombaum & Sala, 2007). Annually, on each individual we will select one leaf to quantify specific leaf area (SLA), a trait which reflects relative growth rate (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013), leaf dry matter content (LDMC) a trait which indicates forage productivity (Pakeman, 2014), and stomatal size and density (*see 'Targeted analysis of drought resistance traits' below*) which reflect drought tolerance (Bertolino et al., 2019; Xu & Zhou, 2008). Mature seed will be collected and weighed at the plant-level for each population*treatment category at each garden to estimate production.

Targeted analysis of drought resistance traits

In Years 3 and 5, we will sample 2 leaves from 10 adult plants from each population and each treatment to determine carbon isotope discrimination and nitrogen concentration, indicators of drought stress (Fravolini et al., 2002), at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University, Flagstaff, AZ using a DELTA V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) which is configured through a Finnigan ConFlo III for automated continuous-flow analysis of $\delta^{13}C$ and %N using a Carlo-Erba NC2100 elemental analyzer for combustion and separation of carbon and nitrogen. Carbon isotope discrimination is related to leaf intercellular CO_2 concentration, which is controlled by the ratio of net photosynthesis to stomatal conductance (Farquhar et al., 1982) and is negatively associated with instantaneous WUE. Stomatal size and density, which are directly linked to WUE (Hardy et al., 1995; Xu & Zhou, 2008), will be measured in Years 3 and 5 using a dissection microscope mounted camera and ImageJ image analysis software. Twice annually, during a dry period (mid-June) and a wet period (mid-August), CO_2 assimilation rate (A), stomatal conductance (g_s), and instantaneous water-use efficiency (WUE, A/g_s) will be measured on one leaf from plants used for carbon isotope discrimination and nitrogen concentration using a portable gas exchange system (LI-6800) that allows control of leaf chamber environment. At the same time, leaves from 5 adult plants from each population and each treatment will be collected to measure leaf osmotic potential at full hydration, a potentially important mechanism of drought tolerance (Bartlett et al., 2012), using a vapor pressure osmometer (VAPRO 5520).

Table 1. Proposed structural and physiological traits and parameter measurements

Measurement	Relevance	Frequency
Mortality	Productivity; resilience	Annually
Aboveground Biomass	Productivity	Annually
Specific Leaf Area	Relative Growth Rate	Annually
Leaf Dry Matter Content	Productivity	Annually
Seed Weight	Fecundity; Productivity	Annually
Seed Viability	Productivity	Year 3
Germination Rates	Productivity	Years 1 & 3
Stomatal Density	Drought tolerance	Years 3 & 5
Stomatal Size	Drought tolerance	Years 3 & 5
Stomatal Conductance	Drought tolerance	Twice Annually
Carbon isotope discrimination	Drought tolerance	Years 3 & 5
Nitrogen Concentration	Forage quality	Years 3 & 5
Instantaneous WUE	Drought tolerance	Twice Annually
Leaf Osmotic Potential	Drought tolerance	Twice Annually
CO_2 Assimilation	Drought tolerance	Twice Annually

Phase 2: Reciprocal transplant experiment to identify TGP; Years 4 and 5

In year three, flower stalks from adult plants from each population*treatment*garden will be bagged to prevent cross-pollination and maintain pure maternal lines. Mature seed will be harvested, cleaned and cold-stratified simulating grower protocols for planting in the following spring. Viability of collected seed will be assessed for 10 individuals from each population*treatment*garden group using tetrazolium staining of 100 seeds following cold stratification. To test for effects of TGP on garden-grown plants, we will propagate seed from the two most productive populations in each treatment*garden. Thirty individuals from each population*treatment*garden will be planted into either the Cameron (hot/dry) or Flagstaff (cool/wet) garden sites in the spring of Year 4 of the project. Plants will be monitored identically to Phase 1 common garden plants.

d) Expected Results

Phase1

We expect that populations sourced from drier, more variable, and hotter sites will display better performance (establishment, growth and fecundity), higher WUE, productivity and nutrition compared to seeds sourced from cooler, wetter, and less variable locations, or cultivars, across all gardens. In addition, we expect populations from the hottest, driest, and most variable populations to experience “release”, with a faster, stronger response to water availability in cooler and wetter gardens, and in response to supplemental watering (Ritter et al., 2020).

Phase 2

Seed Viability
We expect seed from populations sourced from hotter, drier, and more variable locations to display higher viability across all gardens and both treatments. We also expect that seed collected from watered plants will have higher viability than seed sourced from unirrigated plants.

Transgenerational Plasticity
We expect that all populations grown in ambient and irrigated garden conditions will display evidence of TGP in seedlings propagated from garden collected seed. We expect that individuals grown from seed sourced from the irrigation treatment will show decreased performance without irrigation in both the hot/dry and cool/wet gardens compared to individuals sourced from unirrigated plots, and compared to Phase 1 plants from the same population. We expect that seedlings derived from Phase 1 maternal plants that experience hotter, drier, unirrigated garden conditions will have better survival and performance across both gardens, and compared with plants from the same population within the same garden

(e) Evaluating Extension and Education Activities

Although the proposed project addresses the needs of diverse stakeholders, we are not directly engaging in Extension or education activities, and thus will not be conducting any evaluation.

(f) How Data Will be Analyzed

Phase 1

Each species will be analyzed separately. Plant performance, productivity, WUE, physiological and trait values, isotopic and nutritional value will be analyzed using linear mixed-effects models. Each of the previously listed factors will be treated as dependent variables, and population, garden, and treatment will be treated as fixed factors. Plot within garden, and any necessary blocking factors to facilitate irrigation will be used as random factors in the models. To assess morality, hurdle models using a similar structure will be applied.

Phase 2

Percent seed viability and garden germination trials will be analyzed using beta regression with % viable or germinated as the dependent factor and population, treatment, and garden as the fixed factors.

Transgenerational plasticity will be analyzed by comparing the performance of individuals from within a population across gardens. Using the same metrics as in Phase 1, we will employ a similar mixed-effects model at the population level, with source garden and source treatment as fixed factors, with plot and any additional blocking factors as random factors.

(g) Plans to Communicate Results to Appropriate Audiences

We expect the proposed project to generate, **at minimum,** deliverables totaling 5 scientific manuscripts and 5 management fact sheets broadly centered on:

1. A quantitative assessment of the effects of collection site environment on performance, WUE and nutritional value of key forage grasses.

2. A quantitative assessment of seed production and viability for garden produced seeds across a range of environmental conditions, both with and without supplemental watering.

3. A quantitative assessment of the impacts of garden cultivation, and particularly supplementary watering, on transgenerational plasticity and resilience in two key perennial forage grasses.

These results will be communicated to diverse audiences in the following ways:

1. Annual stakeholder meetings with representatives from the BLM-NM, NMLC, and the SWSP.

2. Presentations to managers, practitioners, extension agents, and scientists at the *Biennial Conference of Science and Management on the Colorado Plateau and Southwest Region* scheduled for 2023, 2025, and 2027.

3. Professional presentations to the Southwestern Chapter of the Society for Ecological Restoration.

4. Informal public presentations via the Flagstaff Festival of Science and Science on Tap.

5. To the scientific and management communities via publication of peer-reviewed scientific manuscripts and fact sheets.

(h) Pitfalls that might be encountered
One of the pitfalls of any experiment in the southwestern United States is the specter of persistent drought during the growing season. Drought may limit the establishment or growth of unirrigated plants, reducing our sample size. We have built in strategies in the proposed project to mitigate this possibility. For example, rather than subjecting Phase 1 plants to ambient garden conditions immediately, we will irrigate all plants throughout the first growing season to establish a viable experimental population. In addition, all gardens have water available for additional supplemental watering if exceptional drought continues to persist in this region beyond year 1 of the experiment. In such an event, "ambient" condition plants would receive weekly watering reflecting typical 30-year normal precipitation for each garden location.

(i) Limitations
A limitation of the proposed work is that we will not be assessing genotypic diversity or performance at the genotype level. Although there is growing interest in gene by environment interactions, this level of analysis is often not feasible for growers and agencies wishing to leverage native seed stock for seed increase and grow-out operations. We feel that our seed sourcing at the collection site/population level is a realistic reflection of how managers and practitioners source and develop seed materials.

3. Project Timetable
A detailed timetable for the project is presented on the following page. This project would be expected to begin in August 2022, thus Q1 begins August 1.

Activities	Q1 YR1	Q2 YR1	Q3 YR1	Q4 YR1	Q1 YR2	Q2 YR2	Q3 YR2	Q4 YR2	Q1 YR3	Q2 YR3	Q3 YR3	Q4 YR3	Q1 YR4	Q2 YR4	Q3 YR4	Q4 YR4	Q1 YR5	Q2 YR5	Q3 YR5	Q4 YR5
Phase 1																				
Identify seed populations and collect seed if required	x																			
Establish Greenhouse Populations		X																		
Outplant to common garden			X																	
Trait and Nutrient Measurements				X				X				X				X				X
Physiological Measurements				X				X				X				X				X
Biomass Estimation				X				X				X				X				X
Seed Harvest				X				X				X				X				X
Statistical Analysis		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phase 2																				
Seed Viability and Greenhouse establishment													X	X						
Outplant															X	X				X
Trait and Nutrient Measurements																X				X
Physiological Measurements																X				X
Biomass Estimation																X				X
Statistical Analyses													X	X	X	X	X	X	X	X
Data Products & Deliverables																				
Scientific Publications				X				X				X				X				X
Stakeholder meeting				X				X				X				X				X
Conference Presentations				X				X				X				X				X
Informal Outreach			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X