DISSERTATION

DIMENSIONS OF DIVERSITY IN DOMINANT PRAIRIE GRASSES

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2019

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ABSTRACT

DIMENSIONS OF DIVERSITY IN DOMINANT PRAIRIE GRASSES

As anthropogenic influences cause climate change to worsen, extreme events such as droughts and heat waves are expected to become more frequent. The native prairies of the Central United States have historically experienced drought, yet continue to support highly productive grassland communities. Dominant species in these grasslands, such as *Andropogon gerardii* in the tallgrass prairie and *Bouteloua gracilis* in the shortgrass steppe, are drivers of productivity in these ecosystems. Thus, it is necessary to quantify diversity within these key species in order to determine how these important grasses have been historically shaped as well as how they will respond to future climate change. This dissertation seeks to answer (1) How do functionally similar coexisting dominant grasses differ at the molecular level?, (2) How does plasticity contribute to intraspecific diversity?, and (3) how does intraspecific diversity vary across the range of one of these less studied grasses?

To determine transcriptional differences between codominant species *A. gerardii* and *Sorghastrum nutans*, I performed RNA-seq on watered and droughted tissues, building both species' transcriptomes using Trinity. These codominant grasses responded differently; specifically, *A. gerardii* had greater regulation of stress alleviation transcripts while *S. nutans* tended to be more sensitive within 10 key gene-groups related to stress and abscisic acid. These results support previous work on the physiological level, and demonstrate functional diversity at the gene level within dominant species in the tallgrass prairie. To explore this community further, I documented variation in plastic traits across a gradient of water availability in three *A. gerardii* genotypes. I found that plasticity, in particular nonlinear plasticity, in morphological and physiological traits was widespread and differed across genotypes, highlighting the influence of relatively small changes in water availability on intraspecific diversity. These genotypes also differed in reproductive strategy (flowering versus

clonal tillering), but all recovered from drought similarly. These results demonstrated that variation in plasticity patterns may help explain intraspecific diversity and patterns of selection within a population. Differences in drought response strategy, particularly in terms of transcription and plasticity diversity, could provide further niche space by which the tallgrass prairie community can mitigate the effects of future drought.

Lastly, I applied an understanding of dominant species diversity in *A. gerardii* to the relatively understudied dominant dry steppe species *B. gracilis*. I performed 2b-RAD genome sequencing and a common garden trait and plasticity analysis across both regionally and locally distributed sites to broadly assess intraspecific diversity in this ecologically and economically important species. I found substantial intraspecific diversity among sites, specifically showing that New Mexico sites were distinct in terms of biomass trait distributions and plasticity. While New Mexico sites were clearly different, all sites were at least somewhat distinct genetically, indicating some limitations to gene flow. As has been shown in *A. gerardii*, comprehensive analysis of intraspecific diversity in this dominant grass will help clarify mechanisms of ecosystem function as well as conservation and management of the shortgrass steppe ecosystem. Overall, these three projects highlight dimensions of diversity in dominant prairie grasses, providing useful information for predicting how these species and their associated communities are likely to respond to changing climate.

ACKNOWLEDGEMENTS

My first thanks are owed to those who have been with me since the beginning of my degree. Thank you to my advisor Mendy Smith, for allowing me the freedom to make mistakes, learn, pursue the ideas most compelling to me, and for trusting me with so many projects. My collaborator Meghan Avolio, thank you for helping me dive headfirst into genetic work, and thanks to all the past and present members of the Smith Lab for their valuable feedback and conversation: Lauren Baur, Francis Chaves, John Dietrich, Andrew Felton, Jesse Gray, Sally Koerner, Nate Lemoine, Wei Mao, Leena Vilonen, and Qiang Yu. Many thanks are also owed to the Graduate Degree Program in Ecology faculty, for superior research, teaching, and mentorship, as well as my fellow students and friends for rich discussion and abundant distractions.

For my research projects, I have been helped by so many along the way, but am especially grateful for the patience and mentorship of my committee members and the troubleshooting guidance of Jacob Snelling and Eli Meyer. Dan Sloan, thank you for generously lending your time, lab resources, and computing cluster, and for serving as an excellent teaching mentor. Julie Bushey, thank you for developing such an ambitious project with me, *Bouteloua* would not be the same without you. Thank you to the USDA NIFA Predoctoral Program and Boulder Open Space and Mountain Parks for their generous sponsorship. I am profoundly grateful to the diligent and motivated undergraduate researchers I've worked with, especially Brigitte Gaudrealt, Brianna Magbual, Holly Perretta, Daniel Robotham, and Maddie Swall.

Thank you to Curt and Leah Hoffman, my siblings and best friends, for lending me strength when I've most needed it and for always making me laugh, as well as my mom and dad, you have been my biggest fans since day one. Lastly, I owe endless gratitude to my partner and fellow ecologist Clif McKee. Thank you for lending a thoughtful and curious ear and for always being open to discussion, science-based or otherwise.

DEDICATION

This dissertation is dedicated to women in science, technology, engineering and mathematics. I am humbled by everything you have accomplished and am so proud to count myself as one of you.

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Chapter 1

Introduction

Climate change represents a significant threat to ecosystems worldwide (Stocker et al., 2013). The effects of climate extremes, such as drought, heat waves, and larger rainfall events, have become more prevalent (Knapp et al., 2015b) and are expected to worsen as global climate shifts (Wuebbles et al., 2014). Exclusive of global temperature increase, climate change is expected to produce more climate extremes that have significant impact on biotic community structure and ecosystem function (Smith, 2011). Of all predicted environmental changes, drought poses the most significant threat to native, managed, and agricultural ecosystems (Breshears et al., 2005; Cook et al., 2015; Zhao and Running, 2010). Drought not only negatively impacts growth of individual species, but has the potential to negatively impact some species and ecosystems more dramatically than others. Specifically, plant species of the tallgrass prairie vary dramatically in drought tolerance (Tucker et al., 2011) and tallgrass and shortgrass prairies vary in drought sensitivity (Knapp et al., 2015a; Sala et al., 2015). On a broader scale, prairies of the American Midwest and Southwest have historically experienced extreme droughts (Cook et al., 2004; Weaver and Albertson, 1936, 1939; Weaver et al., 1935) and are likely to experience more extreme droughts in the future (Ault et al., 2014; Cook et al., 2015; NOAA, 2012). While the negative effects of drought and water limitation on ecosystems are well studied (Knapp and Smith, 2001; Knapp et al., 2018), mechanisms of response to drought are not always clear. Indeed, plant responses to drought are complex, involving both chemical and hydraulic signaling and complex metabolic and transcriptional pathways that lead to organism-level responses (Chaves et al., 2003; McDowell et al., 2008; McDowell, 2011; Pinheiro and Chaves, 2011). Understanding drought's impact on plant species physiology and morphology, and ultimately communities, remains a pressing challenge (Smith, 2011; Van Loon et al., 2016) and will be crucial for predicting effects of climate change. In this dissertation, I will focus primarily on the effects of water availability on dimensions of plant diversity.

At the community level, diversity is a major component of drought response. This diversity appears at different biological scales, from genomics, gene expression, metabolomics, and phys-

iology, to morphology, demography, and metacommunity levels. Such dimensions are important for deciphering mechanisms of drought response (Craine et al., 2013; Felton and Smith, 2017). Interspecific plant diversity, or diversity among species, significantly impacts ecosystem function (Cardinale et al., 2013; Naeem et al., 1994), stability (Cadotte et al., 2012; Cardinale et al., 2013; Gross et al., 2014; Hutchinson, 1959), resistance to invasion (Naeem et al., 2000), and unsurprisingly, can mitigate the effects of drought (Van Ruijven and Berendse, 2010). This can occur through niche partitioning (i.e., more resources can be used more efficiently among species) and species' ability to shift in abundance in response to changing conditions, a phenomenon called the "portfolio effect" (Hallett et al., 2014; Tilman et al., 1998). Communities and ecosystems might then appear resistant and/or resilient to climate change through species turnover (Alstad et al., 2016; Jones et al., 2017; Lewthwaite et al., 2017; Schwalm et al., 2017). In the tallgrass prairies of the American Midwest for example, forbs increased in abundance relative to grasses under precipitation manipulation (Jones et al., 2016) while a dominant forb declined compared to other species under experimental climate extremes (Hoover et al., 2014b). It is important not only to conserve and manage existing interspecific diversity, but also to understand where diversity changes ecosystem function and how it contributes to mitigating the effects of abiotic stressors.

Interspecific diversity may help buffer the effects of droughts, but a fundamental pattern in communities is that plants vary in abundance, with one or a few common/dominant species and many rare species (MacArthur, 1957; Magurran and Henderson, 2003; McGill et al., 2007; Preston, 1948; Rabinowitz, 1981). If a particular plant species is more abundant in the community, then it follows that this species plays a greater role in ecosystem function, including production (Smith and Knapp, 2003), stability (Sasaki and Lauenroth, 2011), invasion resistance (Smith et al., 2004), and other functions. Just as interspecific diversity in communities can mitigate against changing conditions, the same logic may be applied to smaller scales of biological diversity within dominant species (Bolnick et al., 2011). For example, given greater genotype diversity within a species, the likelihood that a genotype is present and has high fitness given a new or fluctuating environment increases. This theory has led to more attention given to intraspecific (within species) diversity. Just

as community evolution (described by Whitham et al., 2003) relies on a diverse regional species pool, individual species rely on intraspecific dimensions like genetic diversity, functional diversity, and phenotypic plasticity, all of which are important for introducing novel traits that may impart higher fitness under different environments or changing conditions (Booy et al., 2000; Harter et al., 2015; Manel et al., 2012; Sexton et al., 2002). Intraspecific diversity has been extensively implicated in mitigating responses to climate change (Anderegg, 2015; Gazol and Camarero, 2016; Jung et al., 2014; Ravenscroft et al., 2014), with dominant plant species playing a key role Avolio et al. (2013); Hughes et al. (2008); Sasaki and Lauenroth (2011). As plant communities are faced with increasingly frequent and severe droughts, we must include intraspecific alongside interspecific diversity, especially in communities with one or a few dominant species.

Because of the complexity of response to drought and the necessity of water for plant function, it is often challenging to select the appropriate trait to measure the diversity of plant responses. It is also often unclear whether the trait in question is an adaptation to drought (e.g., smaller plants are less susceptible to drought damage) or a symptom of it (e.g., plants are smaller because they lack water). Within plant biology, it is widely recognized that examining multiple traits contributes to a holistic understanding of drought response (e.g., Mitchell et al., 2008; Skelton et al., 2015), and that gene expression and molecular signaling play an important role (Chaves et al., 2003). Recently, community ecology has widely adopted multiple traits-based approaches to understanding community assembly (Griffin-Nolan et al., 2018a; McGill et al., 2006), but there is a recognized need to quantify diversity (especially intraspecific diversity) across scales (Avolio et al., 2018) and connect functional diversity to patterns at the genetic level (Violle et al., 2012).

Differences among traits may be important for adaptation and response to drought, but differences in plasticity, in terms of trait variance and timing, may also allow plants to survive under water limited conditions (Gargallo-Garriga et al., 2015; Hoffmann and Sgrò, 2011; Lázaro-Nogales et al., 2015; Meyer et al., 2014; Wilkins et al., 2009). Yet, diversity in plasticity is often neglected in studies of functional diversity, whether inter- or intraspecific. Plasticity may be especially important in dominant, clonally reproducing plants compared to fixed locally adapted traits by allowing

it to thrive in multiple environments (Liu et al., 2015). Outside of diversity literature, substantial work has been done examining plasticity as a trait that can be under selection (Agrawal, 1999; Scheiner, 1993). Unlike selection, gene flow, or mutation, the effect of plasticity as a metric of diversity is not necessarily intuitive, and can serve either to constrain or promote diversity. Changes in plasticity within species can be adaptive or non-adaptive, making it difficult to predict its importance (Ghalambor et al., 2007; Handelsman et al., 2013). For example, plasticity may constrain diversity if a single genotype is plastic for a certain trait and is able to fill two niches that could potentially be filled by two separate genotypes. Thus, it is critical to document both species (interspecific) and genotype (intraspecific) plasticity, as variation in plasticity among genotypes may lead to different ability to persist under drought.

Assessing multivariate data pertaining to traits and trait plasticity often requires generalizations, such as classifying species or genotypes into drought coping strategies. A common tool for categorizing these strategies is the isohydric - anisohydric spectrum (Konings et al., 2016). Specifically, very isohydric plants exhibit strict stomatal regulation to avoid water loss and pause CO_2 and other gas exchange, which is sometimes referred to as "drought avoidance", (McDowell et al., 2008; McDowell, 2011). Anisohydric plants on the other hand, exhibit little stomatal regulation but risk damage to vessel anatomy ("drought tolerance"). I extend this categorization of drought coping strategy more broadly to incorporate other dimensions of functional diversity. For example, gene expression related to damage repair might be associated with a drought tolerance strategy of continuing to function during water stress. Although these strategies are typically described with respect to interspecific diversity, previous work has shown that avoidance and tolerance strategies are present within single species (Carvajal et al., 2017).

This dissertation seeks to broadly improve our understanding of diversity within dominant species as it pertains to water limitation. I selected the dominant C_4 grasses of the central United States, *Andropogon gerardii*, *Bouteloua gracilis*, and *Sorghastrum nutans*as the focal study systems because they are widespread, phenotypically diverse, important for ecosystem function, and have evolved in a region frequently plagued by droughts (Anderson, 2006; Borchert, 1950). Fur-

thermore, these plants have shown the capacity to buffer climate change impacts within their respective ecological communities (Collins et al., 2017; Hoover et al., 2014b). Different questions were focused on each species to account for prior differences in research effort among these three grasses. Generally, I addressed (1) interspecific transcriptional responses to water limitation, (2) intraspecific variation in plasticity, and (3) intraspecific spatiotemporal variation in these key species.

More specifically, in Chapter 2, I address interspecific diversity responses to drought by the often codominant species *A. gerardii* and *S. nutans*, seeking to answer the question (1) How do codominant species differ in gene expression under drought? I found that the codominant species *A. gerardii* and *S. nutans* differ in these strategies when we consider their gene expression under water limitation, which has never been shown before in codominant species. In Chapter 3, I focus on intraspecific diversity of well-studied *A. gerardii* genotypes from a single population, asking (2) How do coexisting *A. gerardii* genotypes differ in functional trait diversity and plasticity? I found that three genotypes diverged across several functional dimensions (particularly the directionality of plasticity), indicating different drought response strategies within this well-studied dominant species. Finally, in Chapter 4, I explore the similarly important, yet heretofore rarely described dominant species *B. gracilis*, asking (3) How does intraspecific genomic and functional diversity vary across space and with climate? This is the first next generation sequencing data from *B. gracilis* and represents the first comprehensive study of its plasticity, variance, and trait distributions across local and regional scales. These chapters provide useful information quantifying the high level of diversity seen in these dominant species, which suggests that they might have the capacity to cope with some droughts caused by climate change.

Chapter 2

Gene expression differs in co-dominant prairie grasses under drought

This chapter has previously been published in full.[1]

2.1 Overview

Grasslands of the Central US are expected to experience severe droughts and other climate extremes in the future, yet we know little about how these grasses will respond in terms of gene expression. We compared gene expression in *Andropogon gerardii* and *Sorghastrum nutans*, two closely related co-dominant C_4 grasses responsible for the majority of ecosystem function, using RNA-seq. We compared Trinity assemblies within each species to determine annotated functions of transcripts responding to drought. Subsequently, we compared homologous annotated gene-groups across the two species using cross-species meta-level analysis and functional clustering based on key terms. The majority of variation was found between species, as opposed to between drought and watered treatments. However, there was evidence for differential responses; *A. gerardii* allocated gene expression differently compared to *S. nutans*, suggesting *A. gerardii* focuses on stress alleviation (such as oxygen radical scavenging) rather than prevention. In contrast, *S. nutans* may employ a drought avoidance strategy by modulating osmotic response, especially with hormonal regulation. We found *S. nutans* tended to be more sensitive within 10 key gene-groups related to stress, abscisic acid, and trichomes, suggesting gene expression may mechanistically parallel sensitivity at the physiological level. Our findings corroborate phenotypic and physiological differences in the field and may help explain the phenotypic mechanisms of these two species in the tallgrass prairie community under future drought scenarios.

2.2 Introduction

Droughts pressure many ecosystems, including the Central US, and are expected to increase in frequency and severity (Wuebbles et al., 2014). Through manipulative experiments, ecologists

[1]Hoffman, AM and MD Smith. Gene expression differs in codominant prairie grasses under drought. Molecular Ecology Resources 18(2):334-346.

have learned what we may expect under these scenarios (Fay et al., 2003; Hoover et al., 2014b; Smith, 2011). Yet, detecting stress within individuals and in ecological communities remains a problem of scale (Levin, 1992), and selecting responses and tools that are most appropriate for measuring drought stress remains a challenge. Ecologists have grown increasingly interested in pairing the molecular responses to drought, such as gene expression and metabolite analysis, with morphological and physiological data (Lovell et al., 2016) in order to reveal underlying mechanisms. An understanding of gene expression is a critical hurdle revealing these stress response mechanisms (Johnson et al., 2014; Leakey et al., 2009; Meyer et al., 2014; Swarbreck et al., 2011). Despite this, ecologists have generated few resources for studying gene expression in non-model plants, such as the native co-dominant tallgrass prairie species *Andropogon gerardii* (big bluestem) and *Sorghastrum nutans* (indiangrass) of the Central U.S.

High aboveground biomass (Smith and Knapp, 2003), C_4 photosynthetic capacity, and community co-dominance describe both *A. gerardii* and *S. nutans*, which are similar in appearance and traits (Forrestel et al., 2015). Yet, these two grasses differ in their physiological responses to stress, with these differential responses ultimately cascading to affect ecosystem functioning (Hoover et al., 2014a). Specifically, *S. nutans* is more sensitive overall to both soil moisture and temperature, while *A. gerardii* is primarily responsive to temperature (Nippert et al., 2009). Under severe water limitation, *S. nutans* suffers a greater loss of function (Hoover et al., 2014b) and grows more gradually than *A. gerardii* (Nippert et al., 2011). Trichome morphology also differs substantially in these species and could be related to drought prevention, particularly in *A. gerardii* (Olsen et al., 2013). These findings suggest that *A. gerardii* and *S. nutans* differ in physiological mechanisms for coping with stress, particularly with respect to avoidance versus tolerance or thresholds of response to climate stress. Investigating gene expression responses in these co-dominant grasses will improve our understanding of drought response through molecular mechanisms.

A. gerardii and *S. nutans* cope with and respond to temperature and water stress differently at the genomic level (Smith et al., 2016; Travers et al., 2007, 2010), reflecting differences in their ecophysiological performance in the field (Hoover et al., 2014a; Nippert et al., 2009, 2011). However,

these previous studies utilized heterologous hybridization to the maize (Zea mays) cDNA microarray; it is unclear how many *A. gerardii* and *S. nutans* genes are not detected due to nucleotide dissimilarity to maize probes. Although one RNA-seq transcriptomic resource exists for the *A. gerardii* genus (Raithel et al., 2016), high genetic diversity in the *A. gerardii* species alone (Avolio et al., 2011) may lead to failed transcript alignments. Next-generation resources are completely lacking for *S. nutans*. Therefore, the goal of this study was to conduct the first RNA-seq analysis of *A. gerardii* and *S. nutans* to more rigorously assess molecular mechanisms of differential response of these species under drought.

We manipulated water availability via soil moisture drying for these two grass species to provide two controlled conditions: drought vs. watered. We aimed to (1) characterize transcriptome homology of *A. gerardii* and *S. nutans*, (2) determine differential expression within *A. gerardii* and *S. nutans* under drought conditions, and (3) compare differential expression responses among homologous gene-groups in the two species. Based on ecophysiology in these species and previous microarray research (Smith et al., 2016; Travers et al., 2007, 2010), we expected expression under drought and watered treatments to differ for both species. Specifically, *S. nutans* would have more extreme differences in gene expression between treatments and more genes overall experiencing significant change. We expected enriched stress alleviation expression within *A. gerardii* (such as heat shock or oxygen scavenging proteins). Conversely, we expected *S. nutans* to exhibit enriched expression of osmotic regulatory processes like aquaporin production, abscisic acid (ABA) production, or stomatal regulation. Thus, we expected differential expression responses among homologous gene-groups to encompass these categories. We also expected trichome-related gene-groups to to be more sensitive within *A. gerardii*, in agreement with morphological differences between the two species. This study provides the first next-generation sequencing resource for comparing these two ecologically important grasses and enhances our depth of knowledge of the molecular phenotype, response mechanisms, and ecosystem genetics in the tallgrass prairie.

2.3 Methods

2.3.1 Study species

Andropogon gerardii and *Sorghastrum nutans* are the most common grass species of the tallgrass prairie ecosystem of eastern Kansas. Both are self-incompatible polyploid species, where *A. gerardii* is typically hexaploid (Keeler, 1990) and *S. nutans* is typically tetraploid (Riley and Vogel, 1982) (these cytotypes are used in this study). Both reproduce primarily through asexual tiller formation and experience comparatively low local recruitment from seed germination. Both have large genomes; hexaploid *A. gerardii* contains approximately seven gigabases while tetraploid *S. nutans* contains approximately six gigabases (Delaney and Baack, 2012; Keeler et al., 1987). Both species persist during experimental rainfall manipulation, drought, and heat waves (Avolio and Smith, 2013b; Hoover et al., 2014b), helping prevent loss of ecosystem function under extreme climatic events in the tallgrass prairie (Smith, 2011).

2.3.2 Plant material, RNA extraction, and sequencing

Common genotypes of both species were collected from the lowlands of Konza Prairie Biological Station in Manhattan, KS and were clonally propagated in tissue culture (cultured by SMK Plants, Billings, MT) following Hoffman and Smith (2018). Tissue culture resulted in plants of approximately the same size and phenological stage. Once rooted, we transplanted plantlets to Premier pro-mix HP (Griffin Greenhouse Supplies, Inc., Tewksbury, MA). These plant clones adapted to 70% relative humidity and light irradiance of 37.5 μmol m-2 s-1 in growth chambers for three weeks. We then transferred plants to the greenhouse with a 28°C daytime temperature, 22°C night temperature, and 16h photoperiod. We initiated drought treatment after one month of greenhouse acclimation. At this stage, plants were under 30 cm in height and were not root bound or elongating for flower production. Soil moisture was kept at field capacity, which is approximately 30% volumetric water content (VWC) in similar experiments using the same media (Figure I.1). Water was withheld in an uncontrolled drydown for six days to produce an extreme drought. While this was a relatively short and extreme drought, soils within native tallgrass prairie

are known to dry down quickly, especially late in the growing season (August) (Nippert and Knapp, 2007). Though droughted and non-droughted treatments were performed distinctly, uncontrolled drydowns may produce significant variation (Lovell et al., 2016) and may be an important source of error in this study. In other words, substantially lower VWC in one pot could lead to incorrect conclusions about more extreme expression responses. After six days of drought, we collected newly emerged leaves from two biological replicates within the watered treatment (water not withheld) and drought treatment (n=2, with two treatments and two species). We immediately flash-froze leaves in liquid nitrogen and stored tissue at -80°C prior to RNA extraction.

We extracted RNA from leaf tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified RNA with the RNeasy kit (Qiagen, Valencia, CA) after on-column DNase treatment (Qiagen, Valencia, CA). Eight total cDNA libraries were multiplexed and sequenced on the Illumina HiSeq 2000 platform to generate 72 bp paired-end reads. Library construction, sequencing, and demultiplexing were performed at the Yale Center for Genome Analysis.

2.3.3 De novo assembly

We examined raw data FastQC reports for anomalies using the iPlant Collaborative HTProcess pipeline, after which we trimmed sequences to suitable length and quality using Trimmomatic (version 0.32) (Bolger et al., 2014) (see Supporting Information for more details). Trimmed reads were assembled in Trinity (version 2.1.1) (Haas et al., 2013) using the Colorado State University Correns computing cluster, creating an assembly for each species. We chose the Trinity pipeline because it has been shown to work well with polyploid species, including non-model grasses (Bushman et al., 2016). To evaluate the quality of the Trinity assemblies, we used (1) Trinity's built-in evaluation scripts, (2) read realignment percentage using Bowtie 2 (version 2.2.7) (Langmead and Salzberg, 2012), and (3) Samtools scripts (Li et al., 2009a).

2.3.4 Differential expression within species

Using two Trinity transcript assemblies as references (one for each species), we realigned reads using Bowtie 2. We then used RSEM (version 1.2.28) to count expression of each transcript (tran-

script per million, TPM) (Li and Yang, 2011). In other analyses, Trinity transcripts may be known as genes or contigs. In our analysis, we retained all isoforms of transcripts produced by Trinity. Although there was weak coverage for many transcripts, we retained all transcripts so as not to discard any genes with potential ecological relevance. We calculated differential expression across treatments by comparing negative binomial distributions of transcript counts within edgeR (edgeR version 3.16.4) (Robinson et al., 2010) using Fisher's exact tests for each transcript. After normalizing across sample coverage using trimmed mean of M-values (TMM), we filtered results for a false discovery rate of <0.05 (Benjamini-Hochberg method) and minimum log fold change of >1 (2x difference in expression). All statistical analyses were performed using R (version 3.3.0) (R Core Team, 2018).

2.3.5 Homology and annotation

We used the BLAST+ tool blastn to compare the assembled *A. gerardii* and *S. nutans* transcriptomes to existing cDNA resources. We downloaded cDNA for *Arabidopsis thaliana*, *Brachypodium distachyon*, *Hordeum vulgare* (barley), *Leersia perrieri* (cutgrass), *Oryza sativa* (rice), *Populus trichocarpa* (black cottonwood), *Setaria italica* (foxtail millet), *Sorghum bicolor* (sorghum), *Triticum aestivum* (wheat), and *Zea mays* (maize) from Ensembl genomes (Kersey et al., 2015). Other species were downloaded from the Joint Genome Institute Genome Portal (Nordberg et al., 2014). We used an evalue of e-10 to parameterize all blastn searches. Homology percentages were determined by calculating the average percentage of identical matches.

We used the Trinotate (version 3.0.1) (Haas et al., 2013) pipeline to annotate transcripts in the assembled transcriptomes to known genes. First, we used TransDecoder (version 2.1.0) to extract potential protein coding regions within long open reading frames (ORFs). We simultaneously aligned these predicted protein regions and Trinity assembled transcriptomes for each species to the Swiss-Prot database using blastx (The UniProt Consortium, 2015) and Pfam (Finn et al., 2015) database using blastp. Specific releases of Swiss-Prot and Pfam were required for use with Trinotate (see Supporting Information). Hmmer (version 3.1b2) (Finn et al., 2011), SignalP (version 4.1) (Petersen et al., 2011), and Tmhmm (version 2.0c) (Möller et al., 2001) were used to further

predict and identify protein domains. Gene GO annotations (The Gene Ontology Consortium, 2015) were also retrieved from the Swiss-Prot database. Finally, we downloaded the transcription factor databases for *Zea mays* and *Sorghum bicolor* (Charoensawan et al., 2010). We searched these databases using Pfam annotations above.

Following annotation, we combined count matrices produced by RSEM with annotations compiled by Trinotate. Transcripts with significantly different expression between drought and watered treatments were examined for Gene Onotology (GO) category enrichment using GOseq (version 1.24.0) (Young et al., 2010) with a P-value cutoff of P<0.05. We also searched for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment (using KEGG Mapper Pathway Search against Z. mays) (Kanehisa et al., 2016), and presence of transcription factors. REVIGO was used to reduce and visually assess GO enrichment categories (Supek et al., 2011). We also contrasted the top 400 highly expressed transcripts (average TMM across samples) against the remaining transcripts for both species (Hoffman and Smith, 2017, Table S4).

2.3.6 Comparative expression between species

We used Swiss-Prot annotations to compare orthologous transcripts between *A. gerardii* and *S. nutans*, hereafter referred to as gene-groups. We chose this approach as opposed to direct sequence comparison for leniency; *A. gerardii* and *S. nutans* transcripts were able to align to different parts of the same Swiss-Prot annotated gene and different splice variants could be combined as long as they matched the same annotation. Similar annotation-based comparisons have been used in other studies (Cui et al., 2015). While using homology to model organisms to compare species is useful (Sudmant et al., 2015), our indirect comparison approach eliminates any transcripts lacking model species annotations (Rowley et al., 2011). Moreover, neither direct nor indirect alignment can account for neofunctionalization, even within species (Duan et al., 2016).

To examine species differences outside of treatment, we compared gene-groups within drought samples separately from watered samples. For this broader analysis, we summed count expression within each unique gene-group and normalized across samples using the TMM method. Comparisons were made using Fisher's exact test within edgeR with a false discovery rate of <0.05 and

minimum log fold change of >1 (2x difference in expression). We analyzed gene-groups for GO category enrichment, KEGG enrichment, and transcription factors (as above). We then determined differentially expressed gene-groups across *A. gerardii* and *S. nutans* with respect to treatment using the meta-level analytic method described by Kristiansson et al. (2013). This technique is powerful for deducing species by environment interactions where multiple orthologs and paralogs are found within each gene-group, as would be expected comparing species of different ploidies. We filtered these results for P-values <0.05 and false discovery rate <0.05.

2.3.7 Functional annotation clustering

We clustered all gene-groups from the above analysis by filtering out keywords "stress", "heat shock", "trichome", "abscisic acid", "stomata", and "aquaporin" from all possible annotations. These categories were determined a priori due to the physiological and morphological differences between *A. gerardii* and *S. nutans*.

2.4 Results

2.4.1 De novo assembly and differential expression

After trimming, we retained 5.33 x 107 total reads for *A. gerardii*, representing 73% of original reads. For *S. nutans*, many more reads were trimmed out, leaving 3.44 x 107 reads (50%). All samples were between 50-52% GC with all reads between 40-64 base pairs (Hoffman and Smith, 2017, Table S1). Trinity de novo transcriptome assembly produced 64,930 transcripts for *A. gerardii* with an N50 = 789. *Sorghastrum nutans* had 47,807 transcripts assembled with an N50=764. High quality transcriptomes typically observe realignment rates between 70-80% using Bowtie 2; our realignments met this criteria, with rates between 75-85% (Table 2.1).

In *A. gerardii*, 106 total transcripts differed significantly between the treatments, with 52 highly expressed under the drought compared to the watered treatment and 54 transcripts highly expressed under the watered treatment (Figure 2.1). *Sorghastrum nutans* regulated slightly fewer transcripts: 85 total transcripts differed, with 39 highly expressed only during drought and 46 only expressed when watered (Figure 2.1). When we regressed the representative subset of transcripts against

their coverage using RSEM, *A. gerardii* expressed an estimated 7300 transcripts, while *S. nutans* expressed an estimated 8419 transcripts (Supporting Information, Figure I.2). The discrepancy between the Trinity transcripts and the transcripts aligned using RSEM indicates many lowly expressed transcripts or transcripts with low coverage. Generally, *A. gerardii* retained more gene isoforms which could be of interest for further studies investigating alternative splicing.

2.4.2 Homology and annotation within species

Andropogon gerardii and *S. nutans* generally showed the same order of relatedness to all other species' transcriptomes at the nucleotide level. The two grasses were most related to other Andropogoneae like *Sorghum bicolor* (93.6-93.8%) and *Zea mays* (91.4-91.6%). More distant relationships were observed with other C_4 ($\sim$89%) and C_3 ($\sim$86%) Poales. Unsurprisingly, eudicots and one distantly related monocot (*Zostera marina*) showed the least homology (78.8-80.9%) (Figure 2.2). Similar order of relatedness was obtained at the protein level (Figure I.5). We matched 81% and 84% of transcripts to annotations or conserved domains for *A. gerardii* and *S. nutans*, respectively. Transmembrane helices (TmHMM) represented 8% of transcripts in *A. gerardii*, but *S. nutans* mapped more (9%), which could correspond to *S. nutans* greater water response or transport. *Andropogon gerardii* had far greater transcription factor expression (8%) versus *S. nutans* (3%) which could be reflective of greater heat response. Overall, 42% of *A. gerardii* transcripts and 45% of *S. nutans* transcripts mapped to conserved protein domains (Pfam) and 1.5% of transcripts in both species mapped to signal peptide cleavage sites (SignalP).

Gene Ontology (GO) enrichment of differentially expressed transcripts varied between the two species (Figure 2.3). Enriched biological processes upregulated under drought in *A. gerardii* included dhurrin biosynthesis (plant defense), amino acid salvage, response to stimulus, abscission, and misfolded protein catabolic processes. In *S. nutans*, dhurrin biosynthesis was also enriched, but response to osmotic stress, hypersalinity response, and response to cytokinin were also represented. *Andropogon gerardii* molecular functions were enriched in tetrapyrrole (chlorophyll) binding, oxygenase activity, and transcription factor activity under drought, while *S. nutans* transcripts were not.

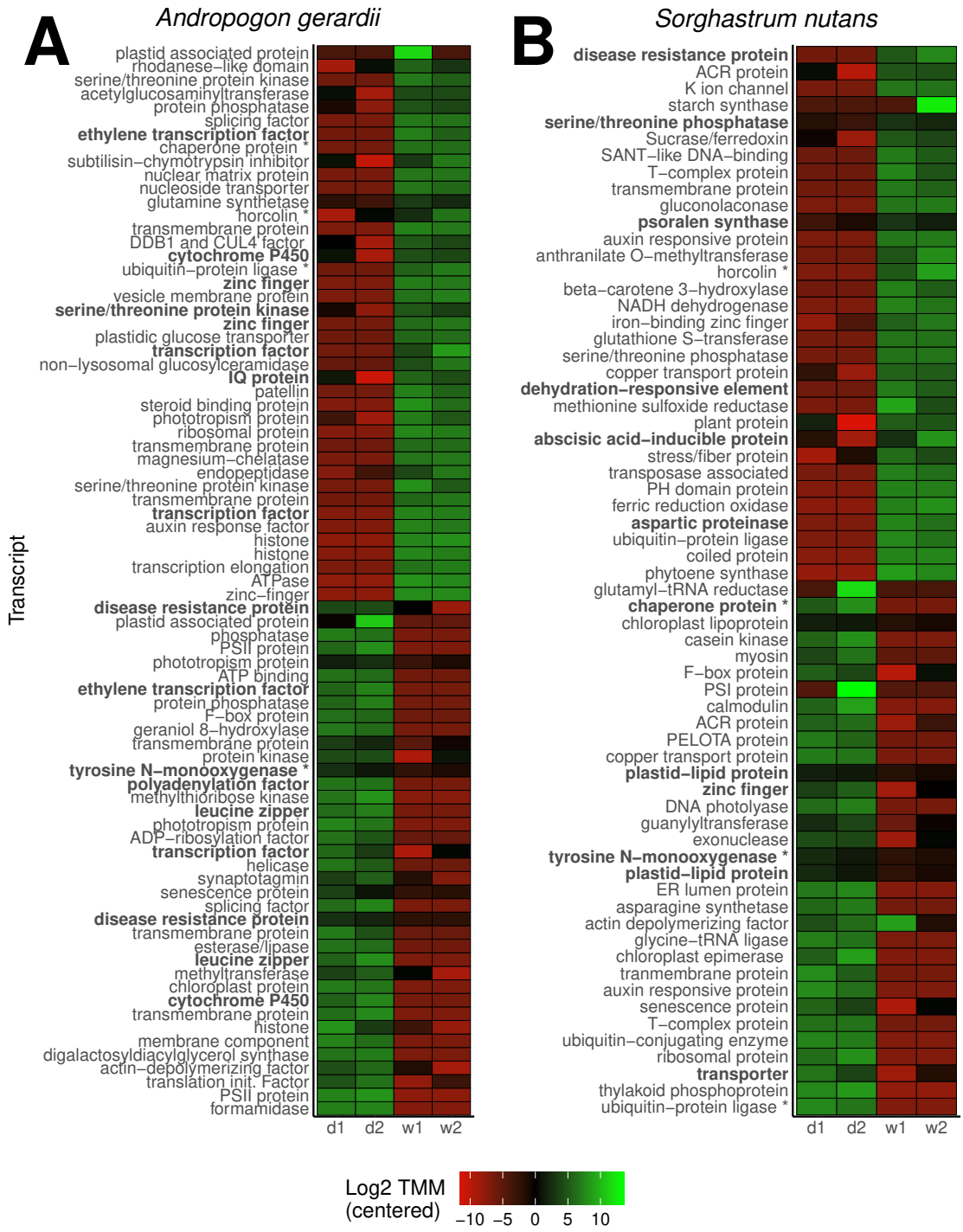

Figure 2.1: Transcripts differentially expressed in (A) *A. gerardii* and (B) *S. nutans*. Drought samples are indicated "d1" or "d2" while watered samples are indicated "w1" or "w2". Units are log2 TMM (trimmed mean of M-values). Bold transcripts map to known transcription factors. Transcripts with asterisk (*) are found in both species.

Table 2.1: Summary of transcriptome assembly and quality checks in both species. Proper pairing indicates both paired end reads aligned together.

		A. gerardii	*S. nutans*
Assembly	total trinity 'genes'	35656	29155
	total trinity 'transcripts'	64930	47807
	percent GC	49.25	49.2
	transcript N50:	789	764
	median transcript length	522	514
	average transcript length	684.96	670.36
	total assembled bases	44474391	32047794
QC	average Bowtie 2 realignment	83%	80%
	average proper pairing	77%	74%

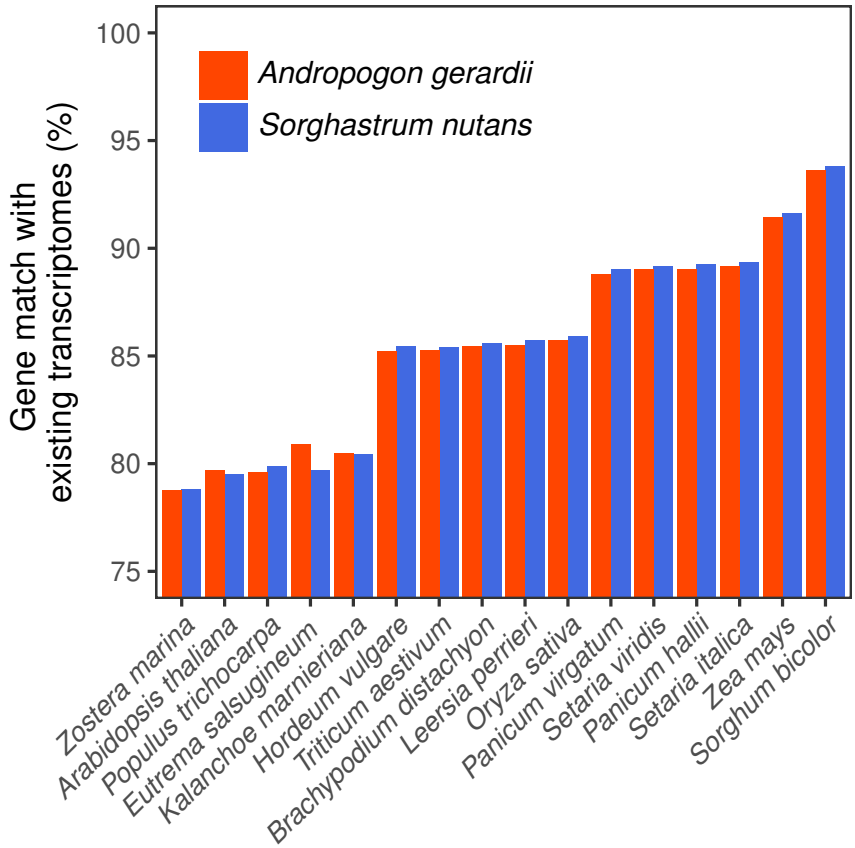

Figure 2.2: Homology of both species to known transcriptomes at a per gene basis. Percent match refers to average percentage of identical matches at the nucleotide level (blastn).

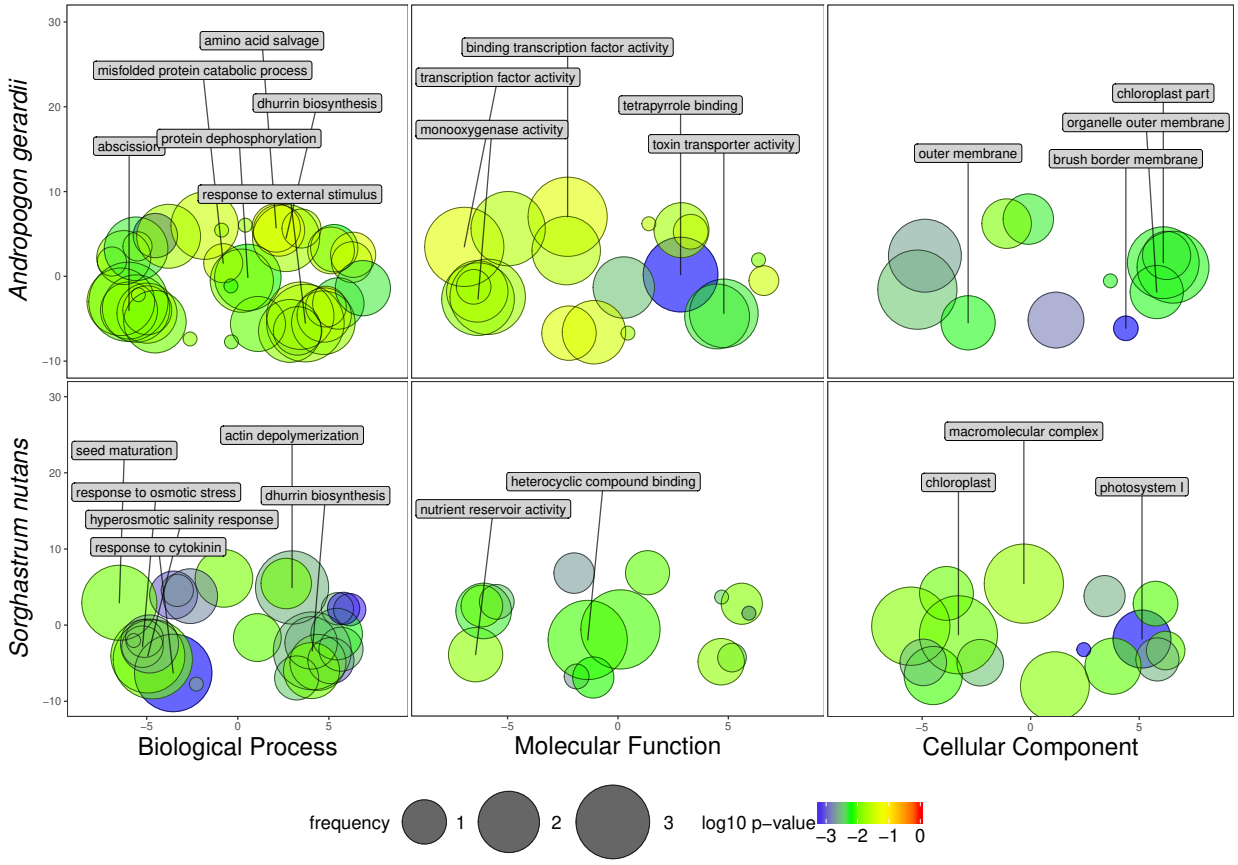

Figure 2.3: Gene Ontology (GO) enrichment in drought-upregulated transcripts within *A. gerardii* and *S. nutans*. Color represents significance of overrepresentation of a specific GO term within the drought upregulated transcripts. Size represents the relative natural log scale frequency of the cluster of transcripts. Horizontal and vertical axes represent semantic space (SimRel similarity measure). Full GO enrichment data is also available within Hoffman and Smith (2017, Table S2).

Using KEGG, we found that for *A. gerardii* drought-upregulated transcripts most represented metabolic pathways, glycerolipid metabolism (stored energy), mRNA surveillance pathway (e.g., degradation of aberrant transcripts), and spliceosome. Transcripts upregulated under the watered treatment also included metabolic pathways and spliceosome, but with more emphasis on amino acid synthesis (Hoffman and Smith, 2017, Table S3). In *S. nutans*, drought transcripts were strongly enriched in plant hormone signal transduction, followed by ubiquitin mediated proteolysis (protein degradation), and RNA degradation. For watered transcripts, plant hormone signal transduction was also most represented (Hoffman and Smith, 2017, Table S3). Using homology to known sequences, we found *A. gerardii* differentially regulated 17 transcription factors while *S. nutans* showed differentially expression of 12 (Figure 2.1). Only four similarly annotated transcripts were found in both species.

2.4.3 Comparative expression between species

Using count data across all shared annotated transcripts, we saw that species separated dramatically in multidimensional space (Figure 2.4). Drought and watered samples were first compared separately in order to focus on species differences. Within the drought treatment, *A. gerardii* and *S. nutans* differed significantly across 4032 gene-groups. *Andropogon gerardii* upregulated gene-groups represented transferase activity, transcription factor activity, flavonoid synthesis and metabolism, glycosylation, and vesicle GO terms compared to *S. nutans*. In contrast, *S. nutans* gene-groups were most represented by DNA-complexes, regulation and response to stress and hormones, and RNA and compound binding (Hoffman and Smith, 2017, Table S5). Metabolic pathways differed in the two species: *A. gerardii* gene-groups represented a greater proportion of secondary metabolite biosynthesis, plant hormone signal transduction, citrate cycle, and amino/nucleotide sugar metabolism pathways (Hoffman and Smith, 2017, Table S6). *Sorghastrum nutans* gene-groups represented a greater proportion of fatty acid degradation, nucleotide excision repair, mismatch repair, and thiamine metabolism pathways (Hoffman and Smith, 2017, Table S6). Significantly upregulated gene-groups in *A. gerardii* matched 321 known transcription factors versus 213 transcription factors in *S. nutans*.

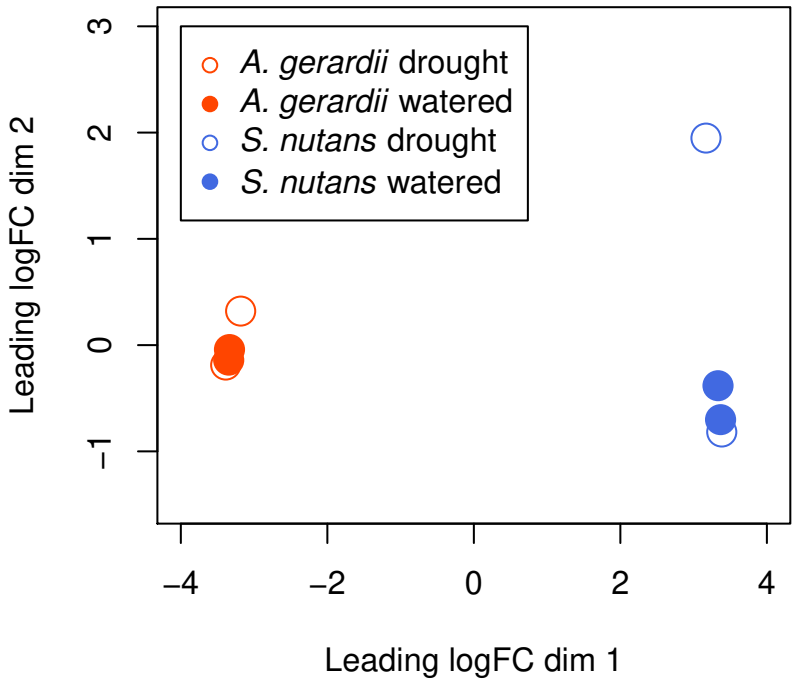

Figure 2.4: Non-metric multidimensional scaling based on annotated genes shared between *A. gerardii* and *S. nutans*. Euclidean distances are based on log2 fold change differences in all genes among samples using transcript counts. Stress = 0.0657.

Compared to the drought treatment, watered plants of the two species diverged significantly across 4929 gene-groups. *Andropogon gerardii* watered gene-groups were enriched in cellular and stem cell proliferation, binding activity, flavonoid synthesis and metabolism, and regulation of biological processes. In contrast, *S. nutans* watered upregulated gene-groups represented primary, nitrogen, and aromatic compound metabolism, negative regulation of transcription, ion binding activity, and nucleoplasm (Hoffman and Smith, 2017, Table S5). Metabolic pathways were largely similar to those represented in droughted plants. *Andropogon gerardii* gene-groups were additionally enriched in the MAPK signaling pathway and glycerolipid metabolism (Hoffman and Smith, 2017, Table S6). *Sorghastrum nutans* watered gene-groups also represented carbon fixation and photosynthesis (Hoffman and Smith, 2017, Table S6). Transcription factor matches stayed consistent across treatments, with only one fewer transcription factor upregulated in *A. gerardii* (320) and three fewer transcription factors upregulated in *S. nutans* (210).

We examined differential responses between *A. gerardii* and *S. nutans* accounting for multiple orthologs and paralogs in a meta-level analysis. Of 11,878 gene-groups, we found significantly different responses in 83 (Figure 2.5) indicating a small but present species x environment interaction overall. These gene-groups were enriched in response to stimulus, response to oxidative stress, membranes and plastoglobule, tetrapyrrole binding, and ER retention sequence binding GO terms (Figure 2.6). Of the 83 significantly changing gene-groups, 17 mapped to known transcription factors.

2.4.4 Functional annotation clusters

Within differentially expressed gene-groups from the meta-level analysis, 10 genes matched the functional annotation clusters. These included seven stress gene-groups, one trichome group, and two ABA groups. *Sorghastrum nutans* appeared more sensitive in five of the seven stress groups and both ABA groups (MSRB1_ORYSJ, USPAL_ARATH, SALT_ORYSJ, NDUS4_ARATH, TIL_ARATH, CUT1B_ARATH, and GRPA_MAIZE, Figure 2.7). *Andropogon gerardii* appeared more sensitive in one stress group and the trichome group (AFG1_YEAST and MYO17_ARATH, Figure 2.7).

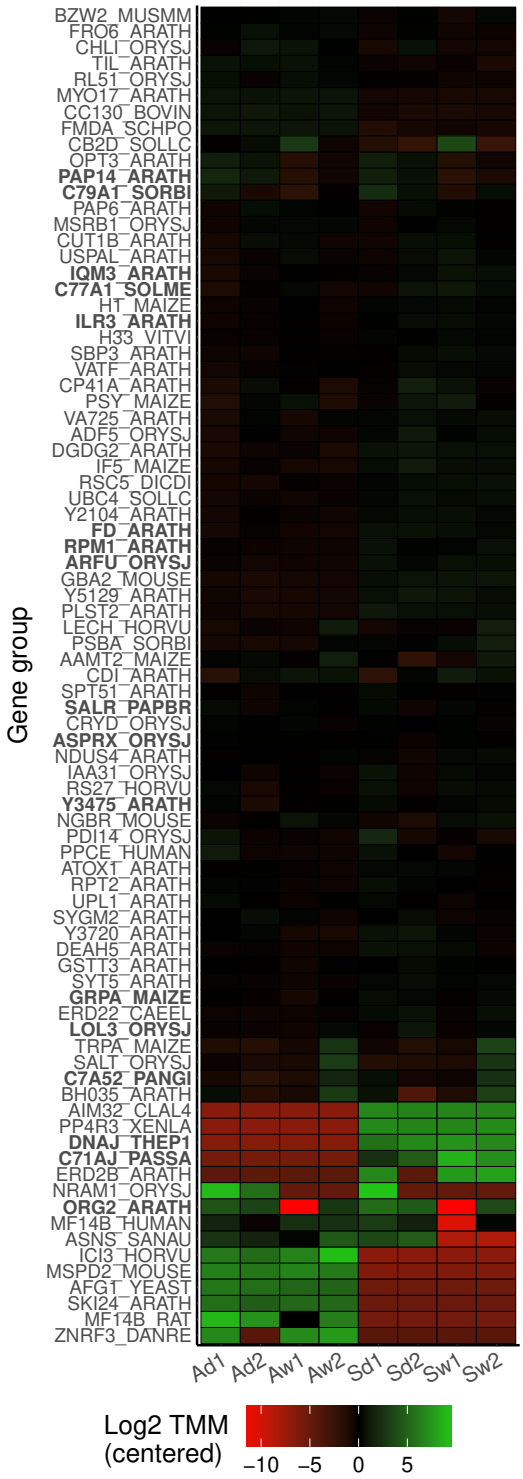

Figure 2.5: Differentially expressed gene-groups representing species by environment interaction between *A. gerardii* and *S. nutans*. Units are log2 TMM (trimmed mean of M-values). Bold transcripts map to known transcription factors.

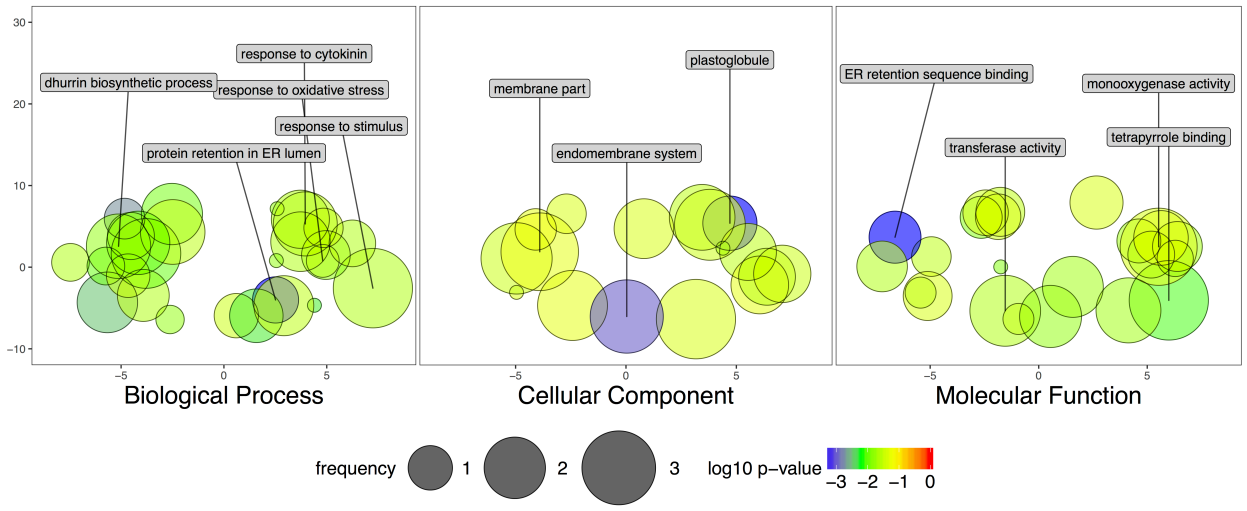

Figure 2.6: Gene Ontology (GO) enrichment for differentially expressed gene-groups between *A. gerardii* and *S. nutans* (gene-groups showing a species x treatment interaction). Color represents significance of over-representation of a specific GO term within these gene-groups upregulated under drought. Size represents the relative natural log scale frequency of the cluster of transcripts. Horizontal and vertical axes represent semantic space (SimRel similarity measure).

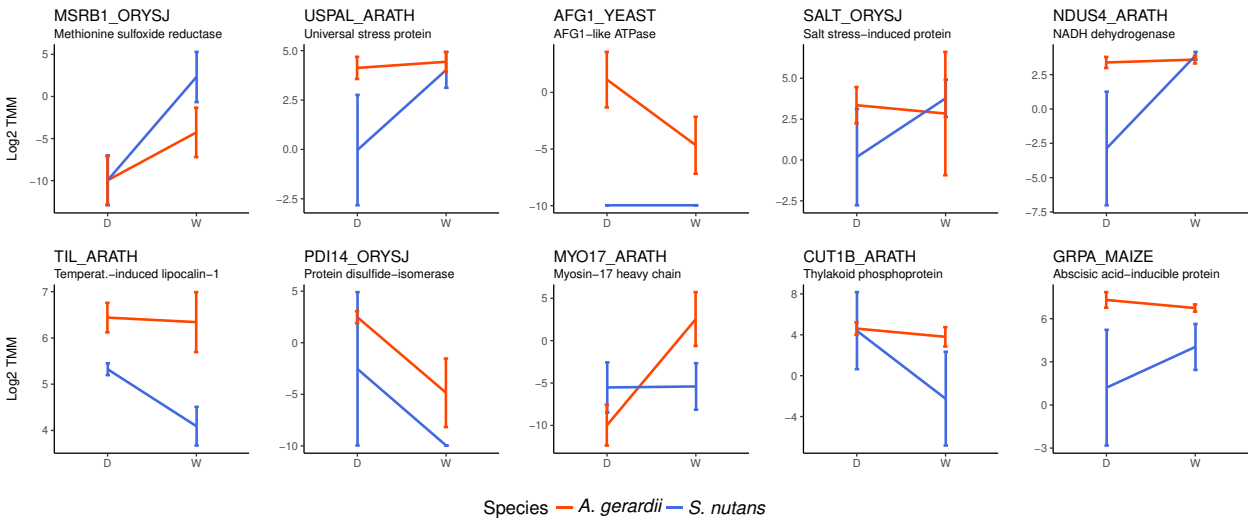

Figure 2.7: Expression change for significant species by environment interaction gene-groups. Figures depict the average expression of the top two most significant paralogs from each gene-group. Bars represent standard error. MSRB1_ORYSJ, USPAL_ARATH, AFG1_YEAST, SALT_ORYSJ, NDUS4_ARATH, TIL_ARATH, and PDI14_ORYSJ belong to the stress category; MYO17_ARATH belongs to the trichome category; CUT1B_ARATH and GRPA_MAIZE belong to the ABA category.

2.5 Discussion

In this study, we assembled the first publicly available and comparative transcriptomes for the tallgrass species *Andropogon gerardii* and *Sorghastrum nutans* as a means to understand molecular phenotypes and pathways. Average realignment of reads back to the transcriptome was greater than 80%, and 50% of the transcriptome consisted of transcripts longer than 700 bp (N50) for both species. Both metrics indicate a successful assembly, despite our use of short sequencing reads. These assemblies will be particularly useful for researchers performing gene expression work on these species as less sequencing depth is needed when a reference transcriptome is available.

Both species shared between 85-86% homology (blastn alignment) with C3 monocot species, but between 89-94% withC_4 monocots, indicating an important divergence in gene structure. *Andropogon gerardii* showed almost consistently lower similarity to model species compared to *S. nutans*, which could be indicative of more unique gene changes in *A. gerardii*. Since *A. gerardii* is hexaploid, it is possible that more paralogous genes have been able to sub- or neofunctionalize and could benefit from paralog network analysis (Gallagher et al., 2016).

Although *A. gerardii* expressed fewer estimated genes in the transcriptome, it also had more lowly expressed genes and a greater percentage matching known transcription factors. *Andropogon gerardii* may only appear less sensitive (fewer genes expressed), while in reality a greater diversity of gene expression could occur via downstream regulation. Greater ploidy and gene duplication has also been previously connected to more important expression differences in regulatory elements like transcription factors and ribosomal proteins (Roulin et al., 2013). *Sorghastrum nutans* mapped a slightly greater percentage of transmembrane helices, lending support to previous findings on the importance of aquaporins (Smith et al., 2016). Overall, 17% and 15% of the transcriptomes of *A. gerardii* and *S. nutans* (respectively) were not annotated, indicating many thousands of transcripts that are unique to either or both species and warrant exploration.

Within species, relatively few transcripts were differentially expressed with the watered treatment. Dhurrin biosynthesis (plant defense) was enriched in the drought treatment for both *A. gerardii* and *S. nutans*. Although metabolism of plant defenses may seem an unusual response

to drought stress, changing dhurrin content in sorghum has been linked to regulation of pre- versus post-flowering senescence (Burke et al., 2013). Other categories were unsurprising given the two species' differing physiology. *Andropogon gerardii* emphasized misfolded protein and amino acid salvage under drought, which could reflect alleviation of the symptoms of drought stress with greater sensitivity to temperature-induced misfolding. Aberrant mRNA surveillance and spliceosome regulation pathways are also implicated in drought tolerance (Lee et al., 2015; Lu et al., 2016). On the other hand, *S. nutans* upregulated transcripts related to osmotic and salinity stress which could reflect its greater sensitivity to soil water content (Nippert et al., 2009). Cytokinin response and general plant hormone signal transduction enrichment by *S. nutans* could indicate a greater emphasis on stomatal regulation through the cytokinin antagonistic relationship with ABA (Pinheiro and Chaves, 2011). Overall, these annotations suggest *A. gerardii* may have modified regulatory elements and tolerates stress symptoms under drought while *S. nutans* avoided stress by focusing on water use. This also corroborates physiological data on *A. gerardii*'s sustained activity under drought (Hoover et al., 2014a; Knapp, 1985). Like the transcriptome, differentially expressed genes in *A. gerardii* also contained more transcription factors, which are widely implicated in drought resistance (Baldoni et al., 2015).

By comparing the annotations of gene-groups in *A. gerardii* and *S. nutans*, we may reveal strategic differences between these two grasses outside of treatment. Nearly 23% more gene-groups differed between the two species under watered conditions than under drought, suggesting that the two species may converge on more similar molecular function when stressed. Only watered *S. nutans* upregulated carbon fixation and photosynthesis, supporting the notion that *S. nutans* is more sensitive in terms of carbon allocation. Greater numbers of transcription factors in *A. gerardii* under both drought and watered conditions suggest enhanced plasticity to maintain more constant carbon acquisition. Compared to *S. nutans*, *A. gerardii* gene-groups were highly represented by transcription factor activity, flavonoid metabolism, and glycosylation (i.e., heat response, Jiang et al., 2015), meaning that this species may divert more resources toward antioxidant and regulatory processes compared to *S. nutans*. In contrast, *S. nutans* gene-groups were represented by RNA

binding (e.g., in response to ABA, Ambrosone et al., 2015) and regulation and response to stress and hormones. Investing greater gene expression dedicated to hormonal signaling could be the mechanism by which *S. nutans* is more sensitive to water availability (Nippert et al., 2009; Silletti and Knapp, 2001). Meanwhile, *A. gerardii*'s lack of sensitivity could be the result of greater emphasis on stress alleviation versus prevention. This difference in allocation of gene expression and strategy could portend community shifts, especially greater variability in production, under future climate change scenarios. On the other hand, *A. gerardii* showed more metabolic pathway hits tied to hormonal regulation and response compared to *S. nutans*. This could indicate that despite no expression enrichment of these gene-groups, signaling pathways may be more complex in *A. gerardii* and involve more modulators (e.g., MicroRNAs, Ding et al., 2013).

When we performed a meta-level analysis across species and treatment, we found 83 differentially expressed gene-groups, including 10 within the previously mentioned functional annotation clusters. These 83 genes were enriched in response to oxidative stress (including tetrapyrrole binding) as well as membranes and plastoglobule, which are tied to osmotic stress. As we suspected, the key differences between *A. gerardii* and *S. nutans* stress response are likely to reflect the abiotic physiological sensitivities (Nippert et al., 2009).

Focusing on functional clusters of gene-groups may add clarity when comparing responses to complex stressors like drought. Selecting clusters a priori according to suspected relevance also prevents bias when data abound. When we explored the 10 differing gene-groups matching our functional annotation clusters, we saw greater sensitivity by *A. gerardii* with MYO17_ARATH (trichome) and AFG1_YEAST (stress) revealed through steeper slope across treatments (Falconer, 1990). Greater sensitivity of MYO17 in *A. gerardii* is unsurprising considering trichomes are visibly more abundant on this species in the field and trichome density is known to vary across populations along a precipitation gradient (Olsen et al., 2013). Trichomes help plants avoid dehydration by increasing the boundary layer, thus reducing transpiration (Schreuder et al., 2001) and could allow less regulation of osmotic balance in *A. gerardii*. AFG1 acts as a chaperone degrading misfolded proteins in yeast and responds to oxidative stress in Arabidopsis (Al Ameri,

2015); it could reflect greater regulation of *A. gerardii* gene-groups to alleviate stress. More gene-groups showed greater sensitivity within *S. nutans*, however. Of these, one was linked with osmotic stress (SALT_ORYSJ), three with general stress (USPAL_ARATH, NSUS4_ARATH, and TIL_ARATH), two with ABA response (CUT1B_ARATH and GRPA_MAIZE), and only one with oxidative stress (MSRB1_ORYSJ) (Abo-Ogiala et al., 2014; Alvarez et al., 2014; Dinakar et al., 2016; Kline et al., 2010; Liu et al., 2014; Roy and Nandi, 2017; Udawat et al., 2016; Zhang et al., 2014). The more sensitive response by these gene-groups in *S. nutans* could indicate greater stress response by this species, especially for gene-groups related to water management.

Our results and conclusions would be incomplete without discussion of sample size in this study. The number of replicates per species and treatment is small (n=2), though not uninformative within ecology (Lemoine et al., 2016). Therefore, despite best efforts to correct for type I and II error, we are likely to have missed or incorrectly characterized expression. The number of differentially expressed genes within species is small compared to other studies (e.g., Bushman et al., 2016; Dong et al., 2014; Meyer et al., 2014; Wilkins et al., 2009), indicating that our power to detect differential expression was low. Although care was taken to minimize differences among plants, we performed an uncontrolled drydown which often leads to variation in water content (Lovell et al., 2016) and has somewhat limited applicability to field studies. As is seen in Fig. 4, there is variation among droughted plants. Uncontrolled drydowns may lead to variation in VWC and could have produced more extreme responses in one droughted pot for *S. nutans*. This must be taken as a caveat for the analyses comparing the two species. Thus, our results should be considered as preliminary evidence for differences in these species.

Overall, variation between these two species far outweighed plasticity in gene expression under different water conditions. This could certainly stem from our limited sample size as well as the particular type of stress we invoked. Previous research revealing greater sensitivity in *S. nutans* (e.g., Hoover et al., 2014b; Nippert et al., 2009; Smith et al., 2016) is corroborated by this study, but specific mechanisms are likely much more complex. For example, *A. gerardii* may actually exhibit greater sensitivity through regulatory elements like transcription factors and may be more

sensitive with regard to oxidative stress and reactive oxygen species scavenging. Most results from this study suggest a more passive drought tolerance strategy by *A. gerardii* versus an active drought avoidance strategy by *S. nutans*. However, this study only accounts for one genotype and a single time point for each species; in the field, plasticity emerging from genotypic diversity and temporal variation may provide more resistance and resilience to drought and other stressors (Avolio and Smith, 2013b; Avolio et al., 2013). Going forward, studies involving these two species should take into account differences in gene expression, including differences in stress, ABA, and trichome gene-groups as well as differences in metabolic pathway allocation. The transcriptome resources generated in this study will also serve as templates for future exploration of the molecular phenotype in these two ecological important grasses, especially as studies using RNA-seq become more important in ecology.

Chapter 3

Nonlinear plasticity improves understanding of intraspecific diversity in an ecological model species

3.1 Overview

Common plant species within ecological communities (dominant species) use intraspecific trait variation to maintain function under both optimal and stressful conditions. Yet, few studies of intraspecific diversity in natural populations account for nonlinear plasticity, variation in plastic traits across a gradient of conditions. We investigated the intraspecific trait differences, plasticity, and nonlinearities in plasticity under a controlled water availability gradient. We applied these concepts in the context of future climate change with three genotypes of *Andropogon gerardii*, the dominant warm-season grass of the North American tallgrass prairie. This ecosystem is likely to face more extreme and frequent droughts in the future. Nonlinear plasticity in morphological and physiological traits was widespread and differed across genotypes, highlighting the influence of relatively small changes in water availability on intraspecific diversity. Genotypes also differed in reproductive strategy, but all recovered similarly following drought. We demonstrate that nonlinear plasticity may help explain intraspecific diversity and patterns of selection within a population. A better understanding of intraspecific diversity and trait variation in this grass species will provide more mechanistic insight into its ability to moderate community changes in the tallgrass prairie under future droughts.

3.2 Introduction

Phenotypic plasticity, or the change in a phenotype exhibited across environments, allows persistence under changing conditions. In plants, phenotypic plasticity is critical for responding to droughts and unpredictable environments (Lázaro-Nogales et al., 2015; Matesanz et al., 2010). Plasticity often varies across genotypes (El-Soda et al., 2014), a phenomenon well-codified using "reaction norms" (Falconer, 1990). Plasticity can also allow species to adapt to novel environments (Ghalambor et al., 2007). Yet, the majority of phenotypic plasticity studies assess traits in two en-

vironments (e.g., wet versus dry), failing to consider trait values under intermediate conditions (Stinchcombe and Kirkpatrick, 2012). In other words, typical plasticity studies must assume linear relationships, while intermediate environments accommodate emergent nonlinearity between environment and trait value (Scheiner, 2002). By failing to consider conditions between extremes (e.g., wet and extreme drought), typical studies may also lack ecological relevance. Detection of linearity versus nonlinearity not only provides more quantitative detail to plasticity (Morrissey and Liefting, 2016; Rocha and Klaczko, 2012), but may also reveal greater breadth of intraspecific diversity. In other words, plasticity is variable across conditions and must also be considered as a quantitatively variable trait (Nussey et al., 2007). By better assessing changes in plasticity, we can more accurately describe the intraspecific diversity that allows plant species to cope selective pressures, such as drought (Benito Garzón et al., 2011; Harter et al., 2015) (Figure 3.1).

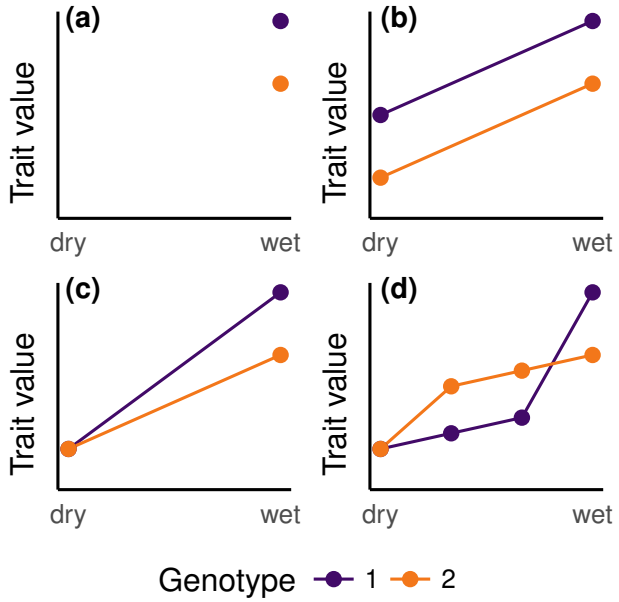

Figure 3.1: Intraspecific trait diversity (a: "genotype effect") and phenotypic plasticity across genotypes (b: "genotype and treatment effect") are critically important for plant drought response. However, it is well understood that genotypes may differ in the extent of their plasticity or respond differently (c: "interaction effect"). Such interactions may be nonlinear; when a finer gradient of water availability is applied to (c) we may observe (d). Plasticity in genotype 1 becomes nonlinear under wetter conditions while plasticity in genotype 2 becomes nonlinear under dry conditions. In this case (d), genotypes vary in the nonlinearity of their trait responses and provide greater intraspecific diversity across conditions.

Overall, intraspecific diversity has widely shown to provide stability in population responses to variable climate (Cook-Patton et al., 2011; Hughes and Stachowicz, 2004; Hughes et al., 2008; Reusch et al., 2005) but see Huber et al. (2016) and is integral to deciphering community assembly (Li et al., 2018). Yet, intraspecific diversity and phenotypic plasticity are less rigorously studied in non-model species (Tack et al., 2012) and may exclude dominant (abundant or foundational) plant species. Dominant species are largely responsible for ecosystem structure and function (Sasaki and Lauenroth, 2011; Smith and Knapp, 2003). Thus, dominant species intraspecific diversity and plasticity is key to understanding how ecosystems will respond to future environmental change. For instance, the dominant tallgrass prairie grass *Andropogon gerardii* can comprise more than 80% of productivity in tallgrass prairie (Smith and Knapp, 2003). This species also exhibits functional diversity and plasticity among genotypes in response to water limitation. A long-term rainfall variability manipulation in native tallgrass prairie produced drier soils, leading to selection against some genotypes of *A. gerardii* (Avolio et al., 2013). Physiological responses and plasticity in biomass allocation also varied among genotypes (Avolio and Smith, 2013b). However, intraspecific diversity emerging from nonlinear responses of traits has not been quantified. Accurately quantifying intraspecific diversity is timely, as selective pressures are only expected to intensify. North American prairies are expected to experience more frequent and extreme droughts with climate change (Cook et al., 2015). We must first elucidate intraspecific variation in order to predict how these unprecedented droughts will influence populations.

While different trait means and variation in plasticity have implications for genotype responses to water availability, differences in recovery following drought may also be important for native populations. Post-drought recovery is a critical means by which plants survive in unpredictable environmental conditions (Carter et al., 2012; Dietrich and Smith, 2016; Lovell et al., 2016; Meyer et al., 2014; VanderWeide et al., 2014). Grasses in particular may resprout or emerge from dormant buds (Carter et al., 2012; McDowell et al., 2008) or recover higher levels of gene expression (Meyer et al., 2014) once favorable conditions return. Like plasticity and other traits, recovery ability is known to differ among genotypes in other grasses (Cui et al., 2015; Okami et al., 2015; Pirnajmedin

et al., 2016). For *A. gerardii*, timing of drought is known to influence biomass and flowering later in the season (Denton et al., 2017; Dietrich and Smith, 2016), so we might expect a legacy effect of water limitation. Yet, it is unknown whether genotypes of *A. gerardii* recover differently following drought or how recovery may be plastic across water availability.

In this study, we better quantified the breadth of intraspecific diversity in drought response by including assessment of nonlinear plasticity. Using *A. gerardii* as the focal ecological species, we used a soil moisture gradient to assess genotype differences and nonlinearity of plasticity in response to drought. We examined differences in genotype recovery after a legacy of different water limitation by restoring water availability at the end of the study. Based on past patterns of selection, we chose two drought resistant and one drought susceptible genotype for comparison (Avolio and Smith, 2013b; Avolio et al., 2013). We hypothesized that genotypes would vary in trait value response to a gradient of soil moisture, broadly indicating intraspecific diversity within-population. We also suspected nonlinear plasticity would be present across a variety of traits and would vary among genotypes. Overall, we expected the drought susceptible genotype to be the most distinct. We also suspected that the two drought resistant genotypes would differ in drought response strategy to minimize competition in the field. Taken together, these multiple hypotheses contribute to our understanding of complex variation in intraspecific responses, which may help maintain ecosystem function in the tallgrass prairie community.

3.3 Methods

3.3.1 Study site and genotype establishment

We performed our study within the context of the Rainfall Manipulation Plots (RaMPs) study at the Konza Prairie LTER site in Manhattan, KS. The long-term RaMPs study altered precipitation patterns to produce fewer larger rainfall events, leading to decreased soil water content in altered plots. This experiment lies within an annually burned remnant tallgrass community by *A. gerardii*, a tall perennial C_4 grass. This species reproduces primarily by clonal tillering and thrives in annually burned areas like the RaMPs experiment. We selected three common genotypes of *A. gerardii* found within the experiment: G2, G5, and G11 (Avolio and Smith, 2013b), where the number cor-

responds to each genotype's ranked abundance. Using flow cytometry, we determined that all three genotypes were hexaploid, with genome sizes similar to previous findings (Keeler, 1990). While G2 and G11 were found within altered plots in RaMPs (drier plots), G5 was selected against. All genotypes were found in ambient plots. In other words, G2 and G11 appear drought resistant while G5 appears drought susceptible. We then performed meristem tissue culture on these genotypes; this allowed us to remove maternal effects and propagate a large number of individuals for use in the greenhouse (Section II.1). Plants were allowed to adjust in misters for 42 days before being placed in 2.65 L cone-tainer style tree pots (Stuewe & Sons, Tangent, OR) (Figure II.2). These pots were placed in a greenhouse bay at 30°C with a 16 hour photoperiod supplemented with overhead lamps. To reduce nutrient limitation, 6g of Osmocote fertilizer was added to each pot. Plants were watered daily for 29 days until the beginning of the drought study (26 August 2014).

3.3.2 Water availability treatments

We selected 10, 15, 20, and 25 percent volumetric water content (%VWC), as well as saturated %VWC, as our water availability treatments. Because %VWC and gravimetric water content (GWC) are nearly equivalent at the Konza location (Wilson et al., 2009), we converted GWC of Konza soils to GWC of Greens Grade media by matching soil water potential (-mPa) of potentiometer curves from both types of soil. In other words, we determined soil water potential at each treatment within Konza soils and matched the corresponding Greens Grade soil water potential. It is worth noting from the potentiometer curve that equal declines in %VWC correspond to similar, but not exactly equal soil water potential values. Thus, we have avoided any sensitivity analyses (Smith et al., 2017).

Following the adjustment period above, pots were weighed daily, and water was added daily to achieve the target water availability treatment. Saturated pots were given 500 mL of water daily, or until water dripped slightly from each pot. The treatment period lasted for 7 weeks for a total of 10 weeks. Pots were rotated weekly to avoid greenhouse microclimate effects. In total, n=20 pots were allocated to each treatment and genotype for a total of 300 pots (3 genotypes by 5 water availability treatments = 15 genotype x treatments each with 20 replicates). Due to the destructive

nature of some measurements, we created subsets of plants for different uses, described below. All water availability treatments were significantly different after day 20 (Figure II.3).

3.3.3 Physiological measurements

Net photosynthetic rate (A_{net}), stomatal conductance (g_s), maximum PSII efficiency (fv/fm), evapotranspiration rate, and instantaneous water use efficiency (WUE_i) provide information related to photosynthesis and water use, a process inhibited by drought and predictive of drought response (Ocheltree et al., 2016). All measurements were performed between 11:00 and 14:00 on a weekly basis during the treatment period using a LI-6400 system (LiCOR Inc., Lincoln, NE, USA) adjusted to a constant CO_2concentration of 400 μmol mol^{-1}. For A_{net} and g_s, the LED light source was maintained at 2000 μmol mol^{-1} s^{-1}. Leaves were dark adapted overnight before performing an fv/fm light saturation flash for 1 s (n=7 replicates per treatment and genotype for all physiological measurements). We calculated WUE_i by dividing A_{net} by evapotranspiration. Because of its destructive nature, midday leaf water potential was measured at the end of the treatment period using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA, n=3 leaves from separate pots per treatment and genotype).

3.3.4 Morphological measurements

For all replicates (n=20 replicates per genotype and treatment), height, leaf number, and tiller number were measured weekly throughout the treatment period to the nearest mm. Relative growth rate was calculated as the natural log of the difference in height between weeks (Philipson et al., 2012). Biomass was harvested at the end of the treatment period and divided into aboveground and belowground (including rhizome) biomass (n=7 per treatment and genotype). From each aboveground biomass sample, a subset of two leaves was removed to measure leaf area (LA) and specific leaf area (SLA).

Root structural traits are key for plant plasticity and adaptation to drought (Comas et al., 2013). From the belowground biomass, a subset of roots was removed from each plant to analyze root architecture. Leaves used for SLA were scanned for area measurement using ImageJ software and

dried. Root architecture, which includes root length, surface area, diameter, volume, and number of root tips, was analyzed using WinRhizo software (Regent Instruments, Quebec City, Canada). Root architecture measurements were scaled according to the proportional weight of the subset roots compared to the total roots. All biomass was weighed fully hydrated and then dried at 60°C for 48 h to determine leaf dry matter content (LDMC) and root dry matter content (RDMC). We also calculated additional metrics: whole plant root to leaf surface area ratio, specific root length (root length / mass), and specific root surface area (root surface area / mass). Root to leaf surface area ratio provides a useful indicator of potential water uptake in proportion to carbon uptake ability (Comas et al., 2013). Although fine roots may be important for resources acquisition, specific root length may be lower in native species with a conservative drought response strategy (Balachowski and Volaire, 2018).

3.3.5 Recovery following water availability treatments

Following the primary treatment period, a subset of plants (n=7 per treatment and genotype) were immediately rewatered to 25% VWC for eight weeks. After this recovery period, aboveground (leaves) and flowering (including stalks) were harvested, dried, and weighed. Plants were allowed to recover from clipping for two weeks at which point height was measured to assess recovery from the rhizome. After recovery from rhizome height measurement, all aboveground and belowground (including rhizome) biomass was harvested, dried, and weighed. Following rewatering, $A_{net}$and leaf water potentials were measured as above. Aboveground, flowering, and belowground biomass were also harvested and dried. Height was measured weekly throughout both recovery periods.

3.3.6 Statistical analyses

Because trait measurements are often correlated (Figure II.4, Figure II.5, Figure II.6, Figure II.7), data were clustered into principal components (PCs) to determine key functional traits for further analysis (Table II.1, Table II.2, Table II.3, Table II.4). Because some measurements are destructive, traits were divided and analyzed in subsets according to the measurements per-

formed (See Table II.1, Table II.2, Table II.3, and Table II.4 for details on PC loadings and all traits measured). Recovery data was clustered separately into PCs since the null hypothesis differs from treatment period data (H0: differences across treatments and genotypes are retained during recovery). Preliminary analyses determined that day was a significant factor in repeated measures analysis, so all days of repeated measurements were included when forming PCs. All PCs were formed using the `prcomp()` function within R (version 3.4.3) to account for the maximum variance across traits. Important traits were determined based on loadings from the top two PCs from each subset (PC1 and PC2, see Table II.1, Table II.2, Table II.3, and Table II.4 for variance explained). We modeled the posterior distribution of these traits ($\hat{y}_i$) as a Bayesian linear regression. The following model was used to test the effects of genotype, treatment, and their interaction on the ith observation of N:

$$\hat{y}_i \sim normal(x_i\beta, \sigma^2)$$

where x is an N x J matrix of predictors (J represents each of 15 unique genotype, treatment, and interactions possibilities) and β is a vector of J parameters. Overall, $x_i\beta$ is a vector of N predictors. This model had the advantage of allowing us to estimate different slopes for each combination of genotype and treatment rather than the overall effect of each. Effects of treatment, genotype, and genotype x treatment interaction were considered significant if the J parameter did not overlap zero. In two cases (recovery flowering biomass and recovery from rhizome), the original data contained many zeros so we allowed additional parameters, probability of flowering ϑ_F and probability of recovery from rhizome ϑ_{Rh} to vary between 0 and 1 across genotypes to inform mixture models. See supplementary material for more details on the statistical models (Text S2). All models were run simultaneously with three chains using Stan (version 2.17.3, Gelman et al., 2015), with 5,000 sampling iterations discarded as burn-in and 5,000 iterations retained. All model parameters converged with Rhat values approaching 1 and normality of residuals maximized (see Section II.1.1); all models were compiled within R (version 3.4.3, R Core Team, 2018). Error bars throughout the study described the 95% CI around the posterior distribution of a given trait.

Plasticity was considered present if there was a treatment effect for a particular genotype. We categorized the difference between CIs of two adjacent treatments, where non-zero differences were considered plastic and zero-overlapping values were considered static. If a genotype demonstrated both static and plastic responses within a particular trait, the plasticity was considered nonlinear.

3.4 Results

3.4.1 Physiological responses to water availability

Physiological traits were typically plastic in response to water availability (significant treatment effect) but trait means did not differ across genotype (Table 3.1). Mean photosynthetic rate showed a significant genotype x treatment interaction, indicating the pattern of genotype plasticity could be distinct even without mean trait differences among genotypes or treatments. Stomatal conductance and evapotranspiration rates sometimes showed significant genotype x treatment interactions, depending on the week (Figure II.8). We also observed large variation in trait value across weeks (Figure II.8).

Nonlinear plasticity was consistent across physiological traits. For example, photosynthetic rate increased sharply between 10 and 15%VWC for all genotypes while leveling off at greater water availability (Figure 3.2a). These patterns differed among genotypes; from 15%VWC, G5 increased photosynthetic rate incrementally, while increases in G2 and G11 continued to show significant plasticity, leveling off between 20 and 25%VWC (Figure 3.2a). Stomatal conductance (Figure 3.2b) and evapotranspiration rate (Figure 3.2c) also showed pronounced plasticity between 10 and 15%VWC for all genotypes. However, Genotype 11 also showed additional plasticity stomatal conductance and evapotranspiration rate plasticity between 15 and 20%VWC not observed in the other two genotypes.

3.4.2 Morphological responses to water availability

All morphological traits showed a significant effect of treatment, indicating morphological plasticity is a key response to water availability in *A. gerardii* (Table 3.1). Aboveground biomass, root complexity, and leaf area tended to increase with increasing water availability (Figure 3.3).

Table 3.1: Important traits, effects, and plasticity types identified from principal component analysis.

Treatment	N	Key PC loadings	Significant effects	Plasticity type: G2	Plasticity type: G5	Plasticity type: G11
7 week water availability treatment	7	Root tips	T	Linear	Nonlinear	None
		Root length	T, GxT	Linear	Nonlinear	None
		Root surface area	T, GxT	Linear	Nonlinear	Linear
		Root volume	T, GxT	Nonlinear	Nonlinear	Nonlinear
		Leaf area	T, GxT	Linear	Nonlinear	Nonlinear
		Aboveground biomass	T, GxT	Nonlinear	Nonlinear	Nonlinear
		Root:leaf surface area	G, T, GxT	Linear	Linear	Linear
		Specific root length	G, T	Nonlinear	Nonlinear	Linear
	7	Mean A_{net}	T, GxT	Nonlinear	Nonlinear	Nonlinear
		Mean g_s	T	Nonlinear	Nonlinear	Nonlinear
		Mean evap. rate	T	Nonlinear	Nonlinear	Nonlinear
	20	Max. height	G, T, GxT	Nonlinear	Nonlinear	Nonlinear
		Max. rel. growth rate	G, T, GxT	Nonlinear	None	Nonlinear
7 week water availability treatment + recovery	7	Max. recovery height	None			
		Max. recovery rel. growth rate	GxT			
		Flowering biomass	G			
		Rhizome recovery	G			

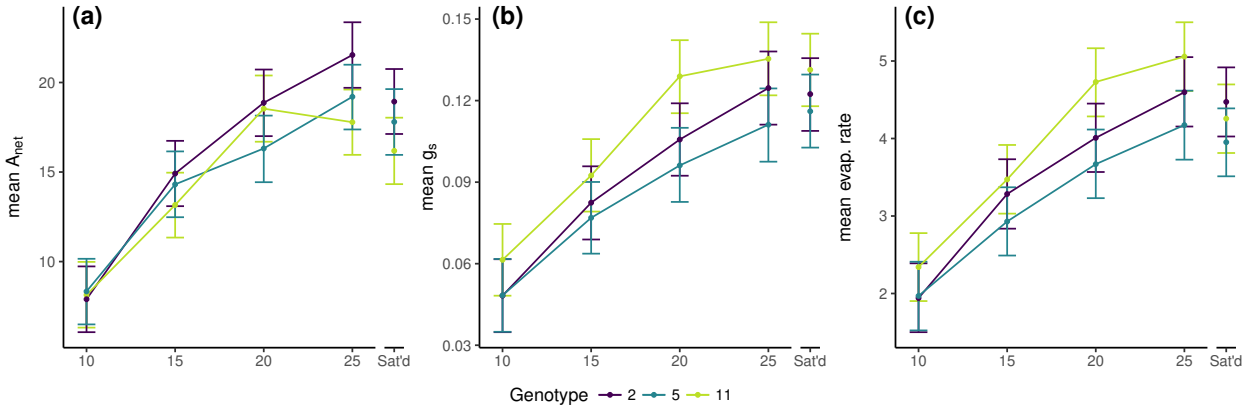

Figure 3.2: Key physiological traits identified through PC analysis show nonlinear plastic responses of (a) mean A_{net}, (b) mean g_s, and (c) mean evapotransipiration rate to a gradient of water availability. See Table 3.1 for significant effects. Error bars represent the 95% CI.

Root to leaf area ratio declined with increasing water, while specific root length (length to mass ratio) was greatest at intermediate water availability. We detected some overall mean differences in genotype trait values; root to leaf surface area ratio tended to be greater overall in G2 and specific root length was smaller in G11 (Figure 3.3g,h). We also detected genotype x treatment interactions in six of the eight key traits, indicating that genotypes commonly responded differently depending on the treatment.

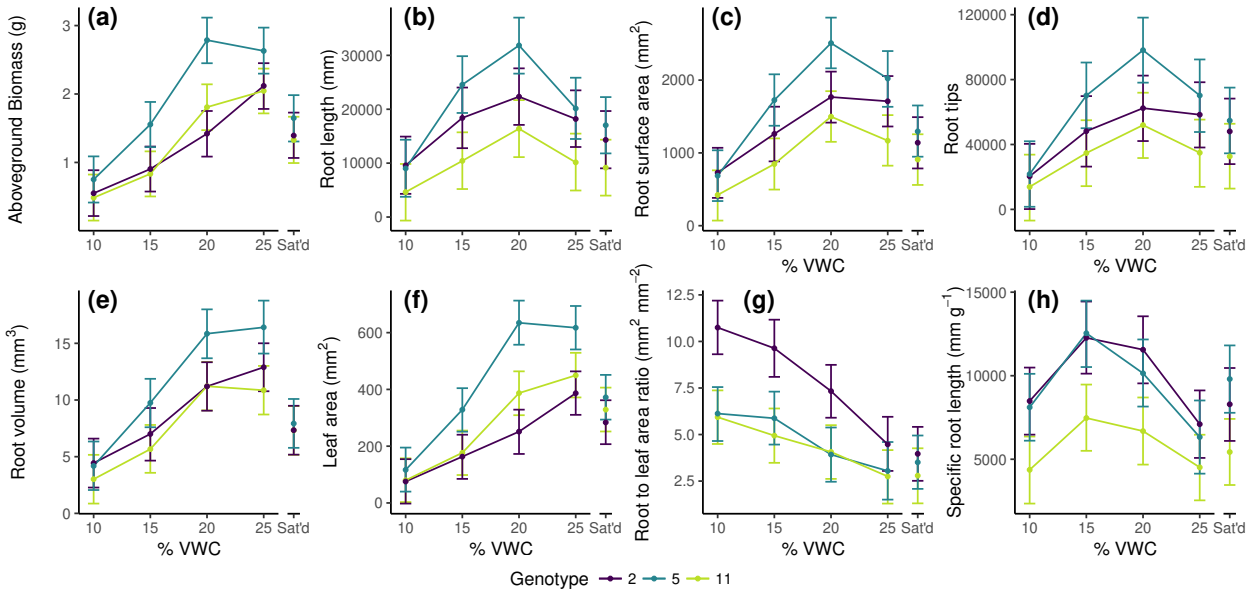

Figure 3.3: Key morphological traits identified through PC analysis show that genotypes and treatments differ and plasticity is also often nonlinear. These include (a) aboveground biomass, (b) root length, (c) root surface area, (d) root tips, (e) root volume, (f) leaf area, (g) root to leaf surface area, and (h) specific root length. See Table 3.1 for significant effects. Error bars represent the 95% CI.

Nonlinear plasticity was also common (Table 3.1). Overall, G5 showed the greatest nonlinear plasticity (7 traits) compared to G2 or G11 (3 traits, Table 3.1). Genotype 5 demonstrated nonlinear plasticity in root tips, root length, and root surface area particularly between 10 and 15%VWC, whereas G2 plasticity was linear throughout and G11 changes were often not significant (Figure 3.3b,c,d). Leaf area plasticity was nonlinear between 15 and 20%VWC for G5 and G11, but not for G2 (Figure 3.3f). Genotypes also experienced nonlinear plasticity at different points on the water availability gradient. Specific root length plasticity was nonlinear between 20 and 25%VWC

for G2 but between 10 and 15% VWC for G5. In contrast, genotype 11 experienced minimal linear plasticity in specific root length (Figure 3.3h). While aboveground biomass plasticity was nonlinear for all genotypes, G5 and G11 experienced the greatest increase in biomass between 15 and 20%VWC while G2 experienced the greatest increase between 20 and 25%VWC (Figure 3.3a). Root volume was plastic for G2 between 25%VWC and saturated conditions, while G5 and G11 root volume was plastic at lower water availability (Figure 3.3e).

We detected genotype, treatment, and genotype x treatment effects for maximum height and relative growth rate. Height differences among genotypes were apparent before water limitation began and persisted throughout much of the experiment, with G11 appearing noticeably shorter (Figure II.9, Figure 3.4a). All genotypes changed little in height between 10 and 15%VWC, but increased height substantially between 15 and 20%VWC. While all genotypes had distinct heights at 10 and 25%VWC, G2 and G5 were similar at intermediate%VWC. Genotype 2 and G11 had the steepest slopes for maximum height plasticity. Relative growth rates differed substantially depending on the genotype (Figure 3.4b). Genotype 5 showed no plasticity in its consistently low growth rate. On the other hand, G2 increased its growth rate between 15 and 20%VWC, while G11 increased consistently between 10 and 20%VWC.

3.4.3 Intraspecific diversity in reproductive strategy and recovery from drought

The most striking difference between genotypes was in their ability to flower during the recovery period. Genotype 2 had consistently greater flowering biomass than the other genotypes, although legacy of low water availability affected ability to flower (Figure 3.5a). The legacy of water treatment was visible in G2, which showed reduced flowering in pots that had been the driest. While there was some flowering by G11 at 20%VWC, it was not statistically different from zero. Genotype 5 almost never flowered. When we modeled the probability of flowering regardless of treatment, we confirmed large genotype differences, where ϑ_F = (0.75,0.96) for G2, ϑ_F = (0.22,0.54) for G11, and ϑ_F = (0.02,0.20) for G5. In other words, the posterior probability of flowering was closer to 1 for G2 but closer to zero for the other genotypes. In contrast, recovery from rhizome was greater in G5 compared to G2 but only at 20%VWC and greater water availability

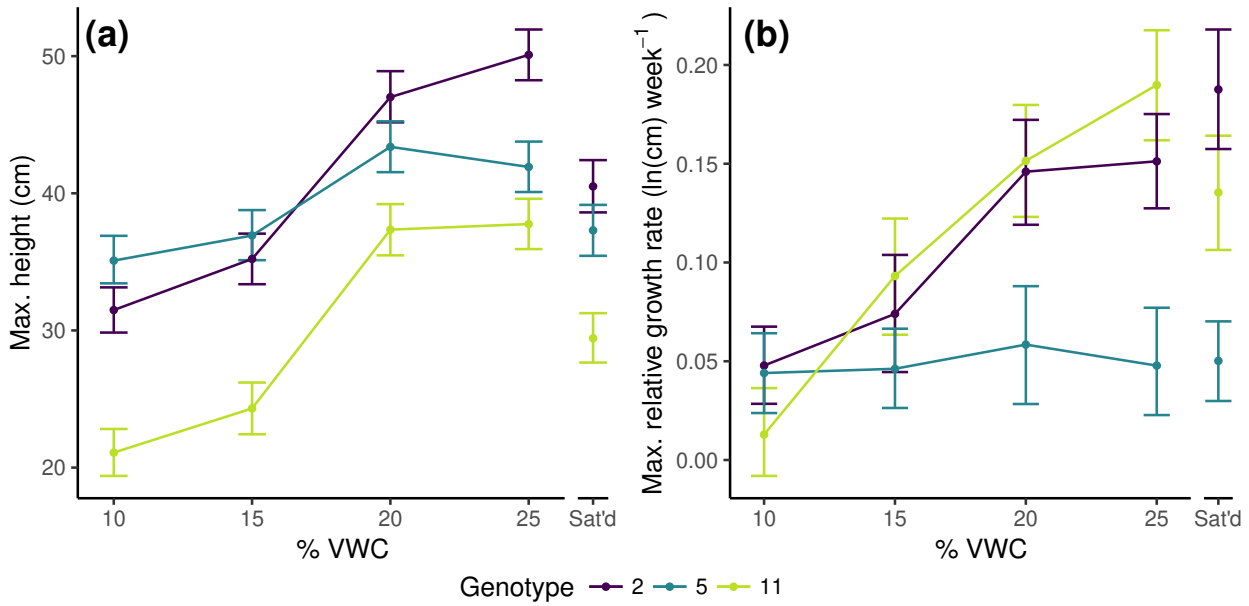

Figure 3.4: Maximum height (a) and (b) relative growth rates differ among genotypes and treatments, where genotypes differ in the pattern of response (GxT) and plasticity is almost always nonlinear. See Table 3.1 for significant effects. Error bars represent the 95% CI.

(Figure 3.5b). When we modeled the probability of recovery from rhizome regardless of treatment, we confirmed large genotype differences, where ϑ_{Rh} = (0.09,0.35) for G2, ϑ_{Rh} = (0.46,0.78) for G11, and ϑ_{Rh} = (0.62,0.89) for G5.

While flowering showed extreme differences after recovery, other traits differed little at the conclusion of the experiment. Maximum heights among genotypes and treatments converged on similar values (Figure 3.6a). This trait convergence may be due in part to increased growth rates by G11 at lower %VWC treatments (Figure 3.6b). Temporal data suggests genotypes and treatments achieved recovery to similar phenotypes by week 12 (Figure II.10). By the end of the experiment (week 15), root biomass, vegetative biomass, height, and relative growth rate recovered to similar values across genotypes and treatments (Table II.4) and suggest a full recovery for droughted treatments. Lack of differences among genotypes post-recovery may indicate a large amount of variation, however.

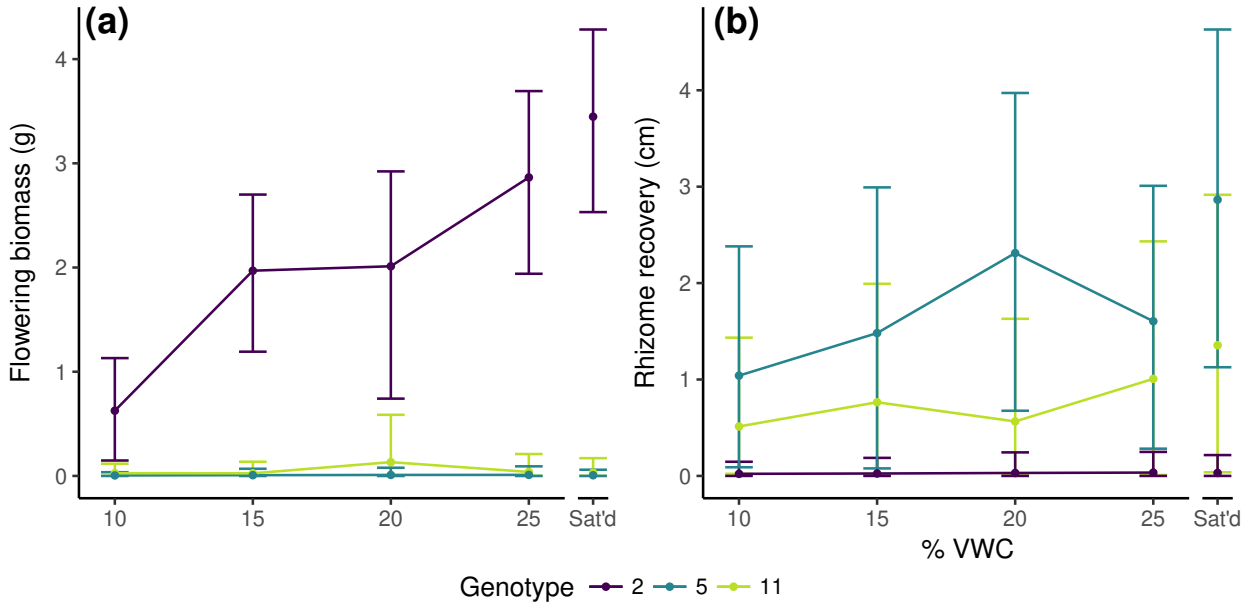

Figure 3.5: Flowering (a) was much greater in genotype 2, while rhizome recovery (b) was greater in genotype 5. Note that treatments on the x-axis refer to the treatments experienced prior to the recovery period. See Table 3.1 for significant effects. Error bars represent the 95% CI.

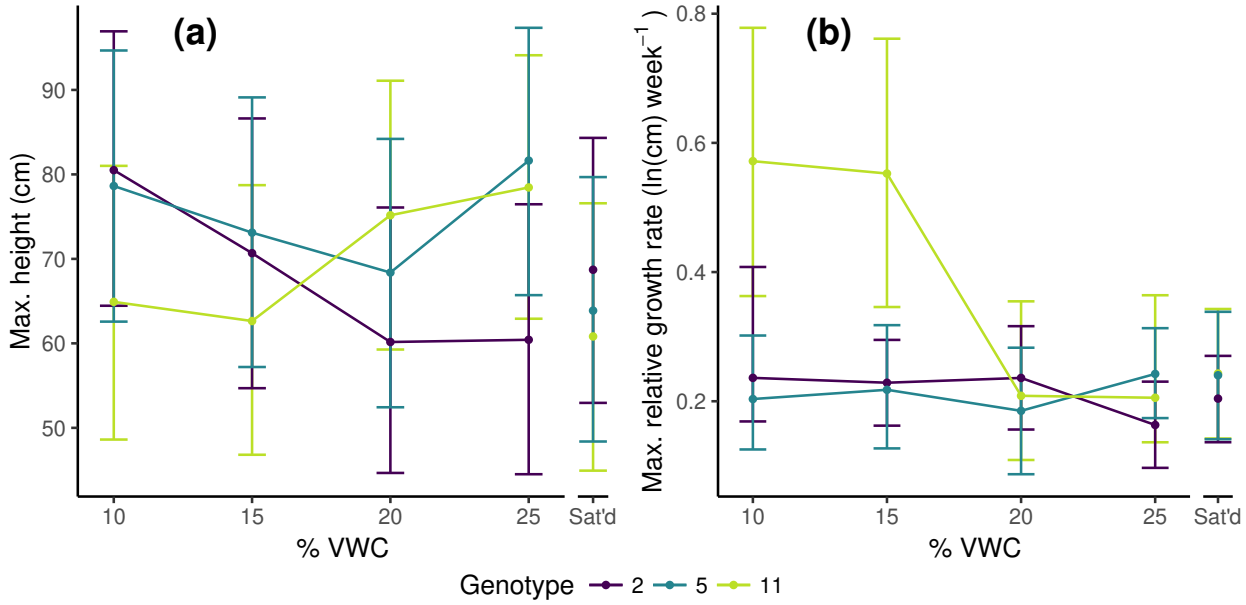

Figure 3.6: All genotypes and treatments had similar heights (a) during recovery. Relative growth rates (b) were larger in G11 at low water availability, allowing this genotype to compensate for prior small stature. Note that %VWC treatments refer to the treatments experienced prior to the recovery period. See Table 3.1 for significant effects. Error bars represent the 95% CI.

3.5 Discussion

In this study, we used the native species *A. gerardii* to compare genotypes with a history of selection, including two drought resistance genotypes and one drought susceptible genotype. We found strong evidence for different strategies employed by each genotype, which could explain the persistence of this dominant species in tallgrass prairie. We also found evidence for the importance of intraspecific diversity within a population, including nonlinear plasticity in drought response and recovery. While research on nonlinear reaction norms is more common in model species like Drosophila (Morrissey and Liefting, 2016; Rocha and Klaczko, 2012), this study demonstrates the utility of quantitative plasticity assessment to further describe intraspecific diversity among coexisting genotypes. Such diversity may contribute to a better understanding of community assembly (Li et al., 2018) and ultimately enhance ecosystem functions like stability under future drought-induced climate change.

Nonlinear plasticity was widespread among our traits. Conditions under which plasticity was significant also depended on the trait (e.g., Figure 3.2a versus Figure 3.4a). However, genotypes also demonstrated differences in the conditions under which nonlinear plasticity occurred, as we detected with stomatal conductance, specific root length, aboveground biomass, root volume, and relative growth rate. This type of intraspecific diversity has likely been overlooked in many genotype x environment studies consisting of two treatments (Figure 3.1) (Stinchcombe and Kirkpatrick, 2012). Because within species functional diversity is constrained under smaller spatial scales (Tack et al., 2012), nonlinear plasticity may be especially important to explain species dynamics under different environmental conditions (Figure 3.1). In contrast to typical studies, our treatments were also precisely controlled to resemble common field conditions. Thus, our study may more accurately reveal the mechanistic responses leading to coexistence of these genotypes in the field. Widespread nonlinear plasticity in our study is supported by threshold-like responses seen in the field at the species level (Hoover et al., 2014a), but here we have demonstrated that these thresholds may help explain intraspecific patterns of diversity.

More broadly, we found strong evidence of genotype x treatment interactions and overall treatment plasticity. Plasticity for many traits is likely to be under convergent selection across the entire species and could be tied to interannual precipitation variability in the field (Vázquez et al., 2017). However, multiple genotype x treatment interactions indicate different trait values are adopted by genotypes depending on the conditions, possibly enhancing functions like stability as has been shown in other species (Ehlers et al., 2008; Reusch et al., 2005). Interestingly, variation among genotypes decreased under the driest treatment and under saturated conditions, whereas there was more variation under intermediate wetter conditions. This suggests that under extreme conditions, genotypes may converge on a common morphological phenotype. Under dry or flooded conditions the effects of intraspecific diversity for maintaining ecosystem function may therefore diminish. This pattern may also cause two-treatment studies to underestimate genotype-based differences in plasticity if the treatments are not carefully chosen.

In our study we detected intraspecific differences, including variation in plasticity, but we also revealed key overall differences among *A. gerardii* genotypes. For example, root:leaf surface area and specific root length appeared to be inherent to genotype and not associated with any treatment. The most substantial difference we detected was a genotype disparity in flowering stalk production, from greater than half of individuals flowering in G2 to almost none flowering within G5. Rhizome recovery appeared inversely related to flowering. These results suggest a possible tradeoff between allocation to sexual and asexual reproduction, as has been observed in other species (Aliyu et al., 2010; Liu et al., 2009; Pluess and Stocklin, 2005; Thompson and Eckert, 2004; Van Drunen and Dorken, 2012). Genotype 2, a drought resistant genotype, appears to prioritize sexual reproduction. This could lead to greater recombination and hypothetically fitter genotypes (heterosis) when environments are stressful (Neiman et al., 2014). In contrast, the drought susceptible genotype G5 prioritizes asexual reproduction through greater biomass under certain conditions and greater recovery from the rhizome. This suggests a bet-hedging strategy (Niklas and Cobb, 2017). If the genotype is well suited for a particular environment, its phenotype could be extremely advantageous for competition and persistence. However, if it is poorly adapted, the asexual genotype could

be selected against. Clonal growth may be advantageous for long term sexual fitness and species persistence (Barrett, 2015; Van Drunen et al., 2015), but may be poorly suited for short, intense droughts if tissue has to be maintained. Alternatively, G5 may have overall delayed flowering, which could also be disadvantageous under drought (Sherrard and Maherali, 2006). In contrast, G11 seemed able to flower and recover from rhizome at intermediate levels, but generally was small in stature. Flexibility in reproductive strategy may be advantageous for G11 but come with costs like reduced overall height (Figure 3.4a). Dominant species are often less phenotypically diverse than rare counterparts (Umaña et al., 2015), but in this case phenotypic differences among genotypes may allow this species to maintain dominance over variable climatic conditions through different reproductive strategies.

As expected, we detected evidence that the drought-susceptible genotype G5 was distinct. Morphological trait analysis suggested that G5 tended to have the greatest biomass across all treatments. While larger size has benefits for fitness and persistence (Younginger et al., 2017), this genotype may be at a disadvantage for cumulative traits as precipitation events vary intrannually within tallgrass prairie (Gibson and Hulbert, 1987). The G5 morphological phenotype may be especially disadvantageous for sudden, late-season droughts. In contrast, the resistant genotypes may persist under a legacy of drought in the field because of a more conservative approach with morphological structures (Lopez-Iglesias et al., 2014). These genotypes may remain small because the likelihood of droughts sometime in the growing season is high and it will be costly to maintain structures. In this case, greater plasticity for biomass and root structures may actually be maladaptive by G5 (Harter et al., 2015; Valladares et al., 2007) or a sign of relaxed selection among non-droughted genotypes from the previous study (Chevin and Lande, 2015). In contrast, G5 showed limited plasticity for height and relative growth rate. The timing of growth by different genotypes has substantial effects on ecosystem function in the tallgrass prairie, since timing of rainfall is known to influence productivity in *A. gerardii* (Denton et al., 2017; Dietrich and Smith, 2016; La Pierre et al., 2011). During typical droughts, it may be more advantageous to grow rapidly but only when sufficient resources are available, as G2 and G11 demonstrated with similar

nonlinear responses over treatments (Figure 3.4) and over time (Figure II.9). These distinct trait patterns, along with prioritization of asexual reproduction, could explain why G5 was unable to persist in the field under drier, more variable conditions.

We found supporting evidence for different strategies between two closely related drought resistant *A. gerardii* genotypes G2 and G11. A fundamental goal of community ecology is explaining why species or genotypes within a species coexist, and much research has been done regarding differential responses and use of resources (Avolio and Smith, 2013a; Chang and Smith, 2014; Tilman, 2004; Tilman et al., 2014). We found large differences in reproductive strategy, but also in root to leaf area ratio, specific root length, overall height, and how nonlinear plasticity was distributed across treatments. These results suggest that these genotypes differ in allocation strategies. For example, G2 tended to have greater root to leaf surface area ratio and specific root length, indicating greater investment in root complexity versus aboveground tissues. Genotype 11 appeared to have less root complexity, which may indicate a more conservative approach to drought (Balachowski and Volaire, 2018). Leaf area and aboveground biomass demonstrated linear plasticity G2 versus nonlinear plasticity in G11, which changed rapidly between 15 and 20%VWC. Coupled with height disparity, these differences suggest that G11 may be more resources conservative, or more drought tolerant versus drought avoidant (Carvajal et al., 2017; McDowell, 2011). Consistent flowering by G2 could also be a means of drought escape rather than tolerance toward the end of the growing season (Nicotra and Davidson, 2010). Moreover, height differences indicate these two genotypes occupy different physical space throughout the tallgrass prairie canopy, which could minimize competition.

Despite the importance of resilience in ecological communities (Hoover et al., 2014b; Mori, 2016), intraspecific differences in recovery are often neglected. Aside from differences in reproductive strategy among genotypes, the recovery portion of this study provided evidence for *A. gerardii*'s ability to recover rapidly once water stress was alleviated (although variation was high). In addition to the findings above, this suggests that plasticity in growth and biomass has been under strong selection in this species overall. With other climatic factors like temperature aside, this

could also indicate that legacy of drought on biomass and height can be alleviated in this population with enough additional water. This directly contradicts findings in field studies, although such quantities of water may be unrealistic in the field (Denton et al., 2017; Dietrich and Smith, 2016) and may be complicated by differences in temperature.

Our results indicate a clear need for intraspecific diversity study looking beyond comparisons of mean trait values. We demonstrate the contribution of nonlinear plasticity to intraspecific variation within a native population of *A. gerardii*. Importantly, nonlinear plasticity was the rule rather than the exception, indicating that multiple conditions may be necessary to accurately quantify trait plasticity. Differences in water use strategy among the genotypes here may have implications for community composition, especially in the context of climate change (Hoover et al., 2017). Our study suggests that these three *A. gerardii* genotypes may increase diversity of canopy structure through timing of growth and plasticity, flowering rates, and other traits. Alongside other studies, this work demonstrates the importance of understanding within-species responses across ecologically relevant treatments, allowing us to mechanistically predict how ecological communities with dominant or foundational species may respond to future drought and selection.

Chapter 4

Genetic and functional variation across regional and local scales is associated with climate in a foundational prairie grass

4.1 Overview

Combating selective pressures caused by climate change requires knowledge of within species diversity, particularly of dominant species within communities. To better understand the fate of the shortgrass steppe ecosystem of the Central US, we quantified genomic diversity from 17 sites of the dominant grass species *Bouteloua gracilis* across regional scales, north-south from New Mexico to South Dakota, and local scales in Northern Colorado. We also quantified trait and plasticity variation within and among sites in order to link genomes to functional diversity and determined the extent to which diversity in *B. gracilis* was related to climate. Genomic sequencing indicated the most pronounced population structure at the regional scale, but sites were also distinct at a finer spatial distances, indicating that gene flow and/or dispersal may be limited within this key species. Within a common environment, we found phenotypic evidence for genetic divergence across traits and plasticity for individual plants, especially for New Mexico sites. Sites also differed in trait variance, indicating different adaptive potential. When comparing regionally and locally distributed sites, both differed according to similar traits, chiefly aboveground and total individual biomass. Finally, we found that traits and plasticity were significantly linked to both temperature and precipitation climatic variables, specifically temperature during drier seasons, precipitation seasonality, and median Palmer Hydrological Drought Index (PHDI). Our results indicate a conclusive link between genomes, phenotypic variation, and plasticity in this species and suggest a possible mechanism explaining differential climate responses in *B. gracilis*-dominated grassland ecosystems. Moreover, our comprehensive analysis of intraspecific diversity across spatial scales in this dominant grass will help conservation and management of the shortgrass steppe ecosystem moving forward.

4.2 Introduction

Global climate change poses a substantial threat to conservation and management of ecosystems worldwide due to its novel evolutionary pressures (IPCC, 2018). Dry grasslands and their ecosystem services will be especially affected (Schlaepfer et al., 2017; Sloat et al., 2018) by droughts, heatwaves, and other drivers (Cook et al., 2015, 2004). Plant communities will be reshaped as they face climate change and other stressors, but generally more diverse communities are more resistant and resilient to change (Hughes and Stachowicz, 2004; Yachi and Loreau, 1999). It is therefore critical to reveal diversity throughout natural systems to predict how they will respond to change worldwide.

While much ecological diversity is distributed among species in plant communities, some ecosystems have pronounced inequality among species abundances, producing one or a few dominant species. These dominant species are often responsible for the majority of ecosystem function (Avolio et al., 2019). For example, *Andropogon gerardii* comprises up to 80% of production in tallgrass prairies (Smith and Knapp, 2003) and *Bouteloua gracilis* makes up nearly 90% in shortgrass steppes (Milchunas et al., 1989; Sasaki and Lauenroth, 2011). While species evenness may be low in such communities, these dominant species can harbor intraspecific diversity that buffers ecosystem change and bolsters ecosystem function (Avolio et al., 2013; Hughes and Stachowicz, 2004; Oney et al., 2013). Effects of intraspecific diversity can even exceed those of species diversity (Cook-Patton et al., 2011; Reusch et al., 2005). Thus, an understanding of intraspecific diversity is essential to quantifying ecological patterns (Albert et al., 2012; Fridley and Grime, 2010). By revealing intraspecific diversity in dominant species, ecologists will better understand the mechanisms leading to community and ecosystem-level responses to global change and climate (Avolio et al., 2019).

In order to conclusively link patterns of intraspecific diversity to climate, observations must be made on spatial scales appropriate to the species and driver in question (Anderson et al., 2010). Species differ in the spatial scale at which they respond to climate variation (Urban et al., 2016), which can make approaching diversity in a non-model species challenging. Because plants will

have limited ability to track climate change through migration (Jump and Peñuelas, 2005; Pearson, 2006), local scales of diversity over which evolution can occur must be examined (Füssel and Klein, 2006). However, continental-scale data will be essential for predicting other features, such as range shifts (Parmesan 2006) and population structure (Manel et al., 2003), indicating that a multi-scale approach might be most appropriate. Alongside different spatial scales, within population variation can reveal the capacity of species to adapt to changing conditions (Hoffmann and Sgrò, 2011; Nicotra et al., 2015) and how such intraspecific variation relates to the local community (Bolnick et al., 2011; Siefert et al., 2015). However, moving beyond single populations to describe among population variation reveals how selection pressures have shaped the species under different environmental conditions (Vergeer and Kunin, 2011). Integrating intraspecific analysis across regional and local scales can provide insight into community structuring (Violle et al., 2012), and thus is key to indicating how a dominant species and its associated communities persist across a wide range of conditions.

Despite the importance of intraspecific diversity, many studies focus solely on genetic diversity, without incorporating functional diversity and plasticity. While measures of neutral genome diversity elucidate evolutionary history and information on population structure, knowledge of phenotype is required to link such diversity to selective pressures and community-level effects (Hughes et al., 2008; McGill et al., 2006). Plasticity must also be considered, as it affects species adaptation to environmental change (Valladares et al., 2014) and can even lead to persistence in changing environments (Ghalambor et al., 2007; Vázquez et al., 2017). Moving forward, synthesizing genomic and functional diversity will be critical to understanding dominant species intraspecific diversity within broader plant communities and applying such knowledge to conservation.

In this study, we quantified intraspecific diversity of the foundational grass species of the Central US shortgrass steppe, *B. gracilis*, in order reveal key dimensions of biodiversity as well as the capacity of *B. gracilis* to adapt and persist under future global change scenarios. We used a novel approach, incorporating within and among site genomic and functional variation across two spatial scales. We examined seven sites along a regional (New Mexico to South Dakota) climate

gradient and ten local (Boulder County, Colorado) sites with climatic variation. At each scale, we assessed diversity by quantifying single nucleotide polymorphisms (SNPs) across the genome and by measuring functional trait means and variance in a common environment. We also assessed plasticity in these traits across two moisture conditions. We addressed the following hypotheses: (1) We expected that sites would be genetically more distinct along the regional versus the local scale. If population structure was present, we would observe genomic variation among sites, as well as differences in genotype richness, evenness, and allelic heterozygosity across sites. (2) If population structure was present in *B. gracilis*, then we also expected phenotypic differences among sites, with regionally distributed sites showing greater distinction. Similarly, if plasticity contributed to local adaptation, then plasticity means and variances would differ among sites. If genetic variation contributed strongly to phenotype diversity in *B. gracilis*, then we would observe correlations between metrics of within-site diversity (such as genotype richness) and phenotypic variance. (3) Finally, if *B. gracilis* is locally adapted with respect to climate, then we would observe trait-climate or plasticity-climate correlations. Specifically, we expected that *B. gracilis*, a drought tolerant species found in water-limited ecosystems, would have functional diversity associated with precipitation and drought indices. Taken together, tests of these hypotheses will improve our understanding of this important species' population structure and phenotypic adaptation to climate. As a key forage species in dry rangelands, this research also elucidates extant resources for managers of this ecologically and economically important grass species.

4.3 Methods

4.3.1 Species and site descriptions

Bouteloua gracilis is the dominant grass species of the shortgrass steppe ecosystem, spanning much of the western Great Plains (Lauenroth and Burke, 2008). A C_4, perennial grass, this species can be long-lived (Fair et al., 1999) and can also occur in other prairies and shrublands throughout the United States, Canada, and Mexico. *Bouteloua gracilis* can grow as a bunch grass or can grow in dense, turf-forming mats via tillering (Wynia, 2007). As the dominant species, *B. gracilis* plays a substantial role in community structure (Milchunas et al., 1990) and stability (Sasaki

and Lauenroth, 2011). With additional economic value, *B. gracilis* is also grazing tolerant and provides forage for livestock and native herbivores (Lauenroth and Burke, 2008). Populations of *B. gracilis* from the Colorado Plateau (Butterfield and Wood, 2015; Tso and Allan, 2018) and Manitoba, Canada (Phan and Smith, 2000; Phan et al., 2003) are known to have genetic variation. Foundational texts described *B. gracilis* as locally adapted to climate (Dayton et al., 1937; Hughes et al., 1952; Stefferud, 1948), yet this assumption has rarely been tested (but see Butterfield and Wood (2015)).

Bouteloua gracilis productivity often responds strongly to climate, but results are mixed. Experimental exclusion of 25% and 50% of precipitation in the shortgrass steppe led to a 40% reduction in *B. gracilis* cover over 10 years (Evans et al., 2011). However a shorter term 50% rainfall exclusion produced greater *B. gracilis* cover (Byrne et al., 2017). *Bouteloua gracilis* also increased production under fewer, larger (more variable) rainfall events (Heisler-White et al., 2008, 2009). In wetter grasslands like mixed grass prairie, *B. gracilis* has been shown to increase in abundance under drought (Knapp et al., 2015a). Conversely *B. gracilis*-dominated grasslands under more arid conditions in New Mexico tend to be most sensitive to changes in precipitation (Knapp et al., 2015a). Such equivocal responses might indicate intraspecific variation for coping with different water availability conditions.

Fifteen sites were selected for *B. gracilis* collection based on variable climate (Table 4.1). These included five sites along a regional north-south gradient in the western Great Plains (large spatial scale) and ten sites along a local elevational gradient within Boulder County in northern Colorado (small spatial scale). These sites represent variation in temperature, precipitation, and aridity that have likely driven *B. gracilis* evolution throughout its range (Table 4.1). Two additional sites, Cedar Point and Konza, were also selected for genomic analysis only due to the sites' status as intact prairies (Table 4.1).

4.3.2 Quantifying genomic diversity

In order to determine diversity across the genome of *B. gracilis*, we quantified neutral single nucleotide polymorphisms using the 2b-RAD method (Wang et al., 2012) on dried leaf tissue.

Table 4.1: Summary of sites and climate characteristics. MAT: mean annual temperature; MDR: mean diurnal range; TS: temperature seasonality; MaxT: maximum temperature of the warmest month; MinT: minimum temperature of the coldest month; TD: mean temperature of the driest quarter; MAP: mean annual precipitation; PS: precipitation seasonality; AI: aridity index; EDM: extreme drought months; mPHDI: median PHDI.

Site	County	Coordinates	MAT (C)	MDR (C)	TS	MaxT (C)
		Regional				
Sevilleta	Socorro, NM	34.3415, -106.622	12.8	12.8	816.2	31.8
Cibola	Torrance, NM	34.4919, -106.417	11.4	11.4	779.5	29.5
Comanche	Otero, CO	37.6556, -103.669	12.0	12.0	934.0	33.5
SGS	Weld, CO	40.8358, -104.763	8.9	8.9	877.0	29.2
Buffalo Gap	Pennington, SD	43.8920, -102.063	8.5	8.5	1081.4	30.8
Cedar Point	Keith, NE	41.2086, -101.646	9.8	9.8	1003.7	30.6
Konza	Riley, KS	39.0754, -96.603	12.1	12.1	1031.1	31.6
		Local				
Andrus	Boulder, CO	40.0462, -105.207	10.2	10.2	857.7	30.2
Rock Creek	Broomfield, CO	39.9360, -105.108	10.3	10.3	855.5	30.0
Steele	Boulder, CO	40.1497, -105.231	9.8	9.8	857.0	29.9
Rabbit Mountain	Boulder, CO	40.2484, -105.217	9.4	9.4	846.2	29.0
Beech Trail	Boulder, CO	40.0993, -105.274	9.8	9.8	842.5	29.4
Davidson Mesa	Boulder, CO	39.9643, -105.206	9.9	9.9	831.7	29.3
Wonderland	Boulder, CO	40.0595, -105.295	9.2	9.2	815.0	28.1
Heil Valley	Boulder, CO	40.1536, -105.298	8.4	8.4	801.1	26.6
Kelsall	Boulder, CO	39.9167, -105.207	9.6	9.6	827.1	29.0
Walker Ranch	Boulder, CO	39.9502, -105.337	6.6	6.6	758.2	24.2

Table 4.1: Summary of sites and climate characteristics (continued).

Site	MinT (C)	TD (C)	MAP (mm)	PS (CoV)	AI	EDM	mPHDI
			Regional				
Sevilleta	-7.5	8.0	277	67.8	0.19	90	-0.46
Cibola	-7.7	3.7	336	62.1	0.24	90	-0.46
Comanche	-9.0	0.7	323	58.7	0.23	63	0.87
SGS	-10.9	-1.3	369	58.9	0.33	88	1.19
Buffalo Gap	-13.8	-4.7	422	65.9	0.37	49	1.46
Cedar Point	-11.7	-2.4	446	62.4	0.39	80	1.04
Konza	-9.3	-1.1	891	51.5	0.76	62	0.84
			Local				
Andrus	-9.8	0.1	454	43.9	0.34	88	1.19
Rock Creek	-9.3	0.4	455	44.4	0.35	88	1.19
Steele	-10.3	-0.3	449	44.7	0.35	88	1.19
Rabbit Mountain	-9.9	-0.5	436	45.7	0.35	88	1.19
Beech Trail	-9.8	0.0	460	44.4	0.36	88	1.19
Davidson Mesa	-9.3	0.2	480	43.5	0.38	88	1.19
Wonderland	-9.3	-0.1	464	42.1	0.38	88	1.19
Heil Valley	-9.7	-0.8	444	41.8	0.38	88	1.19
Kelsall	-9.5	0.1	482	43.2	0.38	88	1.19
Walker Ranch	-11.0	-2.0	455	39.1	0.43	88	1.19

Briefly, this technique uses IIB restriction enzymes that extract small genomic fragments of equal bp length, rather than producing long fragments between enzyme restriction sites. We chose this technique because of its cost effectiveness and ability to produce greater coverage over *B. gracilis*' large genome (estimated 36 billion bp, Bennett and Leitch (1997)). Because this technique has never previously been used on *B. gracilis*, we also performed an extensive sequencing validation using 12 genotypes collected from the SGS site with several clonal tillers each. For this sequencing technique to be valid, clones would need to cluster together with genotypes emerging as separate branches (Figure III.1). Our final data consisted of 283 individuals from 17 sites.

To prepare libraries for Illumina sequencing, we made several modifications to the original method, including restriction fragment reduction for large genomes using custom Illumina adapters (Section III.1). Following sequencing, we processed the genomic fragment data to produce SNPs. We utilized scripts generated by E. Meyer for SNP processing (http://eli-meyer.github.io/2bRAD_utilities/#top). Briefly, we clustered reads into distinct loci based on maximum likelihood similarity. We then called genotypes based on frequencies of alleles; major allele frequency >0.995 was considered homozygous and <0.85 was considered heterozygous, with intermediate values considered ambiguous. After considering only polymorphic loci, we removed any samples with >85% missing data and narrowed our selection of SNPs to only one per 36bp tag to maximize locus independence, providing a final dataset of 9,469 SNPs.

4.3.3 Common environment experiment

To quantify within-site diversity in a common environment, we collected crown tissue from individual clumps of *B. gracilis* from each site in June 2016 for use in trait and genomic analysis. Within each site, seventeen individuals were collected >10m apart to avoid collecting clones (Butterfield and Wood, 2015). Individuals were placed in plastic bags and hydrated with approximately 100mL of water to avoid desiccation. Before treatments began, individuals were transplanted into homogenized 50% Green's Grade Fritted Clay and Sun Pro potting mix inside 2.65L "cone-tainer" style pots (Stuewe & Sons, Tangent, OR). Individuals were clipped to 1cm to equalize early season growth across all samples, with leaf tissue stored on silica prior to genomic analysis. Pots were

maintained at water holding capacity and covered with cotton fabric for two weeks to facilitate successful rooting. See (Bushey et al., in review) for more details on plant collection prior to treatment.

We performed both a water-limited and a water-abundant treatment in our common environment to test for plasticity to water availability. Because individuals consist of many clonal tillers that separate easily, we divided individuals equally into the two distinct water availability treatments, allowing us to balance any genetic differences between treatments. After an acclimation period (20 days), we allowed pots in the water-limited treatment to dry down to a 10% target volumetric water content (VWC) while water-abundant pots were held at water-holding capacity. Water was supplied every two days to reach the treatment target; water addition volumes were determined using a pot mass by VWC regression (Bushey et al., in review) and were confirmed using a Campbell Scientific HydroSense II Water Content Sensor with 20 cm probes (Figure III.2). These treatments were maintained for 14 weeks for n=17 individuals per site (17 x 2 treatments x 15 sites = 510 total).

4.3.4 Phenotypic traits

During the 14-week treatment period, we repeatedly collected both height and flowering data. Height was measured from the soil surface to the nearest mm (with leaves stretched) approximately every five days. Because *B. gracilis* height from each site followed a logistic curve, we retained the maximum height per individual for statistical analysis. Flowers were trimmed and collected from flowering stalks as they emerged throughout the experiment because they rapidly desiccate. Flowers were dried at 55°C for three days after which we measured weight and length of each flower. We retained total flowering biomass, total flower count, average flower biomass, and average flower length per individual throughout the experiment.

Productivity of *B. gracilis* is central to its role as a dominant species in xeric grasslands. Following the 14-week water availability treatment, all individuals were rewatered to pot capacity and then allowed to dry down completely over approximately 3 weeks. We then harvested individual plant biomass and divided tissue into aboveground, flower, rhizome, and root categories. Soil was

rinsed off of rhizome and root tissue using a mesh screen and low-pressure water nozzle. Biomass was allowed to dry at 55°C for three days prior to weighing. Because some individuals were retained for other analyses (e.g., metabolism or transpiration rates) biomass sample size was lower for some sites.

In order to determine the effects of site and treatment on plant physiology, we quantified both predawn and midday leaf water potential (LWP). Predawn leaf samples were cut at approximately 4:00am, acclimated in a plastic bag at full humidity, and measured using a Scholander-type pressure chamber (PMS Instruments). Predawn samples were followed by a paired midday sampling on the same plant between 12:00pm and 2:00pm on a sunny day. Paired predawn and midday samples were collected from both treatments to account for any treatment variation. Using these values, we calculated hydroscape area for each site according to Meinzer et al. (2016). Briefly, hydroscape area corresponds to the graphical space where the predawn and midday LWP relationship deviates from the 1:1 line and represents the conditions over which plant stomata can effectively control water status. Increasing hydroscape area thus indicates greater anisohydry (drought tolerance) on the anisohydry to isohydry (drought avoidance) spectrum Meinzer et al. (2016). In order to obtain more extreme values of LWP needed to quantify the hydroscape, we also sampled leaves during the dry down prior to biomass sampling.

4.3.5 Climate variables

To test for climate adaptation, we used long-term data related to temperature and water status of the sites where tissue samples were collected. Climate data spanning 1970-2000 was collected from WorldClim (http://worldclim.org/version2) (Fick and Hijmans, 2017). We limited WorldClim data to several relevant variables to avoid model overfitting: annual mean temperature (C), mean diurnal range (mean of monthly maximum temperature - minimum temperature, C), temperature seasonality (temperature standard deviation * 100), maximum temperature of the warmest month (C), minimum temperature of the coldest month (C), mean temperature of the driest quarter (C), annual precipitation (mm), and precipitation seasonality (coefficient of variation). We also used an aridity index derived from 1950-2000 WorldClim data (http://csi.cgiar.org/aridity/) (Trabucco

and Zomer, 2010). Lastly, we incorporated a longer-term dataset using the Palmer Hydrological Drought Index (PHDI) from 1895-2015 (https://www1.ncdc.noaa.gov/pub/data/cirs/climdiv/). From this dataset, we calculated the number of months a site experienced extreme drought during this period (extreme drought months) and the median PHDI.

4.3.6 Statistical analysis

We quantified allelic differences among sites by performing discriminant analysis of principal components (DAPC, Jombart et al., 2010), a powerful method for discerning diversity among pre-determined groups (sites). This required stratified cross-validation of DAPC using increasing numbers of PCs to avoid overfitting, leaving us with 60 PCs and 66% of the variance retained along the regional gradient and 60% of the variance retained along the local gradient (discriminant functions). Using these discriminant functions, we calculated posterior proportions of successful individual reassignment to original sites, where high probabilities indicate distinct sites and low probabilities indicate admixture. In order to determine hierarchical genetic relationships among sites, we generated a UPGMA dendrogram with bootstrap support using the R package adegenet (Jombart, 2008) utilizing Nei's distance (Nei, 1978) and 10,000 bootstrap iterations.

Because genomic data can be susceptible to false signals emerging from large datasets, we used several metrics to robustly quantify the *B. gracilis* genome. To determine genetic diversity among sites, we calculated Nei's Expected Heterozygosity (H_{exp}, Nei, 1978) and the multilocus genotype richness at each site using the R package poppr with Multilocus Style Permutation (Kamvar et al., 2014). Site genotype evenness was calculated according to Smith and Wilson (1996) using the codyn package in R (Hallett et al., 2016). We also calculated pairwise allelic distances among samples at each locus to quantify within-site genotype distance. Genotype distances were compared between sites using student's t-test with Bonferroni adjustment.

We used Bayesian hierarchical linear models to determine the effect of pot-level water availability and site on traits and trait plasticity of individuals. We used the following variable intercept α and slope β linear model:

$$\hat{y}_i = \alpha_{i[j]} + \beta_{i[j]} x_i + \epsilon_i$$

for each individual i within site j, with pot-level VWC x used to determine the trait y response. These models allowed us to better estimate site-level trait data by accounting for individual and species-level variance (Gelman, 2006). We used the 95% credible interval (CI) to determine site level overlap in traits at the average pot water content. The above model also allowed us to estimate site-level variance emerging from the trait posterior distribution. To assess trait plasticity, we used a similar model where y represented the difference between treatments and x was the actual pot-level VWC difference. Traits measured at the site level (e.g., hydroscape) were not input into these models. See Section III.1 for more details on these models. We used linear discriminant analysis (LDA) to describe trait differences holistically. In order to link within site genomic variation to trait variation, we ran linear regressions of genotype richness, evenness, and heterozygosity against trait and plasticity variance for key traits emerging from the LDA using Bonferroni adjustment.

Lastly, we determined which climate variables were important for explaining intraspecific variation in *B. gracilis*. Due to covariance among phenotypic traits, we used a partial Pearson correlation matrix for each climate variable, starting with aboveground biomass and progressively analyzing all phenotypic traits. We performed this analysis on genome characteristics (richness, evenness, and heterozygosity) as well as trait means and trait plasticity. Correlation p-values were adjusted using the Benjamini-Hochberg false discovery rate method.

4.4 Results

4.4.1 Genomic diversity in *B. gracilis*

Using DAPC, we determined if sites clustered distinctly across the genome. Regionally, we found that the New Mexico sites, Cibola and Sevilleta, were most distinct across discriminant functions (Figure 4.1a). To validate these clusters, we calculated the posterior probability of cluster reassignment of each individual, where high probabilities indicate distinct site identity and low probabilities indicate an admixed individual. Sevilleta was the most distinct (1.00) and contained

no admixed individuals (Table 4.2, Figure 4.1b,c). While still largely distinct, all other sites along the regional gradient contained at least one ambiguous individual (Figure 4.1b,c). Konza in eastern Kansas in particular had several individuals resembling the Cedar Point site in western Nebraska. *Bouteloua gracilis* also showed evidence of population structure at the local level, although differences were less extreme. Walker Ranch emerged as a distinct cluster across discriminant functions (Figure 4.1d) and had a high reassignment probability (0.94, Table 4.2, Figure 4.1e,f). Rock Creek, Steele, Davidson Mesa, and Heil Valley were also distinct, while Kelsall and Wonderland were more admixed (Table 4.2, Figure 4.1e,f). These differences were retained when regional and local gradients were combined, with Sevilleta and Cibola emerging as the most different sites overall (Figure III.3). When we considered regionally distributed sites, the hierarchical relationship demonstrated strong bootstrap support for New Mexico sites Sevilleta and Cibola as the basal lineages, followed by Comanche, then more northern sites (Figure 4.2, Figure III.4).

We also quantified within-site diversity at the genomic level. Using sequence data, we determined SGS to have the greatest richness and evenness at the regional scale compared to other sites (Table 4.2). The most northern (Buffalo Gap) and southern (Sevilleta) sites had the lowest richness and evenness, indicating dominance by one or a few genotypes (Table 4.2, Table III.1). Heterozygosity was highest for Sevilleta and Cibola (Table 4.2). Local genotype richness tended to be higher than regional, except at Walker Ranch, which consisted of only three distinct genotypes (Table 4.2). Most sites had high evenness except for Walker Ranch, which was dominated by a single genotype (Table 4.2). The Walker Ranch genotype not common at other sites and was only detected at Cibola and Rabbit Mountain (Table III.1). Local sites shared similar heterozygosity (Table 4.2). Pairwise distances within site closely paralleled genotype richness, with much lower pairwise distance observed for Walker Ranch (multiple corrected p-values: <0.0001, Figure III.5).

4.4.2 Phenotype and plasticity diversity

We observed widespread trait diversity and plasticity within *B. gracilis*. Specifically, we analyzed 12 traits and trait plasticity among *B. gracilis* sites in a common environment to further determine if sites were genetically distinct. When we considered all traits and sites, we found

Table 4.2: Genomic characteristics varied among sites. N: sample size of sequenced individuals (not all sites achieved a goal sample of 17 due to thresholds for low coverage; one randomly selected individual of each clone was included in SGS); MLG: number of multi-locus genotypes detected (richness); Standardized MLG: number of multi-locus genotypes detected correcting for sample size; E_{var}: genotype evenness; H_{exp}: Nei's expected heterozygosity; Reassignment probability: average probability of individuals being reassigned to their home site.

Site	**N**	**MLG**	**Standardized MLG**	E_{var}	H_{exp}	**Reassignment probability**
			Regional			
Sevilleta	17	6	5.05	0.53	0.023	1.00
Cibola	17	10	8.11	0.76	0.025	0.94
Comanche	17	7	6.01	0.64	0.015	0.88
SGS	28	25	12.08	0.95	0.017	0.89
Buffalo Gap	16	6	5.44	0.63	0.013	0.94
Cedar Point	16	8	7.36	0.83	0.013	1.00
Konza	16	8	7.17	0.76	0.015	0.75
			Local			
Andrus	17	14	11.13	0.93	0.015	0.76
Rock Creek	15	15	13.00	1.0	0.017	0.93
Steele	15	11	9.91	0.90	0.013	0.93
Rabbit Mountain	15	12	10.77	0.94	0.013	0.87
Beech Trail	17	12	9.98	0.90	0.013	1.00
Davidson Mesa	17	16	12.43	0.98	0.016	0.94
Wonderland	16	9	7.99	0.81	0.012	0.63
Heil Valley	15	13	11.51	0.96	0.014	0.93
Kelsall	13	11	11.00	0.93	0.016	0.69
Walker Ranch	16	3	2.62	0.26	0.012	0.94

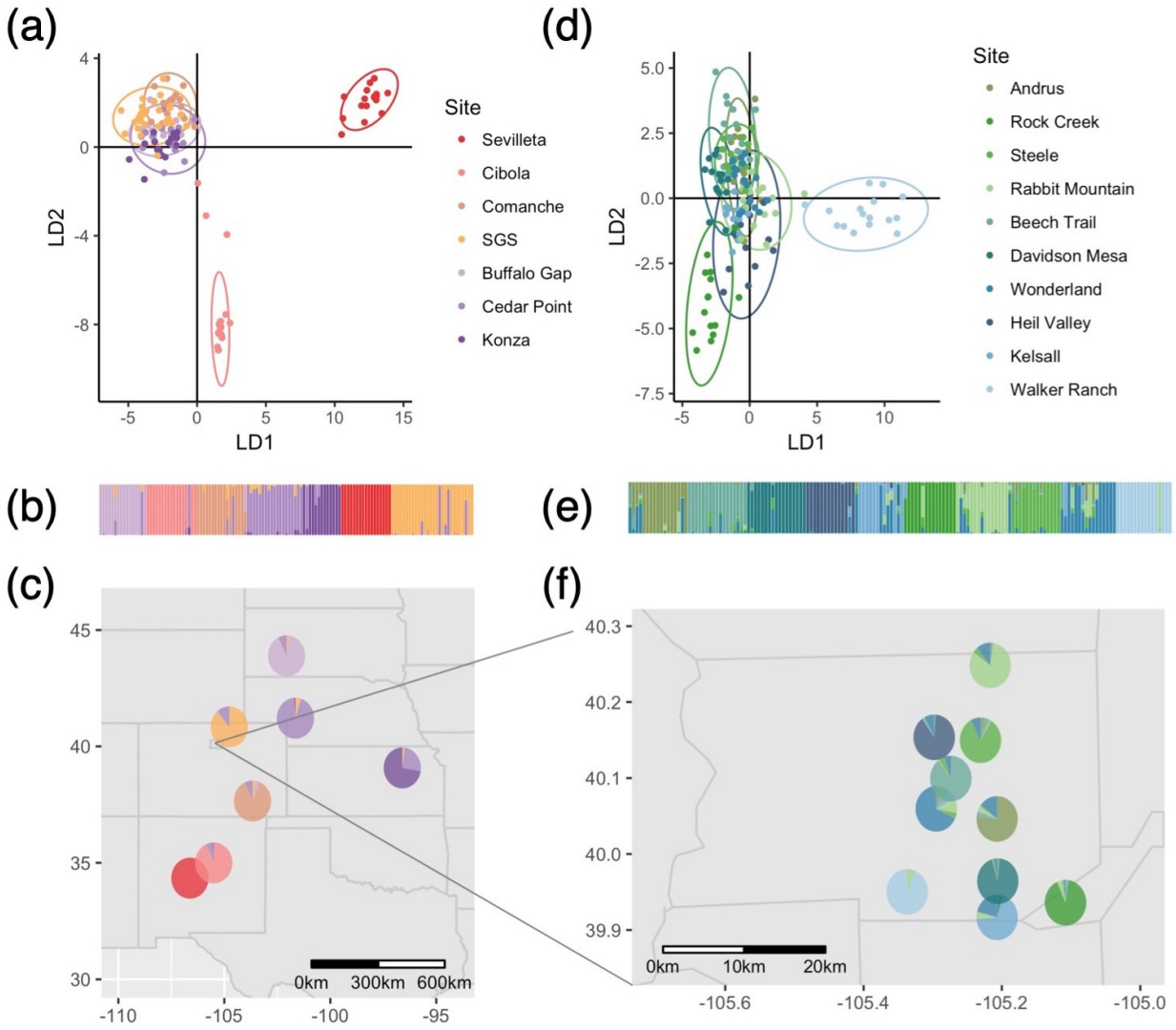

Figure 4.1: *Bouteloua gracilis* sites clustered distinctly in space based on the genome. At the regional scale, (a), and local scale, (d) Discriminant functions LD1 and LD2 from DAPC accounted for 60% of the variance regionally (a), showing distinct New Mexico sites and 66% of the variance locally (d), showing distinct sites within Boulder County. Sites with greater than 0s.8 reassignment probability are encircled. (b,e) "Structure" lines reveal the posterior probability of site assignment of each individual, where solid lines indicate clear site assignment and mixed lines indicate admixed individuals. Posterior probabilities are plotted by location across the regional (c) and local (f) gradient.

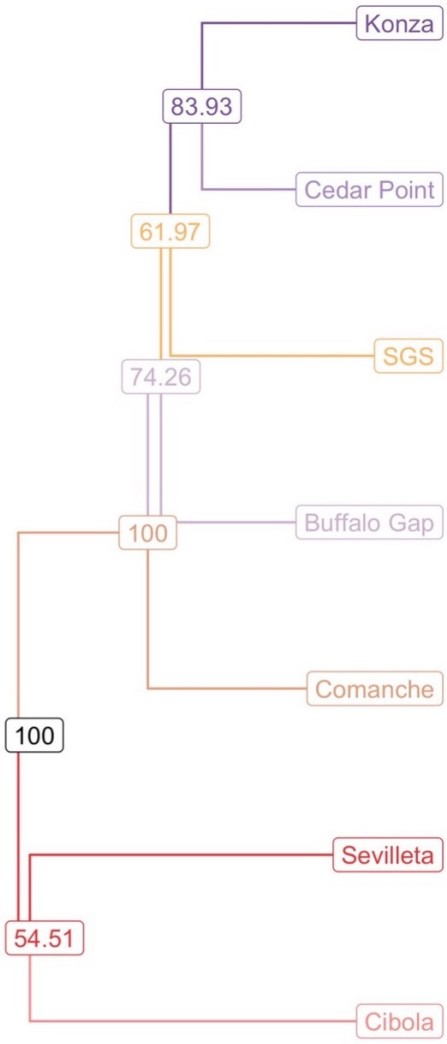

Figure 4.2: Hierarchical relationship among regionally distributed *B. gracilis* sites, where numbers indicate bootstrapped support for each node.

non-overlapping trait distributions indicating all traits had a genetic component and that there is evidence for site evolution within *B. gracilis* (Figure III.6). Regionally, sites diverged strongly across biomass traits, namely total and aboveground biomass (Figure 4.3a). Sevilleta and Cibola had greater total biomass, aboveground biomass, belowground biomass (Figure 4.3b), and maximum height compared to other sites. These sites also had among the lowest root:shoot ratios, lowest predawn and midday LWP, highest flowering mass, and highest belowground biomass (Figure III.6). Comanche, a site in southern Colorado close to the New Mexico border, tended to be ranked just below the New Mexico sites for aboveground and total biomass and maximum height, but had the greatest overall belowground biomass (Figure III.6). Comanche also had greater water potentials (i.e., closer to zero) compared to Sevilleta and Cibola. Northern sites Buffalo Gap (South Dakota) and SGS (northern Colorado) had lower biomass compared to other sites. Buffalo Gap was the shortest, had the lowest average flower mass, and had significantly shorter length flowers (Figure III.6).

Traits distinguishing sites at the local scale in Boulder County were similar to those differentiating sites at the regional level (total and aboveground biomass), but local sites were more similar compared to regional sites (Figure 4.4a). Despite being geographically close, total biomass distributions in a common environment differed among Boulder sites (Figure 4.4b). This pattern was driven largely by one Boulder site, Andrus, which had the lowest aboveground, belowground, rhizome, flowering, and total biomass as well as lowest flower count. A drier site, Rock Creek, had the greatest biomass in most categories and the lowest water potentials (Figure III.6). The wetter site Wonderland was the tallest and had the heaviest flowers, while the coldest, wettest site Walker Ranch was shortest and had the greatest number of flowers. Only root:shoot ratio was similar among all Boulder sites (Figure III.6).

Nearly every trait (except average flower length) was plastic when we paired genotypes across our water availability treatment, but plasticity depended on the site (Figure III.7). Trait plasticity differences among sites were generally less discernable compared to trait means (Figure III.7). However, sites showed differences in biomass and height plasticity at the regional scale (Fig-

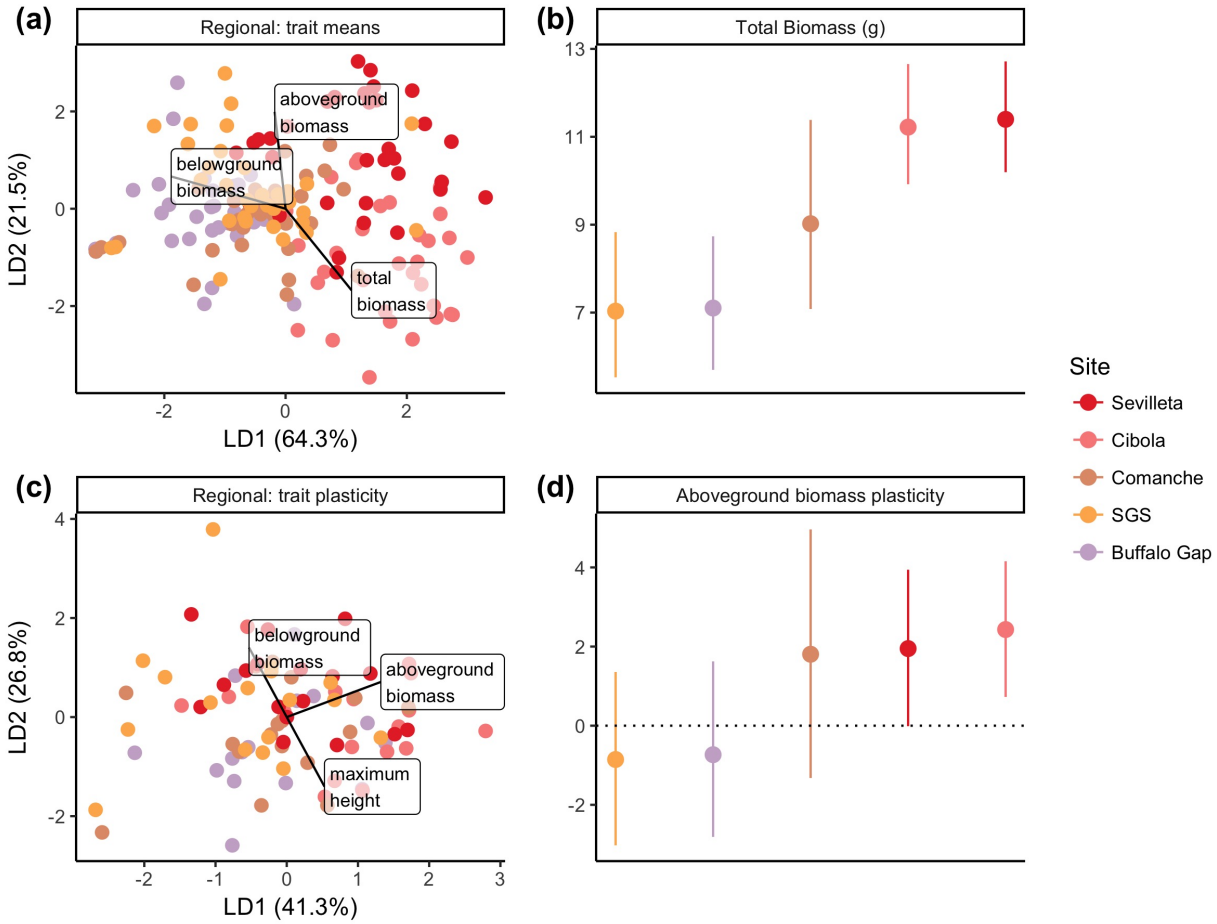

Figure 4.3: At the regional scale, (a) *B. gracilis* traits were differentiated by biomass metrics, where (b) New Mexico sites Sevilleta and Cibola tended to have greater total biomass (sites arranged from smallest to largest mean values). (c) Regional site plasticity differentiated on belowground and aboveground biomass as well as maximum height; (d) drier southern sites tended to be more plastic (sites arranged from smallest to largest mean values). Circular points represent the mean and error bars represent the 95% CI. All intervals represent the trait means at average experimental pot water content.

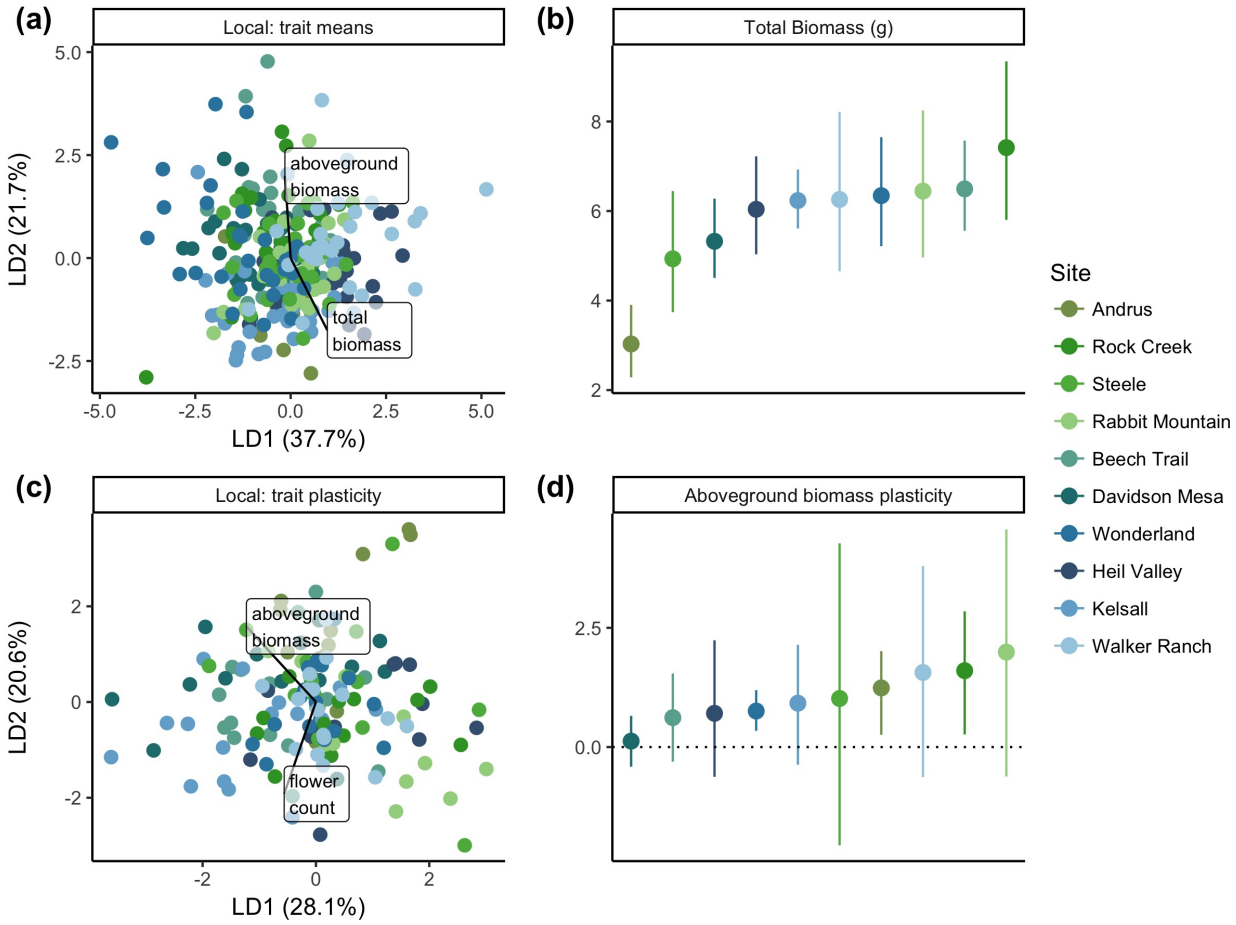

Figure 4.4: At the local scale, (a) *B. gracilis* traits were differentiated by aboveground and total biomass metrics, where (b) Andrus had lower total biomass compared to other sites (sites arranged from smallest to largest mean values). (c) Local site plasticity differentiated on aboveground biomass and flower count; (d) some sites exhibited non-zero plasticity, but all populations overlapped somewhat in plasticity (sites arranged from smallest to largest mean values). Circular points represent the mean and error bars represent the 95% CI. All intervals represent the trait means at average experimental pot water content.

ure 4.3c). Sevilleta and Cibola had significant (non-zero) plasticity leading to greater aboveground/total biomass (Figure 4.3d) and height with greater water availability. Boulder sites also demonstrated some variation in trait plasticity, particularly for aboveground biomass and flower count (Figure 4.4c) as well as total biomass (Figure III.7). Drier Boulder sites like Rock Creek and Rabbit Mountain tended to have more plasticity in aboveground biomass, although plasticity intervals overlapped for all sites (Figure 4.4d); Rock Creek also had the greatest flower count plasticity (Figure III.7). Total biomass was plastic across all locally distributed sites except Andrus.

4.4.3 Phenotype and plasticity variance

In order to assess within-site variation, we modeled trait variance at each site, where greater variance might implicate greater capacity to adapt to changing conditions. When we considered all sites, we found unequal variance for each trait except average flower length (Figure III.8). Comanche tended to have among the greatest variation in biomass traits (Figure III.8). Sevilleta had among the greatest variation in predawn and midday LWP. At the local scale, we detected significantly different variances among sites for most traits (Figure III.8). Rock Creek and Walker Ranch had among the greatest trait variance (Figure III.8). Andrus and Kelsall had the smallest variance in aboveground, belowground, rhizome, and total biomass (Figure III.8). When we assessed all sites, genomic diversity (richness, evenness, heterozygosity) was not correlated with aboveground or total biomass variance (adjusted $p>0.5$, Table III.2).

All traits except predawn LWP exhibited different plasticity variance across sites, indicating different capacities for plasticity to evolve (Figure III.9). Similar to trait variance, Comanche had among the largest variance for plasticity of aboveground biomass, belowground biomass, and maximum height (Figure III.9). New Mexico sites had among the lowest plasticity variance in height and individual flower mass. Northern site Buffalo Gap had among the greatest plasticity variance for rhizome biomass, total biomass, and root:shoot ratio (Figure III.9). Among all sites, Genomic diversity (richness, evenness, heterozygosity) was not correlated with variation in aboveground or total biomass plasticity (adjusted $p>0.5$, Table III.2).

4.4.4 Local adaptation to climate

Using partial correlation matrices, we found that the *B. gracilis* genome, mean trait values, and mean trait plasticity were correlated with climate variables, suggesting local adaptation. While genotype richness was not related to climate, genotype evenness increased with temperature seasonality (Table 4.3). Heterozygosity increased with increasing mean annual temperature, minimum temperature, temperature of the driest quarter, and precipitation seasonality, but decreased with increasing median PDHI (Table 4.3). However, these patterns were largely driven by low evenness at Walker Ranch and high heterozygosity at Sevilleta and Cibola.

Mean trait values were correlated primarily with precipitation-related climate variables. Aboveground biomass increased with lower annual precipitation (Table 4.3) and with greater temperatures in the driest quarter, lower aridity index, and lower median PDHI (Table 4.3). Although many traits were correlated, additional variation in rhizome biomass was explained by median PDHI, where rhizome biomass increased for wetter sites (Table 4.3). Likewise, sites with taller individuals tended to be hotter and drier while accounting for biomass variation (Table 4.3). Hotter and drier sites also tended to have lower midday leaf water potentials.

Bouteloua gracilis plasticity was also locally adapted to climate. Like trait means, greater capacity for water availability plasticity was sometimes linked to temperature. Greater plasticity in aboveground biomass was found in sites with greater minimum temperatures, while greater plasticity in rhizome biomass came from sites with a larger diurnal range in temperature (Table 4.3). Total biomass plasticity was greater in drier sites with greater precipitation seasonality (Table 4.3). Flowers from more arid or seasonal sites also tended to have greater plasticity in length and count when accounting for variation in other traits (Table 4.3).

4.5 Discussion

4.5.1 Genomic population structure is present across scales

We used trait, trait plasticity, and genomic analysis to quantify intraspecific diversity across a foundational species, *Bouteloua gracilis*. We found evidence supporting our first hypothesis that *B.*

Table 4.3: *Bouteloua gracilis* genome, traits, and trait plasticity are correlated with climate variables. MAT: mean annual temperature; MDR: mean diurnal range; TS: temperature seasonality; MaxT: maximum temperature of the warmest month; MinT: minimum temperature of the coldest month; TD: mean temperature of the driest quarter; MAP: mean annual precipitation; PS: precipitation seasonality; AI: aridity index; EDM: extreme drought months; mPHDI: median PHDI. Within cell, above numbers indicate the Pearson partial correlation coefficient. Below numbers indicate the corrected p-values. Significant (<0.05) p-values are bolded.

	MAT	**MDR**	**TS**	**MaxT**	**MinT**	**TD**	**MAP**	**PS**	**AI**	**EDM**	**mPDHI**
Genomic characteristics											
Standardized MLG	-0.033	0.218	-0.216	-0.03	0.154	-0.049	0.054	-0.412	-0.029	0.441	0.322
	0.9	0.4	0.508	0.908	0.555	0.853	0.836	0.15	0.913	0.129	0.311
Evenness	0.34	-0.32	0.627	0.517	-0.166	-0.19	0.264	0.351	0.222	-0.442	0.034
	0.296	0.4	**0.028**	0.092	0.555	0.722	0.484	0.183	0.612	0.129	0.9
Heterozygosity	0.744	0.236	-0.186	0.494	0.625	0.772	-0.318	0.704	-0.405	0.179	-0.94
	0.004	0.4	0.508	0.092	**0.038**	**0.002**	0.484	**0.01**	0.404	0.524	**<0.001**
Mean traits											
Aboveground biomass	0.572	0.202	-0.186	0.262	0.534	0.731	-0.823	0.682	-0.771	0.027	-0.9
	0.136	0.863	0.765	0.499	0.261	**0.026**	**0.002**	0.066	**0.01**	0.991	**<0.001**
Belowground biomass	0.224	0.258	0.558	0.509	-0.238	-0.373	-0.426	0.393	-0.337	-0.641	0.398
	0.641	0.863	0.206	0.399	0.672	0.351	0.417	0.357	0.387	0.083	0.28
Rhizome biomass	-0.183	-0.244	0.192	-0.039	-0.176	-0.261	0.362	-0.06	0.394	-0.067	0.705
	0.641	0.863	0.765	0.957	0.745	0.506	0.583	0.959	0.387	0.991	**0.033**
Total biomass	0.218	0.036	0.435	0.36	-0.14	-0.142	-0.163	0.596	-0.244	-0.343	-0.421
	0.641	0.972	0.41	0.406	0.745	0.715	0.854	0.177	0.6	0.714	0.28
Root:shoot	0.647	0.604	-0.033	0.57	0.418	0.651	0.026	0.032	-0.436	0.254	-0.675
	0.136	0.318	0.923	0.399	0.436	0.098	0.94	0.959	0.387	0.798	0.067
Max. height	0.681	0.668	-0.331	0.43	0.68	0.799	-0.564	-0.019	-0.765	0.432	-0.782
	0.136	0.318	0.684	0.399	0.261	**0.029**	0.389	0.959	0.065	0.691	**0.033**
Predawn LWP	-0.181	-0.097	0.118	-0.021	-0.156	-0.209	-0.038	0.071	0.188	-0.346	0.73
	0.641	0.972	0.826	0.957	0.745	0.697	0.94	0.959	0.678	0.783	0.067
Midday LWP	-0.66	-0.358	0.189	-0.272	-0.569	-0.856	-0.375	0.318	0.175	-0.287	0.469
	0.21	0.863	0.773	0.608	0.436	**0.029**	0.779	0.72	0.678	0.798	0.319
Lifetime flower mass	-0.698	-0.05	-0.646	-0.653	0.25	-0.456	0.309	-0.403	0.527	0.836	0.091
	0.21	0.972	0.381	0.399	0.745	0.439	0.821	0.688	0.387	0.083	0.847
Flower count	-0.35	-0.019	-0.883	-0.691	0.671	0.822	0.343	-0.653	0.377	0.924	-0.805
	0.641	0.972	0.206	0.399	0.436	0.117	0.821	0.357	0.6	0.083	0.116
Average flower mass	-0.301	-0.571	-0.882	-0.691	0.554	-0.823	0.926	-0.92	0.818	-0.166	-0.263
	0.641	0.863	0.206	0.399	0.618	0.188	0.156	0.173	0.323	0.991	0.725
Average flower length	0.836	0.313	-0.398	0.602	0.826	0.229	-0.242	-0.84	-0.901	0.132	0.75
	0.355	0.972	0.773	0.518	0.436	0.771	0.896	0.357	0.323	0.991	0.319
Hydroscape area	0.942	0.672	0.837	0.965	-0.164	-0.935	0.513	-0.384	-0.699	-0.014	0.911
	0.404	0.863	0.684	0.399	0.895	0.375	0.854	0.959	0.6	0.991	0.319

Table 4.3: *B. gracilis* genome, traits, trait plasticity, and climate (continued).

	MAT	MDR	TS	MaxT	MinT	TD	MAP	PS	AI	EDM	mPDHI
					Trait plasticity						
Aboveground biomass	0.468	0.325	-0.515	0.076	0.698	0.617	-0.317	-0.041	-0.463	0.337	-0.584
	0.339	0.475	0.378	0.862	**0.046**	0.086	0.528	0.965	0.165	0.759	0.133
Belowground biomass	0.307	0.485	0.094	0.327	0.151	0.125	-0.294	0.275	-0.335	-0.134	-0.203
	0.42	0.365	0.947	0.501	0.765	0.782	0.528	0.584	0.331	0.9	0.906
Rhizome biomass	0.445	0.768	-0.051	0.403	0.396	0.26	0.011	-0.113	-0.234	0.249	0.055
	0.339	**0.026**	0.947	0.501	0.362	0.722	0.971	0.855	0.481	0.9	0.906
Total biomass	0.422	0.115	0.142	0.286	0.233	0.494	-0.821	0.776	-0.709	-0.22	-0.719
	0.343	0.807	0.947	0.501	0.765	0.308	**0.013**	**0.02**	0.059	0.9	0.101
Root:shoot	-0.312	-0.298	-0.49	-0.496	0.131	0.069	0.626	-0.557	0.504	0.43	-0.067
	0.42	0.613	0.378	0.482	0.765	0.839	0.203	0.287	0.195	0.759	0.906
Max. height	0.764	0.545	-0.006	0.535	0.609	0.659	-0.212	0.292	-0.583	0.116	-0.5
	0.122	0.365	0.987	0.482	0.362	0.152	0.743	0.619	0.165	0.9	0.423
Predawn LWP	0.571	0.273	-0.249	0.104	0.573	0.785	-0.665	0.227	-0.67	0.191	-0.577
	0.339	0.637	0.947	0.862	0.362	0.086	0.203	0.743	0.165	0.9	0.414
Midday LWP	0.569	0.341	0.647	0.629	-0.546	-0.27	-0.205	0.628	-0.455	-0.459	-0.05
	0.339	0.613	0.378	0.482	0.362	0.722	0.751	0.287	0.331	0.759	0.906
Liftime flower mass	0.333	-0.114	0.657	0.453	-0.598	-0.302	-0.141	0.628	-0.302	-0.616	-0.239
	0.508	0.807	0.378	0.501	0.362	0.722	0.833	0.315	0.51	0.759	0.906
Flower count	-0.491	-0.7	-0.293	-0.445	0.28	-0.562	0.732	-0.952	0.765	0.095	0.498
	0.42	0.365	0.947	0.501	0.765	0.59	0.293	**0.02**	0.165	0.936	0.757
Average flower mass	-0.566	-0.715	-0.311	-0.572	0.057	-0.224	0.379	-0.676	0.609	-0.223	-0.213
	0.42	0.42	0.947	0.501	0.928	0.782	0.743	0.421	0.331	0.9	0.906
Average flower length	0.095	0.215	-0.204	-0.035	0.323	-0.458	-0.696	-0.023	-0.998	0.062	-0.149
	0.905	0.807	0.947	0.965	0.765	0.722	0.528	0.977	**0.025**	0.938	0.906

gracilis would exhibit genomic population structure. Specifically, we found that New Mexico sites emerged as the most genomically distinct. These findings support very recent molecular evidence for two distinct groups of *B. gracilis* in the western USA and Central Plains (Avendaño-González et al., 2019). Avendaño-González et al. (2019) also found that Central Plains *B. gracilis* was less diverse and phylogenetically younger, which is supported by our phylogenetic tree (Figure 4.2, Figure III.4) and heterozygosity measurements (Table 4.2). Our analysis expands on these findings to provide more detailed resolution at the local and site level. As expected, we found less admixture at the regional versus the local scale. However, few sites within Boulder County exhibited admixture with other sites, indicating that sites separated by as few as 6 km could be genetically distinct. Despite some admixture at the local level, our results are consistent with limited gene flow and seed dispersal in *B. gracilis* (Anderson, 2003). Many of the Boulder sites are prairie remnants, so sites should be managed carefully to avoid the negative effects of fragmentation on diversity (Haddad et al., 2015).

Fine scale genomic analysis can reveal differences that may be cryptic from trait analysis alone. At the local level, Walker Ranch emerged as a distinct, but genotypically depauperate site. These findings support anecdotal evidence that this site was revegetated following a wildfire (BASIN, 2000). Although we do not know the origin of seeds used in the Walker Ranch restoration, our results are consistent with seed sources outside of the local area (Figure III.4). Indeed, commercial sources of *B. gracilis* often originate from the southern edge of New Mexico (Robins et al., 2009), Texas (Butterfield and Wood, 2015) or as far away as Minnesota (Mintenko et al., 2002). Our findings suggest that outsourcing seeds may not be appropriate to maintain diversity (Gustafson et al. 2004, but see Winkler et al. 2018).

4.5.2 Genetic differences lead to functional variation among sites

In a common environment, we found that the distributions of traits and plasticity by site could be non-overlapping, indicating genetic divergence of our sites and a genetic component to the traits and plasticity we analyzed. Intraspecific variation can account for nearly a third of the variation among communities (Siefert et al., 2015) which for dominant species, can strongly affect ecosys-

tem function (Breza et al., 2012). In conjunction with previous research on *B. gracilis* diversity (Butterfield and Wood, 2015; Giuliani et al., 2013; Phan and Smith, 2000), our findings suggest that a proportion of functional variation among semi-arid prairies in North America could be due to differences within this dominant species.

In support of our second hypotheses, we found that genomic differences were complemented by functional differences for New Mexico sites. These sites demonstrated greater biomass related trait distributions in a common environment. Interestingly, similar traits distinguished sites at both regional and local scales (primarily aboveground and total biomass, Figure 4.3 and Figure 4.4). As was expected, we observed that intraspecific functional diversity was less pronounced at the local level compared to regional sites. Much of the variation in total biomass at the local scale was driven by reduced biomass in the Andrus site. At the time of sampling, Andrus was heavily invaded by *Bromus tectorum*. Because we directly transplanted clones from field to greenhouse, it is possible that the Andrus individuals were susceptible to negative effects of coexistence with *B. tectorum* (such as fewer rhizome resources). Indeed, *B. tectorum* has been shown to affect evolutionary trajectories in native plant species (Leger and Goergen, 2017). Despite overlapping distributions for some traits, some sites (e.g., Rock Creek) had nearly twice the total biomass of other sites (e.g., Steele), suggesting genomic structure at the local scale could have functional consequences.

Paralleling trait distributions, we observed that New Mexico sites also tended to have greater plasticity. Variation in plasticity lends further support for functional differences distinguishing New Mexico sites from others throughout the Great Plains. Interestingly, we found plasticity tended to differ less among sites than trait means. While this could mean that there are greater costs associated with the evolution of plasticity (Valladares et al., 2007) compared to trait means in *B. gracilis*, we also acknowledge that our paired design, while accounting for genotype, had lower sample size which could ultimately obfuscate plasticity differences. Our treatment also neglects the full range of conditions possible for *B. gracilis*. Despite these caveats, site level plasticity was more similar at the local level compared to the regional level, indicating that drivers of plasticity evolution may take place on a larger spatial scale. Our analyses of functional traits and plasticity

indicate that diversity in *B. gracilis* is present across different spatiotemporal scales and that a single scale approach might not completely reveal adaptive patterns (Richardson et al., 2014).

4.5.3 Sites have different adaptive capacity

As the climate changes, plants will rely largely on within-site variation to adapt to new conditions (Nicotra et al., 2015). To quantify differences in ability of *B. gracilis* sites to adapt, we assessed within-site variance alongside trait means. We found that trait and plasticity variance depended on the trait in question. Few regional patterns were present with the exception of the southern Colorado site Comanche, where plants demonstrated greater trait mean and plasticity variance for biomass traits. Greater variance at Comanche could be linked to its distinct monsoon-like precipitation and high interannual variability (Comrie and Glenn, 1998) and could afford greater adaptive potential. However, the lack of consistent patterns with respect to trait and trait plasticity variance indicate that site level responses to climate change could depend strongly on the traits under selection. For example, as droughts become more extreme and unpredictable in the Midwest, variance in other traits like the ability to desiccate and resprout may be fundamental (Pausas et al., 2016). Nevertheless, differences in variance at the local scale (e.g., Boulder County) suggests that even closely situated sites may evolve in response to stress very differently without management.

We found no relationship with genotype richness, evenness, or heterozygosity and biomass and biomass plasticity variance. Genetic variation is often used as a proxy for adaptive potential, where more genotypes represent broader trait space (e.g., Avolio and Smith, 2013b; Collins et al., 2018). However in practice, molecular and phenotypic variation are often poorly correlated (Reed and Frankham, 2001). Indeed, despite the genotype richness and evenness bottleneck within Walker Ranch, it maintained substantial phenotypic variance in the traits we measured. It is possible that variation has been retained primarily in loci contributing to functional variance in this site and others. Greater heterozygosity in New Mexico sites Sevilleta and Cibola, while not contributing to trait variance, may however allow greater outcrossing heterosis, useful for coping with future droughts (Schnable et al. 2013, but see Prill et al. 2014).

4.5.4 *B. gracilis* is locally adapted to climate

We used Pearson partial correlation coefficients to examine climate predictors against traits and trait plasticity to quantify evidence of local adaptation. Generally, drier and hotter sites tended to have greater biomass and/or height with a greater capacity for plasticity. Similarly, Butterfield and Wood (2015) found that *B. gracilis* leaf traits were related to temperature and precipitation. Intraspecific variation in other dominant species has also been largely driven by climate (Brabec et al., 2017; Gibson et al., 2013; Giuliani et al., 2013; Johnson et al., 2015; Roybal et al., 2018). In contrast to species level analysis (Roybal et al., 2018), we observed greater biomass within drier sites, indicating that different plasticity could play a role in this pattern. Intuitively, trait means (such as average aboveground biomass) tended to be predicted by climate means (mean temperature of the driest quarter or median PDHI) while plasticity was better predicted by precipitation seasonality. Similar findings linking plasticity and climate variation have been documented in other plant species (Scheepens et al., 2018). Genomic heterozygosity was strongly linked to climate, however this pattern was driven largely by New Mexico sites with a longer evolutionary history. Thus, more work is needed (particularly in the Chihuahuan Desert) to determine if the substantial effects of climate on traits subsequently affect the genomes of *B. gracilis*.

While plasticity increased in drier, more variable sites (particularly with biomass), many cooler sites had high plasticity, indicating that drivers of plasticity might vary by site. For example, adaptive plasticity in cooler climates could be driven by freezing (e.g., Nicotra and Davidson, 2010; Sexton et al., 2002) or grazing (Coughenour, 1985). Our results indicate that despite high stress tolerance generally (Levitt, 1972), *B. gracilis* evolution is driven by climate and will likely be susceptible to future climate change, as has already been shown in field experiments (Evans et al., 2011; Knapp et al., 2015a). Although diversity among sites may enhance resistance and resilience to changing climate, additional studies are needed to determine the relative effects of heterosis and outbreeding depression on stress tolerance (e.g., Prill et al., 2014).

4.5.5 Ecosystem implications

Interest in grassland responses to global change has grown substantially in the last decade (Cherwin and Knapp, 2012; Hsu et al., 2012; Knapp et al., 2015a; Reichmann et al., 2013; Sala et al., 2012). Specifically, the Shortgrass steppe (SGS) shows greater community turnover with precipitation variability (Cleland et al., 2013) but Sevilleta exhibits greater sensitivity for production with water limitation (Knapp et al., 2015a). Given that both sites are highly dominated by *B. gracilis*, mechanisms leading to these differential responses have been elusive. However, this study provides a possible evolutionary mechanism by which these two ecosystems differ: Genetic divergence in the dominant species, especially with regard to biomass plasticity, produces distinct ecosystem level responses. Ecosystem-level effects emerging from intraspecific variation of dominant plant species has been well documented in dominant seagrasses, Populus, and other systems (Hughes et al., 2008; Schweitzer et al., 2004). Future community biodiversity-ecosystem function work should consider intraspecific variation (including functional trait variation) alongside other mechanisms, such as positive or negative species interactions.

The shortgrass steppe is widely considered to be a water-limited and water driven ecosystem (Lauenroth et al., 1978; Noy-Meir, 1973; Sala et al., 1988, 1992). For example, previous analyses showed less temperature sensitivity compared to precipitation sensitivity along a latitudinal gradient through the shortgrass and mixed-grass steppes (Mowll et al., 2015) and many experimental and observational droughts have shown dramatic decreases in production relative to control conditions (e.g., Evans et al., 2011; Knapp et al., 2015a; Rondeau et al., 2013). Yet, our results indicate that temperature could be significant for driving *B. gracilis* adaptation. These contrasting findings suggest that the drivers of ecosystem, community, and evolutionary processes are distinct but should be considered simultaneously. For example, ecosystem level studies may fail to detect dominant species genetic bottlenecks arising from heat stress while evolutionary studies may fail to detect production compensation by other species during droughts. Comprehensive understanding of semi-arid grasslands dominated by *B. gracilis* requires both dimensions, thus future

studies would benefit from examining community and species level responses to precipitation and temperature stress simultaneously (e.g., Hoover et al., 2014b).

Finally, *B. gracilis* phenotypic and genetic diversity must be taken into consideration for restoration and forage production. We expanded on findings by Avendaño-González et al. (2019), showing that New Mexico sites were genetically distinct, but we also show for the first time that these populations are phenotypically distinct from other *B. gracilis* sites, suggesting that reciprocal transplant to Great Plains sites might not be appropriate. However, as droughts become more frequent and intense in the Midwest (Cook et al., 2015), introducing novel alleles between New Mexico and elsewhere could produce greater likelihood of successful evolution in the face of climate change. Overall, this study provides substantial evidence for genetic and phenotypic diversity in a key species across both regional and local scales, with important implications for future ecological research, restoration, conservation, and management of the shortgrass steppe ecosystem.

Chapter 5

Concluding remarks

A great deal of research effort lies ahead to link dominant species diversity to climate change drivers. Ecologists have been forward-thinking, designing many manipulative experiments to test the effects of droughts (Byrne et al., 2017), heat waves (Bergmann et al., 2010), combined stressors (Hoffman et al., 2018; Hoover et al., 2014b; Smith et al., 2016), and other drivers (Avolio et al., 2014). Ensuring these are realistically designed to mimic the actual effects of climate change will be essential going forward (Knapp et al., 2015b, 2017, 2018). Because acquiring resources to design and construct such experiments remains a challenge, future studies should take advantage of existing infrastructure to document species level patterns alongside ecosystem effects (e.g., Hoffman et al., 2018; Smith et al., 2016). Maintaining these experiments is essential to furthering our understanding of climate change's effects on dominant species.

In addition to climate change experiments, resources should be allocated to document diversity within other dominant species, especially in ecosystems outside of North America. Hundreds of grass species are ecologically dominant (Edwards et al., 2010), but most of have only been anecdotally documented (Hoffman et al., unpublished data). For example, *Themeda triandra*, a dominant C_4 grass of African savannas, has never been assessed for genetic diversity or population structure. While some dominant species, including *Leymus chinensis* and *B. gracilis*, have been assessed for genetic diversity and population structure (Liu et al., 2015, 2007), less is known about how genetic diversity in these important species might be impacted by climate change, as was shown in *A. gerardii* by Avolio et al. (2013). Future work must synthesize spatiotemporal variation and structure in these species at multiple levels of biological organization (e.g., morphological and genetic, Avolio et al., 2018; Meyer et al., 2014) and importantly, in response to climate change drivers. Although diversity of different species can be a major component of ecosystem function (Jain et al., 2014), focusing research efforts on dominant species might make ecosystem function research efforts in understudied systems more feasible (i.e., less domain expertise needed to identify species from a large species pool). This might be especially true in communities with a high degree of dominance.

The extensive work by Avolio, Smith, and colleagues on *A. gerardii* suggests ample future endeavors for lesser studied species like *B. gracilis*. For example, Avolio et al. (2013) found that neutral genetic diversity was reduced following a field climate change experiment where rainfall timing was manipulated, resulting in drier soils. Similar analyses should take place with *B. gracilis* in the context of existing infrastructure, such as DroughtNet (https://drought-net.colostate.edu/ Knapp et al., 2017) or the Extreme Drought in Grasslands Experiment (http://edge.biology.colostate.edu/). As with Avolio et al. (2013), we might expect genotype richness of *B. gracilis* to decline under extreme drought, with greater differences among genotypes. Experimental sites should also be regularly sampled and monitored to determine if and when diversity recovers following drought (Griffin-Nolan et al., 2018b).

Interestingly, Avolio et al. (2013) found that some genotypes of *A. gerardii* were more common than others. In Section 4.4.1 we discovered a similar pattern for *B. gracilis*. As future work examines how climate change affects *B. gracilis* genetic and functional diversity, close attention should be paid to changes in the genotype abundance distribution, or GADs (analogous to species abundance distributions, or SADs, Magurran, 2007; Magurran and Henderson, 2003; McGill et al., 2007). Future research on GADs will benefit from clear hypotheses, such as testing the sampling effect (Wardle et al., 1999), portfolio effect (Tilman, 1999; Tilman et al., 2006), or multiple processes (Vellend, 2010). In general, future work must carefully develop concrete ideas, especially as ecology becomes integrated with big data, highly multivariate response variables, and data science methods (Hampton et al., 2013; Marquet et al., 2014; Michener and Jones, 2012).

Following their field studies, Avolio, Smith, and colleagues performed common garden greenhouse studies and discovered trait variation among focal genotypes (Avolio and Smith, 2013b). Following these results, they constructed mesocosm manipulations of genetic diversity (Avolio et al., 2015). While I found that *B. gracilis* varied across its range and to some extent locally, similar experiments to Avolio and Smith (2013b) would be useful in *B. gracilis*, specifically for determining the trait and genetic variation more thoroughly within single populations, since *B. gracilis*'s exchange of genetic information appears somewhat limited even at small scales (Fig-

ure 4.1d-f). For example, the Shortgrass Steppe site (SGS) is among the sites with the greatest *B. gracilis* dominance (Milchunas et al., 1989), with the greatest negative implications for its local decline. I found this site to be comparatively diverse (25 distinct genotypes) compared to some nearby sites in Boulder County. These unique genotypes demonstrated variance in a common environment (broad sense heritability), which could indicate some genotypes are more productive than others and contribute more to ecosystem function (i.e., the sampling effect). However, we do not know how these genotypes are distributed in communities and whether diverse assemblages "over yield" in terms of ecosystem function. We also do not know if genetic diversity in *B. gracilis* is related to biotic factors, such as presence of invasive species. In Chapter 4, I found that one *B. gracilis* site (Andrus) was heavily invaded by *Bromus tectorum*, but a causal relationship was not tested. This particular site had the most distinct phenotype of all the Boulder sites. Such findings suggest that the relationship between species in the community (e.g., invasive species, codominant plants, presence of herbivores) and *B. gracilis* genetic and phenotypic diversity in *B. gracilis* are worth testing.

Dimensions of biodiversity have been well explored in *A. gerardii*, including with the addition of Chapter 2 and Chapter 3 here. These dimensions are worth exploring in *B. gracilis*. For example, gene expression has never been documented in *B. gracilis*, especially in an ecological context (although some work on MicroRNAs has been performed, Ordóñez-Baquera et al., 2017). In Chapter 2, I found that codominant grasses *A. gerardii* and *S. nutans* differed in gene expression despite functional similarity. Similarly in Chapter 4, I found that *B. gracilis* individuals from different sites were quite distinct. Given their different climates of origin, we might expect New Mexico and Colorado populations to differ substantially in gene expression responses to water limitation. Gene expression analyses provide insight into the drought response mechanisms used by individuals in either population, which will provide more clear explanations of patterns observed in the Sevilleta and Shortgrass Steppe communities under drought. In Chapter 3, I explored the details of plasticity by describing nonlinearity in *A. gerardii*'s responses. Those findings validated and clarified findings by Hoover et al. (2014a), showing that "threshold" responses to water avail-

ability could vary among genotypes. It is unknown whether such thresholds or nonlinear plasticity are present in *B. gracilis*, but given that *A. gerardii* and *B. gracilis* have adapted to cope with similar pressures (e.g., drought, high temperatures, freezing, and grazing), I hypothesize that similar results are likely. Finally, while there is some precedent for work on *B. gracilis* cytotype diversity (Butterfield and Wood, 2015; Tso and Allan, 2018), cytotype diversity across the center of *B. gracilis*'s dominance (shortgrass steppes within Colorado and New Mexico) is unknown, presenting a major obstacle for more in-depth genomic analysis. Research on *B. gracilis* intraspecific diversity would benefit greatly from range-wide cytotype + genotype analysis, as has been performed in *A. gerardii* (McAllister et al., 2014; McAllister and Miller, 2016).

While most prairies are considered to be water limited rather than temperature sensitive (Mowll et al., 2015), *A. gerardii* has been found to be relatively temperature sensitive compared to *S. nutans* (Nippert et al., 2009; Silletti and Knapp, 2002; Travers et al., 2007, 2010) although differences are not always present (Hoffman et al., 2018; Hoover et al., 2014a; Smith et al., 2016). An unexpected finding from Chapter 4 was *B. gracilis*'s simultaneous response to water and temperature variables (Table 4.3). We found that among sites, traits were most often with correlated with mean temperature during the driest quarter, and other metrics of both temperature and aridity (e.g., median PDHI). Similarly, previous research in the Shortgrass Steppe site (SGS) suggests that over time, *B. gracilis* productivity has declined with increasing minimum temperatures (Alward et al., 1999). These findings not only highlight the immediate need for manipulative temperature experiments for *B. gracilis*, but also the concept that drivers within site over time (e.g., minimum temperatures) may be different from species-level patterns across spatial scales.

Future research must utilize improved knowledge and data collection of intraspecific diversity in dominant species and diversity among codominants to develop predictive models. Given trait distributions and the environmental conditions under such distributions, it is possible to project where species might occur (Webb et al., 2010). This could be particularly important for the dominant grasses described in this dissertation, since their respective ecosystems are quite literally named after them, and shifting occurrences of these grasses would result in a shifting ecosystem

landscape. With comprehensive knowledge, intraspecific diversity and trait variation can be incorporated into dynamic vegetation models (DVMs) to predict how distributions of species might change (Snell et al., 2014). Such models will benefit from both niche-based (i.e., traits) and process-based (i.e., adaptation or fitness) approaches (Morin and Thuiller, 2009). Detailed intraspecific analyses will continue to improve models; for example, as models are developed for *B. gracilis*, researchers might consider splitting the species into two subspecies based on regional differences highlighted in Chapter 4 and by Anderson (2006). Likewise, *A. gerardii* models should account for thresholds of function described in Chapter 3 and by Hoover et al. (2014a). Concrete predictions of distributions of these important dominant grasses will help provide stakeholders with actionable insights, allowing conservation and management of native North American prairies even in a more variable future.

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Appendix I

I.1 Supplementary methods

I.1.1 Drought experimental design

Pots were saturated before drydown of half the pots to perform the drought treatment. Typically, saturated pots are approximately 30% volumetric water content (VWC). Figure I.1 comes from another study using the same media shows that saturated pots are 29.78 $\pm$ 1.86% VWC.

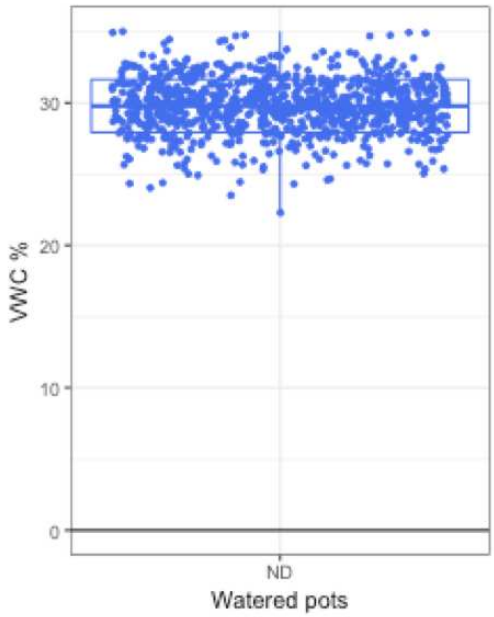

Figure I.1: Volumetric water content (% VWC) for a typical experiment using Premier pro-mix HP.

I.1.2 RNA extraction

We extracted RNA from leaf tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) (following McCarty, 1986) RNA quantity was measured by a NanoDrop spectrophotometer (NanoDrop products, Thermo Scientific, Wilmington, DE). Only RNA samples with A260/A280 from 2.0 to 2.2, A260/A230 from 2.2 to 2.5, rRNA ratio (28s/18s) from 1.1 to 1.4 were used in the sequencing (analyzed via Bioanalyzer by Yale Center for Genome Analysis).

I.1.3 De novo assembly

Sequences were uploaded to the iPlant Collaborative database (www.iplantcollaborative.org) (Goff et al., 2011) using the FTP client Cyberduck (version 4.7.2). Sequences were trimmed using the HTProcess pipeline through iPlant's Discovery Environment. The first 12 bases were cropped (adapters) and any read with a quality score lower than 25 or length shorter than 40 bases was discarded. Trimmomatic (version 0.32, Bolger et al., 2014) parameters were the same across all samples: head crop: 12, leading: 3, trailing: 3, sliding window of 4, and average quality cutoff of 25. Trinity was run on the Colorado State University Correns cluster, using 8 CPUs and 64 GB memory. For further details regarding specific scripts and commands used, please see our online guide at http://avahoffman.com. To evaluate the quality of the Trinity assemblies, we used (1) Trinity's built-in evaluation scripts, (2) read realignment percentage using Bowtie 2 (version 2.2.7, Langmead and Salzberg, 2012), and (3) Samtools scripts (Li et al., 2009b).

Post-assembly Nx statistics were obtained from the *A. gerardii* and *S. nutans* Trinity assemblies using the TrinityStats.pl script provided by the Trinity install. Bowtie 2 was then used to individually align transcripts back to the species-specific assembly, allowing us to assess the proportion of transcripts that realigned. In many cases, Bowtie 1 will detect a high proportion of improper alignments if there is a higher redundancy among transcripts in the samples. Because the two species here are polyploid, we chose to use Bowtie 2 despite Trinity's default use of Bowtie 1. We also used BLAST+ tool blastx to align assemblies to the Swiss-Prot and TrEMBL databases, allowing us to assess the completeness of transcripts to known proteins with an evalue cutoff of e-10 (The UniProt Consortium, 2015). Databases were limited to higher land plants (Euphyllophytes) to maximize computing efficiency at this step. These Swiss-Prot and TrEMBL databases used for assessing transcript completeness and relationship to known proteins was downloaded from ftp.uniprot.org on 13 April 2016. Downloaded Swiss-Prot and TrEMBL represented 35,215 and 3,406,188 annotated protein sequences, respectively. Top hit coverage was determined with a Trinity perl script. In downstream analysis, if no matches were determined, results were examined with slightly relaxed parameters (e-value of 1e-5 and universal blastx search).

I.1.4 Differential expression within species

We used RSEM (version 1.2.28, Li and Yang, 2011) to count expression of each transcript (transcripts per million, TPM). Because many transcripts have weak support from the expression data (few realignments, or counts), we estimated overall expression by regressing a subset of coverage (between 10 and 250 TPM) against the number of transcripts at each coverage level using R (version 3.3.0, R Core Team, 2018). This regression's intercept provides an estimate of total transcripts expressed from the Trinity assembly (Haas et al., 2013) (Figure I.2). We normalized across sample coverage using trimmed mean of M-values (TMM, Robinson et al., 2010).

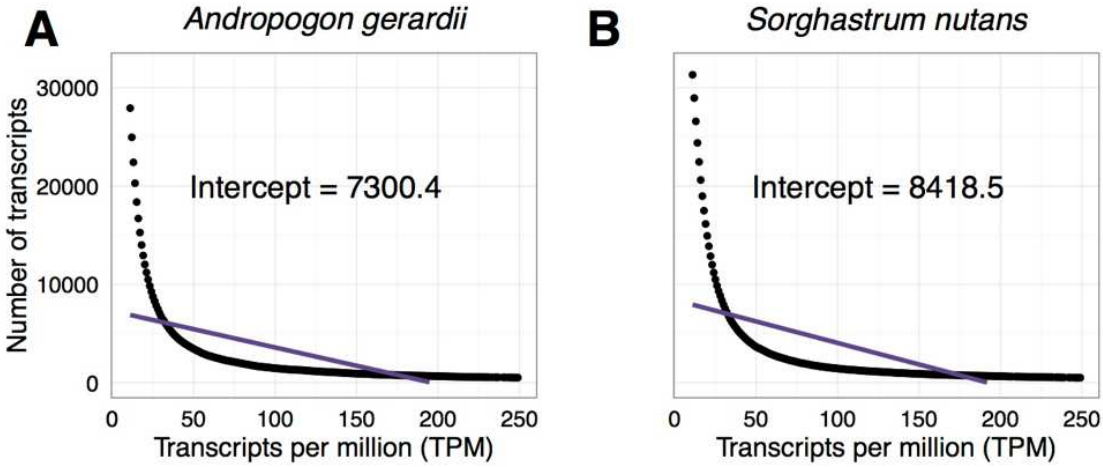

Figure I.2: Regression of the representative subset of transcripts against their coverage using RSEM.

I.1.5 Homology

Transcriptomes were downloaded as gunzipped fasta files on 21 April 2016. Trinity assemblies were blasted against these at an e-value cutoff of 1e-10.

I.1.6 Differential Expression and Annotation

Because Trinotate requires specific editions of SWISS-PROT and Pfam databases, we downloaded both from the trinotate website at https://data.broadinstitute.org/Trinity/Trinotate_v3_RESOURCES/uniprot_sprot.pep.gz and https://data.broadinstitute.org/Trinity/Trinotate_v3_

RESOURCES/Pfam-A.hmm.gz. Transcriptionfactor.org databases for Zea mays and Sorghum bicolor were downloaded on 15 May 2016. RSEM version 1.2.28 was used to determine expression levels among samples. RSEM references were built using Bowtie 2. RSEM expression calculations were estimated using Bowtie 2 with:

```
--bowtie2-sensitivity-level = very_sensitive
--bowtie2-mismatch-rate = 0.2|
--bowtie2-k = 10000
```

Trinity script count_matrix_features_given_MIN_TPM_threshold.pl was used to generate a list of number of features falling into coverage (transcripts per million, or TPM) bins. This output was used to perform linear regression of number of features vs. coverage, and estimate overall expression. We ran edgeR version 3.2 through Trinity using the run_DE_analysis.pl and analyze_diff_expr.pl scripts. Hmmer (version 3.1b2, Finn et al., 2011), SignalP (version 4.1, Petersen et al., 2011), and Tmhmm (version 2.0c, Möller et al., 2001) were used to predict and identify protein domains.

I.2 Supplementary results

I.2.1 De novo assembly

Andropogon gerardii and *S. nutans* had 1338 and 1161 transcripts that matched reviewed and annotated proteins by >90% (Swiss-prot), respectively. 7839 transcripts in *A. gerardii* and 7109 in *S. nutans* had at least some alignment (>10%) to annotated proteins. When we expanded the database to include hypothetical unreviewed proteins (TrEMBL), 24,880 total matches were found for *A. gerardii* and 20,749 for *S. nutans*. When we regressed the representative subset of transcripts against their coverage using RSEM, *A. gerardii* expressed an estimated 7300 transcripts, while *S. nutans* expressed an estimated 8419 transcripts (Figure I.2).

I.2.2 Highly expressed transcripts

We were unsurprised that highly expressed transcripts (>400 TPM) within *A. gerardii* were similar among the drought and watered treatments considering the basic metabolic needs of plants.

However, droughted *A. gerardii* expressed multiple transcripts related to polyamine biosynthesis, as well as tetrapyrrole metabolism and biosynthesis that watered transcripts did not. In contrast, watered *A. gerardii* exhibited high expression of ribonucleoprotein movement and saccharide metabolism, catabolism, and biosynthesis. Compared to *A. gerardii*, we observed more differences among highly expressed drought and watered transcripts in *S. nutans*. Highly expressed drought transcripts in *S. nutans* were related to aminotransferases (component of amino acid synthesis), phosphoribulokinases (carbon fixation), ribonucleases (RNA degradation), and aspartate metabolism. Watered *S. nutans* highly expressed peptidase and endopeptidase regulation (amino acid catabolism), photoprotection, and protein modification transcripts.

I.2.3 Comparative expression between species

We performed a non-metric multidimensional scaling among samples (shown plotted in Figure 2.4 of the main manuscript). The stress value, 0.0657 indicates a good fit (Figure I.3). All volcano plots are shown in Figure I.4.

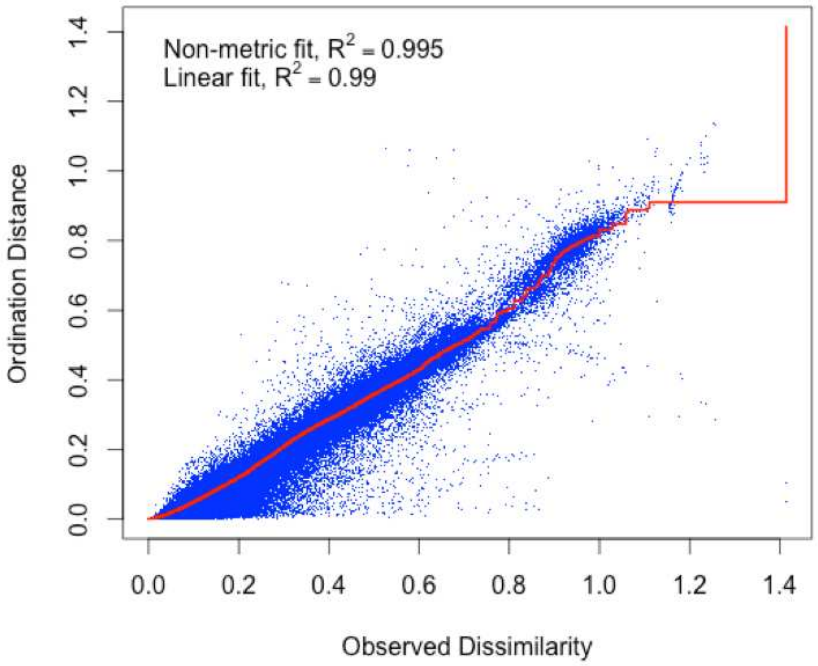

Figure I.3: Stress plot for NMDS of all samples performed in the main manuscript.

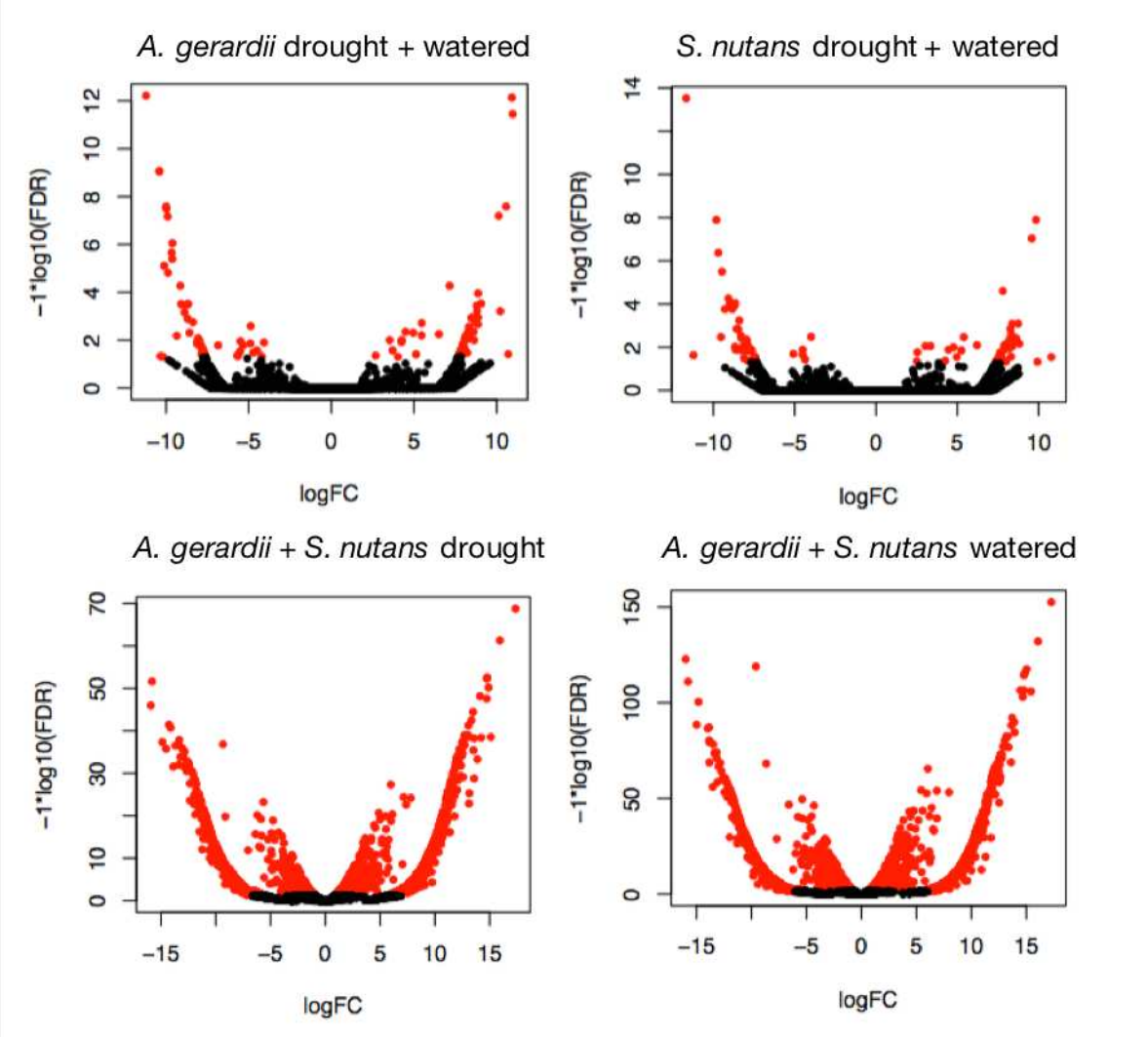

Figure I.4: Volcano plots showing transcripts or gene groups differentially expressed. Significantly different transcripts (*A. gerardii* drought + watered, *S. nutans* drought + watered) or gene groups (*A. gerardii* + *S. nutans* drought, *A. gerardii* + *S. nutans* watered) are colored red.

I.2.4 Homology

In addition to percent transcript match at the nucleotide level, we also performed an amino acid similarity search using the tblastx tool. Order of relatedness was similar to the nucleotide matches, but with lower percentage for proteins (Figure I.5). While percent transcript match at the nucleotide level can be found in the main text, the proportion of genes with any match over the

total found in *A. gerardii* or *S. nutans*are shown below (Figure I.6). As an example, this shows the percent of *A. gerardii* transcripts out of ∼65,000 total with a match to *Arabidopsis*.

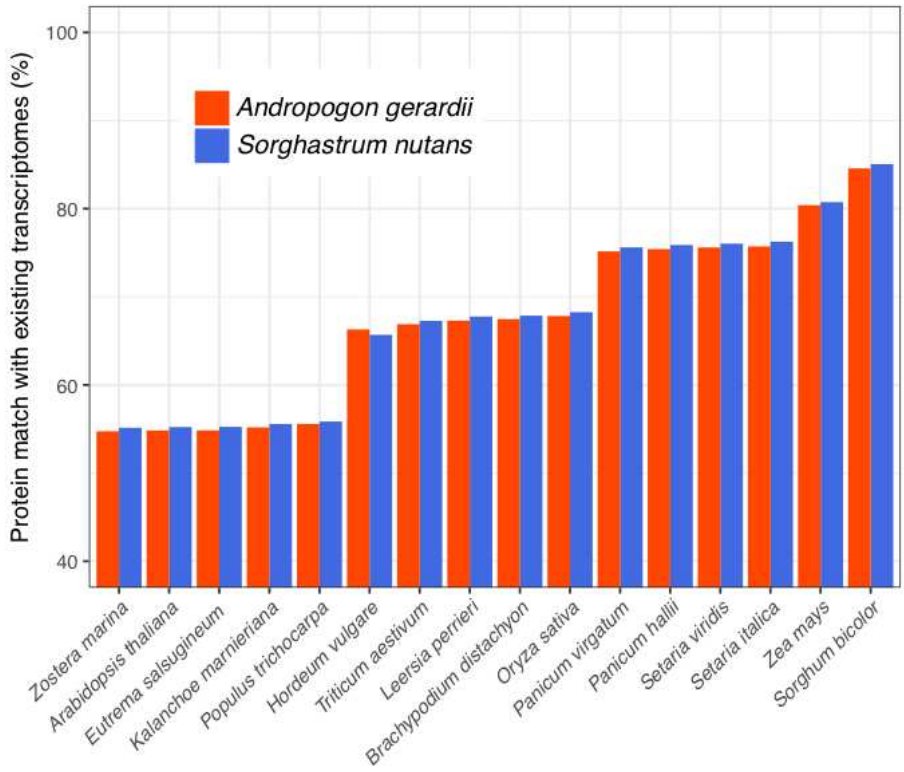

Figure I.5: Homology of both species to known transcriptomes. Percent match refers to average percentage of identical matches at the protein level (tblastx).

I.3 Supplementary discussion

Within RNA-seq data there is more statistical power when comparing genes with higher expression. In *A. gerardii*, polyamine, porphyrin, and tetrapyrrole biosynthesis were highly represented under drought. All are related to drought tolerance: polyamine biosynthesis enhances photosynthetic efficiency and leaf water status in rice under drought (Farooq et al., 2009), porphyrins help maintain less negative xylem water potential and oxidative stress (Phung et al., 2011), and some tetrapyrroles help signal oxidative stress under drought (Nagahatenna et al., 2015; Phung et al., 2011). In droughted *S. nutans*, aminotransferases and aspartate metabolism may be linked to accumulation of amino acids under drought (Berdeja et al., 2015; Malatrasi et al., 2006; Szabados

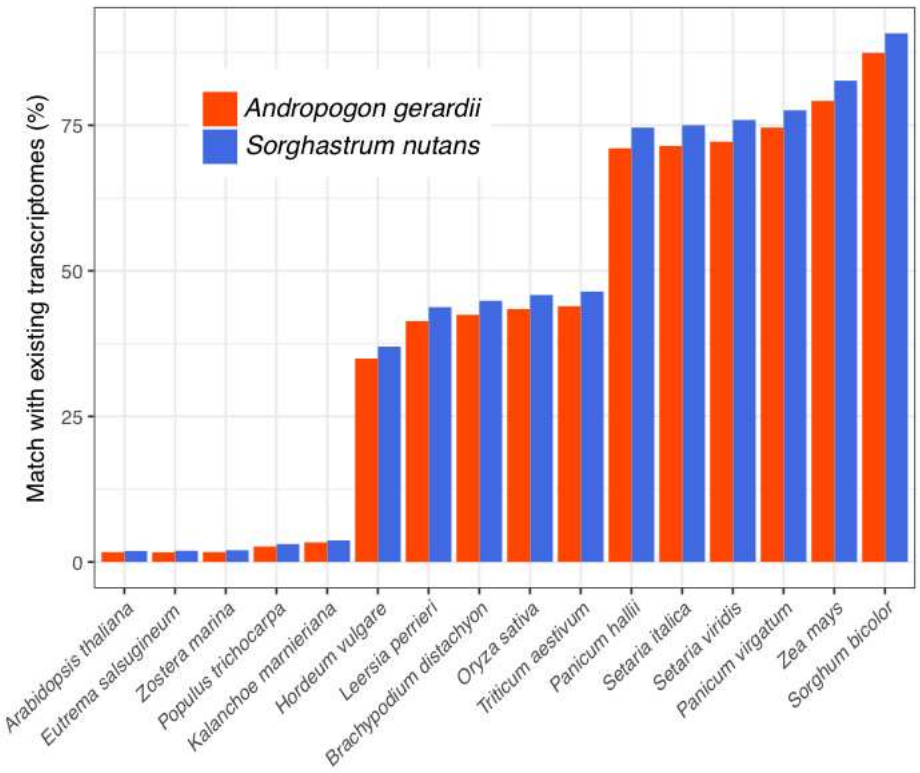

Figure I.6: Homology of both species to known transcriptomes. Percent match refers to a proportion of the number of genes with blastn alignment when comparing two transcriptomes at the nucleotide level.

and Savouré, 2010), phosphoribulokinase is linked to stabilizing photosynthesis under drought in sugarcane (Khueychai et al., 2015), and ribonucleases serve a regulatory role under drought in switchgrass (Hivrale et al., 2015). These differences suggest *A. gerardii* approached drought within highly expressed transcripts by coping with reactive oxygen species byproducts, while *S. nutans* took a more cautious approach via regulating key metabolic processes differently.

Appendix II

II.1 Supplementary methods

After establishment of sterile genotype lines, plantlets were propagated at the U.S. Department of Agriculture National Center for Genomic Resources Preservation (USDA-NCGRP), in Fort Collins, CO. Boxes were placed in a walk-in growth chamber at the USDA- NCGRP, and provided a 16 hour photoperiod at 25°C and 20-30% humidity. Plants were allowed to propagate on high-nutrient M.S. media for 24 days, during which clumps of individuals were produced. Individuals were then transferred with forceps to boxes containing rooting media. Plants grew roots for 50 days before being transferred to misting benches at Plant Growth Facilities, Colorado State University. Transfer to the greenhouse involved rinsing residual media from plant roots with water, weighing individual fresh plants for covariate analysis, and placing individuals into moist fritted clay media (Porous Ceramic "Greens Grade", Profile Products, Buffalo Grove, IL). Height and tiller number measurements were collected on these individuals for covariate analysis on day 6 (early) and day 32 (late) following transfer (Hoffman and Smith, 2018).

II.1.1 Model details

We chose distributions for data based on whether residuals met normality assumptions. Unless otherwise noted, we used the likelihood for the ith observation of N total observations:

$$y_i \sim normal(\hat{y}_i, \sigma^2)$$

$$\hat{y}_i = x_i\beta$$

where x is an NxJ matrix of predictors (J represents each of 15 unique genotype, treatment, and interactions possibilities) and β is a vector of J parameters. Overall, $x_i\beta$ is a vector of N predictors. We used the following priors unless otherwise specified:

$$\beta \sim normal(0, 10^6)$$

$$\sigma^2 \sim cauchy(0, 5)$$

Code for models is available at http://www.avahoffman.com/resources.

Figure II.1: Genotypes of *A. gerardii* emergent from tissue culture and adjusting prior to the primary treatment.

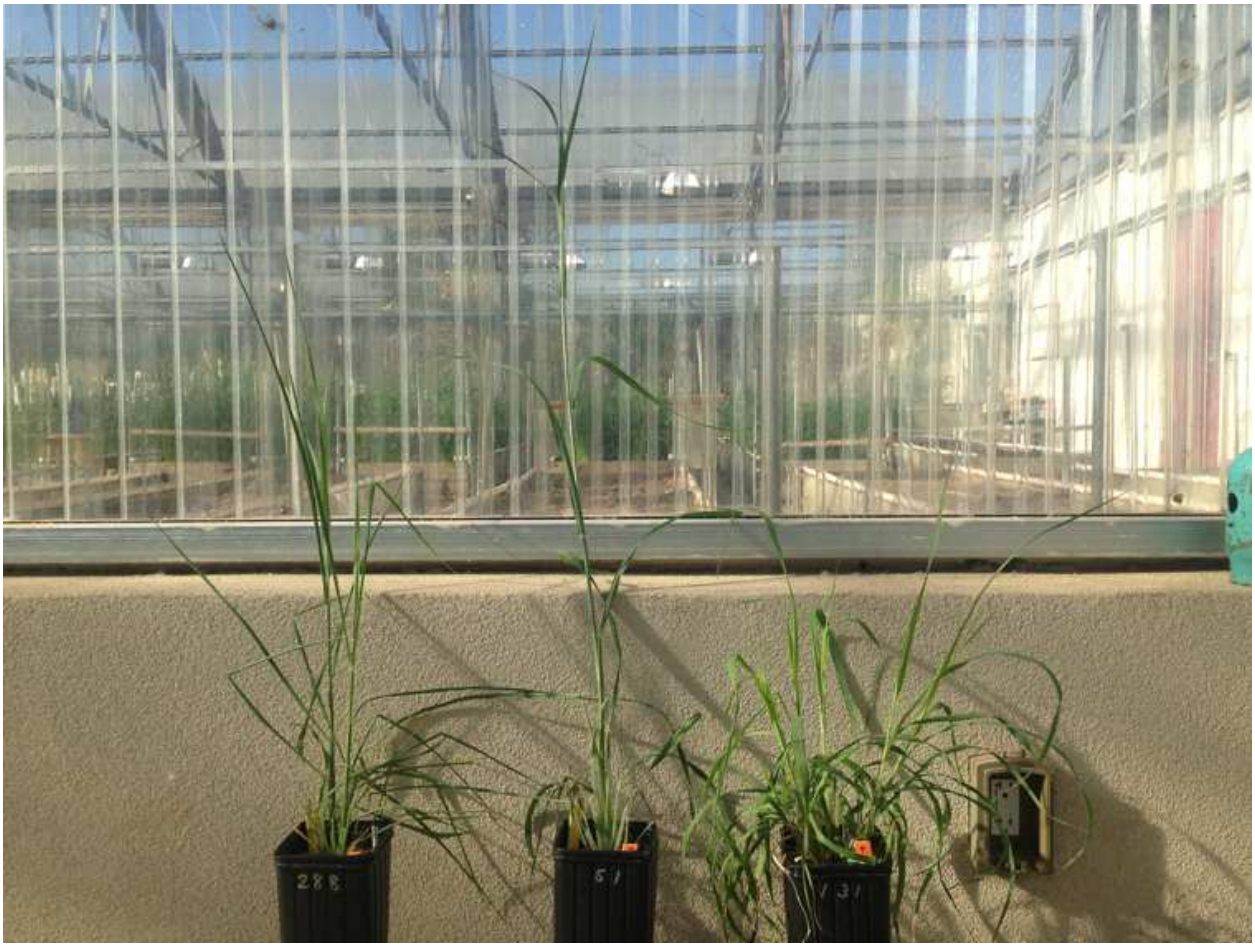

Figure II.2: Genotypes of *A. gerardii* from left to right: G11, G2, and G5.

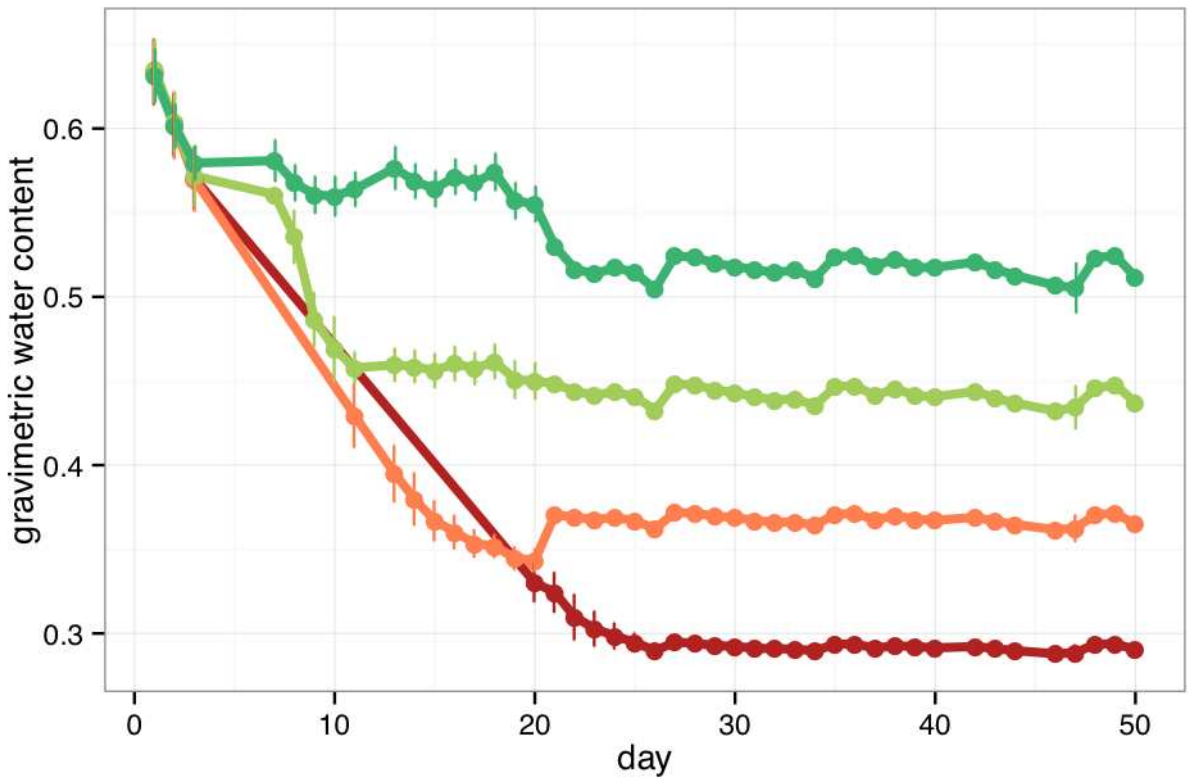

Figure II.3: Gravimetric water content over the course of the primary treatment. Dark green represents the 25% volumetric water content (VWC), light green represents 20%, orange represents 15%, and red represents 10%. Calculations from VWC to GWC were performed by comparing soil water potential across potentiometer curves for Konza soil and fritted clay media.

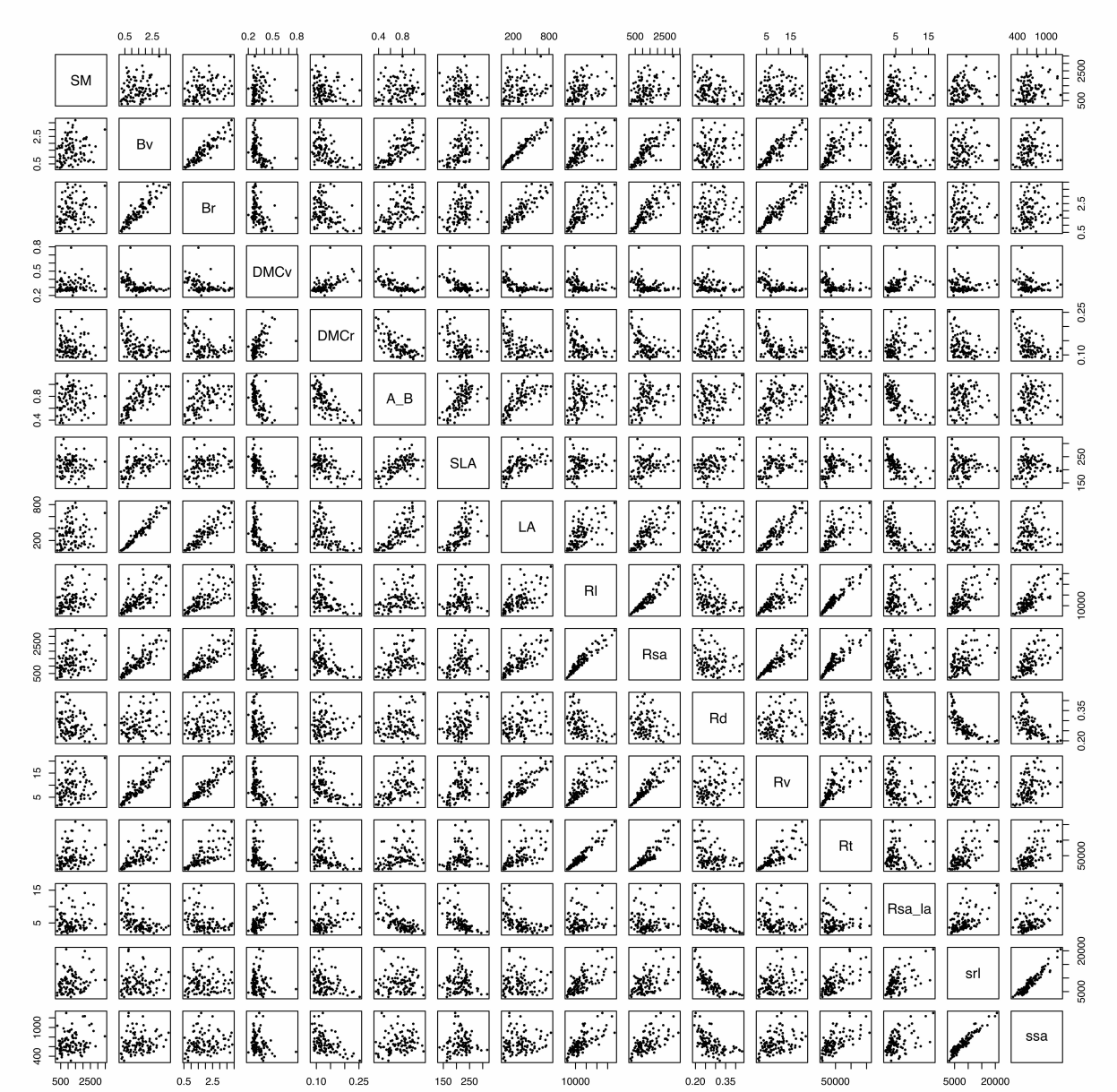

Figure II.4: Correlation among *A. gerardii* morphological traits. See Table II.1 for trait abbreviations.

Table II.1: Morphological traits used in the principal components analysis. Traits explored further in Chapter 3 are in bold. Proportion of variances is as follows for PC1 and PC2: 0.596, 0.218. Treatment: 7 week water limitation; n=7 (105 total).

Response variables	**PC1 loading**	**PC2 loading**
SM (starting mass, mg)	0.100065945	0.10480153
Bv (vegetative biomass, g)	**0.347638871**	-0.24997025
Br (root biomass, g)	0.269300800	-0.13492212
DMCv (leaf dry matter content, mg g^{-1})	-0.078938760	0.07964500
DMCr (root dry matter content, mg g^{-1})	-0.099545253	0.02126078
A:B (shoot:root ratio, g g^{-1})	0.097004269	-0.13886521
SLA (specific leaf area, mm^2 g^{-1})	0.045515648	-0.08555616
LA (leaf area [scaled], mm^2)	**0.387546795**	-0.33644767
Rl (root length, mm)	**0.382717823**	0.29707395
Rsa (root surface area, mm^2)	**0.362234772**	0.11047337
Rd (mean root diameter, mm)	-0.009574089	-0.17321892
Rv (root volume, mm^3)	**0.341019127**	-0.08252271
Rt (root tips)	**0.440063299**	0.27032713
Rsa:LA (root to leaf surface area, mm^2 mm^{-2})	-0.091265866	**0.55415942**
SRL (specific root length, mm g^{-1})	0.114642300	**0.42785644**
SSA (specific root surface area, mm^2 g^{-1})	0.097958168	0.24744516

Table II.2: Physiological traits used in the principal components analysis. Traits explored further in Chapter 3 are in bold. Proportion of variances is as follows for PC1 and PC2: 0.391, 0.125. Treatment: 7 week water limitation; n=7 (105 total).

Response variables	**PC1 loading**	**PC2 loading**
SM	0.048162439	0.059002621
μA_{net} (mean net photosynthetic rate, μmol m^{-2} s^{-1})	**-0.187165271**	0.005494838
μg_s (mean stomatal conductance, mol H2O m^{-2} s^{-1})	**-0.204531364**	-0.027373901
μfo (mean minimal dark adapted fluorescence)	-0.070223351	-0.003880752
μfm (mean maximal dark adapted fluorescence)	-0.079381770	0.004529034
μfv (mean Fv/Fm or maximum PSII efficiency)	-0.003835177	0.003405529
μE (mean evapotranspiration rate, mmol H2O m^{-2} s^{-1})	**-0.181656247**	-0.038410593
μWUE$_i$ (mean instantaneous water use efficiency, μmol $mmol^{-1}$)	-0.014171862	0.050279510

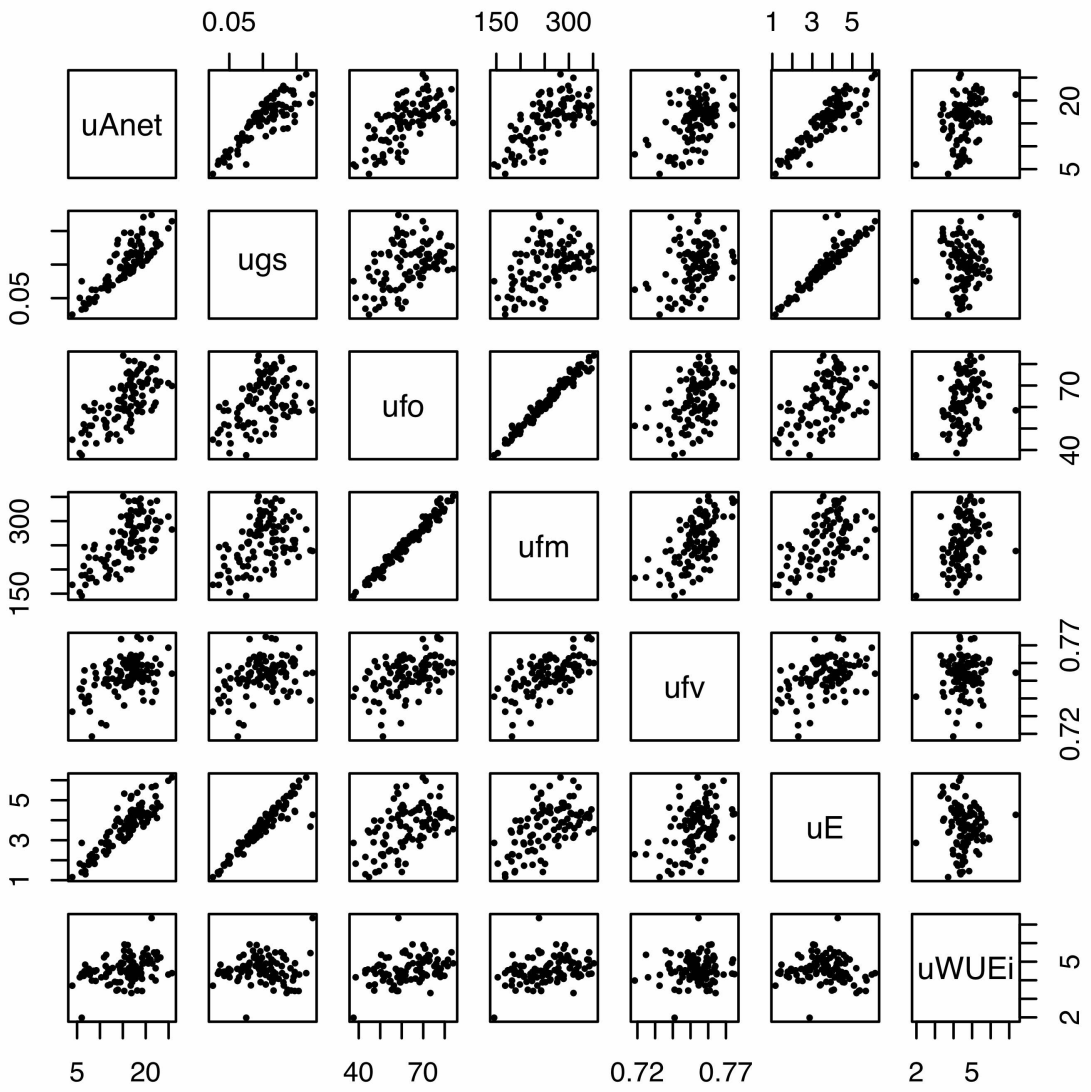

Figure II.5: Correlation among *A. gerardii* physiological traits. See Table II.2 for trait abbreviations.

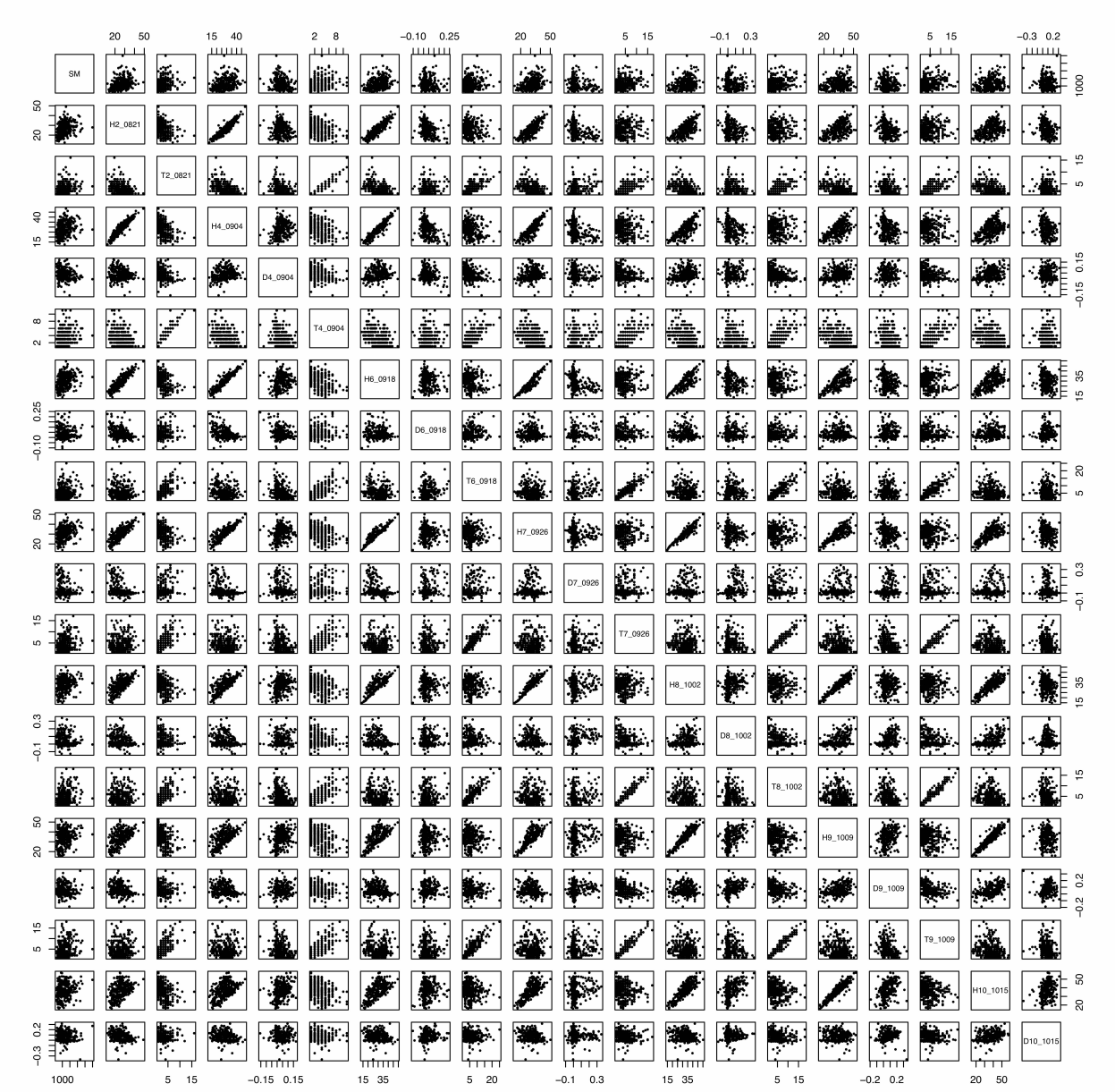

Figure II.6: Correlation among *A. gerardii* growth related traits. See Table II.3 for trait abbreviations.

Table II.3: Growth traits used in the principal components analysis. Bolded traits were modeled, but the maximum value of all weeks for each trait was used in the main text (Chapter 3). Subscripts for response variables indicate the week of measurement. Proportion of variances is as follows for PC1 and PC2: 0.344, 0.187. Treatment: 7 week water limitation; n=20 (300 total).

Response variables	**PC1 loading**	**PC2 loading**
SM	0.03367078	-0.05693340
H_2 (height, week 2, cm)	0.04638048	-0.00278920
T_2 (tiller count, week 2)	-0.07114830	-0.21779420
H_4	0.04960002	0.02445134
Δ_4 (relative growth rate, week 4, ln(cm) week^{-1})	0.06219766	0.42250066
T_4	-0.05466700	**-0.19689180**
H_6	0.03986469	0.01454532
Δ_6	-0.18791060	-0.17524370
T_6	-0.07511290	-0.20861400
H_7	0.01255380	0.00563312
Δ_7	**-0.78597650**	-0.28081100
T_7	-0.03612060	-0.22916480
H_8	-0.00823540	0.02571861
Δ_8	**-0.48748400**	**0.45953188**
T_8	-0.04098640	-0.24131600
H_9	-0.02316700	0.04758371
Δ_9	-0.26332880	**0.39221767**
T_9	-0.03763280	-0.24054730
H_{10}	-0.02896350	0.06196142
Δ_{10}	-0.09513580	0.19425520

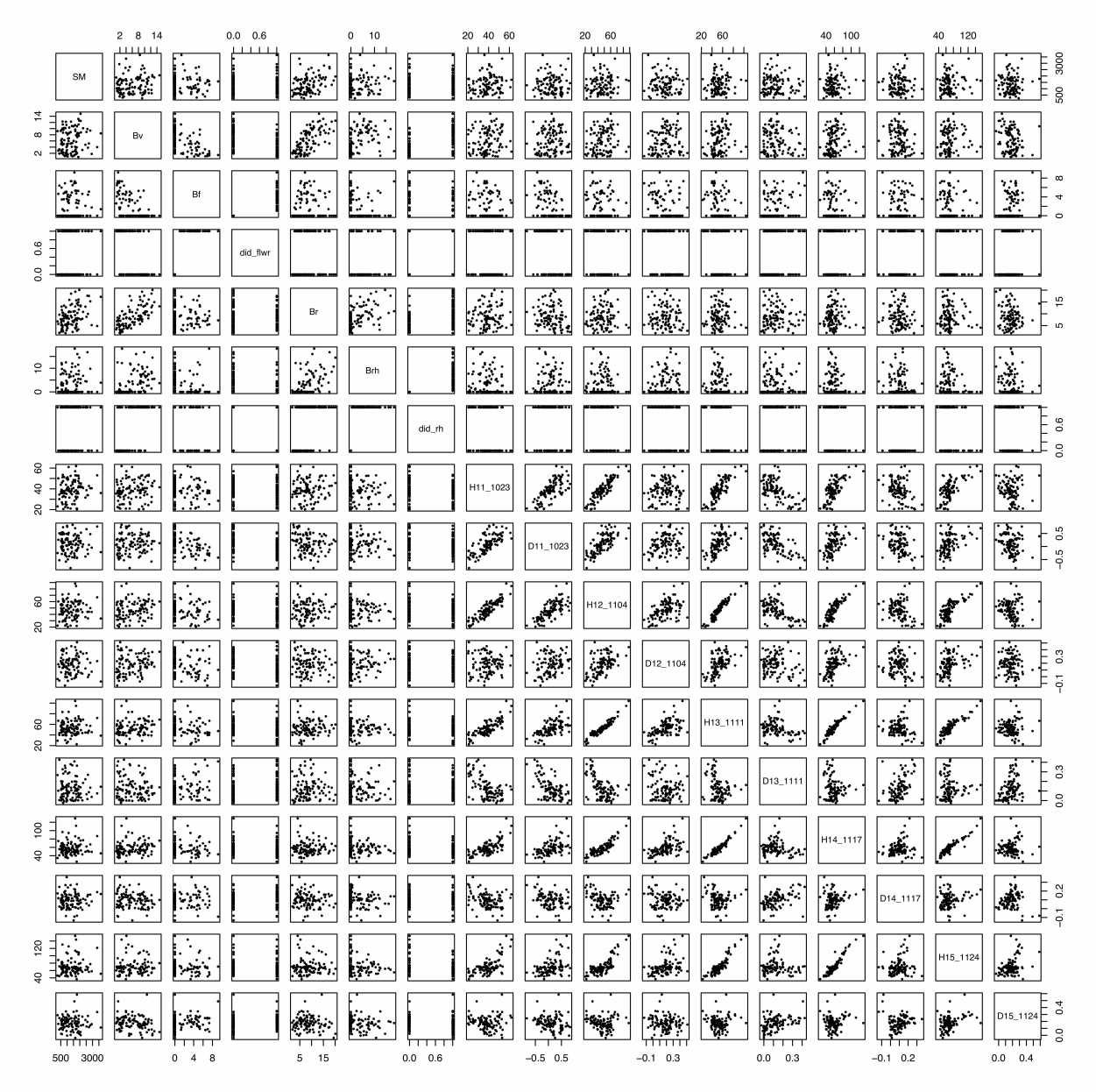

Figure II.7: Correlation among *A. gerardii* recovery traits. See Table II.4 for trait abbreviations.

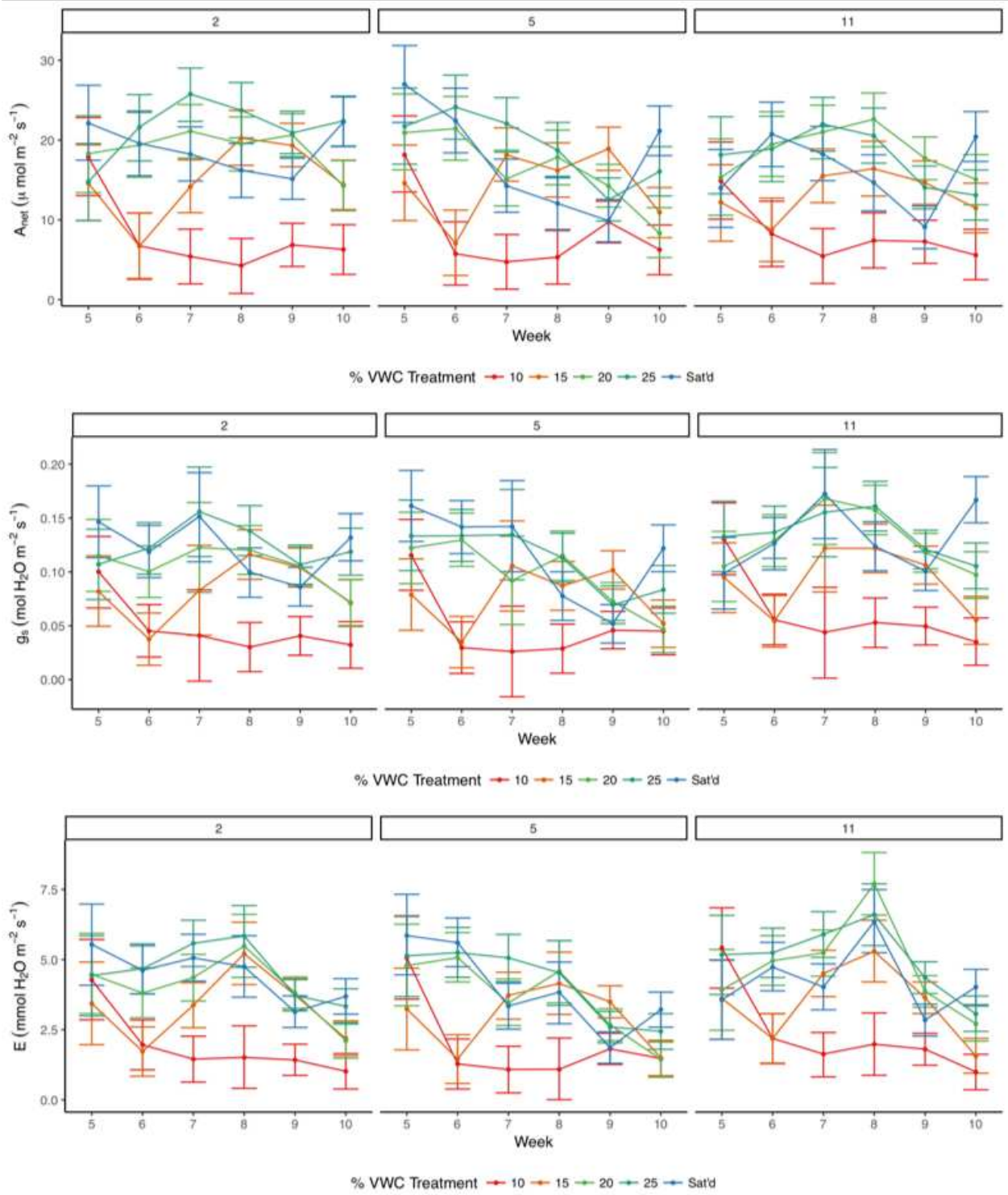

Figure II.8: Physiological measurements by *A. gerardii* genotype and treatment over time. Heading number indicates genotype.

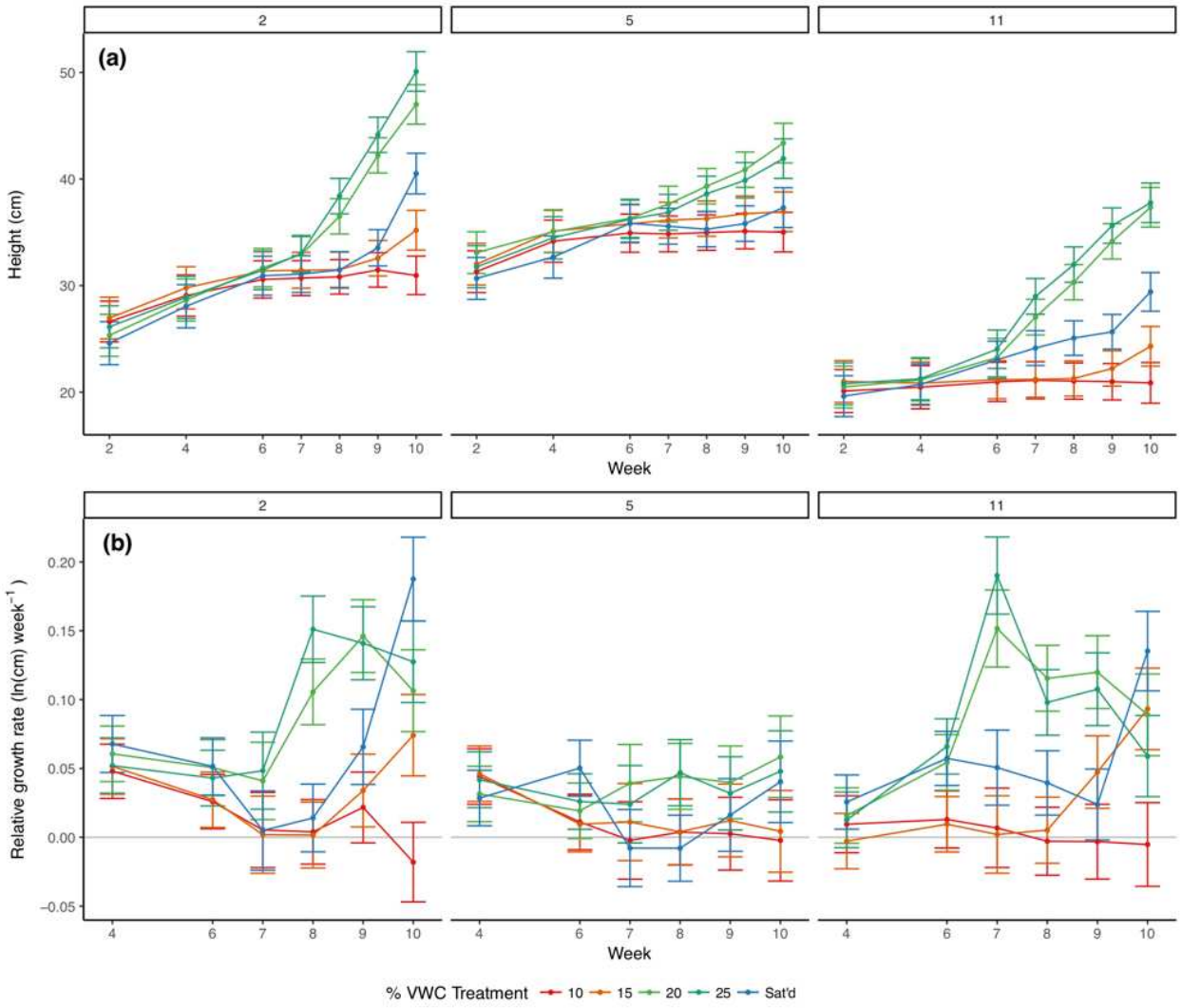

Figure II.9: Height (a) and (b) relative growth rates differ among *A. gerardii* genotypes, treatments, and over time. Error bars represent the 95% CI.

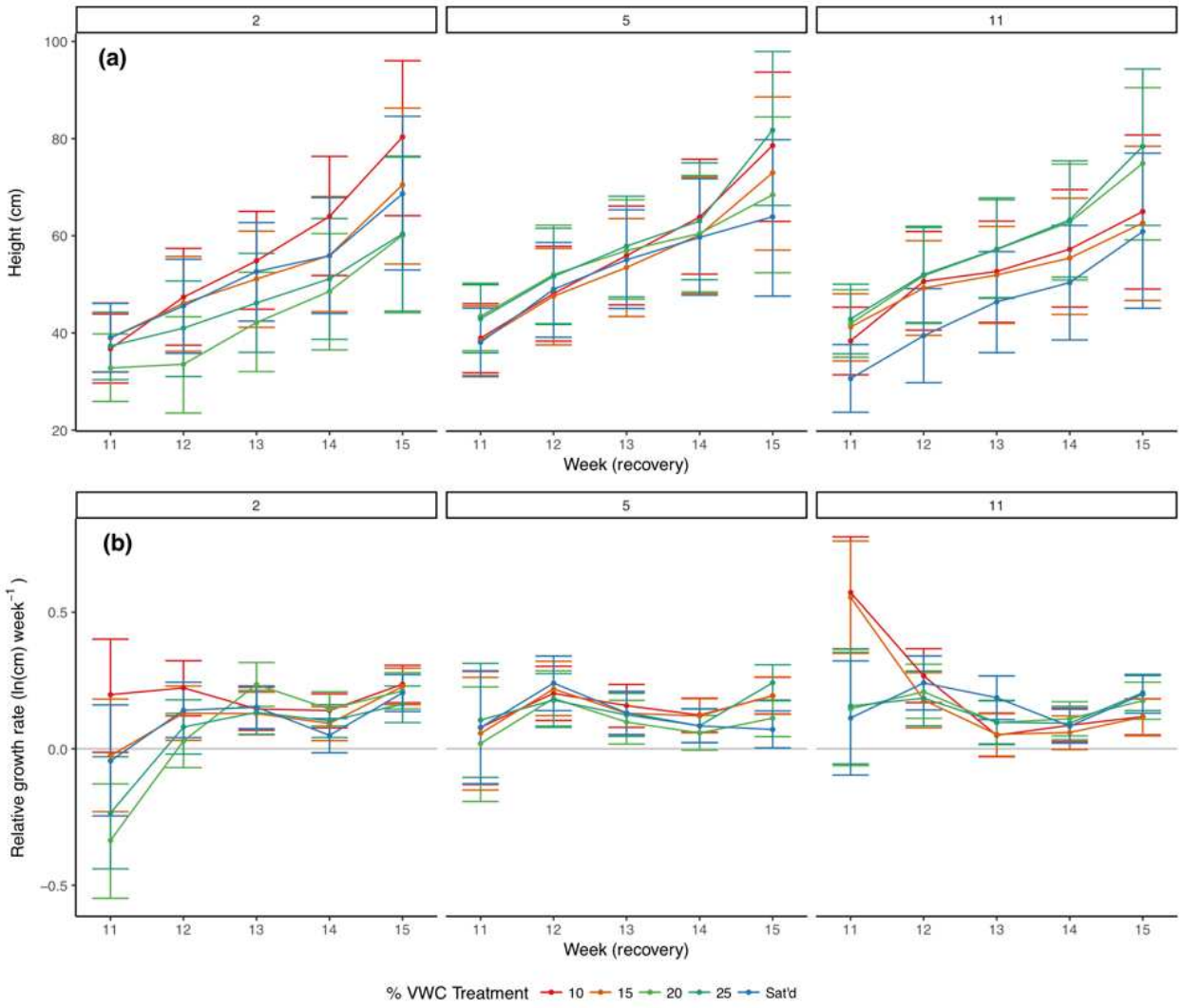

Figure II.10: All *A. gerardii* genotypes and treatments had similar heights (a) during recovery. Relative growth rates (b) became similar across genotypes after week 11. Note that treatments (different colors) refer to the treatments experienced prior to the recovery period. See Table 3.1 for significant effects. Error bars represent the 95% CI

Table II.4: Recovery traits used in the principal components analysis. Traits explored further in Chapter 3 are in bold. Subscripts for response variables indicate the week of measurement. Proportion of variances is as follows for PC1 and PC2: 0.634, 0.156. Treatment: 7 week water limitation + recovery; n=7 (105 total).

Response variables	**PC1 loading**	**PC2 loading**
SM	0.00119545	-0.04316790
Bv	-0.02387650	-0.27561150
Bf (flowering biomass, g)	**0.18807746**	**0.51723909**
Br	0.02383496	-0.17768430
Brh (rhizome recovery, cm)	0.01623755	**-0.76701500**
H_{11}	-0.04799900	0.02432013
Δ_{11}	**-0.95975100**	0.10138805
H_{12}	-0.05769140	0.01096925
Δ_{12}	-0.05969250	-0.11557920
H_{13}	-0.04260700	0.01185929
Δ_{13}	0.14610361	0.01165506
H_{14}	-0.03788540	0.01660715
Δ_{14}	0.07759748	0.03697981
H_{15}	-0.03466380	0.03385888
Δ_{15}	0.03295917	0.08566641

Appendix III

III.1 Supplementary methods

See guide prepared by E. Meyer (http://people.oregonstate.edu/~meyere/docs/Preparing2bRAD.pdf) and Wang et al. (2012) for detail in addition to notes below. Prior to DNA extraction, we dried leaf samples in coin envelopes on silica gel and processed samples into a fine powder using a Qiagen TissueLyser II. With DNA extraction, we produced the highest quality DNA using the Omega Bio-tek E.Z.N.A. Plant DNA DS kit (for samples high in polysaccharides) compared to higher throughput kits. Because high quality, concentrated DNA is required for successful library preparation, we quantified our samples using a Qubit II, followed by an air-drying concentrating step where samples were allowed to air dry overnight with a porous cover followed by rehydration to 125 ng μl^{-1}. This DNA was then digested overnight using the AlfI IIB enzyme (Fisher Scientific, www.fishersci.com) followed by adapter ligation for Illumina sequencing. In order to further improve coverage and reduce sequencing costs in the ligation step, we ligated specific Illumina adapters to target only 1/256 of the AlfI enzyme cut site fragments. In other words, instead of targeting DNA fragments ending in NN, we targeted only fragments ending in GG for sequencing. We annealed single-stranded adapters by heating to 97°C and cooling to 25°C in 1°C min^{-1} increments. Finally, we amplified adapter-ligated fragments using 22 PCR cycles and dual-indexed Illumina barcodes. After electrophoresis gel size selection of fragments, we quantified samples using qPCR (BioRad) and pooled samples according to concentration into six "lanes" followed by an Omega Bio-tek Mag-Bind Total Pure NGS magnet cleanup and concentration step. Pooled lanes were sequenced on an Illumina HiSeq 4000 and de-multiplexed at the Genomics and Cell Characterization Core Facility at the University of Oregon in January 2018.

Following sequencing, we processed the genomic fragment data to produce SNPs. We relied extensively on scripts generated by E. Meyer for SNP processing (http://eli-meyer.github.io/2bRAD_utilities). First, we truncated sequencing reads to 36bp according to the fragment size produced by the AlfI restriction enzyme, followed by quality filtering of any reads containing more than 4 bases with a quality score lower than 20. We also removed any reads containing more than

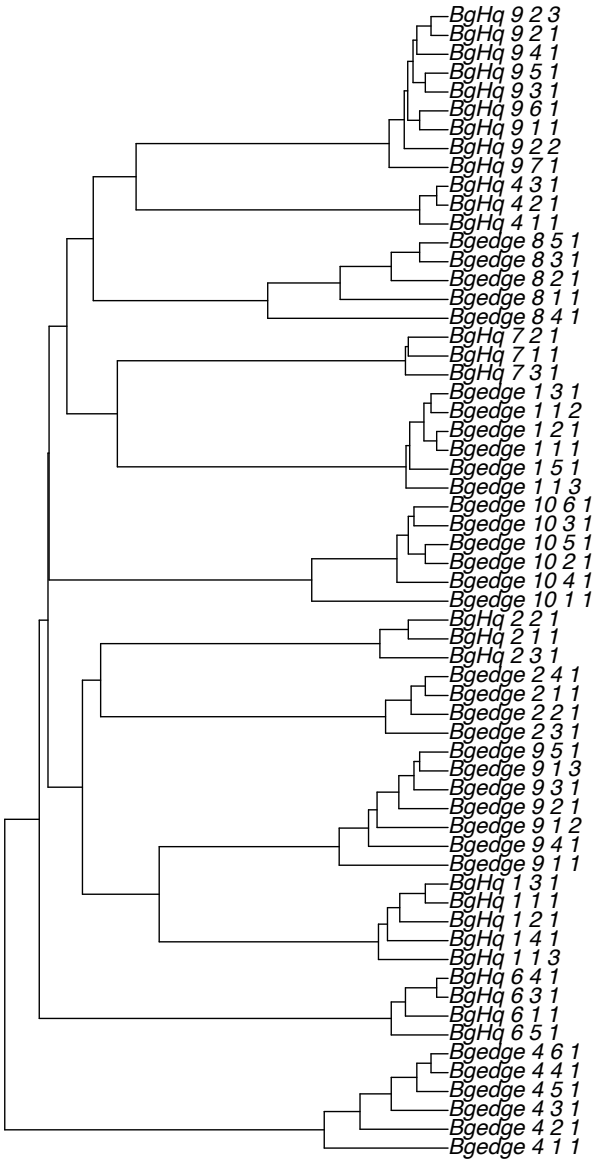

Figure III.1: *B. gracilis* clones clustered distinctly, indicating the efficacy of the sequencing technique for this species. The first number index on each branch tip indicates clone number, second index indicates tiller replicate, and third index indicates leaf replicate. Dendrogram was generated using the UPGMA method.

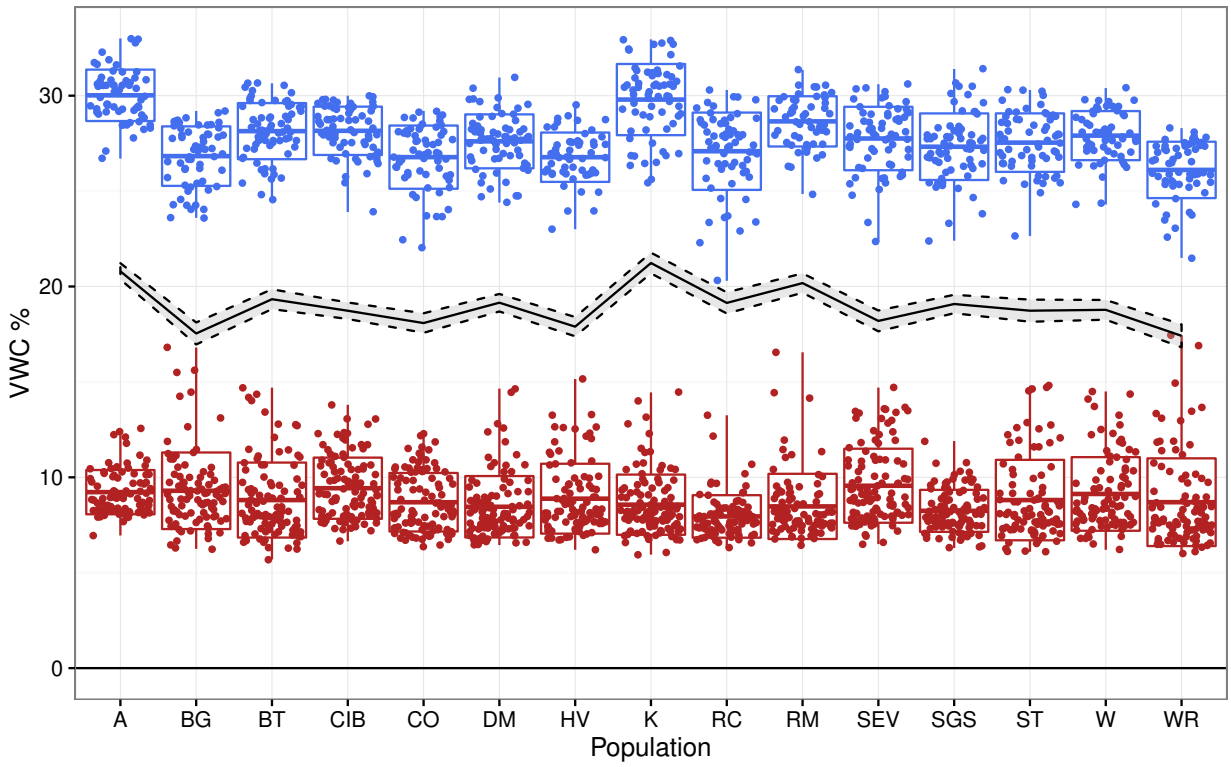

Figure III.2: Water treatments (water limited and non-water limited) were distinct. Data points in the figure below were collected from 20 August - 18 September.

12 bases in common with the Illumina adapters since adapter amplification during library preparation is possible. We then used filtered reads from the highest-coverage sample per site to assemble a representative de novo reference using RAxML clustering of similar tags. We then aligned all reads to this reference using SHRiMP, followed by a step to filter out any reads aligning with fewer than 32 bases to avoid ambiguity arising from matching with multiple tags.

We ran all models using Stan (Gelman et al., 2015) within R (R Core Team, 2018), discarding 25,000 iterations as burn-in and retaining 25,000 model iterations for the posterior distribution. We confirmed convergence of all parameters with Rhat values of $\sim$1. We also validated our model with a posterior predictive check; all observed data and posterior distributions overlapped appropriately (figures of these checks available upon request).

Table III.1: Common genotypes found across *B. gracilis* sites.

	MLG 126	**MLG 14**	**MLG 70**	**MLG 159**	**MLG 204**	**MLG 18**	**MLG 168**	**MLG 45**
SGS	4	0	0	0	0	0	0	0
Andrus	3	0	2	0	0	0	0	0
Buffalo Gap	1	0	0	0	0	0	0	1
Beech Trail	2	0	1	0	0	0	0	0
Cibola	0	3	0	0	0	0	6	0
Comanche	0	0	0	0	0	7	0	0
Cedar Point	4	0	0	0	2	0	0	2
Davidson Mesa	0	0	0	0	0	0	0	0
Heil Valley	2	0	0	0	0	0	0	0
Kelsall	3	0	0	0	0	0	0	0
Konza	2	0	0	0	6	0	0	0
Rock Creek	1	0	1	0	0	0	0	0
Rabbit Mountain	1	1	2	0	0	0	0	0
Sevilleta	0	0	0	10	0	0	0	0
Steele	2	0	0	0	0	0	0	3
Wonderland	2	0	5	0	0	0	0	0
Walker Ranch	0	14	0	0	0	0	1	0
Total	27	18	11	10	8	7	7	6

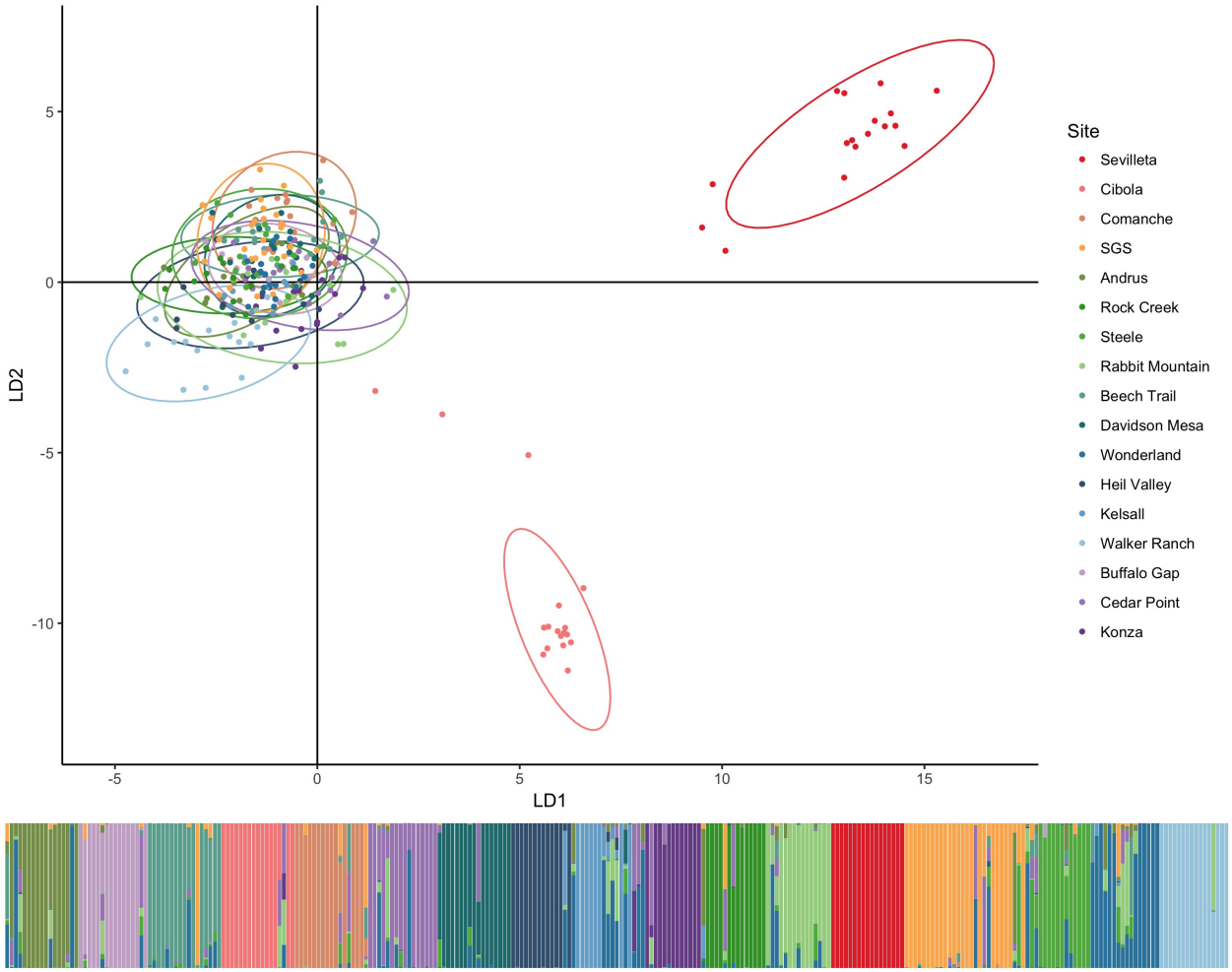

Figure III.3: *Bouteloua gracilis* sites clustered distinctly in space based on the genome. Plot space was determined using DAPC, where discriminant functions LD1 and LD2 account for 57% of the variance. "Structure" lines reveal the posterior probability of site assignment of each individual, where solid lines indicate clear site assignment and mixed lines indicate admixed individuals. Probabilities of assignment by site; Sevilleta: 1.00, Cibola: 0.94, Comanche: 0.88, SGS: 0.82, Andrus: 0.82, Rock Creek: 0.82, Steele: 0.80, Rabbit Mountain: 0.87, Beech Trail: 0.88, Davidson Mesa: 0.94, Wonderland: 0.75, Heil Valley: 0.87, Kelsall: 0.62, Walker Ranch: 0.94, Buffalo Gap: 0.94, Cedar Point: 0.81, Konza: 0.75.

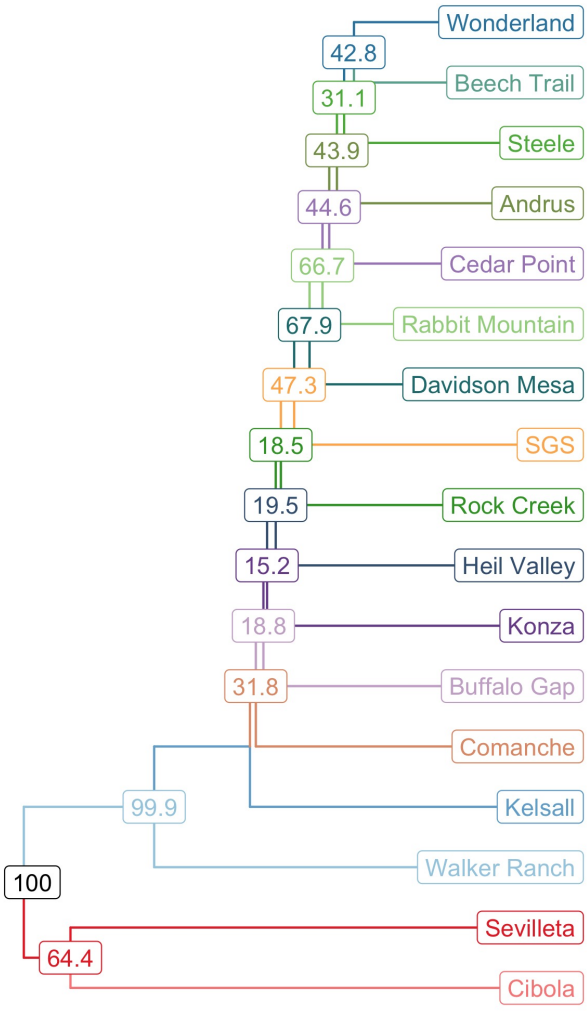

Figure III.4: Hierarchical relationship among *B. gracilis* sites based on the genome. Numbers indicate bootstrapped support (percent) for each individual node.

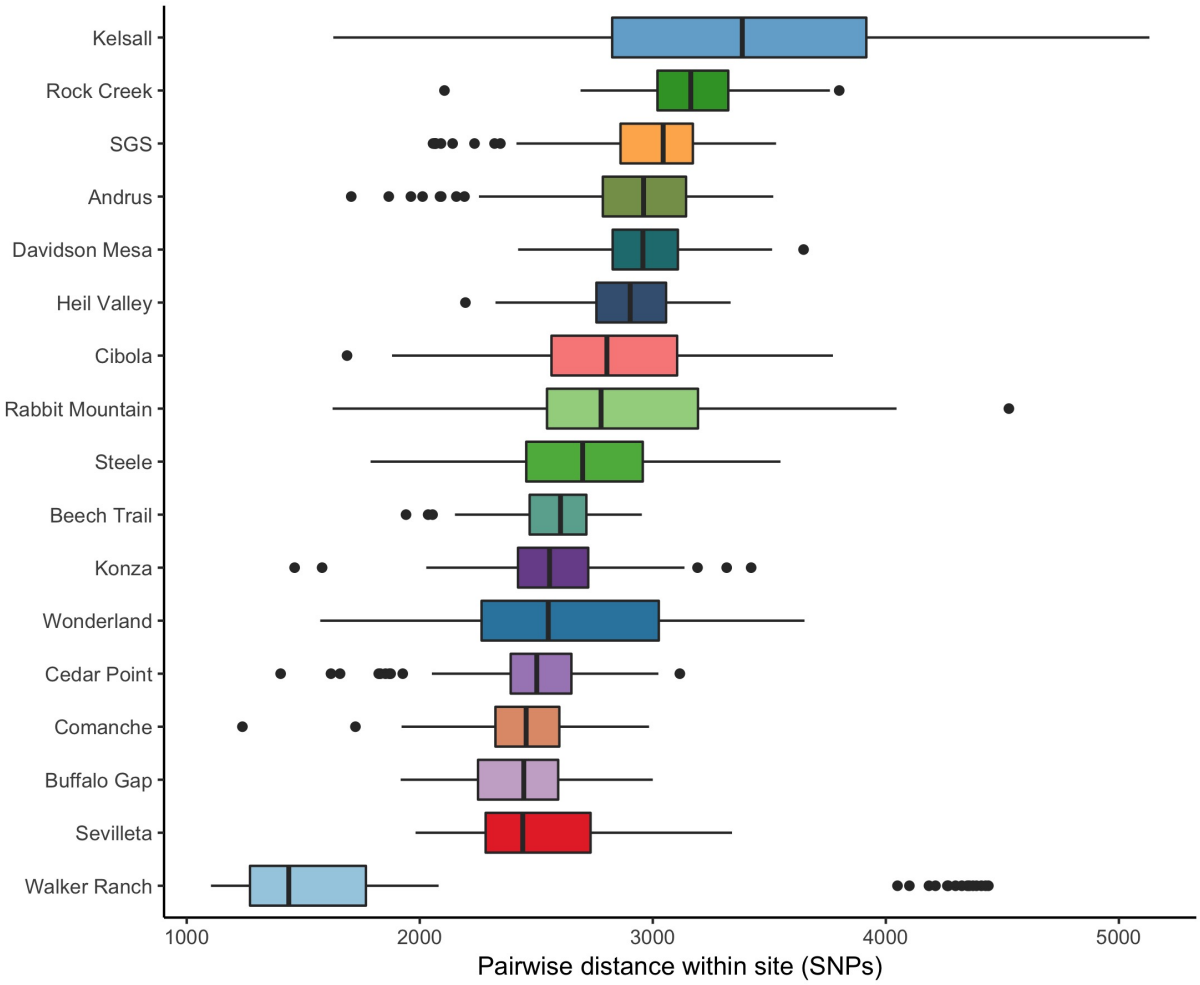

Figure III.5: Pairwise genomic distance within site.

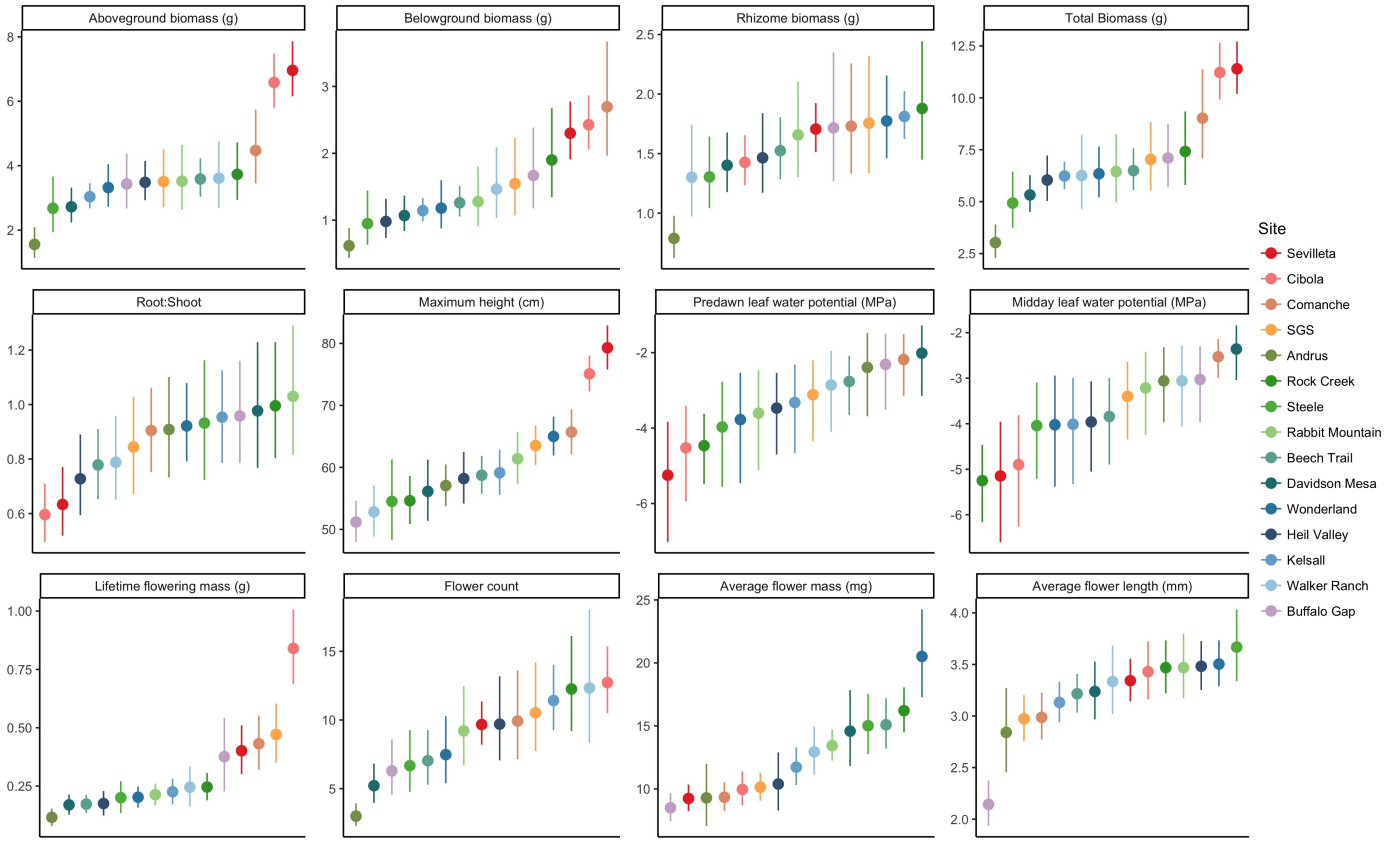

Figure III.6: Traits were distinct across sites in a common environment, indicating genetic divergence. Bars represent trait distributions at the mean soil %VWC. Circular points represent the mean and error bars represent the 95% CI. Shades of green and blue represent Boulder, CO sites. Sites are organized from red-purple based approximately on aridity index.

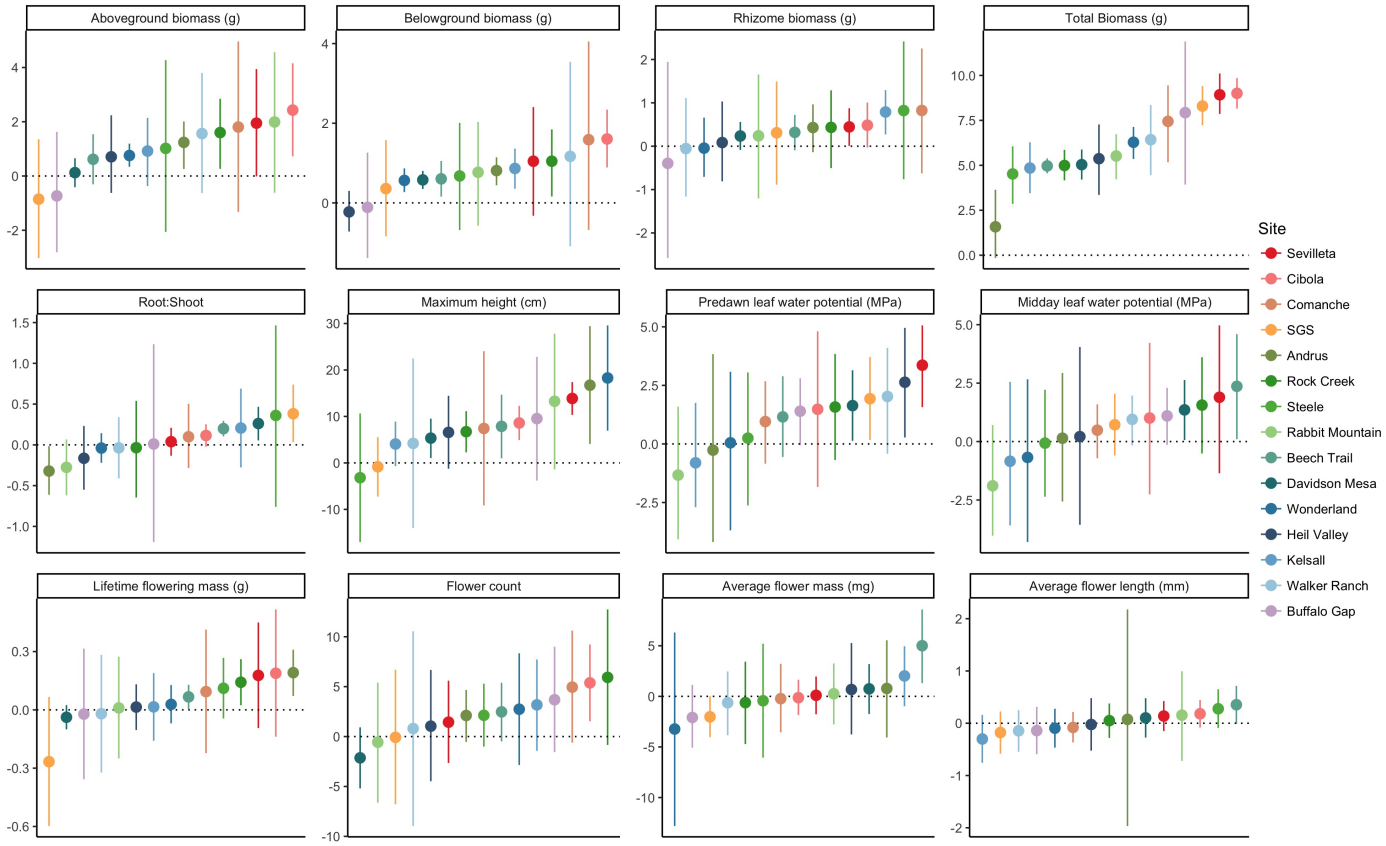

Figure III.7: Plasticity of traits was distinct across sites in a common environment, indicating genetic divergence. Circular points represent the mean and error bars represent the 95% CI. Credible intervals deviating from zero indicate significant plasticity. Shades of green and blue represent Boulder, CO sites. Sites are roughly organized from red-purple based on aridity index.

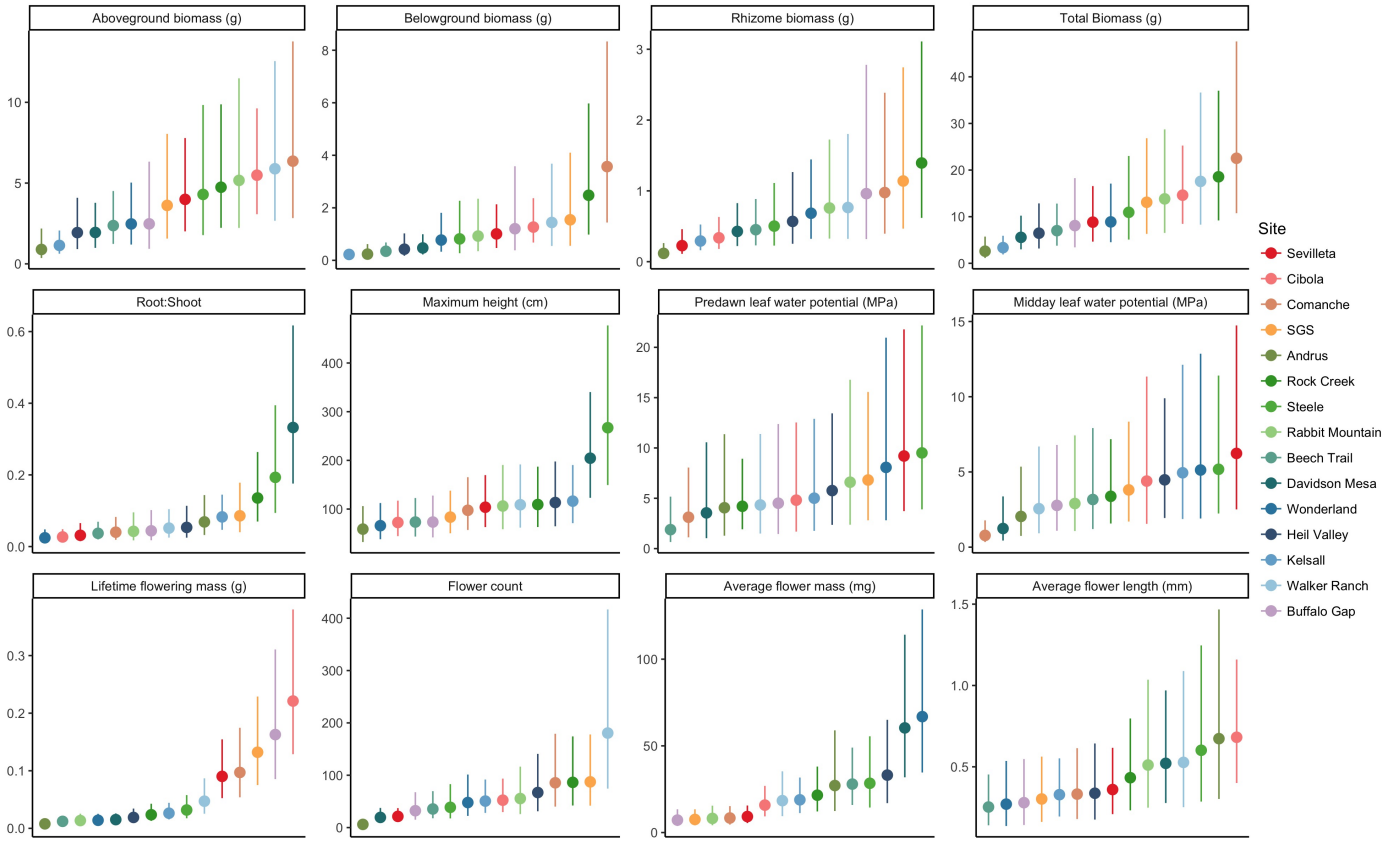

Figure III.8: Trait variance within site was distinct across sites in a common environment. Circular points represent the mean and error bars represent the 95% CI. Shades of green and blue represent Boulder, CO sites. Sites are roughly organized from red-purple based on aridity index.

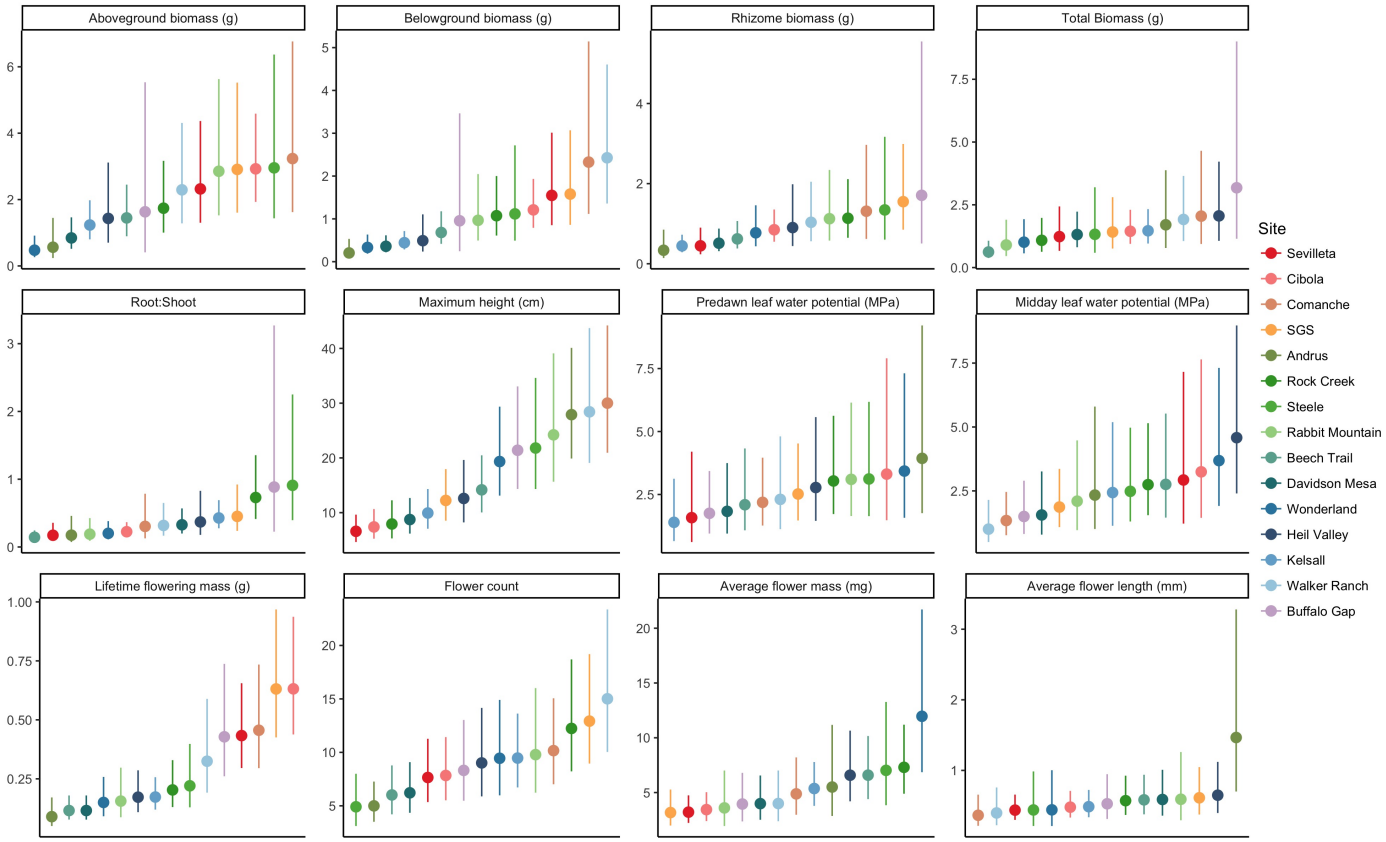

Figure III.9: Trait plasticity variance within site was distinct across sites in a common environment. Circular points represent the mean and error bars represent the 95% CI. Shades of green and blue represent Boulder, CO sites. Sites are roughly organized from red-purple based on aridity index.

Table III.2: Results of linear models comparing genome variance and trait or plasticity variance. Adjusted p-values were generated using Bonferroni correction.

Predict	Response	Estimate	t value	p-adj
genotype evenness	total biomass variance	-10.530	-1.480	1.000
genotype richness	total biomass variance	-0.612	-1.259	1.000
heterozygosity	total biomass variance	139.054	0.334	1.000
genotype evenness	aboveground biomass variance	-4.102	-2.003	0.797
genotype richness	aboveground biomass variance	-0.252	-1.797	1.000
heterozygosity	aboveground biomass variance	96.204	0.772	1.000
genotype evenness	total biomass plasticity variance	-1.195	-1.597	1.000
genotype richness	total biomass plasticity variance	-0.085	-1.714	1.000
heterozygosity	total biomass plasticity variance	-17.169	-0.387	1.000
genotype evenness	aboveground biomass plasticity variance	-1.247	-1.054	1.000
genotype richness	aboveground biomass plasticity variance	-0.081	-1.015	1.000
heterozygosity	aboveground biomass plasticity variance	70.576	1.099	1.000