GETTING TO THE ROOT OF CHANGE:

HOW PLANTS RESPOND TO NOVEL CLIMATES, SOILS, AND SOIL BIOTA

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ABSTRACT

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HOW PLANTS RESPOND TO NOVEL CLIMATES, SOILS, AND SOIL BIOTA

Global climate change is having profound and widespread effects on plant growth and survival. For the southwestern United States, warmer temperatures, more variable precipitation and more extreme droughts are expected. As plant populations experience these changes they may adapt and persist in place or may experience increasing environmental stress, eventually leading to mortality. An interesting component of environmental change is that different edaphic conditions may mitigate or exacerbate changes in the environment. As an example, coarse soils with low water holding capacity may exacerbate a change in water availability. Additionally, soil biota may play a critical role in facilitating plant survival during environmental change. Mycorrhizal fungi and plant growth promoting rhizobacteria both have been shown to have an impact on plant water uptake and physiological regulation. Interestingly, plants migrating to new locations maybe experiencing different novel environments by migrating across edaphic boundaries. Novel edaphic environments may have vastly different physical and chemical properties to which plant populations are adapted to. Furthermore, plant migration often occurs independently of the migration of associated soil microbes, including mycorrhizal fungi. Both arbuscular mycorrhizal (AM) fungi and ecto-mycorrhizal (EM) fungi play important roles in plant nutrient and water uptake. While plant responses to changes in climate, or even soils are fairly well understood, few studies have examined the impact of simultaneous change in climate, soil, and soil biota on plant performance

To better understand adaptation to novel environments, the grass *Bouteloua gracilis* was grown at six field sites: two natal source sites, a +2°C site, a +3°C site, a -2°C site and -3°C site where the warmer sites simulate *in situ* warming and precipitation changes whereas the cooler sites simulate plant migration. In these papers we define home as soil communities from the plants site of origin, and away as soil communities from the transplant site. Plants at all of the transplant sites were then grown in the following combinations of soil and soil biota: 1) home soil, home soil biota, 2) away soil, home soil biota, 3) home soil, away soil biota and 4) away soil, away soil biota. Home refers to soil or soil biota from the same site as the plant, whereas away represents soil or soil biota from the transplant site. We found plants generally grew more in cooler/wetter environments than in warm/dry environments. In warm/dry environments, we also found that home soil biota generally facilitated plant growth and plants were larger than those grown with away soil biota. Away soils originating from one site in particular, had a dramatic negative effect on plant growth. In general, our results demonstrate that warmer temperatures have a negative effect on plant growth that can be mitigated partly by plant associated soil biota.

In order to better understand plant physiological responses to changes in environment, we conducted a similar, parallel study with the tree *Pinus ponderosa* where we grew *P. ponderosa* at three field sites: one natal source site, a +2°C site and a -2°C site. We used the same treatment combinations described above. We monitored plant growth and leaf physiology metrics during the monsoon season. Trees grown at the +2°C site grew as large as those grown at the home site when they had their home soil biota, but not when they had their away soil biota. Trees with their home soil biota maintained nearly 2× the maximum net photosynthetic rate and stomatal conductance rate than those grown with their away soil biota. These results imply that

home soil biota play a critical role in either water uptake or physiological regulation and away soil biota do not have the same effect.

Lastly, we conducted a third experiment to more closely examine how the plant symbiosis with home soil biota influence plant growth differs from that with away soil biota. In this experiment, we grew the grass *Bouteloua gracilis* from a relatively wet and relatively dry site with either home or away soil biota. We then subjected plants to a watering regime that simulated or moderate drying or extreme drying and monitored plant growth. At the termination of the experiment we recorded fungal structures colonizing plant roots. We observed that home plant-soil biota combinations grew larger and had a greater portion of roots colonized by AM fungi structures for nutrient exchange and uptake (hyphae and arbuscules). In contrast, away plant-soil biota combinations resulted in a greater portion of roots colonized by less beneficial AM fungi structures that are used for fungal carbon storage (vesicles). These results may indicate that home plant-fungal pairings generally have greater mutualistic function, partially due to fungal allocation.

Plants responding to changes in their environment will be exposed to a wide array of scenarios and thus exhibit a wide range of responses. In general, our studies indicate that soil biota mitigate some of the negative effects of warmer drier environments on plant growth. We also demonstrate that plants migrating to novel cooler and wetter environments are much less dependent on these soil biota, however, edaphic boundaries are likely to be a barrier to plant growth with certain soil environments a greater barrier than others.

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Dedicated to

Nathan Nieto

Who has always had positive spirts while battling cancer and who has been a friend and an adviser in life and academia

Preface

This dissertation is presented in journal format and consists of three chapters which will be submitted for publication to scientific journals. Some redundancy between chapters was necessary for cohesion of data and results. The pronoun "we" instead of "I" was applied to data chapters II, III, IV to denote multiple authors for each publication.

Chapter II is entitled "*Bouteloua gracilis* responses to novel climates, soils, and soil biota: Using an environmental gradient to better understand plant responses to change," and is formatted for submission to the journal, *New Phytologist*. Chapter III is entitled "Familiar soil conditions help Pinus ponderosa seedlings cope with warming and drying climates," and is formatted for submission to the journal, *New Phytologist*. Chapter IV is entitled "Sympatric pairings of dryland grass populations, mycorrhizal fungi, and associated soil biota enhance mutualism and ameliorate drought stress," and is formatted for submission to the journal, *New Phytologist*. Based on reviewer feedback, Chapter IV uses the terminology sympatric in place of home and allopatric in place of away.

Chapter I

Introduction

Climate change is resulting in plants experiencing novel environments relative to the environment to which they are currently adapted (Thompson, 2000). For the Southwest, global climate change is expected to manifest warmer temperatures and more variable precipitation with an overall trend towards drier conditions and more extreme droughts (Seager *et al.*, 2007; Cayan *et al.*, 2010). Given that many plants are adapted to their local climate, climate change creates a major change for vegetation (Miller & Fowler, 1994; Joshi *et al.*, 2001; Richardson *et al.*, 2014; Kraemer & Kassen, 2016; Peterson *et al.*, 2016; Bucharova *et al.*, 2017). As a result of climate change, plant populations can either adapt in place, migrate to a new environment, or die (Breshears *et al.*, 2005, 2009; Allen *et al.*, 2010; Hällfors *et al.*, 2016; Bjorkman *et al.*, 2017; Tietjen *et al.*, 2017). Plants are also sometimes locally adapted to their soil environment (Pregitzer *et al.*, 2010; Smith *et al.*, 2012; Rúa *et al.*, 2016). In some cases, plant migration may result in plants crossing edaphic boundaries in order to seek climate refuge (Damschen *et al.*, 2012; Roberts & Hamann, 2016). Soil properties influence plant growth in a variety of ways including nutrient availability and water availability, and as a result certain soil types may be barriers to plant migration (Johnson *et al.*, 2010; Bowker *et al.*, 2012; Bjorkman *et al.*, 2017). Likewise, different soil types may exacerbate or mitigate the effects of a warming environment by influencing plant available water or nutrients. Furthermore, biotic interactions are often not regarded in studies of plant local adaptation to climate and soil, thus soil biota may also exacerbate or mitigate the effects of climate change on plant communities (Rúa *et al.*, 2016;

Gehring *et al.*, 2017). Indeed, plant migration often occurs independently of soil microbe migration resulting in migrating plants experiencing a novel suite of soil biota (Mangan & Adler, 2002; Lekberg *et al.*, 2007; Abbott *et al.*, 2015; Bucharova, 2017). Of course, plants adapting to climate change *in situ* may also experience varying effects of soil biota from negative to positive (van der Putten *et al.*, 2016; Revillini *et al.*, 2016; David *et al.*, 2018; Fry *et al.*, 2018).

Negative effects of soil biota on plant growth are often observed as the result of pathogens. Plants grown in their own soil may accumulate pathogens, which in turn negatively impact the plant, thus limiting growth relative to its potential in sterile soil (Mills & Bever, 1998; Klironomos, 2002; Stoel *et al.*, 2002; Reinhart *et al.*, 2005; Reinhart & Callaway, 2006; Mangan *et al.*, 2010a,b; Bezemer *et al.*, 2013; Pizano *et al.*, 2014; Maron *et al.*, 2016). Plant-soil biota interactions are becoming a widely accepted phenomenon as a process that promotes biodiversity independently of plant-plant competition (Mangan *et al.* 2010a, 2010b; Mack & Bever 2014; Schaminée *et al.* 2015) . Simultaneously, plants also associate with beneficial soil biota such as arbuscular mycorrhizal (AM) fungi and ectomycorrhizal (EM) fungi (Gerdemann, 1968; Daniels & Trappe, 1980; Krikun & Levy, 1980; Laret *et al.*, 1980; Levy & Jakrikun, 1980; Pang & Paul, 1980; Parke *et al.*, 1983; Osonubi *et al.*, 1991; Gehring *et al.*, 1998; Lehto & Zwiazek, 2011; Reininger & Sieber, 2012). These interactions may facilitate plant adaptation and survival in high stress environments where resource availability is low (Johnson, 2010; Johnson *et al.*, 2010; van der Putten *et al.*, 2016; Revillini *et al.*, 2016; David *et al.*, 2018; Fry *et al.*, 2018). Furthermore, a complex suite of bacteria known as plant growth promoting rhizo-bacteria also interact with mycorrhizas and plants and have been shown to mitigate drought stress (Rubin *et al.*, 2017). Studies have shown that plants and their associated mutualists tend to have a greater mutualistic function when they share a evolutionary history and are co-adapted to one another

and their environment (Piculell *et al.*, 2008; Hoeksema, 2010; Johnson *et al.*, 2010; Revillini *et al.*, 2016; Rúa *et al.*, 2016; Gehring *et al.*, 2017; Hoeksema *et al.*, 2018). These microbes tend to influence plant growth by altering plant nutrient status, water availability and plant hormonal regulation of photosynthesis (Safir *et al.*, 1971, 1972; Levy & Jakrikun, 1980; Allen *et al.*, 1981; Allen, 1982; Elen and Allen, 1986; Sanchez-Díaz & Honrubia, 1994; Johnson *et al.*, 1997, 2003; Augé, 2001, 2004; Lehto & Zwiazek, 2011; Birhane *et al.*, 2012; Hodge & Fitter, 2013; Castle *et al.*, 2016). Furthermore, studies have also shown that like plants, soil microbes may also be locally adapted to their soil and climatic environments (Vos *et al.*, 2009; Rúa *et al.*, 2016; Kraemer & Boynton, 2017).

Many of these plant-soil biota interactions are well understood in controlled experiments, however, few studies have closely examined ideas of plant adaptation to climate and soil while also simultaneously examining soil microbe-plant coadaptation (Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2013; Revillini *et al.*, 2016; Rúa *et al.*, 2016). In this dissertation, we conduct three field experiments to better understand plant adaptation to climate, soil and soil biota and plant physiological responses to novel environments and novel soil biotic communities, we also closely examine how plant mycorrhizal function varies in novel symbioses.

In chapter II, we conducted a field study to investigate the responses of *Bouteloua gracilis* growing in novel environments. We grew plants from seed collected at two source, or 'home' sites and outplanted plants to sites that vary in temperature from +2°C warmer and +3°C warmer to -2°C cooler an -2°C cooler than the home site. At each outplant (away) site we grew plants in the following treatment combinations of soil and soil biota: 1) home soil, home soil biota, 2) away soil, home soil biota, 3) home soil, away soil biota and 4) away soil, away soil biota. We closely monitored plant growth over three growing seasons to determine their overall

responses to these novel environments. At the termination of the experiment, we collected final biomass and root biomass to more thoroughly understand plant responses.

In chapter III, we conducted a field study on *Pinus ponderosa* growing in novel environments. We grew trees from seed collected at one home site and transplanted trees to sites +2°C warmer and -2°C cooler than the home site. At each away site we grew plants in the following treatment combinations of soil and soil biota: 1) home soil, home soil biota, 2) away soil, home soil biota, 3) home soil, away soil biota and 4) away soil, away soil biota. In this study, in addition to closely monitoring plant growth, we also monitor net photosynthetic rate, stomatal conductance, and florescence change in novel environments and with novel soil biota.

In chapter IV, we conducted a greenhouse study with *Bouteloua gracilis* to more closely examine how plants respond to novel soil biota. In this study, we grew *B. gracilis* individuals from seed from a relatively wet and a relatively dry population. We then inoculated plants with soil biota communities from each site in home and away combinations. During the experiment we monitored plant growth, and at the termination of the experiment we closely examined fungal growth in the soil while also examining the portion of plant roots colonized by fungal structures.

In chapter V, I present the overall conclusions of chapters II, III, and IV, and address the implications of these results in the context of climate change and land management. These studies provide insight into how plants respond to novel climatic, edaphic and biotic environments while expanding our understanding of how many facets of a changing environment influence plant growth.

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Chapter II

Novel climates, soils, and soil biota interact to diminish performance of Bouteloua gracilis

Abstract

Climate change is altering temperature and precipitation resulting in widespread plant mortality and shifts in plant distribution. Plants responding to such changes may experience exacerbated effects of shifts in available water in soil types with less water holding capacity. Furthermore, complex biotic interactions between plants and soil organisms may mitigate or exacerbate the changes experienced as a result of climate change. We grew *Bouteloua gracilis* ecotypes from two sites and outplanted individuals across an environmental gradient with either their original home soil or with the different soil of the transplant site. We also moved plants with their soil biotic communities or forced them to grow in the different soil biotic community of the transplant site to test 1) how changes in climate alone influence plant growth, 2) how soil types interact with climate to influence plant growth, and 3) the role soil biota play in facilitating plant growth in novel environments. At warmer drier sites, we observed general decreases in plant growth, plant specific leaf area and plant fitness, however, home soil biota often mitigated these negative effects. At cooler, wetter sites……We also found some soil types to have strong negative effects on plant growth regardless of soil biota. One soil type in particular, was derived from basalt cinders and was particularly harsh for plant growth.

Introduction

Plant populations are being confronted with emerging novel environments throughout the world. Climate change is the major driver of this phenomenon, already creating, and expected to continue to create novel environments for plants as warmer temperatures and more variable precipitation patterns arise in most regions (Kharin *et al.*, 2013; Sillmann *et al.*, 2013). One possible response of plant populations growing in novel climates as a result of climate forcing is to migrate to environments more similar to those they are adapted to (Aitken *et al.*, 2008; Roberts & Hamann, 2016). Plant species distributions have already demonstrated an upward shift or "lean" in altitude in response to warming (Breshears *et al.*, 2008; Feeley *et al.*, 2011). However, novel climates are not the only relevant facet of emerging novel environments. Interestingly, the migration response can help plants maintain a climate niche that is similar to that of their evolutionary history, but also may expose plants to novel edaphic conditions or novel biotic environments (Bucharova *et al.*, 2016; Butterfield *et al.*, 2016; Bucharova, 2017).

Seeds of plant populations in the process of shifting their distributions will may cross edaphic boundaries, thus plant movement to maintain a similar climate niche could induce exposure to different soils (Hoopes & Hall, 2002; Bowker *et al.*, 2012; Sanderson *et al.*, 2015). In many regions, a complex history of geological processes lead to a mosaic of widely different soil parent materials that change at scales ranging from meters to kilometers (Jenny, 1941). Distinct parent materials give rise to soils with distinct physical and chemical properties. Novel edaphic environments are not constrained only to changes in the abiotic soil environment, but also encompass distinct soil biotic communities from those of the plant population's evolutionary environment. Plant migration often occurs independently of plant associated microbes, including

co-adapted symbionts, and other biotic interactions (Knevel *et al.*, 2004; Eppinga *et al.*, 2006; Andonian *et al.*, 2011; Bagchi *et al.*, 2014; Müller *et al.*, 2016b,a). Thus, colonizing individuals at the migration front of plant population must establish interactions with biota with they may have recent history of co-occurrence. While the effects of novel climatic environments are being widely explored, we have a paucity of studies able to separate the relative influence of multiple facets of emerging novel environments, spanning climate and soil.

Plant populations may become strongly locally adapted to their environment and often perform best in climates and soil environments that resemble their evolutionary environment (their "home" conditions) than in novel ("away") environments (Byars *et al.*, 2007; Pregitzer *et al.*, 2010, 2013). The role of soil biota in determining plant success is widely documented, ranging from beneficial effects of root symbionts such as mycorrhizal fungi, negative effects of pathogens and a variety of influences arising from complex soil food webs (Pineda *et al.*, 2010; Smith *et al.*, 2010; Johnson & Graham, 2013; Paz *et al.*, 2015). Plants and home soil biota generally interact in a more mutually beneficial way than plants interacting with away soil biota (Johnson *et al.*, 2010; Rúa *et al.*, 2016; Gehring *et al.*, 2017). Evidence suggests that biotic interactions are often the product of coevolution, thus plants and their associated home soil biota often perform better as a whole when the environment is most similar to how the interaction evolved (Piculell *et al.*, 2008; Hoeksema *et al.*, 2010, 2018; Brockhurst & Koskella, 2013; Rúa *et al.*, 2016). Just as abiotic environmental factors are a selective agent in plant evolution, they also may be selective of the interactions between plants and soil biota.

The emergence of any facet of a novel environment may put a plant population, adapted to a different set of conditions, under stress due to its potential maladaptation to the new conditions. Warming, for example, could induce greater levels of stress resulting in a more harsh

environment (Kharin *et al.*, 2013; Yamori *et al.*, 2014; Gremer *et al.*, 2015). Alternatively, plant migration could introduce plants to a novel soil environment or to an environment that is slightly cooler and wetter than their home environment. Plants adapting to novel environments may alter their traits in order to acclimate to the new environment. Stress imposed by a novel environment could be offset by more beneficial interactions between plants and soil biota, including plant growth promoting rhizobacteria and mycorrhizal fungi (Sanchez-Díaz & Honrubia, 1994; Revillini *et al.*, 2016; Rubin *et al.*, 2017). These soil biota are known to contribute to plant water uptake and alleviate drought stress with greater mutualistic function in coevolved mutualisms (Ruiz-Lozano & Azcón, 1995; Ruiz-Lozano & Azcón, 1996; Nilsen *et al.*, 1998; Warren *et al.*, 2008; Smith *et al.*, 2010; Ruth *et al.*, 2011; Bárzana *et al.*, 2012). These studies suggest that soil microbes may be critical facilitators for plant growth in a novel climate induced by global change, however, they may have less of a role for plants alleviating stress by migrating to more benign environments (Revillini *et al.*, 2016). A key factor in a plant's ability to acclimate to novel environments could be a plant's associated soil biota.

To test plant responses to a variety of novel environments we designed a field experiment in Northern Arizona using the Southwest Experimental Garden Array (SEGA) (https://sega.nau.edu/home). We selected *Bouteloua gracilis* as our focal species because of its widespread distribution and its known associations with arbuscular mycorrhizal (AM) fungi as well as it being documented as locally adapted (Wood et al. 2016). We identified two natal sites where *Bouteloua gracilis* was abundant and four transplant sites that were approximately two and three degrees centigrade warmer to simulate warming and two and three °C cooler to simulate plant migration to cooler environments. We grew plants at all sites with all possible combinations of natal and novel edaphic environment, and natal and novel soil biota. This

allowed us to simultaneously manipulate soil, soil biota, and climatic environments to better understand how plants respond to various facets of novel environments. We generated predictions in regards to *1) Plant growth phenology and 2) Plant traits and fitness.* We hypothesized that plants grown in novel climatic environments would consistently be smaller than plants grown in their home climatic environment, and that plants grown in novel environments would be less green during the growing season. We predicted that plants grown in novel soil environments will be smaller than plants grown in their home soil environment and that soil biota may mitigate some of these effects in novel environments resulting in plants more similar in size and greenness to plants at their home site. We also measured a morphological trait, specific leaf area, as specific leaf area is often a correlate with precipitation (Westoby, 1994). We also predicted that specific leaf area will be correlated with available water and thus be lowest at the warmest driest sites and in soils that have poor water holding capacities. We also hypothesize that seed mass, a metric of plant fitness, will be lowest in novel environments.

Methods

Plant and soil source sites

We conducted our study using Northern Arizona University's Southwest Experimental Garden Array (SEGA). The SEGA is a collection of experimental sites situated on a climate gradient spanning six degrees centigrade. We used the Seeds of Success (http://www.nps.gov/planTs/sos/protocol/index.htm) protocol to collect seeds of *Bouteloua gracilis* from two home sites, while soil was collected from the same two home sites and an

additional four away sites, creating a total of six sites. Detailed information about each site is listed below:

Blue Chute (Home Site One):

Blue Chute (BC) (35.58°, -111.97°) is a Piñon-Juniper woodland with an understory dominated by *Bouteloa gracilis* located adjacent to Red Mountain (Coconino County, AZ) at an elevation of 1,930m. BC receives approximately 478 mm of precipitation a year with an average minimum annual temperature of 0.9°C and average maximum annual temperature of 18.6°C (PRISM Climate Group, Oregon State University). These soils are ulstalfs with a clay loam texture (Soil Survey Staff, Natural Resource Conservation Service).

White Pockets Canyon (Home Site Two):

White Pockets Canyon (WPC) (36.61°,-112.41) is a Piñon-Juniper woodland with an understory dominated by a mix of perennial C4 grasses including *Bouteloua gracilis* located on the west side of the Kaibab Plateau (Coconino County, AZ) at an elevation of 2,057m. WPC receives approximately 443 mm of precipitation a year with an average minimum annual temperature of 4.0°C and average maximum annual temperature of 19.0°C (PRISM Climate Group, Oregon State University). The soil at WPC is derived from Kaibab limestone and is an argid soil with a gravelly loam texture (Soil Survey Staff, Natural Resource Conservation Service).

Black Point (Away site):

Black Point (BP) (35.68°, -111.48°) is a desert shrubland dominated by *Atriplex canecens* located adjacent to Grey Mountain Quarry (Coconino County, AZ) at an elevation of 1,566m). BP has *Bouteloua eriopoda* and a very small population of *B. gracilis*. BP receives an average of approximately 152 mm of precipitation a year with an average minimum temperature of 5.0°C

and average maximum annual temperature of 21.0°C (PRISM Climate Group, Oregon State University). The soils at BP are derived from a mix of young basalt cinders and Moenkopi Sandstone. These soils are orthents and have a loamy sand texture (Soil Survey Staff, Natural Resource Conservation Service).

Walnut Creek (Away site):

Walnut Creek (WC) (34.92°, -112.84°) is a upland riparian habitat dominated by *Juglans major* located adjacent to Hyde Mountain (Yavapai County, AZ) at an elevation of 1,567m. WC understory species are dominated by *B. gracilis* and several other C4 grasses. WC receives approximately 397mm of precipitation a year with an average minimum annual temperature of 3.0°C and an average maximum annual temperature of 22°C (PRISM Climate Group, Oregon State University). Soils at WC are derived from a mixed alluvium. These soils are argids and have a sandy loam texture (Soil Survey Staff, Natural Resource Conservation Service).

Little Mountain (Away Site):

Little Mountain (LM) (36.58, -112.36) is a Ponderosa Pine forest with an understory dominated by a mix of C3 and C4 grasses located adjacent to Little Mountain (Coconino County, AZ) at an elevation of 2,276m. LM receives 502 mm of precipitation annually with an average minimum annual temperature of 1.0°C and average annual maximum temperature of 16.0°C (PRISM Climate Group, Oregon State University). Soils at LM are derived from Kaibab limestone and are ustolls with a loam to gravelly clay loam texture (Soil Survey Staff, Natural Resource Conservation Service).

Arboretum at Flagstaff (Away site):

The Arboretum at Flagstaff (ARB) (35.16, -111.73) is an open meadow surrounded by Ponderosa Pine forest with a diverse mix of perennial grasses and forbs. ARB is located adjacent

to Woody Mountain (Coconino County, AZ) at an elevation of 2,179m). ARB receives approximately 556mm of precipitation annually with an average minimum annual temperature of -1.0°C and an average maximum annual temperature of 16.0°C (PRISM Climate Group, Oregon State University). Soils at ARB are derived from basalt. These soils are ustolls with a sandy clay loam texture (Soil Survey Staff, Natural Resource Conservation Service).

Preparation of Experimental Units

We focused on two home populations of *Bouteloua gracilis* from BC and WPC. These sites were identified as source sites because *B. gracilis* is native and abundant and these sites are in the middle of the climate gradient on SEGA, thus allowing us to easily manipulate climate in both a warming and cooling direction by transplantation. In addition to seed collections, we made two types of soil collection from all of the sites: background soil, and live inoculum soil. Background soil was collected in bulk at depths of 0-60 cm from each site, away from the rhizosphere of living plants, and homogenized. This soil was s sterilized at 125°C for 24 hours two times. Live inoculum soil was collected from all sites by picking a random starting point and collecting soil every five meters for 90m in each cardinal direction. This soil was stored refrigerated for six weeks until it was used. Live inoculum soil was collected from the rhizosphere of target plants at depths of 0-30 cm and later homogenized. Target plants were *Bouteloua gracilis* at the home sites and when present at the away sites; however, if *Bouteloua gracilis* was absent or uncommon at the away site, soil was collected from rhizospheres of any living herbaceous vegetation, such as *Hillaria jamesii* and *Ipomopsis reacemosa*. To prepare each experimental unit, we filled Steuwe & Sons TP812 7.8L tree pots with 7.5L of sterile

background soil. This pot size was selected because it could accommodate years of growth of *Bouteloua gracilis* in semi-arid environments. Each pot was then topped with a 2 cm thick band of inoculum soil. The source of the background and inoculum soils were varied as experimental factors (see *Creating novel edaphic environments below)*. We sprinkled *Bouteloua gracilis* seed collected from the two home sites onto inoculum soil at a density of 20 seeds per pot and covered them with one cm of background soil and later thinned to 1 seedling per mesocosm. We grew seedlings from November 2014 until Late April 2015 under a standard nursery watering regime that maintained soil that was damp to the touch. This meant watering approximately every 48-72 hours in the greenhouse prior to out-planting to field sites to ensure that we did not stress seedlings and induce premature mortality.

Creating home and away edaphic environments

We designated each experimental unit to be field-planted either in its home site, or one of four possible away sites with novel climatic environments (detailed in Creating novel climates, below). Based on these designations, we created pots featuring multiple combinations of soil and soil biota relative to each plant population and transplant site. Each plant population was grown in experimental units with home background soil, and home inoculum soil (and therefore home soil biota) to create a home edaphic environment. We also created various away combinations, supplanting either background soil or inoculum soil, or both, from away sites to generate novel soil environments and novel soil biota. This design created three types of novel edaphic environment to compare to the home edaphic environment: home soil and away soil biota, away soil and home soil biota, and away soil and soil biota. We replicated each combination of these

four types of soil environment 10 times for each away site for each plant population, in addition to preparing another 10 units of the home edaphic environment for planting at the home site. This resulted in 170 experimental units for each of the two plant populations and a total of 340 experimental units.

Creating home and away climates

To expose plant populations to home and away climates, we selected our out-planting sites along the climatic gradient of the SEGA. Ten replicates of each plant source, growing in a home edaphic environment, were planted back into their respective homesite. We planted each unit, without removing plant and soil from the pot, into the ground so that soil level within and outside of each pot was approximately equal. These units represented home climate and home soil environments and were used as a frame of reference for all other treatments. The rest of the replicates were subjected to away climates by planting them in one of four sites that are approximately 2°C (BP) and 3°C (WAL) warmer, and 2°C (LM) and 3°C (ARB) cooler than the home sites (BC and WPC). All field plantings occurred on consecutive days in early May 2015.

Monitoring plant performance

We measured plant height and basal area, and ocular estimates of percent of plant tissue that was green in all experimental units three times per year, in spring, summer, and fall in 2015, 2016, and 2017. The spring 2015 sampling immediately followed out-planting to the field sites. If plants produced inflorescences, we counted the number inflorescences prior to seed set and then once seed was established we clipped each inflorescence and massed seeds.. At the

termination of the experiment all aboveground biomass was clipped, dried at 60°C for 24 hours and weighed. Root biomass was sampled by drying and weighing after excess soil was carefully cleaned from each sample using soaking and wet sieving. At the termination of the experiment we used final shoot biomass to develop our own allometric equations based on day of harvest height and basal area measurements in a multiple regression. The formula for allometric biomass is as follows:

$$B = -1.694 + 0.64(h) + 4.08\ (d)$$ ($r^2 = 0.92$).

Where B= biomass, h= height (cm) and d = basal diameter (cm).

This allowed us to back-estimate biomass through time, allometrically.

Statistical analysis

Four-way repeated measures ANOVA was used to compare the effects of plant origin, soil inoculum origin, soil origin, and climate on plant greenness for the duration of the field experiment. Three-way ANOVA was used to compare final plant biomass, soil biota, density of external AM hyphae, and percent root length colonized by AM fungi and DSEs. Differences within groups were determine using Tukey's HSD test. Model assumptions were checked using the Shapiro-Wilk test of normality and the Levene's test of heterogeneity of variance. All statistics were conducted in R (version 3.3.1). All data sets met all of the assumptions and no transformations were made.

Results

Plant Greenness

Plant greenness varied by season and year. Spring 2015 had the highest percentage of green plant tissue since this was immediately following the growing period in the nursery. During subsequent years, plant greenness was generally close to 0 in the spring with the exception of plants from the Blue Chute population grown in their home soil with away soil biota at the 2C warmer site, which attained > 40% greenness (Figure 2.1). Summer greenness was generally high (>75%) in 2015, 2016, however in 2017 summer greenness for all treatments was close to 15%. Away climates interacted with away edaphic environments, such that for the Blue Chute population, summer greenness was lowest for plants grown in away soil regardless of soil biota in the +2°C site whereas there was no climate, soil interaction effect for plants from the White Pockets population. Fall greenness was low in all years at all sites and for all treatment combinations (<25%). The magnitude of difference between treatments and years is also quite low. Interestingly, plants from the Blue Chute population at -2°C and -3°C sites had slightly higher fall greenness in away soil and away soil biota treatment combinations.

Plant Growth

Overall, allometric plant shoot biomass increased through time for most treatments as plants became mature, although some exceptions were observed as detailed below (Figure 2.2). Cooling climates generated comparable, or slightly greater growth compared to home climates. Differences among edaphic treatments were subtle under cooling climates. In direct contrast, there were stark differences among home and novel soil environments under warming climates and some instances of neutral biomass change of even loss of biomass through time. Under warmed climates, some treatments performed better, and some performed worse than plants

grown under both home climate and edaphic environment. Two distinctly different patterns emerged. First, regardless of plant population, edaphic environments featuring home soils outperformed those featuring away soils, under the +2°C novel climate in the final two years. Similarly, but more subtly, the White Pockets plant population performed best in home soils under the +3°C novel climate in the final year. The second major pattern was that the Blue Chute population produced more biomass in the away soil and home soil biota treatment in the +3°C, as early as summer of year 1 of the experiment.

Final plant shoot biomass was lowest at the +2° and +3°C warmed sites and largest at the cooler sites ($F=6.68$, $p<0.001$) (Figure 2.3). Plants from the Blue Chute population generally were 10-20% larger than plants from the White Pockets Population ($F=8.79$, $p=0.03$). Additionally, plants grown in away soil were generally smaller than plants grown in home soil with some soils decreasing plant growth as much as 60% and other soils having only subtle decreases ($F=23.34$, $p<0.01$). Within the +2°C site, away soil resulted in plants with 60% less biomass than those grown in home soil ($F=15.8$, $p<0.001$). Lastly, plants grown at the +3°C site from the Blue Chute populations grew 20% larger when paired with their home soil biota, regardless of the soil source ($F=24.674$, $p<0.001$).

Final root biomass also varied by site with the lowest root biomasses occurring at the +2°C and +3°C sites and the highest root biomass at the -3°C site ($F=24.66$, $p<0.001$). Root biomass was also 10% higher for the plants from the Blue Chute population ($F=13.2$, $p<0.001$). Similar to shoot biomass, root biomass was also lower for plants grown in away soil than home soil ($F=27.45$, $p<0.001$). Plants also had 30% more root biomass in home soil home soil biota treatment combinations than other treatments ($F=6.57$, $p=0.01$). There were no statistical

differences in root:shoot ratios, however, the variation was high in some treatment combinations (Figure 2.4.)

Specific Leaf Area

Specific leaf area generally decreased with decreasing precipitation with the +3C warmer site having the lowest SLA ($F=202.913$, $p<0.001$). We also observed a main effect of soil ($F=26.112$, $p<0.001$) as well as an interaction between soil and site ($F=90.0$, $p<0.001$). The soil effect, whereby home soils are associated with greater SLA, is most pronounced at the +2°C site (SLA is approximately doubled in association with home soils).. Further, there was an interaction effect of site, soil source, and source of biotic community ($F= 2.8$, $p=0.04$). This interaction appears related to larger than expected SLA values for plants paired with home soil and soil biota at +3°C sites. Finally, the plants from White Pockets had a 4% higher specific leaf area than those from Blue Chute ($F=6.816$, $p=0.01$) (Figure 2.5).

Inflorescence mass

There was no difference in the inflorescence mass among populations ($F=2.97$, $p=0.09$). Site had a significant impact on seed production with the +2°C site producing the smallest amount of seed ($F=3.7$, $p= 0.002$). The interactions between plant and site ($F=4.73$, $p=0.003$) and soil source and site ($F=4.78$, $p=0.002$) show that this pattern is at least partially driven by lower seed production in plants grown in the soil of the +2°C. There was also a significant interaction between site and soil biota source ($F=3.65$, $p=0.02$) where home soil biota resulted in up to 250% more inflorescence mass produced at +2C (Figure 2.6).

Discussion

Our results indicate that *Bouteloua gracilis* is responsive both to its climatic and edaphic environment. When plants were grown in warm and dry environments (+2°C and +3°C), plant biomass, specific leaf area, and plant fitness were generally lower than plants grown in their home environment or at away sites that are cooler and wetter (All figures). Some response variables, including SLA and seed mass, suggested a preference for cooler, less arid environments. Novel soil types often resulted in lower plant growth and fitness than home soils, especially under warming climates. Lastly, the home soil biota generally had a positive effect on plant growth at warm dry sites and only sometimes at cool wet sites. This observation suggests that soil biota may have an important role in facilitating plant growth in novel, and potentially stressful environments.

Response to home and away climates

Overall, vegetative growth either was unaffected by cooler away climates, or enhanced. In contrast, vegetative growth was generally reduced under warmer novel climates. Seed mass, an indicator of fecundity, was less clearly affected by novel climate *per se*, but instead was determined idiosyncratically by the combination of plant population, and novelty of climate and edaphic environments. Our study allows us to examine local adaptation to climate in *B. gracilis* by only comparing home soil and home soil biota treatments to the home site. Despite that climate is a strong driver in local adaptation in plants, our study demonstrates *B. gracilis* can tolerate a wide array of temperatures (Bowker *et al.*, 2012; Butterfield & Wood, 2015; Wood *et al.*, 2015). We observed that plants growing at cooler and wetter sites performed just as well or

better than plants at their home site. This could be the result of relieving plants of stress brought about by water limitation (Allen *et al.*, 1984). An alternative explanation is that these sites actually more closely represent the climatic environment in which our focal populations evolved (Fischer *et al.*, 2014; Grady *et al.*, 2015).

At the warmer, drier away sites we observed lower plant biomass than at home sites. This is likely the result of plants both growing outside of an environment in which they evolved and under conditions that likely enhance environmental stress and water limitation (DeWitt *et al.*, 1998; Grady *et al.*, 2015). At these warmer, drier sites we also observed lower plant fitness with one exception where plants produced more seed at the warmest site, which could be indicative of a stress-induced seed production (Westoby, 1998; Mueller *et al.*, 2005). It is important to note these results are contingent upon the simultaneous effects of plants and their associated soil biota, i.e., it is difficult to interpret whether maladaptation to the environment is the result of soil biota being poorly adapted or plant populations being poorly adapted to any given environment (Revillini *et al.*, 2016; Rúa *et al.*, 2016; Bjorkman *et al.*, 2017; Kraemer & Boynton, 2017). An alternative concept is that local adaptation to any given environment is driven by biotic interactions. The coevolution of mutualists results in greater plant plasticity suggesting that local adaptation is driven by coadaptation (Ehrlich & Raven, 1964; Tomiolo *et al.*, 2015).

Response to home and away soils

Soils are another strong driver in plant adaptation to the environment (Johnson *et al.*, 2010; Bowker *et al.*, 2012; Bjorkman *et al.*, 2017). Our study reveals a clear importance of soil novelty, when climates are warmer and drier. Away soils adversely impacted all indicators of plant performance, including vegetative growth, fecundity and phenology. The magnitudes of some of these effects are comparable to climate effects, suggesting that emerging novel edaphic environments (induced by plant migration or human-assisted movement of plant materials) may be as influential to future plant productivity as the changing climate. Furthermore, if plant distributions shift to novel edaphic environments, plant productivity could dramatically change based on the soil conditions in which plant distributions move to (Damschen *et al.*, 2012; Sedlacek *et al.*, 2014; Bucharova, 2017). It is important to note that the majority of, and the most extreme of, the adverse effects of novel soils were observed in the +2°C site. Here, plants grown in novel soils exhibited 60% less growth and had minimal development of roots. Plants at this site and in this soil also had very small specific leaf areas and very low fitness, and at times, suppressed greenness. It is possible that the soil properties at this site in particular exacerbated the already existing climatic factors of being warmer and drier than the natal sites (Fry *et al.*, 2017). The soils at the +2°C site are derived from young volcanic cinders that are dark in color and extremely coarse and thus have low water holding capacity and absorb a tremendous amount of solar radiation making them a harsh soil environment for plants, tending to exacerbate drought conditions (Bowker *et al.*, 2012; Meador *et al.*, 2017). Perhaps these soils were so extremely different from the home sites – thus, more novel – that plant performance was poor. Soil nutrients are well understood to have profound impacts on plant growth, however other soil

factors that could exacerbate plant responses to global change or possibly inhibit plant migration (Bucharova *et al.*, 2016, 2017; Bjorkman *et al.*, 2017; Bucharova, 2017; Kraemer & Boynton, 2017). Soil texture, as an example could have dramatic impacts on soil water holding capacity with coarse grained soils exacerbating water limitation experienced by a subtle shift in precipitation (Bowker *et al.*, 2008; Jansa *et al.*, 2014). An equally plausible explanation is simply that this away soil, in particular, was of low quality to sustain plant growth. An intriguing future study would aim to parse the effects of away soil, compared to soil water holding capacity and fertility, within and among climate manipulations.

Response to home and away soil biota

Several studies have demonstrated greater mutualistic function in coevolved symbioses between plants and associated soil biota than in recently assembled plant-soil biota communities (Hoeksema & Forde, 2008; Hoeksema *et al.*, 2010, 2018; Johnson *et al.*, 2010; Gehring *et al.*, 2017). Some research suggests that such effects may be contingent on the novelty of the soil (Johnson et al. 2010), or the novelty of the soil water environment (Chapter IV). In our study, we detected instances where away soil biota impacted plant performance negatively, and fewer instances where away soil biota exerted a positive influence. More commonly than a main effect of soil biota, we found that the combination of home soils and soil biota were likely to induce a positive influence on plant performance under warmed environments. The magnitude of some of these effects is strong. For example, seed mass is more than double the mean of other treatments when both soils and soil biota are sourced from the home site in multiple contexts: Blue Chute populations grown at +2° and + 3°C, and White Pockets population grown at +2°C. Further, the

White Pockets population grown with home soil biota, regardless of the origin of the soils, produced about twice as much seed at +3°C. This suggest that plant fecundity persistence in environments prone to global change may be dependent upon the intact home plant-soil biota relationship (Wubs *et al.*, 2016). In support of this interpretation, SLA and biomass are also sometimes positively influenced either by home soil biota or home soil biota in combination with home soils under warmed climates.

In the cooler, wetter sites, the effect of soil biota source was more variable (including some negative effects of home soil biota, or home soil biota in combination with home soils) and much less pronounced. This contingency of the effects of joint soil and soil biota novelty on plant performance could be related to the generalization that in more stressful environments, soil biota play a greater role in mitigating environmental stress whereas more benign environments results in a lesser need for soil biota and perhaps even a negative effect of soil biota (Revillini *et al.*, 2016; David *et al.*, 2018). Such patterns have been observed in systems where phosphorous is limiting. Mycorrhizal fungi have a less positive effect on plant growth if phosphorous is added (Johnson, 2010; Johnson *et al.*, 2010). Ecological stoichiometry and the law of the minimum suggests that the most limiting resource drives ecological function and it is likely in semi-arid to arid systems that water is the most limiting nutrient (Antoninka *et al.*, 2015; Johnson *et al.*, 2015; Tietjen *et al.*, 2017). Some evidence has shown the importance of soil biota, in particular arbuscular mycorrhizal fungi on soil nutrient and water uptake in plants (Wu & Xia, 2006; Birhane *et al.*, 2012; Worchel *et al.*, 2013; Revillini *et al.*, 2016; Rubin *et al.*, 2017). Collectively, these results represent a joint temperature-water availabilitygradient along whichsoilbiota contribute more to plant growth in warmer drier environments then in cooler wet environments. The ability of soil biota to improve plant water uptake and their contribution to

hormonal regulation of plant growth could be important factors in plant soil-biota interactions in a changing world (Augé, 2001; Estrada-Luna & Davies, 2003; Warren *et al.*, 2008; Smith *et al.*, 2010, 2018; Augé *et al.*, 2015). The general superior quality of these home symbioses may be enhanced or diminished by novel conditions within the environment. For example, Johnson *et al.* (2010) demonstrates that if the environment is altered so that the most limiting resource is no longer limiting then the function of the mutualisms is also degraded. Alternatively, different phenotypes or populations may be associated with different soil biotic communities that have different functions. Gehring *et al.* (2017) has also demonstrated that different phenotypes of *Pinus edulis* correspond to different mycorrhizal communities and confer varying degrees of resistance to disease or drought.

Management Implications and Future Directions

Our findings demonstrate the complexity of plant responses to novel environments as a result of both migration and *in situ* climate change. The strong edaphic influence on plant growth in our study shows the need to strongly consider edaphic boundaries when assessing plant responses to global change or in intentional manipulation of ecosystems. Specifically, certain soil types may exacerbate the stresses of warmer temperatures and more variable precipitation caused by climate change while other soil types may mitigate such changes. Given that soil is a strong driver in plant adaptation, local adaptation to specific soil types may result in undesirable results should land management practices introduce a plant genotype to a novel soil environment. An additional factor seldom considered in land management is soil biota. Our study indicates that soil biota can be strong facilitators of plant growth in warm and dry environments, but only when

paired with plant hosts from the same home site. The implications of such observations are that local soil biota sources may be equally important to local seed sources, and the practice of pairing the two may be a viable strategy for generally enhancing restoration of native plant communities (Emam, 2016; Wubs *et al.*, 2016; Zhang *et al.*, 2018).

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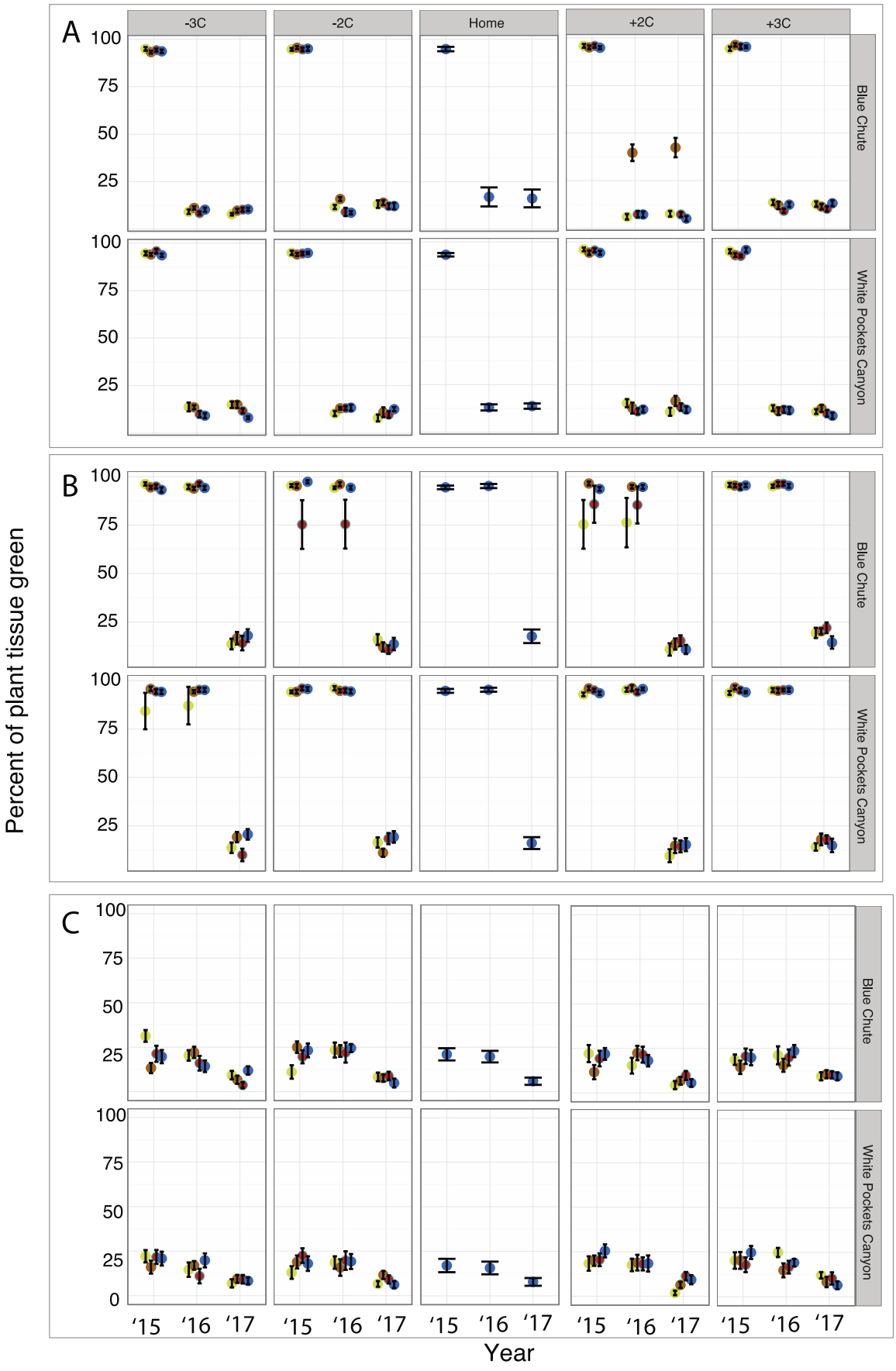
A
B
C
-3C
-2C
Home
+2C
+3C
Blue Chute
White Pockets Canyon
Percent of plant tissue green
100
75
50
25
0
'15
'16
'17
Year

Figure 2.1: Percent of plant tissue green for the three years of growth for *Bouteloua gracilis* for spring(A), summer (B), and fall (C). Light green dots represent plants grown in away soil and away soil biota, brown represent home soil, awaysoil biota, red represents away soil, home soil biota and blue represents home soil and home soil biota. Error bars are the standard error of the mean.

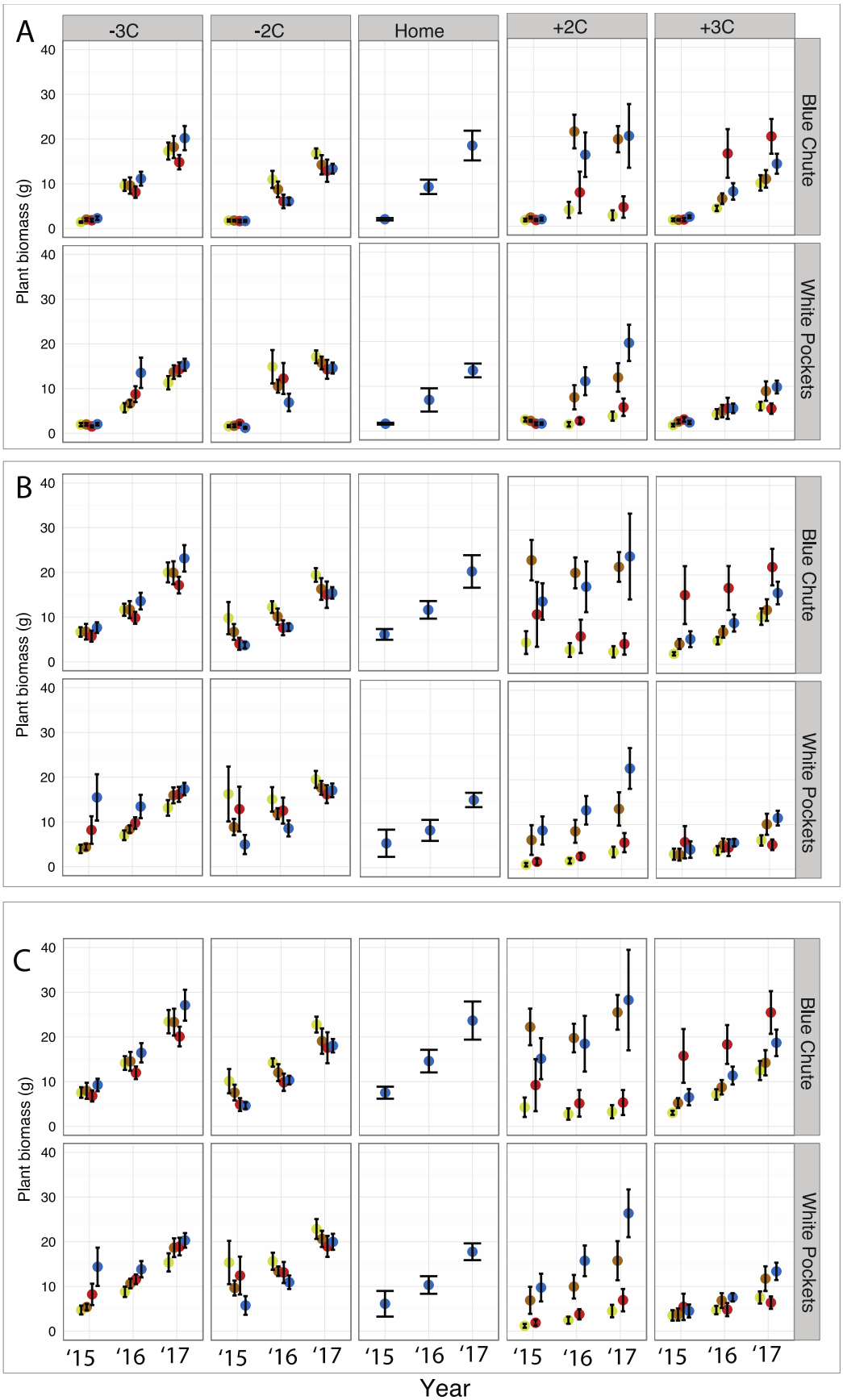
A
B
C
-3C
-2C
Home
+2C
+3C
Blue Chute
White Pockets
Plant biomass (g)
0
10
20
30
40
'15
'16
'17
Year

Figure 2.2: Allometric biomass for the three years of growth for *Bouteloua gracilis* for spring (A), summer (B), and fall (C).. Light green dots represent plants grown in away soil and away soil biota, brown represent home soil, away soil biota, red represents away soil, home soil biota and blue represents home soil and home soil biota. Error bars are the standard error of the mean.

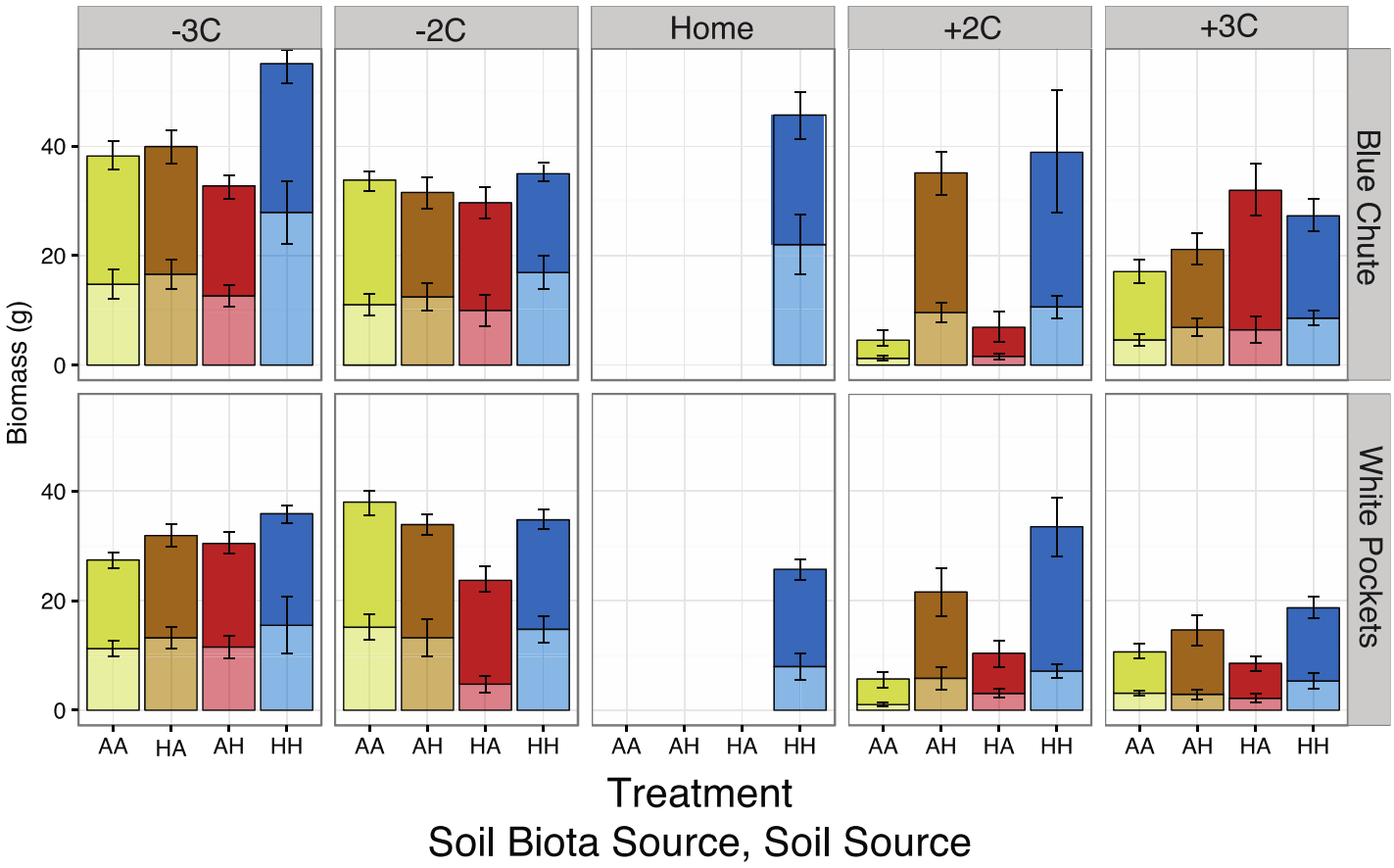

Figure 2.3: Above, belowground and total final biomass after three years of growth of two populations of *Bouteloua gracilis*). Light green bars represent plants grown in away soil and away soil biota, brown represent home soil, away soil biota, red represents away soil, home soil biota and blue represents home soil and home soil biota. The dark shades of each color represent shoot biomass while the lighter iterations of each color represent root biomass. A is short hand for away and H short hand for home. Error bars are the standard error of the mean.

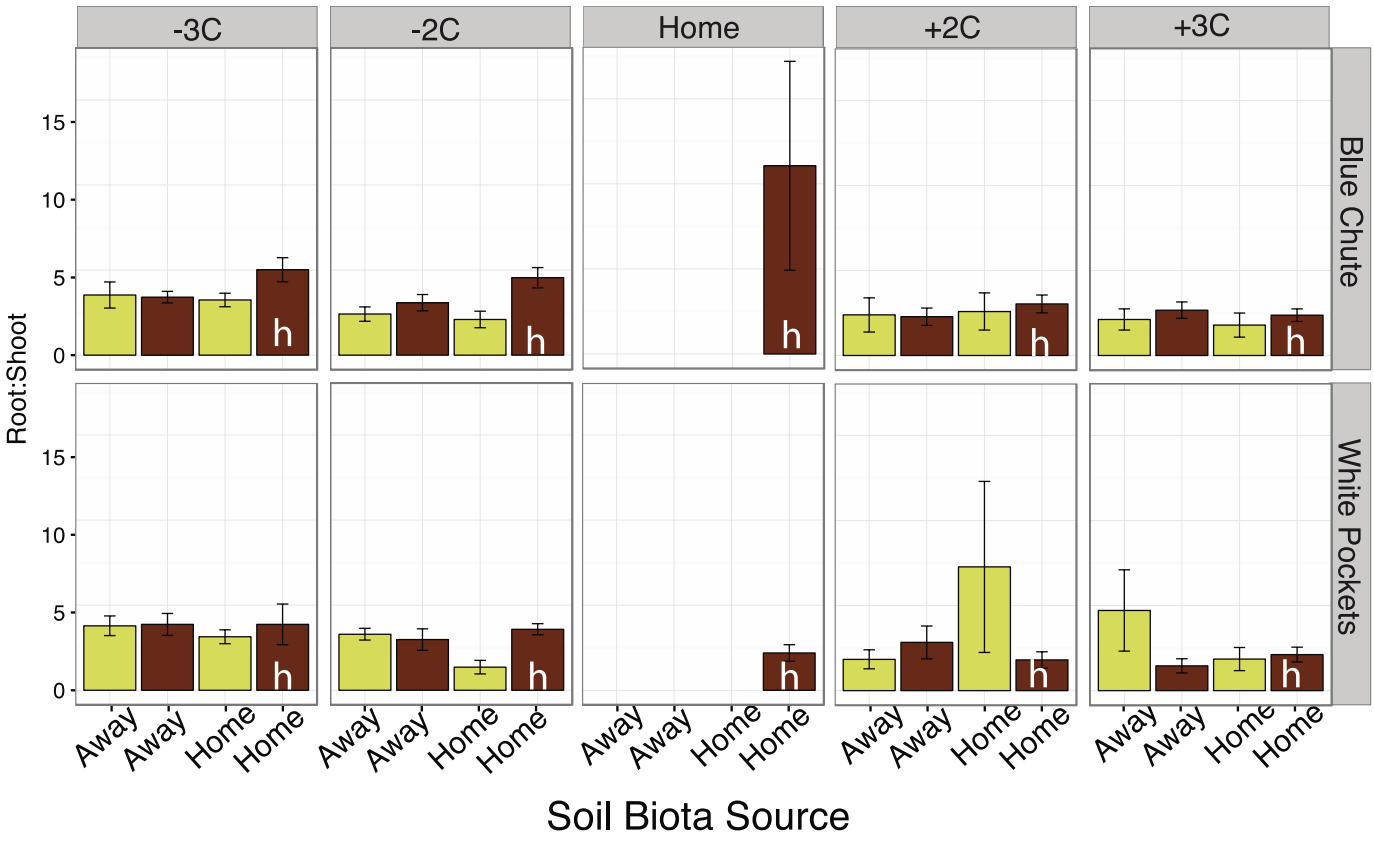

Figure 3.4: Root:Shoot ratio for the three year study of *Bouteloua gracilis* for each study site and population. Dark colors represent plants grown in their home soil and light colors represent plants grown in away soil. The 'h' represents the home soil biota and home soil pairing. Different letters represent statistically different ($p<0.05$) results according to Tukey's Test of Honestly significant differences.

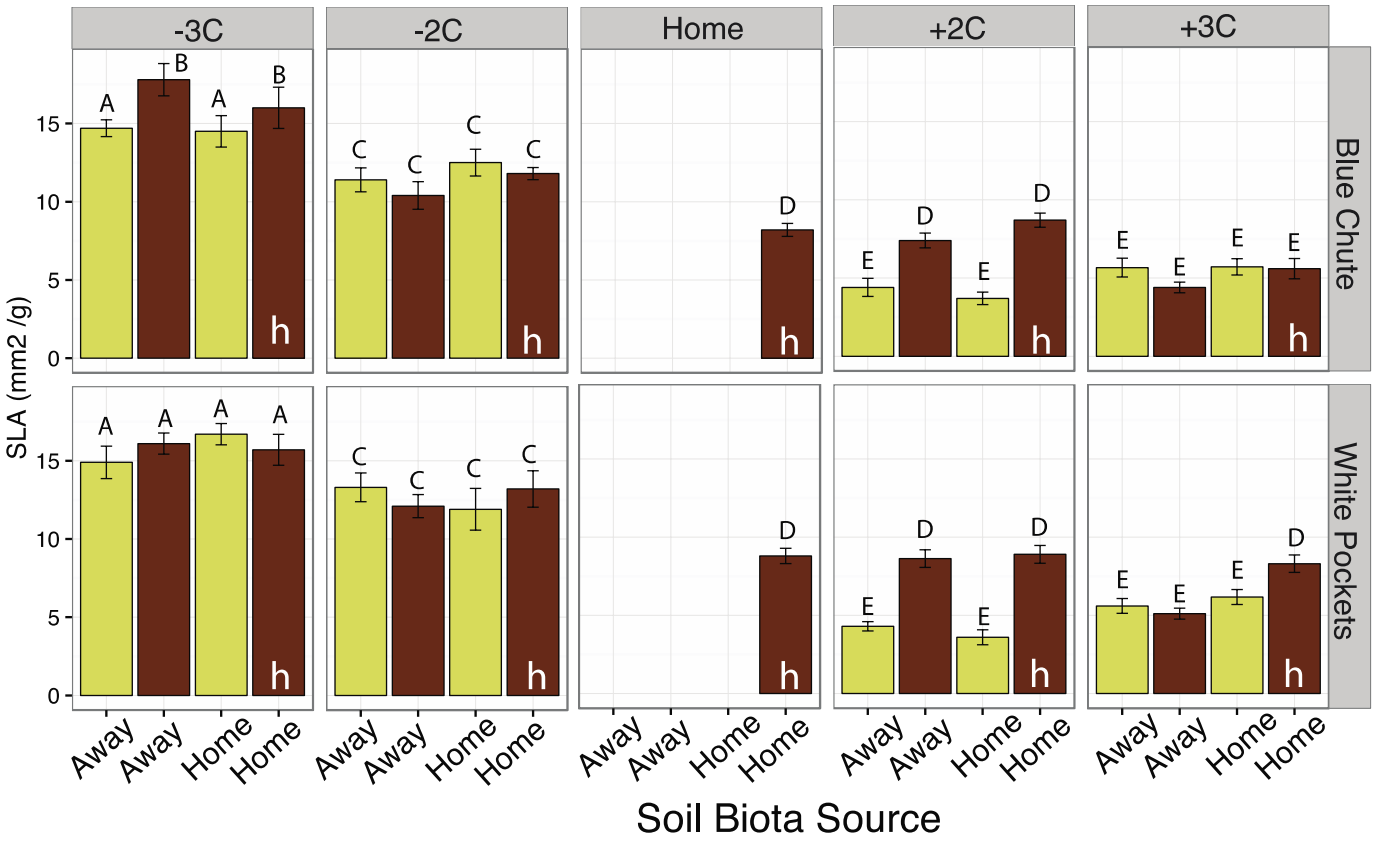

Figure 2.5: Specific leaf area (mm^2/g) for *Bouteloua gracilis* for each study site and population. Dark colors represent plants grown in their home soil and light colors represent plants grown in "away" soil. The 'h' represents the home soil biota and home soil pairing. Different letters represent statistically different ($p<0.05$) results according to Tukey's Test of Honestly significant differences.

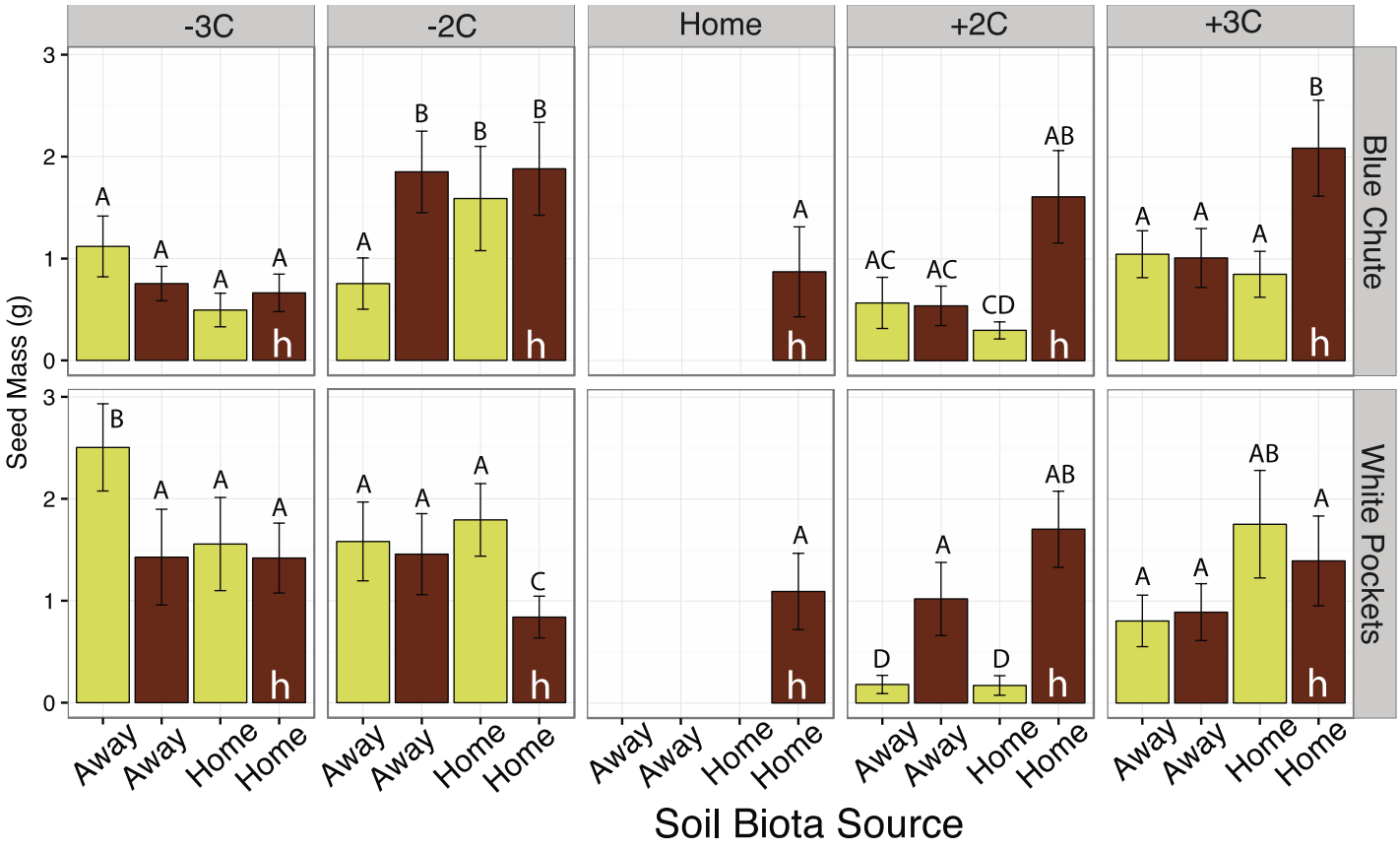

Figure 4.6: Total seed mass (g) produced for the three year study of *Bouteloua gracilis* for each study site and population. Dark colors represent plants grown in their home soil and light colors represent plants grown in away soil. The 'h' represents the home soil biota and home soil pairing. Different letters represent statistically different ($p<0.05$) results according to Tukey's Test of Honestly significant differences.

Chapter III

Familiar soil conditions help *Pinus ponderosa* seedlings cope with warming and drying climates

Abstract

Changes in temperature and available moisture as a result of climate forcing can dramatically change tree physiological processes and have subsequent impacts on tree growth and survival. Tree performance may also be sensitive to new soil conditions, for example, brought about by seeds germinating in soils different from those that the ancestral population grows in. Such "edaphic forcing" may come about through natural migration of plant populations, or human assisted planting and seeding, and has both abiotic and biotic components. Abiotic components of edaphic forcing might include distinct soil moisture environments, while biotic components include key symbionts such as mycorrhizal fungi, and other soil biota, which can both mitigate or exacerbate environmental stresses. We grew *Pinus ponderosa* individuals from seed, sourced from one location (the "home" site), and allowed them to grow across an environmental gradient with either their original home soil or with the soil of a different "away" site. We also moved plants with their soil biotic communities or forced them to grow in the soil biotic community of an "away" site to test 1) how changes in climate alone influence plant growth, 2) how soil types interact with climate to influence plant growth, and 3) the role soil biota play in facilitating plant growth in novel environments. At warmer drier sites, we observed no change in tree photosynthetic rate or physiology compared to plants growing at the home site, as long as trees were grown in their home soil with their home soil biota; however, in away soil and with away soil biota we observed a strong decrease in in plant growth. Plants growing in a cooler, wetter

away site grew similarly to plants grown at the home site, and soil treatments were less influential. We complemented these observations with physiological measurements of the *a priori* strongest contrasts (home soil biota and soil, away soil biota and soil). As a possible mechanistic explanation of the suppressed growth under the novel condition, we found that the photosynthetic rate was lower when plants were subjected to edaphic forcing. Because edaphic forcing did not suppress efficiency of photosystem II (Fv/Fm), but did decrease stomatal conductance, we hypothesize that edaphic forcing exacerbated water stress in the trees. Our results suggest that success of plantings of *Ponderosa pine* seedlings into warming environments may be enhanced by their associated soil biota.

Introduction

Tree populations are experiencing widespread mortality as a result of global change, drought, and heightened pressure by native and non-native pathogens and insects (Adams *et al.*, 2009; Breshears *et al.*, 2009; Allen *et al.*, 2010; Anderegg *et al.*, 2012, 2015). Trees experiencing these phenomena are showing a variety of physiological and adaptive strategies to coping with novel environments that are emerging due to climate forcing (Adams *et al.*, 2009; Breshears *et al.*, 2009; Allen *et al.*, 2010; Anderegg *et al.*, 2012b, 2015). Some of these strategies occur over many generations and result in the evolution of traits such as the ability to regulate stomatal conductance to maintain water status, known as isohydric physiology (Kolb & Stone, 2000; Adams & Kolb, 2005; McDowell *et al.*, 2008; Roman *et al.*, 2015). The ability to regulate stomata may result in the immediate survival of a short-term drought in that it allows a plant to conserve water during a drought event, however, by also reducing photosynthetic rate this

responsel may also result in reduced growth or possibly carbon starvation in extreme cases, and may not be beneficial during long-term drought or prolonged warming-drying trends (McDowell *et al.*, 2008; Adams *et al.*, 2009; Breshears *et al.*, 2009).

Because tree populations can migrate *via* seed dispersal, or intentional movement of nursery stock and seed by humans, they can also be said to experience edaphic forcing in addition to climate forcing when they are forced to grow in soil conditions different (away) from those of the seed source (home). Soils compose several additional factors, abiotic and biotic, that influence tree growth and physiology (Bowker *et al.*, 2012; Laliberté *et al.*, 2013; Laliberté, 2016; Bjorkman *et al.*, 2017). Soils vary greatly in their composition of mineral nutrients with nutrient rich soils generally facilitating higher photosynthetic rates and thus greater plant growth (Bailey *et al.*, 2004; Pasquini & Santiago, 2012). In addition, soil texture may also have a strong influence on plant growth and physiology by influencing soil water holding capacity and availability or by influencing root morphology (Koepke *et al.*, 2010; Pregitzer *et al.*, 2010; Bowker *et al.*, 2012; Smith *et al.*, 2012). Thus, trees living in different soil environments may respond differently to an environmental change, such as drought, with soil properties either mitigating or exacerbating the effects of drought (Bowker *et al.*, 2012).

Beyond the physical and chemical components of soil, soil biota can also have profound influences on tree growth and potentially contribute to differences in plant growth as a result of different soils, or edaphic forcing (Näsholm *et al.*, 2013; Pizano *et al.*, 2014). Soil biota include a complex suite of microorganisms including pathogens, saprotrophs and mutualists such as mycorrhizal fungi. Studies have shown that the accumulation of species-specific pathogens can hinder plant growth while others have suggested that specific species of ectomycorrhizal fungi confer drought resistance in members of *Pinaceae* (Mangan *et al.*, 2010a,b; Rúa *et al.*, 2016;

Gehring *et al.*, 2017). These contradicting examples of the influence of soil biota on plant growth are likely dependent on the environmental context (David *et al.*, 2018). In more benign, resource rich environments, soil microbes may have a more negative influence on plant growth whereas in more stressful, resource poor environments soil microbes may have a more positive influence on plant growth, David *et al.* (2018) coined this idea the *microbial mitigation-exacerbation* hypothesis providing a plant-microbe analog to the plant-plant stress gradient hypothesis (Callaway & Aschehoug, 2000; van der Putten *et al.*, 2016; Revillini *et al.*, 2016). Many studies have suggested that plant-microbe relationships are more functional when they are comprised of potentially coadapted plant hosts and soil biota community that have shared an evolutionary environment (home pairings), as opposed to recently assembled plant-microbial consortia (away pairings) (Hoeksema, 2010; Hoeksema *et al.*, 2010, 2018; Johnson *et al.*, 2010; Peters *et al.*, 2013; van der Heijden *et al.*, 2015). This hypothesis is known as the sympatric advantage hypothesis (Johnson *et al.* 2013).

To test how tree growth and physiology responds to changes in climate, soil and soil biota we designed a field experiment in Northern Arizona using the Southwest Experimental Garden Array (SEGA). We grew *Pinus ponderosa* from seeds collected at a home site near Flagstaff, Arizona and out-planted them grown from seed to two "away" sites: one warmer and drier than the home site, and another cooler and wetter. This environmental gradient effectively simulates changes that may be encountered by establishing individual trees as a result of global change or plant migration (including migration assisted by humans), while also creating a natural gradient to examine how physiology changes across an environmental gradient. We grew trees in either home or away soil to better understand how soil influences plant growth. To test how soil

biota influences plant growth, prior to transplant, we inoculated treatments with either a home or away soil biotic community. We tested the following non-mutually exclusive hypotheses:

1) *Local preference:* Local preference would suggest that plant growth will be highest at the home site. We also expect to see highest net photosynthetic rates at home. We also expect to see greater plant growth at the away sites when plants are grown in their home edaphic conditions.
2) *Co-adapted microbial mitigation:* Our predictions follow from synthesizing the *microbial mitigation-exacerbation* hypothesis and the *sympatric advantage* hypothesis. We anticipated that putatively co-adapted home soil microbes would be more beneficial for trees than away soil microbes at the warm dry site (more stressful) and thus mitigate some of the stress induced by warming. At the cooler-wetter site (less stressful) we predict than home microbes wouldexert a lesser difference with regards to plant growth relative to away soil microbe populations.

Gaining a better understanding of these hypotheses will help us better understand how trees will respond to warming environments, and exposure to other climatic or edaphic novelties in their environments as a result of migration, and to determine which facets of their environment are most important in regulating growth. These interactions will help inform land and forest managers with important context in planning any type of tree planting, including silviculture, assisted migration, and ecological restoration.

Methods

Plant and soil source sites

We conducted our study using Northern Arizona University's Southwest Experimental Garden Array (SEGA). The SEGA is a collection of experimental sites situated on a climate gradient spanning 4°C. Seeds were collected from ten mature trees using pole pruners to clip cones at the home site. Cones were then air dried and seeds were extracted and stored at -4°C. Soil was collected from the same home site and an additional two away sites, creating a total of three sites. Detailed information about each site is listed below:

Arboretum at Flagstaff (Home Site):

The Arboretum at Flagstaff (ARB) (35.16, -111.73) is an open meadow surrounded by Ponderosa Pine forest with a diverse mix of perennial grasses and forbs. ARB is located adjacent to Woody Mountain (Coconino County, AZ) at an elevation of 2,179m). ARB receives approximately 556mm of precipitation annually with an average minimum annual temperature of -1.0°C and an average maximum annual temperature of 16.0°C (PRISM Climate Group, Oregon State University). Soils at ARB are ustolls derived from basalt, with a sandy clay loam texture (Soil Survey Staff, Natural Resource Conservation Service).

White Pockets Canyon (Warmer, drier away site):

White Pockets Canyon (WPC) (36.61°,-112.41°) is a Piñon-Juniper woodland with an understory dominated by a mix of perennial C4 grasses, located on the west side of the Kaibab Plateau (Coconino County, AZ) at an elevation of 2,057m. WPC receives approximately 443 mm of precipitation a year with an average minimum annual temperature of 4.0°C and average maximum annual temperature of 19.0°C (PRISM Climate Group, Oregon State University). The

soils at WPC are argids derived from Kaibab limestone with a gravelly loam texture. (Soil Survey Staff, Natural Resource Conservation Service).

Bear Springs (Cooler, wetter away site):

Bear springs (BS) (36.37°, -112.18°) is a warm-dry Mixed Conifer forest co-dominated by Douglas fir and Ponderosa Pine located near the high point of the Kaibab Plateau (Coconino County, AZ) at an elevation of 2,668m. BS receives approximately 772 mm of precipitation a year with an average minimum temperature of -1°C and an average maximum annual temperature of 14°C (PRISM Climate Group, Oregon State University). BS soils are ustalfs derived from Kaibab limestone with a clay-loam to gravelly silty clay loam texture (Soil Survey Staff, Natural Resource Conservation Service).

Preparation of Experimental Units

We focused on one natal population of *Pinus ponderosa* from ARB, and selected WPC and BS as additional out-planting sites. To prepare our experimental units, we first made soil collections in the summer of 2014. Inoculum soil was collected from all sites by picking a random starting point and collecting soil every five meters for 90m in each cardinal direction. We collected inoculum soil from the rhizosphere of target plants and later homogenized it. Target plants were *Pinus ponderosa* at the home site and overstory conifers, including but not limited to *Pinus ponderosa*, at away sites. This soil was stored refrigerated until it could be used within 60 days. To inoculate seedlings with soil biotic communities, we placed four *Pinus ponderosa* seeds from the home population into Steuwe & Sons RL200 conetainers filled with 50mL live soil inoculum collected from each site, creating one home and two away combinations

of plant and soil biota. As seeds germinated, they were thinned to one seedling per container, always keeping the largest seedling.

Seedlings were grown in the NAU Research Greenhouse until roots occupied most of the conetainer, at which point the seedlings were transplanted into Steuwe & Sons TP1124R tree pots filled with 30L of background soil. Background soil was previously collected from each site, away from the rhizosphere of living plants and homogenized. Prior to use, this background soil was s sterilized at 125°C for 24 hours twice. In transplanting conetainers into tree pots, we were able to create custom treatments of the soil biota and soil. We created seven combinations varying in degree of environmental novelty for the ARB-sourced plants: plants inoculated with home soil biota in home, away (WPC), or away (BS) soil or one of two away soils (, plants inoculated with away (WPC) soil biota in either home or away (WPC) soil, and plants inoculated with away (BS) soil biota in either home (BC) or away (BS) soil. We then grew trees in the greenhouse in the large pots for an additional four months and then hardened plants outside under a shade cloth for another month. This gave time for trees to establish roots into the background soil.

Field planting

To expose plant populations to varying climates we out planted experimental units to the field by excavating planting holes and inserting the plants, still in pots, into the holes so that the soil levels inside and outside of pots were similar. We retained the pots as a method to maximize exposure to the manipulated abiotic and biotic soil environments, and decrease and delay influences from the surrounding soil. The volume of our pots was adequate to accommodate seedling growth up to a few years, simulating the crucial establishment phase.

As a frame of reference, we outplanted a set of experimental units with home soil biota and soil back into the home site. The rest of the plants were planted into climates that are approximately 2°C (WPC) warmer, or 2°C (BS) cooler than the home site. BS was planted with experimental units featuring all four combinations of BS and ARB soil and soil biota. Likewise, WPC was planted with experimental units featuring all four combinations of WPC and ARB soil and soil biota. The full design creates situations where ARB plants are exposed to a new climate, either warmer-drier or cooler-wetter, while varying the novelty of the soil and soil biota. Each treatment combination had ten replicates creating a total of 90 experimental units, including the 10 reference units at ARB.

Plant performance

We measured plant height, diameter at root collar and the number of branches on all trees three times, in spring, summer, and fall. This allowed us to estimate biomass with allometric equations. We destructively harvested seedlings grown in the greenhouse adjacent to our field sites to construct allometric equations based on field measurements of root collar diameter, number of branches, tree height, and canopy diameter. We used a multiple regression to fit an allometric equation to estimate biomass where B= above ground biomass, h= height, d = diameter at root collar and b= number of branches.

$$B = -151.38 + 1.97h + 4.5221d + 2.90b.\ (F=81.67),\ p=0.0001,\ R^2=0.9167)$$

To ensure that measurements could be taken on the same day, we conducted physiological measurements on only a subset of trees, focusing on expected maximal contrasts:

plants growing with both home soil and soil biota at all sites including the home site, and plants growing with both away soil and soil biota at each of the away sites. We used an Integrated Fluorometer (ADC BioScientfic, Ltd., Hoddesdon, United Kingdom) to measure stomatal conductance, net photosynthetic rate, and florescence ratios of dark adapted leaves. Stomatal conductance and net photosynthetic rate were captured along light response curves. These data were collected during the summer monsoon season of 2016 at approximately noon repeated with multiple PAR values ranging from 0 to 2000 and a fixed CO_2 level of 400 ppm with a fixed temperature of 25°C and 30% relative humidity.

Statistical analysis

A repeated measures ANOVA was used to test differences in plant biomass during the three sampling periods, spring, summer, and fall. Three-way ANOVA was used to compare the effects of site, soil origin, and soil inoculum origin on allometric final plant biomass. Differences within groups were determined using Tukey's HSD test. Repeated measures three-way ANOVA was used to compare stomatal conductance and photosynthetic rate at different light levels. Model assumptions were checked using the Shapiro-Wilk test of normality and the Levene's test of heterogeneity of variance. All statistics were conducted in R (version 3.3.1).

Results

Tree Growth

There were no differences in biomass after planting trees at the field sites in the spring across site ($F=0.78$, $p=0.34$), soil source ($F=0.67$, $p=0.38$), or soil biota ($F=0.82$, $p=0.29$). By

summer, no differences between treatments at the -2°C site and between the -2°C site and the home site had emerged: however, at the +2°C site plants grown in home soil with their home soil biota were 15% larger than plants grown at their home site ($F=3.6$, $p=0.03$) and 150% larger than plants grown at the +2°C site in away soil with away soil biota ($F=5.8$, $p=0.001$). This pattern is perpetuated in the fall sampling with all plants about 20% larger than they were in July ($F=2.8$, $p=0.04$) (Figure 3.1).

Final tree biomass after one year was surprisingly not influenced by site, suggesting only a minor or a highly contingent influence of climate ($F=1.87$, $p=0.16$) (Figure 3.2). In contrast, edaphic factors were much more influential. Plants grown in home soil with their home soil biota at the +2°C site were 15% larger than plants grown in away soil ($F=6.68$, $p=0.002$) and 10% larger than plants grown at the home site ($F=5.32$, $p=0.006$). Also at the +2°C site, plants grown with either home soil biota and away soil, or home soil and away soil biota grew 100% larger than plants grown with both away soil and soil biota and away soil ($F=8.23$, $p<0.001$). These mixed treatment combinations were also the same size as plants grown at their home site. At the -2C site, plants grown with away soil biota but in home soil were 50% smaller than any other treatment combination ($F=5.4$, $p=0.03$). There were no observed differences between home soil and away soil or home and away in the site x soil x soil biota interaction term ($F=1.2$, $p=0.19$).

Physiology

Overall similar photosynthetic rates were achieved at all sites in the best performing treatments, but soils and soil biota differently affected photosynthetic rate at the warmer and cooler sites. At the warm, dry site there was a significant difference in light saturation point and

net photosynthetic rate at any light level greater than 200 with home soils and soil biota combinations having 40% greater net photosynthetic rates than away soils and soil biota ($F = 24.6$, $p<0.001$). Home treatment combinations also had 20% higher dark respiration rates ($F = 8.76$, $p=0.002$). At the cool, wet site there was no statistical difference in photosynthetic rate at any light level.

Stomatal conductance was similarly influenced differently by soil treatments under contrasting warming and cooling scenarios. At the warm dry site, stomatal conductance was up to 100% higher for the home soil and soil biota treatment at light levels less than 200 and more than 1000, compared to away soil and soil biota ($F = 18.4$, $p<0.001$). It is also notable that the home soil and soil biota treatment in the warm dry site exhibited stomatal conductance values 10% higher than those at the home site ($F = 3.4$, $p= 0.01$). In contrast, the away soil and soil biota treatment achieved stomatal conductance well below that of the home site (F 12.6, $p<0.001$). Stomatal conductance in home and away soil and soil biota treatments were nearly identical at the cool wet site ($F = 0.48$, $p=0.54$). Lastly, Fv/Fm ratios at the warm dry site were 20% lower at the warm dry site than either the home site or the cool wet site ($F=3.6$, $p=0.02$) (Figure 3.5). There were no differences in Fv/Fm ratios in different soil type ($F=0.28$, $p=.67$) or in different soil biota treatments ($F=0.34$, $p=0.61$).

Discussion

Our study shows a stronger effect of edaphic forcing than climate forcing on plant growth of *P. ponderosa* with soil biota as an important factor in growth regardless of climate or soil type. We documented some clear differences in final plant biomass after a half year of growing trees in the nursery and a full growing season in the field, such that under warmer, drier climates,

plants grow larger with home soil conditions and therefore less edaphic forcing. Soil conditions were much less influential under wetter, cooler conditions. We demonstrate that in part these differences are likely due to differences in physiological performance and water stress experienced by the plants.

Local Preference

Interestingly, our study documents little evidence of local preference with *respect to climate* because trees grew to approximately the same biomass across all sites along the elevation gradient given an averaging across all soil and soil biota treatments. A likely explanation for the lack of local preference observed in our study is the unusual weather patterns experienced during the field portion of our study. During our study period, all of our sites experienced unusually high temperatures and an unusually wet spring. Compared to long-term modeled averages (PRISM Climate Group, Oregon State University), BS was about 3°C warmer and 100mm wetter with the ARB only 1°C warmer. WPC was 2°C warmer than expected and received approximately 120mm greater than average precipitation. These weather patterns resulted in a warmer growing season with more available water that likely facilitated more plant growth at all sites (Dreesen *et al.*, 2012).

In contrast, our study does provide evidence for local preference with respect to soil, that appears to be modulated by climate. Possibly the best overall indicator of preference for home or away soil biota is the final biomass data after the entire growing season. These results indicated better plant performance with either home soil, home soil biota or both in the warmest site. In contrast, there was no such preference for home soil or soil biota in the coolest site. Local adaptation to soil has been demonstrated in tree species before with soil chemical and physical

properties being principal drivers of adaptation (Pregitzer *et al.*, 2010; Bucharova *et al.*, 2017). Interestingly, in this study the home soil type is not the most fertile soil, suggesting that a simple preference for greater fertility is not a complete explanation of the patterns. Possibly the effect of home soil biota resulted in the greatest plant growth because the combination of individuals from the plant population and the soil biota with which they shared an evolutionary environment was the most efficient in gathering soil resources, and the result was heightened mutualistic function (Johnson *et al.*, 2010; Rúa *et al.*, 2016; Bjorkman *et al.*, 2017). These findings demonstrate the importance of soil boundaries and soil biota in determining local adaptation of a species to a specific geography and environment (Gibson *et al.*, 2016; Bjorkman *et al.*, 2017; Bucharova, 2017; Bucharova *et al.*, 2017).

Synthesizing microbial mitigation-exacerbation and the sympatric advantage

Why are the positive effects of home soil biota most clearly observed at the warmer-drier site? We must synthesize two hypotheses to develop a new working hypothesis that explains these results. The microbial mitigation-exacerbation hypothesis proposes that in response to a stressor, soil microbes may either mitigate or exacerbate the level of stress. The net effect of soil biota is predicted to be more likely to shift in the direction of mitigation as stress increases, and in the direction of exacerbation in benign, low-stress sites (David *et al.*, 2018). This prediction is based upon comparing the effect of live soil biota to sterile soils. Possibly, the sympatric advantage hypothesis follows a similar dynamic in that: as a generality home soil biota are likely to be more beneficial (mitigating environmental stresses) than away soil biota, with this difference becoming more pronounced under increasing stress.

Our study supports this assertion, *but only with regard* to the home soil biota; we do not compare the same away soil biota under different climates. Along the SEGA gradient and in the region, suboptimal soil moisture is the most common source of plant stress and mortality (Adams *et al.* 2009; Gitlin *et al.* 2006). Although the stressfulness of the site was somewhat dampened in this wet year, the soil moisture regime was more limiting in the warmest, driest site than the other sites.

At the wetter end of the environmental gradient, home soil biota did not appear to mitigate stress more than away soil biota at all, i.e. the effect was neither a mitigating or exacerbating one. This suggests that perhaps because the environment was unlikely to be water-limited, that plants growing there were less dependent on mutualisms and beneficial soil microbes. Had our gradient been broader, extending into even more benign climates, we could plausibly have observed microbial exacerbation by home soil biota, but we would posit that in order for this to happen our study system would had to have been richer in antagonistic interactions (van der Putten *et al.*, 2016; Revillini *et al.*, 2016).

How might home soil biota mitigate stress?

Some work has shown that specific phenotypes of trees are associated with specific groups of ectomycorrhizas which confer resistance to drought stress (Gehring *et al.*, 2017). Other studies have shown that the mutualistic function of such associations is higher in co-evolved partnerships suggesting that microbial mitigation of environmental stress is dependent on intact coevolved partnerships (Johnson *et al.*, 2010; Rúa *et al.*, 2016). While our study did not control for host tree genetics we can support that we only observed microbial mitigation in home partnerships, though we cannot determine whether this was the result of microbial community

compositions, as opposed to shared evolutionary history of the plant-microbe relationship, or both. We also demonstrate that the effect of the home soil biota was most effective in home soil, particularly at +2°C suggesting the plant-microbe relationship is perhaps locally adapted to soil type (Rúa *et al.*, 2016, Pánková *et al.*, 2014). In either case, we do have evidence that superior performance of plants growing with home soil biota is linked to water-limitation and its effect on photosynthesis in this isohydric species.

Overall, our physiological data support the interpretation that stomatal closure and lower photosynthetic rates are the result of varying water availability (Frey-Klett *et al.*, 2005; Warren *et al.*, 2008; Lehto & Zwiazek, 2011; Goltsev *et al.*, 2012), which may in turn be influenced by soil biota (Warren *et al.*, 2008; Lehto & Zwiazek, 2011). Plants grown with home soil and soil biota in the most water-limited (WPC, +2°C) site clearly exhibited higher photosynthetic rates than plants grown in away soil and soil biota. Given that Fv/Fm ratios were about the same among home and way soil treatments under warmer, drier conditions, differences in photosynthetic rate are unlikely to be explained by photosynthetic efficiency. Instead, they appear to mirror patterns in stomatal conductance data. Because isohydric plants regulate water loss through stomatal conductance, higher stomatal conductance strongly indicates greater relative water availability which is influenced by soil microbial communities. Because restriction in stomatal conductance also restricts gas exchange, we believe this is the key reason why photosynthetic rate was higher in plants grown with home soil and soil biota and lower with away soil and soil biota. Gradients in water availability could be the result of different soil types holding water differently, or ectomycorrhizas could be influencing tree water relations by hydraulic redistribution (Warren *et al.*, 2008; Lehto & Zwiazek, 2011; Bowker *et al.*, 2012). Ectomycorrhizas are well known to redistribute water in the soil profile making it more available

in the rhizosphere (Warren *et al.*, 2008; Lehto & Zwiazek, 2011). If water limitation was mitigated by home soil biota, trees may have been able to maintain their hydration status under the home soil and soil biota condition with a lesser degree of stomatal closure, and therefore higher photosynthetic rate and ultimately biomass than plants grown under the away soil and soil biota treatment.

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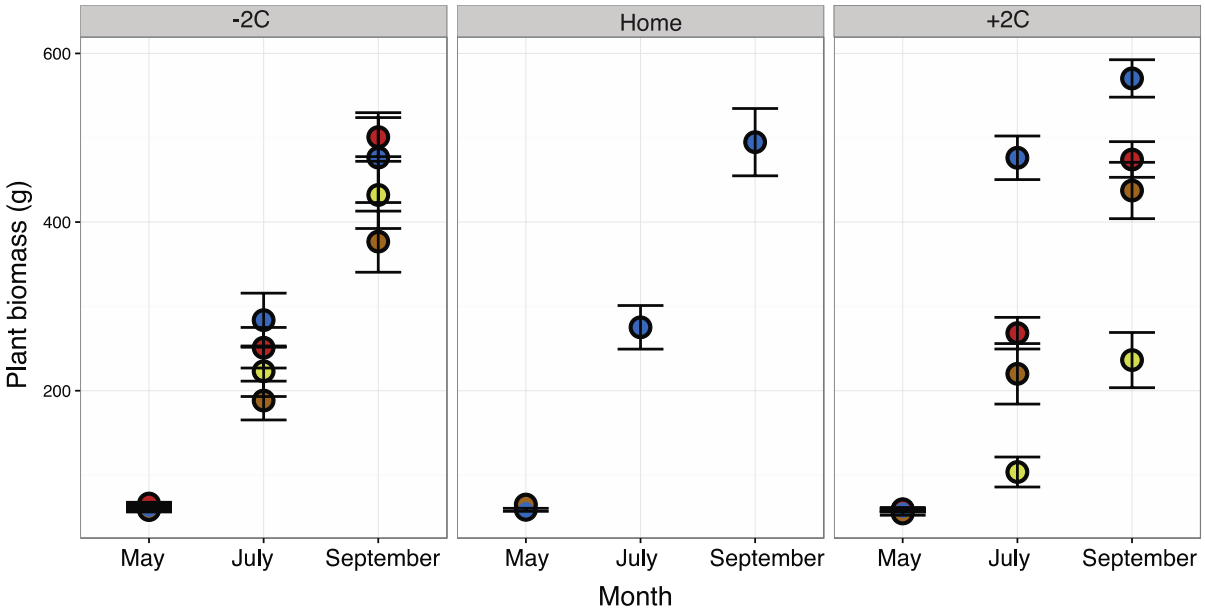

Figure 3.1: Above ground allometric biomass for *Pinus ponderosa* for each sampling period in the field. Light green dots represent plants grown in away soil and away soil biota, brown represent home soil, away soil biota, red represents away soil, home soil biota and blue represents home soil and home soil biota. Error bars are the standard error of the mean.

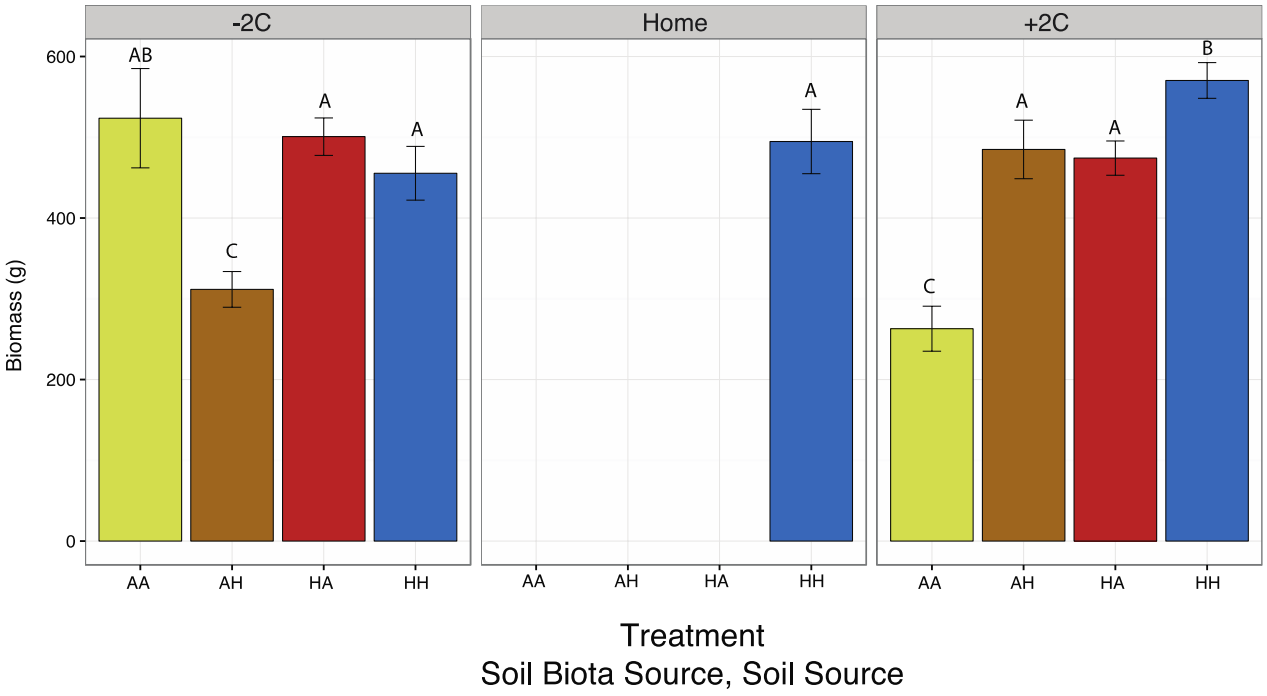

Figure 3.2: Above ground allometric biomass for *Pinus ponderosa* after one year of growing in the field. Light yellow bars represent plants grown in away soil and away soil biota, brown represent home soil, away soil biota, red represents away soil, home soil biota and blue represents home soil and home soil biota. Error bars are the standard error of the mean.

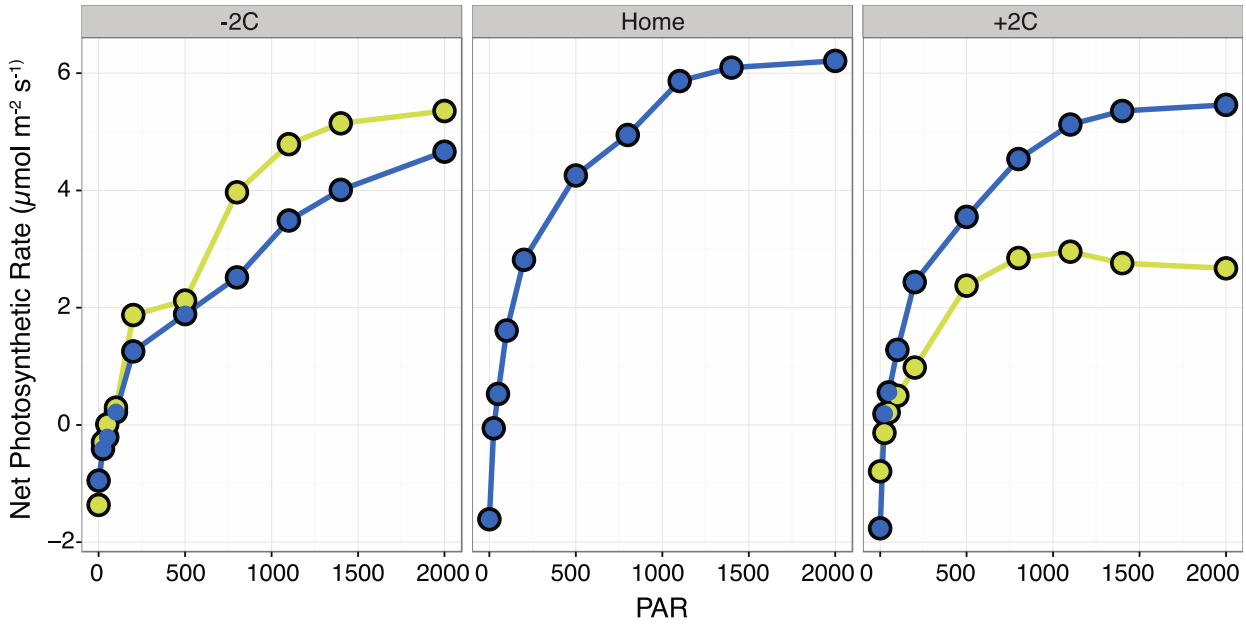

Figure 3.3: Net photosynthetic rate for *Pinus ponderosa* at varying levels of photosynthetic active radiation (PAR). Dark blue dots represent trees grown in their home soil with home soil biota, light green dots represent trees grown in away soil biota. Error bars are excluded in order to better visualize curves.

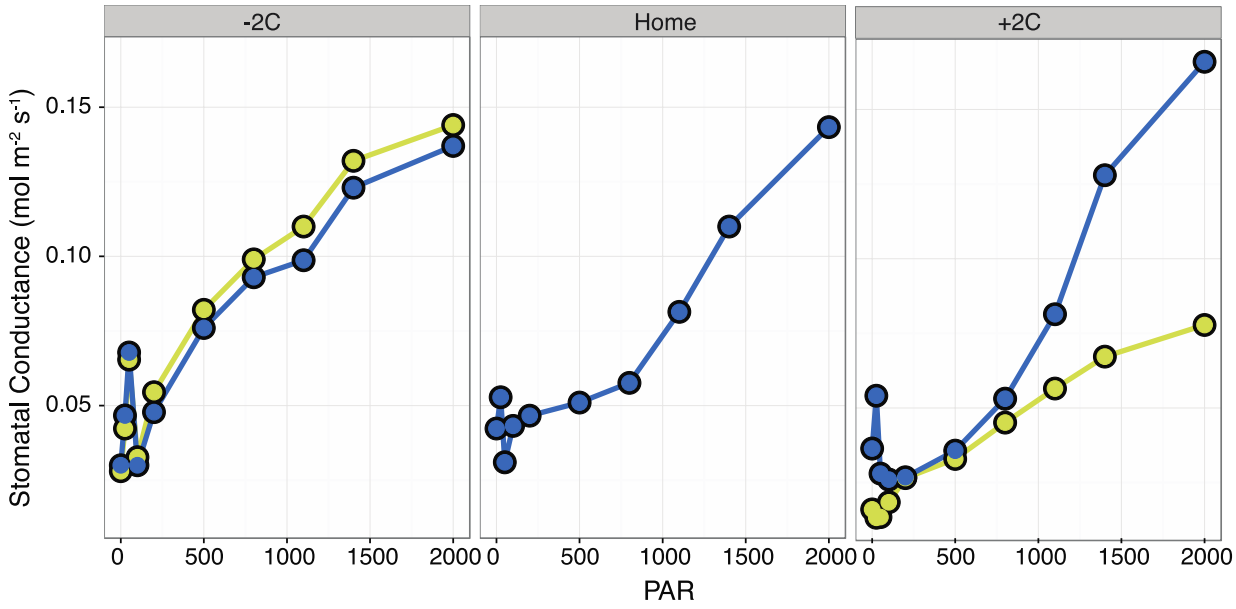

Figure 3.4: Stomatal conductance for *Pinus ponderosa* at varying levels of photosynthetic active radiation (PAR). Dark blue dots represent trees grown in their home soil with home soil biota, light green dots represent trees grown in away soil biota. Error bars are excluded in order to better visualize curves.

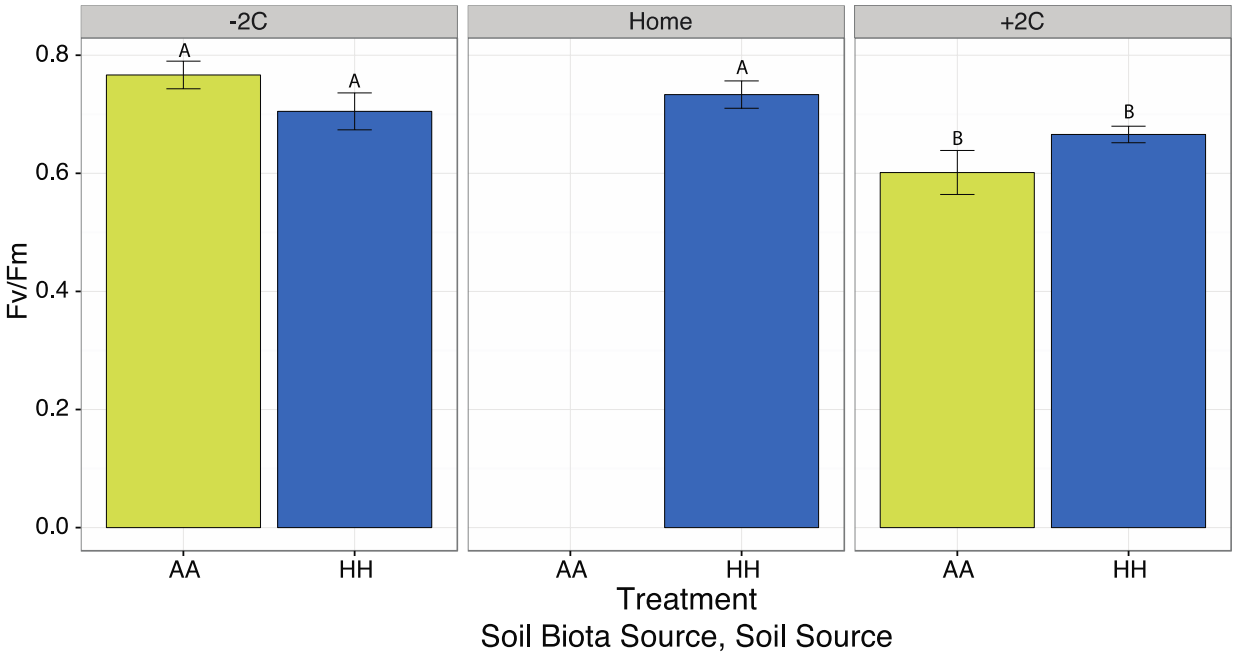

Figure 3.5: Fv/Fm ratios for *Pinus ponderosa* during the July sampling period. Dark blue bars represent trees grown in their home soil with home soil biota, light green bars represent trees grown in away soil biota. Error bars represent the standard error of the mean. Different letters indicate significant differences from Tukey's Test of Honestly Significant Differences at a critical value of p=0.05.

Chapter IV

Sympatric pairings of dryland grass populations, mycorrhizal fungi, and associated soil biota enhance mutualism and ameliorate drought stress

Abstract

There is evidence that the distribution of ecotypes of plants and their symbiotic arbuscular mycorrhizal (AM) fungi and other associated soil biota may be structured by the availability of essential soil nutrients; and locally adapted partnerships most successfully acquire nutrients that are in limited supply. Much less is known about how water availability may influence the geographic structure of symbioses among plants and soil biota. We grew *Bouteloua gracilis* ecotypes from wet and dry sites, with either sympatric or allopatric soil inoculum under moderate and extreme soil drying treatments to examine 1) how varying degrees of water limitation influences grass responses to soil biota, and 2) the relationship between AM fungal structures and these responses. Under extreme soil drying the dry-site ecotype tended to perform better than the wet-site ecotype. Both ecotypes performed best when inoculated with their sympatric soil biota. Sympatric pairings produced more AM fungal hyphae, arbuscules and dark septate fungi. In contrast, allopatric pairings produced more vesicles. Extreme soil drying tended to accentuate these patterns. As water became increasingly limited, sympatric partnerships produced more resource harvesting and exchange structures than allopatric ones.

Introduction

Plants are often locally adapted to their abiotic environment (Leimu & Fischer, 2008; Smith *et al.*, 2012; Richardson *et al.*, 2014) . However, abiotic variables are not the only factors that define an organism's niche, plants may also be strongly adapted to their local biotic environment, including soil biota (Gehring *et al.*, 2006; Johnson *et al.*, 2010; Waller *et al.*, 2016) . Plants respond variably to soil biota, in part because soil biota can both enhance and inhibit plant growth and survivorship via the activities of beneficial mycorrhizal fungi, harmful pathogenic fungi, saprotrophic fungi, a suite of bacterial species, and food webs of soil fauna (Hendriks *et al.*, 2015; van Grunsven *et al.*, 2009; van der Putten *et al.*, 2013) . In turn, plants can shape soil communities, for example evolving features that attract beneficial biota such as mycorrhizal fungi or repel detrimental biota such as pathogens (Venturi & Keel, 2016). Plant associations with arbuscular mycorrhizal (AM) fungi are known to facilitate soil nutrient and water acquisition as well as buffer plants against a variety of stresses (Stahl & Smith, 1984; Rowe *et al.*, 2007; Reininger & Sieber, 2012) . There is evidence that, like their plant partners, these fungal symbionts are also locally adapted to the abiotic and biotic environments (Stahl *et al.*, 1990a,b; Johnson *et al.*, 2010) .

Many AM fungal species have a nearly global distribution (Davison *et al.*, 2015) demonstrating physiological variation within species (Ehinger *et al.*, 2012) that may display differing functional attributes contingent upon the environmental context (Johnson *et al.*, 1997; Hoeksema *et al.*, 2010; Antoninka *et al.*, 2015; Revillini *et al.*, 2016) . Mycorrhizas from resource limited and stressful environments tend to show greater mutualistic function (Revillini *et al.*, 2016), reminiscent of the stress-gradient hypothesis in that greater abiotic stress favors more facilitative interactions (Callaway *et al.*, 2002) . Additionally, AM fungi and plants that originated from a common location and potentially share a co-evolutionary history, tend to have

a greater mutualistic function (Johnson *et al.*, 2010) . We call this the sympatric advantage. Some evidence suggests that plants and co-occurring soil microbes, including mycorrhizal fungi, rapidly adapt to changes in the environment and thus co-adaptation creates greater mutualistic function regardless of environment (Lau & Lennon, 2011, 2012; Vurukonda *et al.*, 2016) . Thus, mycorrhizal function is documented to vary based on *both* environment and sympatry (Johnson *et al.*, 1997, 2015) . The need for a better understanding of the mechanisms of these joint influences is becoming increasingly poignant as climate change modifies the abiotic environments of plants and their fungal partners.

The functional equilibrium model might serve as a reasonable expectation of the outcome of increasing environmental stresses in drylands. This model predicts that plant allocation of photosynthate and biomass varies to optimize acquisition of the most limiting resource (Briske & Wilson, 1980; Johnson 2010; de Vries *et al.*, 2012) . When a soil resource such as phosphorus or water is added to a resource limited system, the need for mycorrhizal delivery of that resource diminishes (Johnson *et al.*, 1997; Ladwig *et al.*, 2012) . As a result, plants invest less in root exudates and fungal symbionts (Orwin *et al.*, 2010) . Simultaneously, fungi allocate less to resource harvesting (hyphae) and exchange (arbuscules) structures and more to storage structures (vesicles) (Johnson *et al.*, 2003) . This shift in allocation to different AM fungal structures may be one possible manifestation of a shift in mycorrhizal function to less mutualistic symbioses (Johnson 1993) . Furthermore, decreasing the supply of the limiting soil resource can increase the mutualistic function of mycorrhizas and allocation to arbuscules and hyphae.

Mycorrhizal fungi are known to contribute to vascular plant water balance both directly and indirectly. Mechanisms for this are observed as active water uptake and delivery (Ruth *et al.*, 2011) , passive water delivery (Allen *et al.*, 1981) , improved plant nutritional status and size

(Augé, 2001, 2004) , and plant hormonal regulation of stomata (Augé *et al.*, 2015) . It follows that plant available water is a soil resource that influences mycorrhizal function (i.e. location on the mutualism-parasitism continuum). Increased frequency and severity of drought in many drylands predicted by many climate change scenarios suggests the potential for increasing the importance of AM mutualisms in the future (van der Putten *et al.*, 2016) . Studies have documented that plants and associated soil organisms are co-adapted in native grasslands and perform best when grown together in nutrient limited systems (Johnson *et al.*, 2010). Given the contributions of mycorrhizas to plant water balance, the importance of co-adaptation among plants and AM fungi in a water limited system should be evaluated. We sought to determine the interactive effects of provenance and dryness on mycorrhizal function, and elucidate how patterns of fungal allocation to resource harvesting and exchange structures versus storage structures are associated with mycorrhizal function.

To examine mycorrhizal functioning and fungal allocation across different environmental and co-adaptation scenarios, we grew two populations of a C4 perennial grass, *Bouteloua gracilis*, with locally occurring (sympatric) or novel (allopatric) soil organisms. The populations were sourced from semi-arid environments at two elevations in close geographic proximity, with strongly contrasting precipitation (28 cm versus 43 cm mean annual precipitation). We hypothesized that more severe limitation of soil moisture wiould favor stronger mycorrhizal mutualisms at the drier site compared to the wetter site. The experimental plants were maintained under moderate (more gradual) or extreme (more abrupt) soil drying conditions to simulate the natural environmental stress caused by limited soil moisture at the wetter and drier sites respectively. This allowed us to simultaneously test predictions of three complementary

hypotheses: local adaptation, co-adaptation and functional equilibrium, and their interactions, as they relate to mycorrhizal function.

Local adaptation hypothesis: regardless of symbiotic partners, plant genotypes and AM fungi collected from the drier site will grow largest under the extreme soil drying and those collected from the wetter site will grow largest under moderate soil drying.

Co-adaptation hypothesis: plants grown with sympatric soil biota will be larger and more tolerant of soil drying compared to allopatric pairings.

Functional equilibrium hypothesis: plant growth and tolerance of soil drying will be associated with greater fungal allocation to structures that facilitate acquisition and exchange of the most limiting soil resource (hyphae and arbuscules) and less allocation to storage structures (vesicles). We further hypothesize that optimal allocation is one of the mechanisms in which the sympatric advantage is expressed.

Testing these hypotheses will help generate a useful framework for predicting the responses of mycorrhizal symbioses to increasingly water limited environments. Also, we expect to evidence that maintenance or re-creation of sympatric pairings of plant genotypes and soil organisms may be important for successful ecological restoration, forestry, assisted plant migration and other applications.

Methods

Sources of plants and soil organism inoculum

Seeds and soil were collected from two sites within 25 km of one another, but with very different annual precipitation. The wetter site (hereafter "wet site") was a semi-arid grassy understory of a piñon-juniper woodland on the west side of the Kaibab Plateau (Coconino County, Arizona, USA) at an elevation of 2,064 m with approximately 43 cm of precipitation annually (PRISM Climate Group). The drier site (hereafter "dry site") was a semi-arid grassland adjacent to an alluvial drainage on the east side of the Kaibab Plateau at an elevation of 1710 m with an average of 28 cm of precipitation annually (PRISM Climate Group). The soil at both sites is derived from Kaibab Limestone and the wet site is an argid while the dry site is a mosaic of orthents and calcids.

Bouteloua gracilis seed was collected from the two sites using the Seeds of Success protocol (http://www.nps.gov/planTs/sos/protocol/index.htm). Live soil inoculum was collected from the rooting zone of *B. gracilis* along three 100 m transects established from a random origin (azimuths of 0˚, 90˚ and 270˚) at the wet and dry sites. Soil subsamples within each site were pooled together and mixed. Inoculum soil was refrigerated 2 weeks until its use in the experiment. The abundance of different soil organisms in the two inoculum soils was determined using phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analysis. Lipids were extracted from 5 g of freeze-dried inoculum soil by vortex mixing in a one-phase mixture of citrate buffer, methanol, and chloroform (0.8:2:1, v/v/v, pH 4.0). The biomass of AM fungi was estimated from the NLFA 16:1 ω5, 20:1 ω9, and 22:1 ω13, biomass of other fungi was estimated from 18:2 ω9,12c, and biomass of bacterial groups was estimated signature PLFAs for gram positive and gram negative bacteria (Olsson *et al.,* 1995). This analysis indicated that the soil

inoculum from the wet and dry sites had similar abundances of various fungal groups, including AM fungi, and bacteria (Supporting Information Table S1).

Experimental design

Mesocosms were prepared with all four possible combinations of plant and inoculum origin: two sympatric combinations (inoculum and plants from the wet site, or inoculum and plants from the dry site) and two allopatric combinations (inoculum from the dry site with plants from the wet site, or inoculum from the wet site with plants from the dry site). These treatments were further crossed with two levels of water availability to mimic the severity of water limitation at the two source sites. To generate a frame of reference for the performance of plants without sympatric or allopatric soil organisms under the soil drying regime that most closely resembles their home site, we created two sterile inoculum treatments in which plants from the wet site were grown with sterile soil under a moderate drying regime and plants from the dry site were grown in sterile soil under extreme drying conditions. Each combination of plant ecotype, inoculum origin and moisture regime was replicated 9 times, resulting in 72 mesocosms, plus, the two sterile inoculum treatments replicated 9 times for a total of 90 experimental units.

Mesocosms were constructed from 21 L plastic containers (43 cm x 28 cm x 18 cm) with six 0.3 cm diameter holes drilled into the bottom for drainage. In order to remove the effects of any variation in soil physical and chemical characteristics at the two different sites, we created a sterilized common soil using a 1:1 mixture of soil from the two sites that was s sterilized at 125°C for 48 hours. Gundale *et al.* (2018) found that composite soil media is appropriate for these types of experiments. Each mesocosm was filled with approximately 15 liters of sterilized soil and topped with a 1 cm thick band of either live or sterilized (dead) inoculum soil. *Bouteloua*

gracilis seed was sprinkled onto the inoculum soil at a rate of 60 seeds per mesocosm and later thinned to 10 seedlings per mesocosm. Mesocosms were placed in fully randomized spatial locations to account for microclimatic variation within the glasshouse.

Watering treatments

Initially, all mesocosms were watered three times each week for eight weeks and then they were watered twice per week for four weeks before starting the drying treatments. Each watering event brought the mesocosms to field capacity to ensure adequate moisture for plant establishment. Rather than simulate an unrealistically abrupt transition from abundant moisture to dry conditions, we simulated a more gradual transition based on percent of field capacity. These transitions simulate what a plant may experience during the growing seasons as soil moisture diminishes after snowmelt or summer monsoons. Mass at field capacity was estimated by weighing ten randomly selected containers 24 hours after watering. Then, the mass of one randomly selected container was measured every other day, until a soil mass threshold indicated it was time to water again to field capacity. For the moderate drying treatment, we used an initial threshold of 60% of mass at field capacity. For the extreme drying treatment, we used an initial threshold of 40%. After each sequential watering, we decreased both of these threshold percentages by 5%. This both gradually decreased the amount of water available to the plants and increased the length of time between watering events. These watering regimes were designed to simulate drying events at each respective site. Eventually, we reached permanent wilting point in both treatments resulting in at least 90% mortality after 8 months when the experiment was terminated.

Plant performance

Every two weeks, we measured plant height in all containers and the percentage of plant tissue that was green was monitored to estimate the length of time until plant senescence. Greenness was based on ocular estimates of color. No plants produced inflorescences. At the termination of the experiment all aboveground biomass was clipped, dried at 60°C for 24 hours and weighed. Root biomass was sampled by taking four soil cores (5 cm diameter and 18 cm deep). Roots were cleaned, dried and weighed and the weight of roots per volume of core was used to estimate root biomass in the total volume of the mesocosm.

AM fungal performance

Soil and root materials obtained from destructive harvesting at the end of the experiment were analyzed from all 90 mesocosms. A 10 g subsample of fresh root material was refrigerated until it could be examined for root colonization by fungi. Root samples were cleared with 5% KOH and stained with ink in vinegar (Vierheilig *et al.*, 1998) . Colonization by AM fungi and other root endophytes was determined using the gridline intersect method at 200 × magnification (McGonigle *et al.*, 1990) . Mycorrhizal root colonization was distinguished as arbuscules, vesicles and hyphae; dark septate endophytes (DSEs) were also quantified.

The soil-borne (external) hyphae of AM fungi were extracted from the soil cores after root removal, using the methods of Sylvia (1992) , and quantified using a gridded eyepiece graticule in an inverse compound microscope at 250 × magnification. At points where hyphae intersected gridlines, hyphae were counted and counts were converted to length of hyphae per gram of soil. Hyphae of AM fungi were distinguished from other fungal hyphae based on their morphology and color.

Statistical analysis

Soil biota effect was calculated to quantify plant biomass responses to AM fungi and other soil organisms relative to plants grown in the absence of living inoculum. Each *B. gracilis* population was compared to its own sterile reference grown under the moisture regime most similar to its site of origin.

$$Soil\ biota\ effect\ = \frac{\mu_{living} - \mu_{sterile}}{\sqrt{\frac{(n_{sterile} - 1)sd^2_{sterile}}{(n_{living} - 1)sd^2_{living}}}}$$

Where μ is the mean final plant biomass, n is the sample size, and sd is the standard deviation of the treatment of interest.

Three-way repeated measures ANOVA was used to compare the effects of plant origin, soil inoculum origin and watering regime on plant height and time until senescence over 24 time points. Three-way ANOVA was used to compare final plant biomass, soil biota effect, density of external AM hyphae, and percent root length colonized by AM fungi and DSEs. Differences within groups were determine using Tukey's HSD test. Linear regressions were used to determine relationships between soil biota effect and density of external AM hyphae, and percent root length colonized by different AM fungal structures and DSEs. Model assumptions were checked using the Shapiro-Wilk test of normality and the Levene's test of heterogeneity of variance. All statistics were conducted in R (version 3.3.1).

Results

Plant responses

Bouteloua gracilis ecotypes from the wet and dry sites differed in their responses to moderate and extreme drying. Ecotypes tended to grow taller and stay green longer when grown under the watering regime most similar to their site of origin (Fig. 1). Plants grew significantly larger and were more tolerant of drying when grown with sympatric soil organisms compared to allopatric soil organisms. Plants from the dry site inoculated with their sympatric soil organisms consistently grew 1.5x taller than those grown in sterile soil or inoculated with allopatric soil organisms (Fig. 1a; $F = 82.9$, $p < 0.001$). Plants from the wet site grew 1.2x taller under moderate drying than under extreme drying with their sympatric soil organisms ((Fig. 1a; $F = 82.9$, $p < 0.001$). Plants grown with allopatric soil organisms were no taller than those grown in sterile controls (Fig. 1a). Plants paired with their sympatric soil organisms maintained green tissue 3 - 4 weeks longer into the drought events than those grown in sterile soil or grown with allopatric soil organisms (Fig. 1b; $F = 128.4$, $p < 0.001$). Sterile controls stayed green up to two weeks longer than plants that were grown with allopatric soil organisms (Fig. 1b).

There were no main effects of plant population, or watering treatment on plant biomass, however, there was a significant effect of inoculum source and an interaction between plant origin and soil inoculum (Supporting Information Table S2). Tukey's HSD shows that plants grown with sympatric soil organisms were consistently larger than allopatric pairings (Fig. 2). Although not statistically significant, the total biomass of plants from the dry site tended to be higher when grown under extreme drying than under moderate drying (Fig. 2).

In both *B. gracilis* populations, the soil biota effect was positive for sympatric inoculum and negative for allopatric inoculum, and this effect was exacerbated in plants from the wet site

grown in extreme drought (Fig. 3). The dry site population exhibited a more positive response in sympatry and a less negative response in allopatry compared to the wet site population. There was no effect of drought treatment alone.

Fungal responses

The initial soil inoculum from the wet and dry sites had nearly equal microbial biomass (Supplemental Information Table S1), but at the end of the experiment, abundance of AM fungal hyphae differed across the experimental treatments. The density of external AM fungal hyphae in the soil responded to watering treatment and provenance. Mesocosms with sympatric pairings of plants and soil inoculum consistently had more external AM fungal hyphae than allopatric ones. The highest density of external AM fungal hyphae was observed in mesocosms with both *B. gracilis* and soil inoculum from the dry site that were grown under the extreme drying treatment (Fig. 4). Under the moderate drying treatment, sympatric pairs of plants and inoculum from the dry site population produced nearly two times more external hyphae than pairs from the wet site (Fig. 4). (Supporting Information Table S5).

Root colonization by different fungal structures was highly responsive to watering treatment and provenance. Mycorrhizal fungal hyphae inside plant roots showed similar patterns as the hyphae outside plant roots with approximately 2.5x greater colonization in extreme drying treatments in sympatric pairings than in allopatric pairings in extreme drying (Fig. 5). In general, there was 10% more root length colonized by hyphae in sympatric pairings regardless of drought treatment (Fig. 5; Supporting Information Table S4). Furthermore, sympatric pairings had three to four times more arbuscular colonization compared to allopatric pairings in moderate and extreme drought respectively (Fig. 5; Supporting Information Table S5. In contrast, vesicular

colonization was more than twice as high in allopatric pairings compared to sympatric pairings. The highest colonization by fungal vesicles was observed in allopatric pairings of the wet population grown under extreme drying (Fig. 5; Supporting Information Table S6*).* In the dry-site *B. gracilis* population provenance of the inoculum did not influence colonization by DSEs but in the wet-site population it did with significantly higher colonization in sympatric pairings (Fig. 5; Supporting Information Table *S7*). There was a strong, positive relationship between the soil biota effect and the abundance of external and internal hyphae and arbuscules, and a strong negative relationship with root length colonized by vesicles (Fig. 6). There was no significant linear relationship between effect size and colonization by DSEs.

Discussion

Our findings show evidence that local adaptation of *B. gracilis* is largely generated by co-adaptation between plants and their associated soil biota. When inoculated with sympatric soil organisms, the dry site population did best in the extreme drying and the wet site population survived longest in the moderate drying treatment (Figs. 1, 2). In contrast, plants inoculated with allopatric soil biota performed no better, or even worse, than plants grown with sterile inoculum, regardless of soil drying regime (Fig. 3). We interpret these results as evidence that local adaptation in our system is the result of co-adaptation between plant ecotypes and their associated root and rhizosphere microorganisms. We also found support for the functional equilibrium hypothesis which we interpret as one of the expressions of co-adaptation.

Environmental Stress Optimizes Co-adaptation Among Plants and Soil Biota

Local adaptation in plants and soil microorganisms has been shown to be driven by several abiotic factors such as climate (Hoeksema and Forde 2008) and soil (Rúa et al. 2016), which are often linked to environmental stress. In our system, severe water limitation at the dry site selected for sympatric soil biota that were more beneficial under extreme drying than moderate drying while sympatric soil biota from the wet site did not show this difference (Fig. 3). The *B. gracilis* population from the dry site appears to have selected for traits that best optimize the benefits of sympatric associations with soil biota and also minimize the detrimental effects of allopatric soil biota. Although both populations experienced growth depressions with allopatric soil biota, growth depression was significantly more negative in the population from the wet site (Fig. 3). One mechanism for the sympatric advantage is that antagonistic relationships are likely selected against (Hoeksema, 2010; Werner & Kiers, 2014) . It is not known if antagonistic relationships are due primarily to the species composition of soil organisms, or the behaviors of different populations of the same plants and soil organisms. In either case, a longer shared history could reduce antagonism through either 1) increased abundance of mutualistic taxa at the expense of commensal or parasitic taxa (Waller *et al.*, 2016; Bennett *et al.*, 2017) , or 2) altered gene frequencies or gene expression within either or both plant and microbial populations, that enhance mutualistic behavior (Hoeksema, 2010) . An equally likely explanation of the sympatric advantage is the positive selection of cooperative traits over many generations, reminiscent of the often highly specialized plant-pollinator interactions (Ehrlich & Raven, 1964; Brundrett, 2002; Burdon & Thrall, 2009) such co-

adaptation could hypothetically play out on a very local scale because one set of partners, the AM fungi and other soil organisms, are more dispersal-limited than their plant partners.

Extension of the Functional Equilibrium Model to Water Limitation

Plants allocate photosynthate to AM fungal symbionts as an alternative strategy to investment in roots for acquiring soil resources, and this may buffer against stress caused by either nutrient or water limitation (Westoby, 1998; Bever et al., 2009; Almaghrabi et al., 2012; Augé et al., 2015; Ji & Bever, 2016) . Compared to allopatric combinations, sympatric pairings of plants and inoculum produced greater growth of external and internal AM hyphae and arbuscules, and less root colonization by vesicles (Figs. 4, 5). This result is important because hyphae and arbuscules are involved in the acquisition and exchange of soil resources between AM fungi and their host while vesicles are fungal storage units that have been associated with less mutualistic or even parasitic AM symbioses (Johnson 1993; Lekberg et al., 2010). The functional equilibrium model suggests that plants invest in structures that most effectively help them forage for the most limiting resource (Brouwer 1983; Bloom et al., 1985). The observed shift in relative allocation between resource harvesting and exchange structures versus storage structures suggests that the functional equilibrium model may be applied to allocation to fungal structures in AM symbioses (Johnson et al., 2003).

Support for functional equilibrium in AM symbioses has been documented in nutrient limited systems (Johnson 2010). Results of this study support the assertion that a functional equilibrium between plants and associated mycorrhizal fungi may also exist in water limited systems. It is well understood that mycorrhizal fungi can alter the water balance of their host plants both directly and indirectly, thus it is logical that the functional equilibrium model can

incorporate water as a soil resource (Augé, 2001; Augé et al., 2015) . Mycorrhizal hyphae in the soil can act as hollow tubes that transport water directly from soil pores to plant root tissue (Allen et al., 1981; Hardie, 1985) . While this topic has been debated over the years, recent experimental evidence supports this claim (Ruth et al., 2011) . Alternatively, AM fungi alter plant water balance by variety of indirect means. First, by improving plant nutritional status, mycorrhizas increase plant size and thus can contribute to increased root surface area for plant uptake of soil water (Ruiz-Lozano & Azcón, 1995) . In our experimental system, water is obviously in limiting supply, but because phosphorous availability is influenced by soil moisture, we cannot rule out the possibility that plants and fungi are allocating resources toward P-foraging, and as a side effect benefiting from enhanced water access. Mycorrhizal fungi also are known to alter the hormonal status of their plant hosts and thus can help plants regulate stomata closure during periods of soil drying (Augé et al., 2015) . Lastly, AM fungi can alter hydraulic conductivity in the soil through increased surface area and soil exploration (Bárzana et al., 2012) . Combined, these mechanisms can have a profound influence on plant water balance in mycorrhizal plants compared to non-mycorrhizal controls (Augé, 2001) . These influences make soil water a direct or indirect resource in the economic market between plant hosts and their associated AM fungi. When soil water is limiting, the functional equilibrium model would suggest that plants and their associated mycorrhizal fungi would invest in structures that optimize the foraging of soil moisture. For a plant that is highly mycorrhizal, this likely means increased investment to external hyphae to explore a greater soil pore volume for soil moisture, as we observed in our study. If, however, a plant is less mycorrhizal or is growing in a soil environment with greater soil water content, plants may alternatively invest in fine root growth rather than in their fungal symbionts.

The whole-soil inoculum used in our study contained complex communities of soil organisms, consequently, our observed inoculum effects arise from the interactions of plants with many soil-dwelling microorganisms, not only AM fungi. Although we acknowledge the potential roles of unmeasured soil organisms, the strong correlations between mycorrhizal structures and plant responses suggest that AM fungi are important drivers of the observed co-adaptation dynamics. Also intriguing in our results were patterns of DSEs being more prevalent in sympatric pairings from the wet site, however the abundance of DSEs was not correlated with plant responses. Although the functions of DSEs in natural ecosystems are still relatively poorly understood, studies suggest that they tend to be more abundant in warmer, drier ecosystems and that they may reduce the pathogenicity of oomyctes (Newsham, 2011; Tellenbach & Sieber, 2012). Also, research shows that DSEs have a positive impact on plant growth in the absence of nitrogen fertilizer (Newsham, 2011). Our results are difficult to discern in the role DSEs played in tandem with mycorrhizal colonization in facilitating plant growth, but we cannot eliminate the possibility of their contribution. Given the strong correlation between AM fungi colonization and the soil biota effect compared to no correlation between DSEs and plant growth it is likely that in our system AM fungi have a more prevalent role in the ecosystem than DSEs.

Conclusions

Moving forward, the frontier of this line of inquiry will be to determine to what degree the sympatric advantage is due to resource availability or co-evolution, and what controls their relative importance. This study shows how fungal allocation, either within species or across species in the community, varies in sympatric vs. allopatric plant-mycorrhizal pairings and provides evidence that fungal allocation, at least in part, determines their function. This work

provides the foundation for the integration of a diversity of techniques from transcriptomics to community genetics to better understand the complex ecology of plant-interactions with soil organisms (Hungate *et al.*, 2015) . It is plausible that both population and community level forces are interacting to determine mycorrhizal allocation and function across resource gradients, and a better understanding of these determinants of fungal allocation is an intriguing next step.

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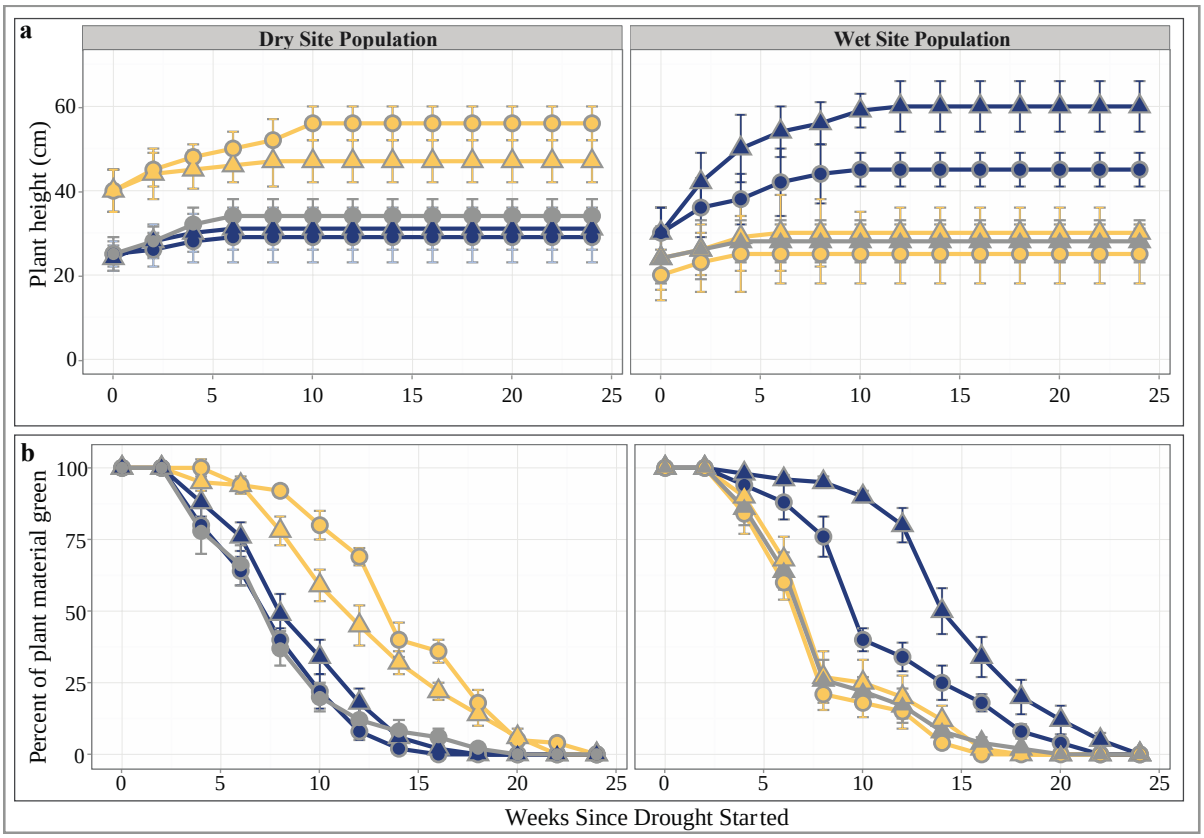

Figure 4.1: Plant height (**a**) and percent of green plant material (**b**) plotted against time since initiation of drought treatments for different plant populations, inoculum sources and watering treatments. Dark blue represents wet-site soil biota and light brown represent dry-site soil biota. Grey represents sterile inoculum. Triangles represent moderate drought and circles represent extreme drought treatments. Error bars represent the standard error of the means.

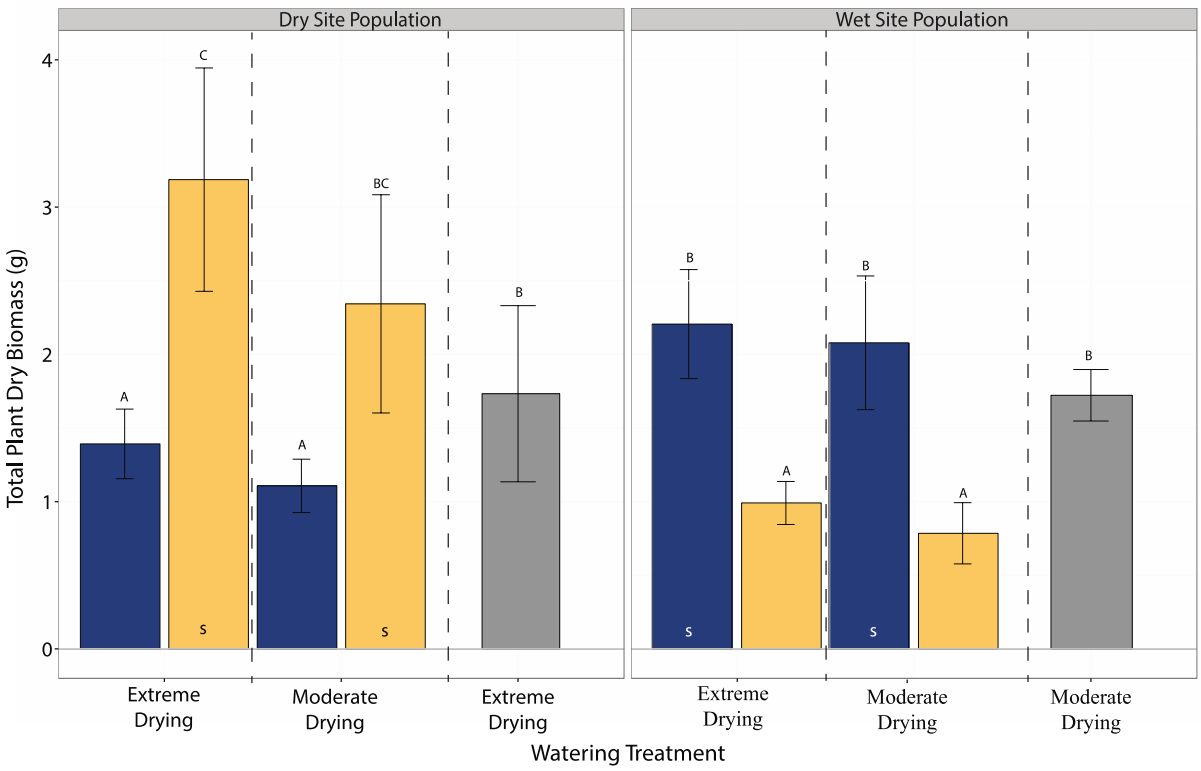

Figure 4.2: Total plant biomass in mesocosms at the termination of the experiment. Dark blue represents wet-site soil biota and light brown represents the dry-site soil biota, grey represents sterile inoculum. Plants were grown under extreme drought and moderate watering treatments for 32 weeks. 'S' represents sympatric pairings of plants and soil biota. Error bars represent the standard error of the means. Different letters indicate significantly different ($p<0.05$) results from Tukey's HSD.

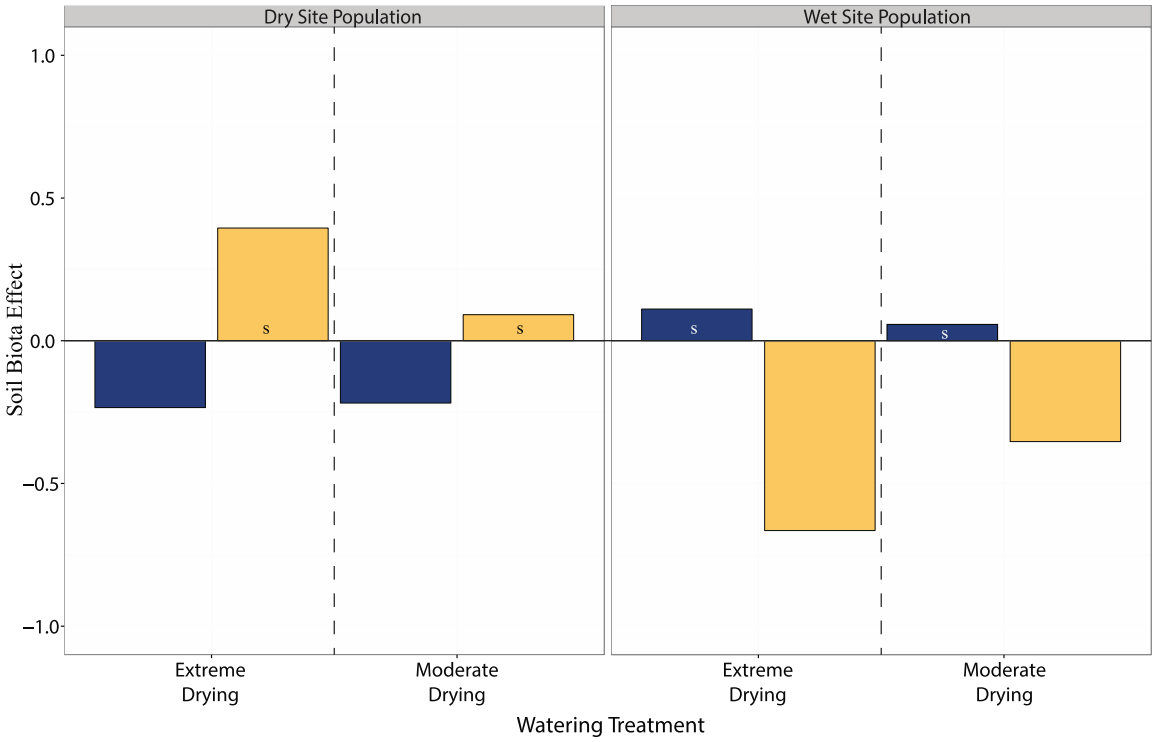

Figure 4.3: Soil Biota Effect for sympatric and allopatric plant-inoculum pairings grown under moderate and extreme drought for wet-site and dry-site plant populations. Dark blue indicates wet-site soil biota and light brown indicates dry-site soil biota. Plants and inoculum were grown under extreme and moderate drought conditions for 32 weeks. 'S' represents sympatric pairings of plants and soil biota. Error bars represent the standard error of the means.

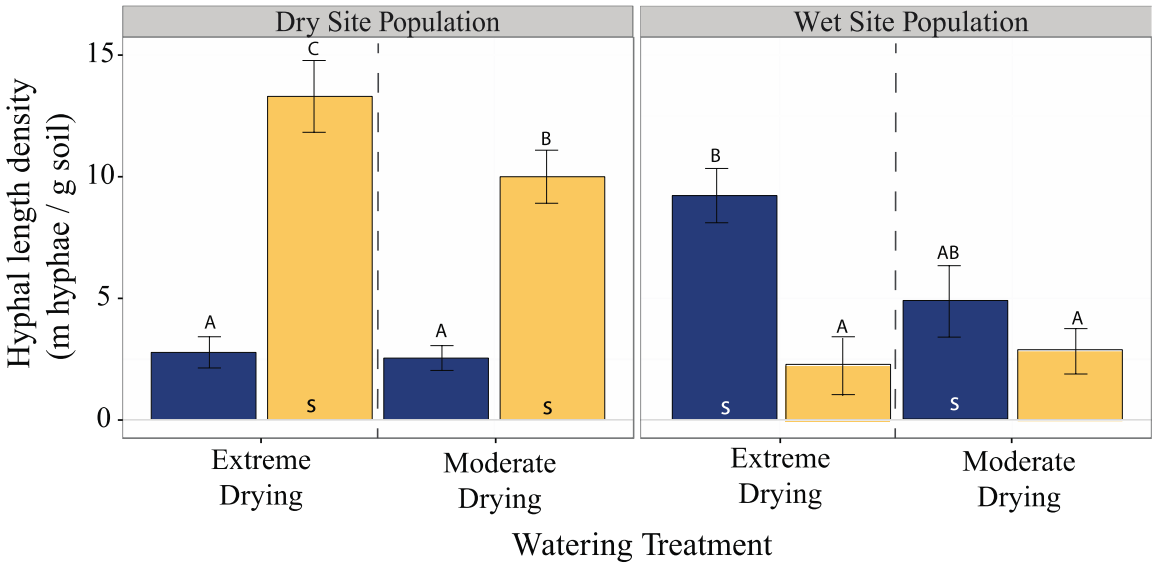

Figure 4.4: External hyphal length density in mesocosm soils with sympatric and allopatric pairings of plants and inoculum grown under moderate and extreme drought. Dark blue indicates wet-site soil biota and light brown represents dry-site soil biota. Plants and inoculum were grown under extreme and moderate drought conditions for 32 weeks. 'S' represents sympatric pairings of plants and soil biota. Error bars represent the standard error of the means. Different letters indicate significantly different ($p<0.05$) results from Tukey's HSD.

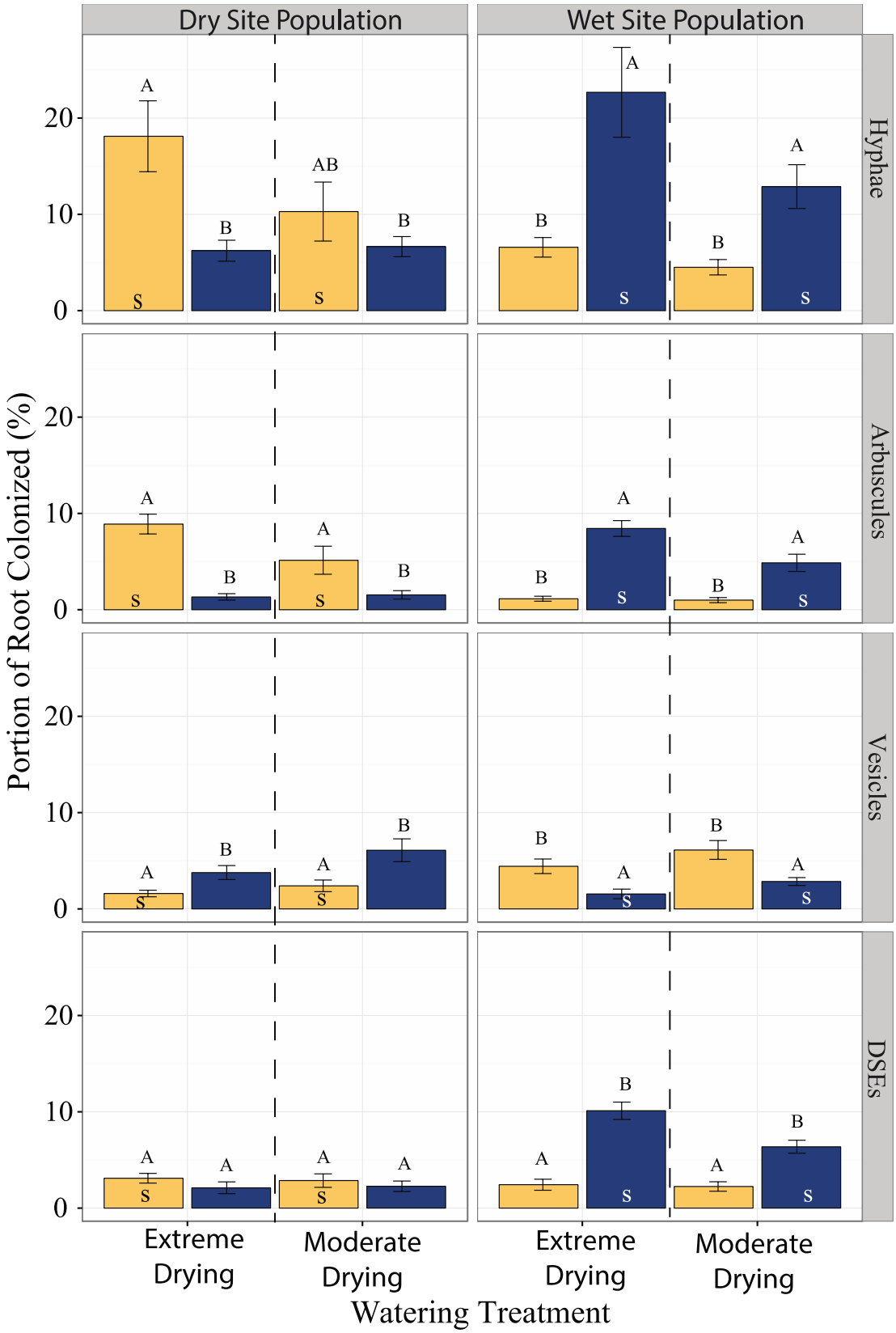
Dry Site Population
Wet Site Population
Hyphae
Arbuscules
Vesicles
DSEs
Portion of Root Colonized (%)
0
10
20
A
B
AB
S
Extreme Drying
Moderate Drying
Watering Treatment

Figure 4.5: Percentage of plant root length colonized by hyphae, arbuscules, vesicles and dark septate endophytes (DSEs) in wet-site and dry-site populations of *Bouteloua gracilis*. Dark blue represents wet-site soil biota and light brown represents dry-site soil biota. Plants and inoculum were grown under extreme and moderate drought conditions for 32 weeks. 'S' represents sympatric pairings of plants and soil biota. Black bars represent the standard error of the means. Different letters indicate significantly different ($p<0.05$) results from Tukey's HSD.

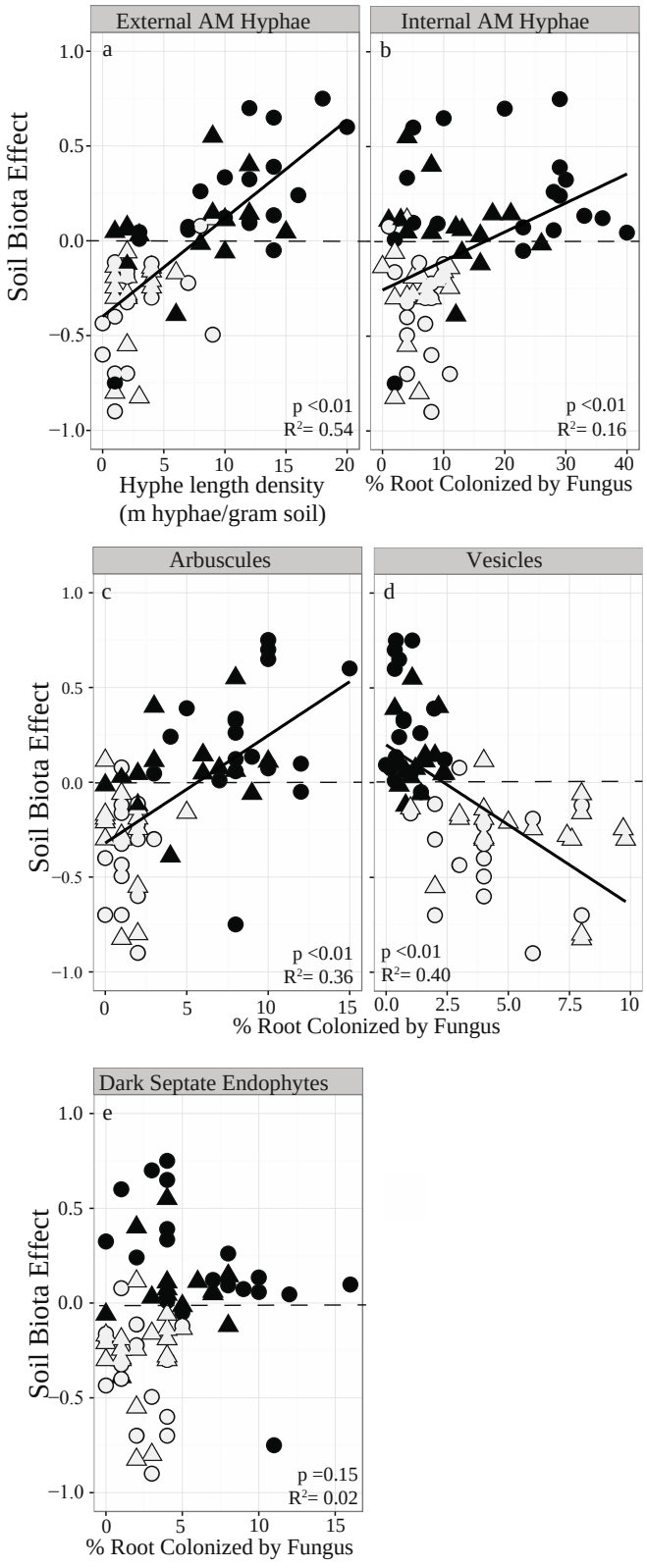
External AM Hyphae
Internal AM Hyphae
a
b
Soil Biota Effect
p <0.01
R²= 0.54
p <0.01
R²= 0.16
Hyphe length density
(m hyphae/gram soil)
% Root Colonized by Fungus
Arbuscules
Vesicles
c
d
p <0.01
R²= 0.36
p <0.01
R²= 0.40
% Root Colonized by Fungus
Dark Septate Endophytes
e
p =0.15
R²= 0.02
% Root Colonized by Fungus

Figure 4.6: Soil Biota Effect size plotted against external hyphal length density (m hyphae / gram soil) (a), and percent root length colonized by AM fungal hyphae (b), arbuscules (c), vesicles (d), and dark septate endophytes (e). Sympatric (black symbols) and allopatric (grey symbols) pairing of plants and inoculum were grown under extreme and moderate drought conditions for 32 weeks. Triangles represent moderate drought and circles represent extreme drought treatments.

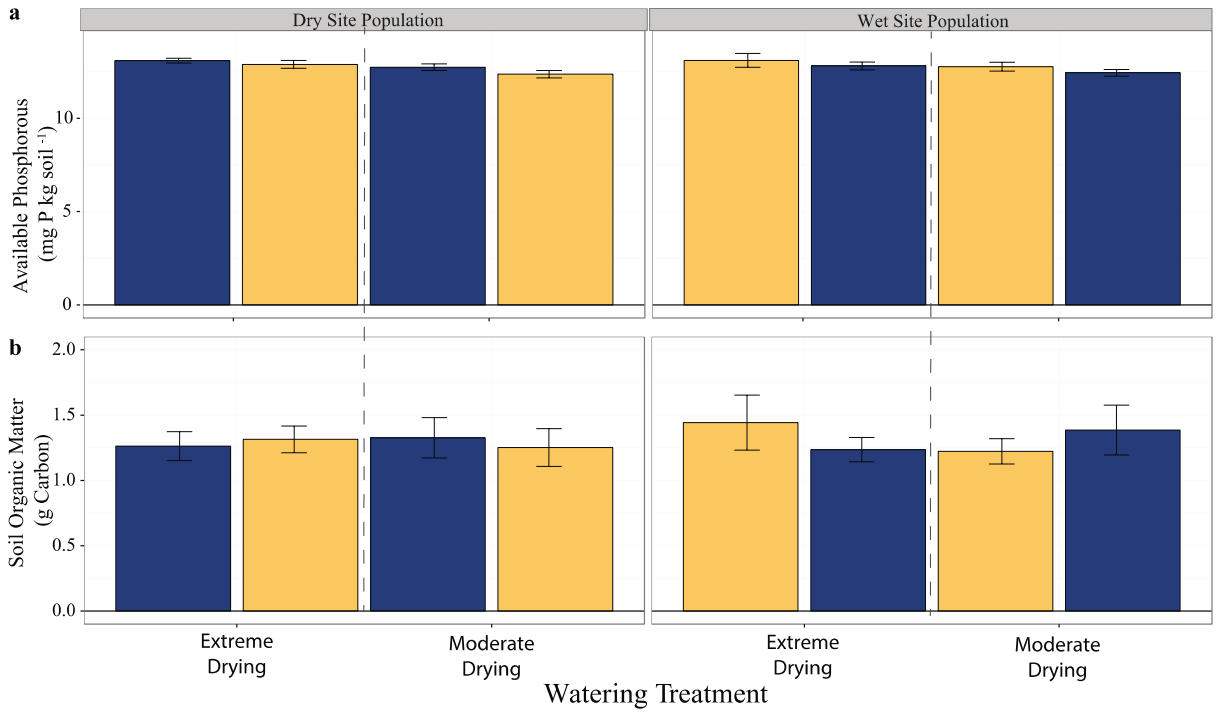

Figure 4.7: Dry Inoculum available phosphorous (panel a) and Inoculum organic matter (panel b) for treatments with the wet site inoculum (dark blue) and the dry site inoculum (light brown) for treatments with plants grown from each site. There are no statistically observed differences observed across any treatments.

Table 4.1: Phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) in the Inoculum inoculum from the wet and dry sites.

Analysis	Signature Organism	Lipid Fatty Acid	Dry Site (nmol/gram)	Wet Site (nmol/gram)
NLFA	AM fungi	16:1w5c	*8.29*	*10.03*
NLFA	AM fungi	20:1w9	*0*	*0*
NLFA	AM fungi	22:1w13	*0*	*0*
NLFA	SAP fungi	18:2w9,12c	*5.48*	*3.48*
NLFA	SAP fungi	18:1w9c	*6.24*	*6.42*
PLFA	Gram + bacteria	i-15:0	*2.65*	*2.12*
PLFA	Gram + bacteria	a-15:0	*15.27*	*13.62*
PLFA	Gram + bacteria	i-17:0	*1.75*	*1.57*
PLFA	Gram + bacteria	i-16:0	*0*	*0*
PLFA	Gram - bacteria	16:1w7	*1.19*	*0.87*
PLFA	Gram - bacteria	cy19:0	*0*	*0*
PLFA	Gram - bacteria	2-OH 16:0	*0*	*0*
PLFA	Gram - bacteria	18:1w9 trans	*2.43*	*3.56*

Table 4.2*: ANOVA table for plant height responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	142.5	142.5	16.96	< 0.001 ***
Plant	1	38.2	38.2	4.55	0.03 *
Water	1	134.5	134.5	16.01	0.001 **
Inoculum * Plant	2	850.2	425.1	50.61	< 0.0001 ***
Inoculum * Water	1	36.2	36.2	4.3	0.04 *
Plant * Water	1	1.2	1.2	0.14	0.84
Inoculum * Plant * Water	1	72.5	72.5	8.6	0.01 **
Residuals	62	543.2	8.4		

Table 4.3*: ANOVA table for plant greenness responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	128.4	128.4	12.57	< 0.001 ***
Plant	1	58.9	58.9	5.76	0.02 *
Water	1	119.9	119.9	11.74	0.001 **
Inoculum * Plant	2	772.6	386.3	37.84	< 0.001 ***
Inoculum * Water	1	0.4	0.4	0.04	0.85
Plant * Water	1	0.0	0.0	0.00	0.99
Inoculum * Plant * Water	1	67.5	67.5	6.61	0.01 **
Residuals	62	623.1	10.2		

Table 4.4*: ANOVA table for plant biomass responses to each experimental treatment.*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	34.27	17.135	10.15	< 0.001 ***
Plant	1	3.74	3.75	2.22	0.14
Water	1	1.00	1.00	0.60	0.44
Inoculum * Plant	2	2.66	1.33	0.79	0.46
Inoculum * Water	1	0.22	0.22	0.13	0.72
Plant * Water	1	0.66	0.66	0.39	0.53
Inoculum * Plant * Water	1	0.43	0.43	0.25	0.62
Residuals	62	129.96	1.69		

Table 4.5*: ANOVA table for external AM Hyphae in the Inoculum responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	139.42	139.42	13.64	0.0005***
Plant	1	61.14	61.14	5.98	0.017*
Water	1	106.67	106.67	10.44	0.0019**
Inoculum * Plant	2	770.30	385.15	37.69	<0.00001***
Inoculum * Water	1	1.99	1.99	0.19	0.66
Plant * Water	1	0.71	0.71	0.07	0.79
Inoculum * Plant * Water	1	67.52	67.52	6.61	0.013*
Residuals	62	613.12	10.22		

Table 4.6*: ANOVA table for root length colonized by AM Hyphae responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	81.2	81.25	0.62	0.43
Plant	1	5.5	5.54	0.04	0.84
Water	1	527.3	527.30	4.01	0.04*
Inoculum * Plant	2	199.l	99.55	0.75	0.45
Inoculum * Water	1	365.9	365.86	2.78	0.10
Plant * Water	1	52.4	52.36	0.40	0.53
Inoculum * Plant * Water	1	39.1	39.13	0.30	0.58
Residuals	62	8023.9	131.54		

Table 4.7*: ANOVA table for root length colonized by AM arbuscules responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	0.71	0.715	0.048	0.83
Plant	1	0.37	0.37	0.03	0.87
Water	1	0.48	0.48	0.03	0.85
Inoculum * Plant	2	9.87	4.94	0.33	0.42
Inoculum * Water	1	17.99	17.99	1.20	0.28
Plant * Water	1	55.56	55.56	3.67	0.05*
Inoculum * Plant * Water	1	136.90	136.90	9.05	0.004**
Residuals	62		15.13		

Table 4.8*: ANOVA table for root length colonized by AM vesicles responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	0.003	0.003	0.0015	0.97
Plant	1	2.30	2.30	0.92	0.34
Water	1	0.05	0.05	0.0019	0.89
Inoculum * Plant	2	96.67	48.49	19.16	<0.001***
Inoculum * Water	1	7.23	7.23	2.86	0.096
Plant * Water	1	23.75	23.75	9.38	0.003**
Inoculum * Plant * Water	1	2.87	2.87	1.13	0.29
Residuals	62	154.6	2.53		

Table 4.9*: ANOVA table for root length colonized by DSEs responses to each experimental treatment*

Factor	Df	Sum of Squares	Mean Squares	F	P value
Inoculum	1	5.0	4.97	0.03	0.85
Plant	1	40.9	40.86	0.29	0.60
Water	1	2.60	2.65	0.02	0.89
Inoculum * Plant	2	345.5	172.75	1.2	0.24
Inoculum * Water	1	101.0	100.96	0.71	0.40
Plant * Water	1	35.60	35.61	0.25	0.62
Inoculum * Plant * Water	1	229.50	229.50	1.6	0.21
Residuals	62	8732.2	143.15		

Chapter V

Conclusions and Management Implications

Our studies show that plants respond to different aspects of novel environments in several ways. If the predictions that the Southwest is likely to experience warmer climates and more variable precipitation with heightened frequency of drought are accurate, then we can expect a general decline in plant productivity within species' current distributions (Breshears *et al.*, 2008; Allen *et al.*, 2010; Cayan *et al.*, 2010; Seager & Vecchi, 2010). Consistent with this expectation, we observed 10-20% less plant growth after three years of simulated warming in a dominant grass and a dominant tree species. We also observed no decrease, or even an increase in plant biomass at sites that are cooler and wetter than their current site, even if the transplant site is outside of the species' current distribution. This could be the result of the climate of these sites better representing the historic conditions in which plant populations evolved, however, it could also be the result of alleviation of plant stress via greater availability of the most limiting resource. These findings are consistent with studies that suggest that plant populations have a tendency to lean upslope in response to climate warming (Breshears *et al.*, 2008; Feeley *et al.*, 2011). These observations also support the idea that assisted plant migration may be a feasible strategy to mitigate plant species loss as a result of climate change in order to maintain productivity of certain target species (Gray *et al.*, 2011; Butterfield *et al.*, 2016; Roberts & Hamann, 2016; Vitt *et al.*, 2016; Bucharova, 2017). We show that plants growing in warmer drier climates tend to have reduced photosynthetic rates relative to those growing in their original environment or at sites that are cooler and wetter, and this may in part explain reductions in plant growth at these sites.

Our studies also highlight that climatic novelty is not the only concern in plants responding to change in their environment. Edaphic conditions can also have profound impacts on plant growth. We observed some soil types to have up to 60% less final plant biomass than when grown in their home soil under +2°C warmer conditions. These results demonstrate how edaphic boundaries can exacerbate the effects of changes in the climate and create harsher growing conditions for plants. For plants migrating to new environments, artificially or naturally, our findings suggest that soil boundaries could create barriers to plant growth and survival (Bucharova *et al.*, 2016; Bjorkman *et al.*, 2017). Changes in soil properties are complex and not all soil types have negative effects on plant growth and it is possible that soil properties that exacerbate already existing stressors have the most negative effect (Bowker *et al.* 2010). As an example, a coarse soil with low water holding capacity is likely to exacerbate the effects of reduced precipitation or drought by having even less available water than soils with greater water holding capacities. These concepts may be further complicated by soil biotic communities which often migrate independently of plants (Allen *et al.*, 1989; Mangan & Adler, 2002; Lekberg *et al.*, 2007).

Our studies demonstrate the importance of co-adapted plants and soil microbes in facilitating plant growth in novel environments. We observed in particular, that in warm dry sites, or in particularly harsh soils that home , or co-adapted soil biota, facilitated plant growth and mitigated against the negative effects observed when plants were grown with different soil biotic communities. These findings suggest that home mutualisms tend to have greater function and provide greater benefit to both the plant host and mutualistic fungal partner (Johnson *et al.*, 2010; Pánková *et al.*, 2014; Revillini *et al.*, 2016; Rúa *et al.*, 2016). At warm dry sites, plants maintained greater photosynthetic rates and grew as much or more than plants at their home site

when grown with co-adapted soil biota than plants growing with different soil biota communities. We observed that in co-adapted mutualisms, plant roots tend to be colonized to a greater extent by fungal structures that contribute to the uptake and exchange of nutrients, whereas in different plant-soil biota partnerships we observed more fungal structures dedicated to storage of nutrients. These results indicate that fungal allocation can contribute to plant growth and fitness, and varies based on the history of mutualism. These findings imply that restoration projects in the context of global change are likely to be more successful and resilient if management practices can also restore soil microbial communities (Koziol & Bever, 2016; Wubs *et al.*, 2016). Our results suggest that plant communities migrating to new environments may be more successful if they also move with their associated microbes, however, plant growth in cooler environments was more variable without their original microbes and it may be a less critical factor for the immediate future (Bucharova, 2017).

Overall, our studies show plant responses to novel environments is dependent upon climatic conditions, edaphic boundaries, and soil biotic communities. If more than one or all of these factors are changed, plant growth may be more dramatically stunted. If only one aspect of the plant environment is altered then plants generally show greater resistance to change. This logically makes sense as local adaptation has been demonstrated to each one of these factors individually and is possibly linked to co-adaptation (Ehlers & Thompson, 2004; Johnson, 2010; Pánková *et al.*, 2014; Sanderson *et al.*, 2015; Hällfors *et al.*, 2016; Revillini *et al.*, 2016; Rúa *et al.*, 2016; Bjorkman *et al.*, 2017; Kraemer & Boynton, 2017).

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