INTEGRATING PLANT FUNCTIONAL TRAITS, GENETICS, PHENOTYPIC PLASTICITY, AND COMMUNITY STRUCTURE TO ASSESS THE IMPACT OF CLIMATE CHANGE ON NATIVE PLANTS IN THE SOUTHWESTERN U.S.

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ABSTRACT

INTEGRATING PLANT FUNCTIONAL TRAITS, GENETICS, PHENOTYPIC PLASTICITY, AND COMMUNITY STRUCTURE TO ASSESS THE IMPACT OF CLIMATE CHANGE ON NATIVE PLANTS IN THE SOUTHWESTERN U.S.

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The southwestern U.S. is a hot spot of extreme climate change, where increasing temperatures and limited water has resulted in more frequent and severe heatwave, drought, and fire events, subsequently affecting species traits, distributions, interactions, and prevalence across the landscape. These climate extremes, combined with competition from exotic species, has resulted in the degradation of many native ecosystems, including large pine forests and riparian corridors throughout the west. In this dissertation, I examine the impact of climate change in the southwestern United States on the ecological and evolutionary processes influencing species occurrence and traits at the plant community, population, and genotype level. First, I analyze the natural climate change event of severe drought in the Northern Arizona cinder fields to understand the response of whole plant communities to the combined effects of drought-stress and overstory tree mortality. In this long-term observational study, I combine traditional taxonomic metrics with community phylogenetic measures. In the next two studies, I utilize an experimental model of climate change through the use of replicated common garden experiments, where populations of Fremont cottonwood were transplanted into environments ranging from 12°C hotter and 10°C colder compared to their origin sites. Using this design, I first explore the degree of genetic (G), environment (E), and GxE effects on two phenology traits, spring bud flush and fall bud set, within and across the three common gardens. The use of cloned genotypes allowed for the study of phenotypic plasticity for these traits, and the degree of genetic variation, and thus evolutionary potential, for plasticity. Lastly, I compared the among-population quantitative variation in phenology, growth, and leaf traits found in each common garden to their neutral genetic variation using Q_{ST}-F_{ST} analysis to understand the role of climate-driven natural selection and the degree of local adaptation in shaping large phenotypic divergences across the species range. Together, these studies can help inform how important native species will respond to extreme climate events, and help shape management guidelines to ensure healthy and productive ecosystems are preserved.

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PREFACE

The manuscript chapters presented here are written in a format for publication in scientific peer-reviewed journals. As such, the chapters may read as slightly redundant in order to adhere to Northern Arizona University's dissertation guidelines. The first dissertation chapter, titled "Shifting trajectories of plant community diversity, composition, and phylogenetics due to extreme drought in the Southwest", is formatted for the journal Ecology. The second chapter, "Genotypic variation in phenological plasticity: reciprocal common gardens reveal adaptive responses to warmer springs but not to fall frost", is formatted for publication in Global Change Biology. The third dissertation chapter, "Climate-driven selection of functional traits in a foundation tree species reveals strong patterns of local adaptation and the potential for future performance declines with climate change" is formatted for the journal Molecular Ecology. Please note that table and figure legends as well as the literature cited are provided in each of the chapters separately.

CHAPTERS

CHAPTER 1: Introduction

Ecosystems throughout southwestern North America have already and will continue to experience the effects of climate change due to warmer temperatures, increasingly severe drought events, and reductions in streamflow and snowpack across the region (Seager & Vecchi 2010, Garfin *et al.* 2013). These climatic shifts have manifested in altered species traits (Parmesan & Yohe 2003) and distributions (Rehfeldt *et al.* 2006, Brusca *et al.* 2013), and extensive forest dieback, including widespread mortality of dominant conifer species, such as ponderosa and pinyon pines (Allen & Breshears 1998, Adams *et al.* 2009), as well as riparian tree species (Gitlin *et al.* 2006; Figure 1). The loss of foundation tree species can have major downstream impacts on local forest conditions, including (often unpredictable) changes in energy and nutrient fluxes, hydrology, pathogens, and biodiversity (Ellison *et al.* 2005, Ikeda *et al.* 2014). As the southwest becomes increasingly arid, with more frequent extreme heatwave, drought, and flooding events forecast through the end of the century (Ganguly *et al.* 2009, Garfin *et al.* 2013), plant populations once highly adapted to their local environmental conditions stand to become increasingly maladapted, exhibiting reduced productivity and fitness (Shaw & Etterson 2012, Aitken & Whitlock 2013). In this dissertation, I utilize a variety of methods to understand the nature of ecological and evolutionary shifts occurring from the whole plant community level down to individual genotypic changes in the context of ongoing climate change in the southwestern US. In my first dissertation chapter, I add to a long-term observational dataset to document plant community changes resulting from the 2002 drought in arid woodlands dominated by one-seed juniper, pinyon-juniper, and ponderosa pine in the nascent cinder fields of Sunset Crater. In the following chapters, I focus on the foundation riparian tree species, Fremont cottonwood (*Populus fremontii*), in an experimental common garden array to explore the genetic variation in important functional traits and show how these traits can change across environmental gradients as a function of phenotypic plasticity. In addition, the variation in quantitative traits observed in the three common gardens, such as tree growth and phenology, was compared to neutral genetic structure using Q_{ST}-F_{ST} comparisons to elucidate the relative roles of drift and natural selection acting to differentiate populations across Arizona. Together, my research explores the consequences of climatic shifts on key native plants of the southwest and informs restoration and management practices to help maintain these important ecosystems in the face of future climate change.

Figure 1.

Plant community changes after extreme drought

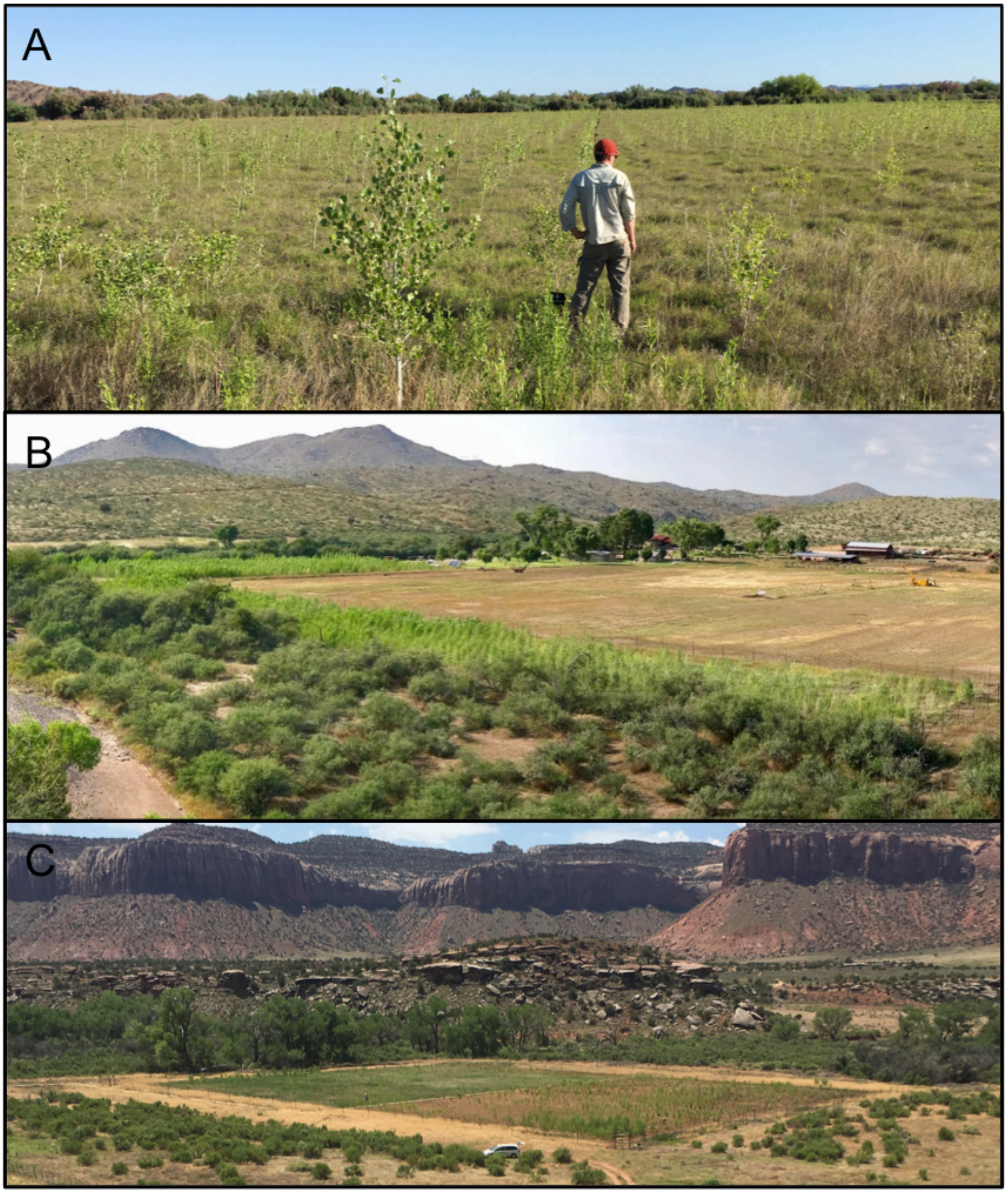
Large-scale tree mortality in the southwest following unusually warm drought conditions (Adams *et al.* 2009) has resulted in shifting ecotone boundaries between forest types (Breshears & Allen 1998) and altered the dominant tree species (Mueller *et al.* 2005) or genotypic composition (Sthultz *et al.* 2009) across the landscape. Drought-induced forest mortality is not confined to the American southwest, but is a global phenomenon (Allen *et al.* 2010), as forests across the world are being increasingly affected by heat and drought stress and associated insect-outbreaks and wildfire. Modeling of climate warming and plant physiology suggests that much of the world's forests will be subject to increasing rates of mortality and replacement by more xeric plants, substantially reducing global carbon storage (McDowell & Allen 2015). The restructuring of forests has the potential to create no-analog communities, combinations of species that have never co-occurred, and consequently new combinations of plant interactions and functionality (Williams & Jackson 2007). Although there has been much research into the effect of severe drought on overstory tree species in the southwest, little is known about the long-term responses of understory plant communities after extensive tree mortality.

The Sunset Crater volcanic field north of Flagstaff, Arizona is an extremely harsh environment for plants, given the low nutrient content, high temperatures, and poor water holding capacity of the young cinder soils (Kuske *et al.* 2003), with facilitative nurse plant associations essential for key species' establishment (Sthultz *et al.* 2007). These already harsh conditions combined with the severe drought of 2002 led to widespread mortality of juniper, pinyon pine, and ponderosa pine (Koepke *et al.* 2010). After almost a decade following the worst of the drought, I resurveyed plant communities in 2011 to determine if they were still changing compared to the 2001-2004 surveys, or if they had returned to pre-drought conditions. I found that plant community composition, regardless of forest type or mortality level, had shifted significantly since 2002, and rather than moving up in elevation as an intact entity, they are diverging in an unpredictable manner. Furthermore, although species diversity and abundance had increased since the drought, the evolutionary distance among community members, as measured using community phylogenetics metrics, had shrunk (i.e. more closely-related species are co-occurring now), thus providing evidence for a diminished role of species interactions compared to environmental filters in shaping community assemblages (Cavender-Bares *et al*. 2009). The reduced phylogenetic breadth of these communities post-drought may indicate that the ecosystem services and overall functionality across this unique landscape has narrowed in scope.

Genetic and environmental effects on phenology shifts

By planting populations into a common garden experiment, environmental variation is eliminated, isolating the effects of genetic variation on phenotype. This allows for the identification of genetically-based differences in important traits between populations and even individuals. Additionally, common gardens are often one of the best experiments for understanding responses to climate change, as researchers can transfer populations from their home sites to a garden site, mimicking a transition to a warmer or cooler climate, depending on the direction of transfer (Franks *et al.* 2014). In the summer and fall of 2014, three common gardens spanning a broad climate gradient (Figure 2) were planted with cloned cuttings of 12 tree genotypes from each of 16 populations of *Populus fremontii*, collected throughout Arizona. By planting the same individual across multiple environments, the effect of phenotypic plasticity, defined as a change in phenotype due to a change in the environment, can be measured (Franks *et al.* 2014). Our experimental design of repeated common gardens in Yuma, Arizona, Agua Fria National Monument, Arizona, and adjacent to Canyonlands National Park, Utah allowed for the evaluation of genetic variation among and within populations in each common garden environment, as well as the effect of phenotypic plasticity on important plant traits across the common garden gradient.

Figure 2.



Phenology, the timing of biological events, is especially sensitive to climate change, with numerous examples of advanced spring timing events in response to warmer temperatures (Parmesan & Yohe 2003), and subsequent fitness advantages in populations that can track warming best (Cleland *et al.* 2012). Additionally, phenology traits are highly differentiated among populations adapted to different climates (Savoleinen *et al.* 2007, Evans *et al.* 2016). In our study, we found high genetic variation in spring bud flush and fall bud set among the 16 populations of *P. fremontii,* correlating to increased survival in the populations' local provenance. In addition to genetic variation, we documented a greater influence of phenotypic plasticity shaping trait differences, as the variation in phenology timing of genotypes transplanted across the three garden sites was often much larger than the trait variation observed in any one garden. We found that the magnitude of plasticity was correlated with provenance climate, with plasticity increasing with increasing temperature and decreasing elevation and moisture of the population's source location. The plastic southern populations experienced high mortality rates when planted in the north, likely due to frost damage from delayed bud set timing. This result of non-adaptive plasticity suggests smaller transfer distances when moving populations from locations with minimal freezing events to areas with shorter growing seasons and predictable fall and winter frosts. Conversely, the less plastic northern populations shifted their phenologies toward the local populations when transferred south, showing a limited adaptive plastic responses under climate warming. Although these northern populations still had reduced growth and survival compared to the local populations, this result suggests movement of more northern populations south could be a viable restoration option into warming locations that still experience freezing.

Q_{ST}-F_{ST} comparisons: Climate as an agent of selection

The phenotypic differences expressed among widely distributed tree species is often linked to a high degree of local adaptation of populations across strong climate gradients

(Alberto *et al.* 2013). The relative roles of adaptive processes like natural selection versus neutral processes, such as gene flow and drift, in shaping these population differences can be assessed using Q_{ST}-F_{ST} comparisons. Here, the neutral genetic variation among populations (F_{ST}) is compared to the genetic variation in quantitative traits (Q_{ST}) of the same populations (Spitze 1993), often ascertained through common garden experiments where environmental variation is eliminated (Franks *et al.* 2014).

In this chapter we analyzed the degree of population and genotype variation in each of the three common garden sites for five functional traits: tree height, basal diameter, specific leaf area, fall bud set phenology, and spring bud flush phenology. The amount of genetic variance among populations relative to total genetic variance (Q_{ST}) was calculated for each trait in each of the common gardens. These values were compared to the F_{ST} value obtained from single nucleotide polymorphism (SNP) markers sequenced using double-digest restriction-associated DNA (ddRAD) of all 192 genotypes planted in the experiment. We found significant within and among population variation for all traits in all common gardens. In general, these traits were correlated to their source provenance climate, providing evidence of local adaptation of populations throughout Arizona. The high levels of among-population variance in traits (average Q_{ST} across traits and garden = 0.48) compared to our moderate F_{ST} estimate of 0.10 suggests that natural selection plays an important role in population divergence. The two most differentiated traits were bud set and bud flush, consistent with other findings of strong phenology differences along environmental clines (Savoleinen *et al.* 2007, Evans *et al.* 2016), while growth traits and specific leaf area showed more moderate levels of differentiation. Finally, we found that traits with the highest correlation to source climate were also the most strongly differentiated (highest Q_{ST}), providing evidence for climate as the agent of divergent selection. Together, these results show that populations are strongly differentiated across the landscape, and transplanting them to new locations via assisted migration might result in performance declines, especially if the transfer results in phenological mismatches. Managing for healthy, productive riparian ecosystems in the context of increasing aridity the southwest will require targeted movement of adaptive genotypes, taking into account the degree of predicted climate change and levels of both trait and climate differences between the source and transfer locations.

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LIST OF FIGURES

Figure 1. Examples of extensive forest mortality in Arizona: A) Large-scale pinyon pine (*Pinus edulis*) dieback following the severe 2002 drought in a pinyon-juniper woodland north of the San Francisco Peaks near Flagstaff, Arizona. Photo taken in December 2003 by Paul Heinrich. B) Fremont cottonwood (*Populus fremontii*) mortality along the Bill Williams River National Wildlife Refuge south of Lake Havasu, Arizona. Photo taken in March 2017 by Hillary Cooper.

Figure 2. The three Fremont cottonwood common gardens used in chapters two and three. A) The common garden in Yuma, Arizona. B) The common garden at Horseshoe Ranch adjacent to Agua Fria National Monument. This garden is shaped like an “L”, whereas the other two are square. Photo by Tom Whitham. C) The Canyonlands Research Center garden.

CHAPTER 2: Shifting trajectories of plant community diversity, composition, and phylogenetics due to extreme drought in the Southwestern U.S.A.

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ABSTRACT

The Southwestern U.S. is an ideal location to assess ecosystem consequences of global climate change, as severe droughts and large-scale tree dieback events have occurred in the recent past. While the mortality of dominant tree species has been well documented, an assessment of the effects of drought and subsequent loss of overstory trees on whole plant communities is lacking. In this study, we investigate the ecological and phylogenetic changes to plant communities across high and low mortality sites in juniper, pinyon-juniper, and ponderosa pine dominated forests spanning nearly a decade since a severe drought in 2002. By combining traditional community ecology measures with community phylogenetics, we can better understand the relative roles of abiotic (environmental filtering) versus biotic (species interactions) processes in shaping community assembly by looking at the evolutionary relationships of co-occurring species. Using these methods before and after disturbance can then inform and how these ecological and evolutionary patterns are affected by extreme climate events. Here, we present three major findings: 1) Species abundances and diversity significantly increased after the mortality event. In high mortality plots, there was an increase in species richness and a shift in the dominant plant functional group from woody shrubs and grasses to herbaceous species. 2) Plant communities did not change as an intact unit after drought, but continue to become compositionally divergent through time. 3) In general, the evolutionary relationships of co-occurring species shifted from phylogenetically overdispersed to significantly phylogenetically clustered after the drought event. We conclude that plant communities are not tracking climate change by moving up in elevation to replace the previous community, but are characterized by individualistic species responses to drought. Although we found increases in species abundance, richness, and diversity, the post-drought assemblages were more closely related than before the

2002 drought and in high mortality areas, suggesting a reduction in the breadth of plant function within the community. These findings have important implications for the future functionality of native ecosystems, as severe drought becomes an increasing phenomenon in the Southwest.

KEYWORDS: drought, climate change, community phylogenetics, overdispersion, clustering, plant community, tree mortality, Southwest

INTRODUCTION

A major goal of ecology and evolutionary biology is to understand the mechanisms underlying shifts in the diversity and composition of communities across environment gradients. This issue is becoming ever more pressing in the face of rapid global change altering the Earth's climate and biota. Increases in the duration, severity, and frequency of droughts across the globe have been identified as an escalating threat to numerous forest ecosystems (Allen et al. 2010). Tree mortality rates throughout the western US are increasing rapidly, likely due to warmer temperatures and water deficits (van Mantgem et al. 2009, Williams et al. 2013). As the diversity, composition, and function of these ecosystems shift or narrow due to global climate change, so too will important ecosystem services that we require of our natural lands (Anderegg et al. 2013). This has been demonstrated in prairie and grassland experiments showing decreases in biodiversity and functional diversity lead to decreased ecosystem productivity and stability (Tilman et al. 1996, 2001, 2006, 2007). In forests with foundation tree species modulating ecosystem properties (Ellison et al. 2005), the expected increases in drought-induced mortality of these species (Allen et al. 2010) will have major downstream effects on associated community members and the overall functionality of the ecosystem (Ellison et al. 2005, Anderegg et al. 2013, McDowell and Allen 2015).

Just as Allen and Breshears (1998) showed an upward migration of the ecotone between ponderosa forest and pinyon-juniper woodlands after the extensive dieback of low elevation ponderosa pines during the 1950's drought, many common montane plants have shifted up in elevation in southern Arizona after five decades of increasing temperature and decreasing precipitation (Brusca et al. 2013). Likewise, Kelly and Goulden (2008) showed rapid upward shifts of ~65 meters for dominant plant species following a 30-year warming trend in Southern

California’s Santa Rosa Mountains. This is not an uncommon phenomenon; a review of widespread distributional changes on over 1,700 species, including plants, mammals, and birds, showed a poleward shift of ~6.1 km per decade (Parmesan and Yohe 2003). Given that plant species respond individually to changes along environmental gradients (Gleason 1926), these upward migrations are often characterized by asymmetrical species responses instead of cohesive communities migrating in concert (Hansen et al. 2001, Brusca et al. 2013). This leads to the potential for no-analog communities, unique combinations of species that have not existed in the past, to form as environmental change continue to affect species differently (Williams and Jackson 2007), thus giving rise to novel species interactions. Species interactions affect the way climate change can impact community structure and dynamics, and at the same time, be directly affected by climate change (Gilman et al. 2010). A review of over 680 studies found that climate change substantially alters both antagonistic and beneficial biotic interactions (Tylianakis et al. 2008). The combination of shifting species assemblages and novel interaction networks may then further shape the trajectory of the biotic community in complex and unanticipated ways (Tylianakis et al. 2008, Gilman et al. 2010, Lau et al. 2016).

In addition to taxonomic ecological metrics such as species abundance and diversity, incorporating phylogenetic information about a community of interacting species can inform the evolutionary underpinnings of community assembly processes. It also addresses the common assumption that species are phylogenetically independent entities present in traditional taxonomic metrics (Swenson et al. 2012). A community phylogenetic approach can elucidate whether species in a particular location are more closely related (phylogenetically clustered) or more distantly related species (phylogenetically overdispersed) than expected by chance (Webb et al. 2002), and how their traits are distributed with respect to phylogeny. In addition, it can lend a new perspective on the role of species interactions in structuring communities by examining the degree to which phylogenetically related, ecologically similar species co-occur. For example, facilitation is often seen between distantly related species, while competition can inhibit closely related species from occupying the same niche space (Verdú and Pausas 2007). Community phylogenetics provides a set of complementary diversity measures to the customary species diversity metric (Faith 1992, Helmus 2007). These can be used as tools in conservation in order to identify sets of taxa that maximize functionality or “feature diversity”, increasing the chances of a having the right feature for an uncertain future (Forest et al. 2007). The benefits of including phylogenetics to understand ecological processes will increase as climate change continues to alter community assemblages and threaten species or populations of conservation concern.

The Southwest is considered one of the most “climate-challenged” regions in the United States due to high natural climatic fluctuations, limited water, extreme drought conditions, and the rapid increase in human population in the last few decades (Garfin et al. 2013). Although the past decade experienced the second most extreme drought in the last century following the 1950’s drought (Garfin et al. 2013), it was the warmest on record (Breshears et al. 2005, Woodhouse et al. 2010). Warmer temperatures intensify drought severity by increasing evapotranspiration and the water-holding capacity of the atmosphere (Weiss et al. 2009, Williams et al. 2013). By August of 2002 in northern Arizona, annual precipitation was 56% below the 1900-2000 average, and the Palmer Drought Severity Index (PDSI, Alley 1984) dropped to -7.4, the lowest value in the last 100 years (Koepke et al. 2010). These extreme drought conditions resulted in the widespread mortality of dominant tree species throughout the Southwest (Adams and Kolb 2005, Breashers et al. 2005, Gitlin et al. 2006, van Mantgem et al. 2009) and altered the genetic composition of the pinyon pines forests (Sthultz et al. 2009). With the expected increases in drought frequency and severity in the upcoming century (Garfin et al. 2013, USGCRP 2017), accelerated rates of forest mortality and a transition to more xeric plant communities are predicted (McDowell and Allen 2015).

The cinder fields of Sunset Crater, the youngest in a string of volcanoes in northern Arizona, are an extremely harsh environment for plants due to poor nutrients and low water holding capacity of the undeveloped igneous soils (Cobb et al. 1997, Kuske et al. 2003). The extreme abiotic conditions of the 2002 drought, combined with the challenges of these undeveloped soils, resulted in massive tree and shrub mortality, reaching 100% in some areas (Fig. 2, Adams and Kolb 2005, Gitlin et al. 2006, Koepke et al. 2010). Vegetation shifts from equal proportion of pinyon and juniper to juniper-dominated have been reported for this area (Mueller et al. 2005, Adams et al. 2009). In addition, Sthultz et al. (2009) observed genotypic variation in pinyon pine response to drought, as differential mortality caused phenotypic frequencies to shift from insect-resistant dominated (3:1) to equality with insect-susceptible trees after the 2002 drought.

The direct effects of drought and the consequent indirect effects of overstory tree mortality on the understory plant communities of the semi-arid Southwest are largely unknown (but see Kane et al. 2011). Research thus far has focused on quantifying the magnitude of water stress and mortality of dominant foundation tree species, such as ponderosa pine (*Pinus ponderosa*), pinyon pine (*Pinus edulis*), and one-seed juniper (*Juniperus monosperma*) (Shaw et al. 2005, Gitlin et al. 2006, McDowell et al. 2008), although Koepke et al. (2010) also surveyed major woody shrub species. In this study we examined how the entire plant community characterized by the above three gymnosperms has changed nearly a decade since the extreme 2002 drought. First, we explored the extent to which the three forest types, and the degree of tree mortality within each forest type, affected components of the community's diversity. We hypothesized that plant abundance, richness, and diversity would be greatest in 2011 relative to all other years surveyed due to the release of severe drought stress, and would be lower in the high mortality sites compared to low mortality sites across years. Additionally, we hypothesized that given the higher mortality rates of pinyon and ponderosa pines compared to juniper recorded after the drought event (Koepke et al. 2010), abundance, richness, and diversity would be highest in the juniper sites. Second, based on Brusca et al. (2013) and earlier studies by Whittaker and Niering (1965), we hypothesized that species assemblages will not respond to the drought event in concert, but changes in community composition will be characterized by individualistic species responses. Climate change has been shown to affect species and species interactions differently, leading to unpredictable community responses (Gilman et al. 2010). Lastly, given the evidence of phylogenetic clustering in post-disturbance communities (Verdú and Pausas 2007, Helmus et al. 2010, Cavender-Bares and Reich 2012, Zhang et al. 2014) where harsher environmental conditions can limit the presence of diverse taxa, we hypothesized that there would be an increase in phylogenetic clustering of plant communities after drought. This long-term, whole-system analysis of plant communities after an extreme disturbance event will provide better evidence of how ecosystems will fare in the face of continued environmental change in the Southwest.

METHODS:

Study area and sampling:

We selected three main study sites in the cinder fields northeast of Sunset Crater National Monument in the Coconino National Forest in northern Arizona. The three sites are characterized by distinct forest types dominated by one-seed juniper (*Juniperus monosperma*), mixed pinyon pine (*Pinus edulis*)-juniper woodlands, and ponderosa pine (*Pinus ponderosa*). Within each of these three forest types, a high and low mortality plot was chosen based on observations of drought-induced tree mortality in 2002. All plots were 5-7 ha in size, within 5 km of one another, and between 1742 and 1947 meters in elevation. In the late summer of 2002, 2003, 2004, and 2011, five random transects of ~ 400 meters were made in each of the six plots, recording species identity and abundance every two meters. Pre-drought communities were extrapolated from the surveys in 2002, assuming all dead vegetation was alive before the drought began. Drought stress was calculated for the broader study region of northeastern Arizona (NOAA Climate Division 2) using annual and August Palmer Drought Severity Index (PDSI) values (Alley 1984) from 1995-2014 (NOAA National Climatic Data Center) (Fig. 2).

Community composition

To examine overall functional changes in plant community, the relative abundances of three major plant functional groups, woody species, forbs, and graminoids, were compared for each year surveyed, with all six plots combined in each year. In addition, Bray-Curtis dissimilarity was computed between all communities and analyzed using PERMANOVA tests in the *vegan* package (Oksanen et al. 2013) in R (R Core team 2014) to assess community compositional changes across all years, mortality levels, and vegetation types. Significance values from these PERMANOVAs are shown in Table 1. Compositional changes were visualized with non-metric multidimensional scaling (NMDS) ordinations. In order to decouple the vegetation zones that track elevation from the effect of drought, three separate NMDS ordinations are presented showing the 2002, 2004, and 2011 plant communities in each of the juniper, pinyon-juniper, and ponderosa woodlands. To test if compositional changes were driven by the abundance of either forbs or annuals in a particular year, as the relative abundance of these plant functional groups were variable across years, we removed each of these sets of species and re-ran the analysis.

Phylogeny construction

A regional phylogeny of all species surveyed was constructed based on the Angiosperm Phylogeny Group III megatree (R20120829) in Phylomatic v3 (Webb and Donoghue 2005) (Fig. 3). The tree was then adjusted for branch lengths using the BLADJ algorithm in Phylocom (Webb et al. 2008) and family divergence estimates (Wikström et al. 2001, Chaw et al. 2004, Bouchenak-Khelladi et al. 2010). A community phylogeny for each of the six plots in each year was constructed by pruning the regional tree to include only those species found in that particular site. Each pruned community phylogeny was used to generate a distribution of null phylogenies for that particular plot and year to assess the significance of phylogenetic measurements.

Community diversity

We used several measures of community diversity including species richness, Shannon's diversity index, phylogenetic diversity (PD; Faith 1992), phylogenetic species variability (PSV; Helmus et al. 2007), and net relatedness index (NRI; Webb et al. 2008). PD, PSV, and NRI were calculated in R using the *picante* package (Kembel et al. 2010). Phylogenetic diversity is defined as the sum of all branch lengths in a phylogeny. This metric has been shown to be highly correlated with species richness, whereas PSV is statistically independent of species richness (Helmus 2007), however Forest et al. (2007) showed in cases of unbalanced phylogenies with strong phylogenetic structure, PD can become uncoupled from taxon richness. Phylogenetic species variability (PSV) is bound between zero and one, with a value of one indicating a completely unrelated community (as in a star phylogeny), with relatedness increasing as the value approaches zero (Helmus et al. 2007). Net Relatedness Index (NRI) is a standard measure of phylogenetic structure that quantifies the level of clustering within a phylogeny. It is defined as the difference in mean pairwise distances (MPD) between the observed and randomly generated null communities, standardized by the standard deviation of the null communities' mean pairwise distance:

$$\mathrm{NRI} = -(\mathrm{MPD} - \mathrm{MPD}_{\mathrm{null}})/\mathrm{sdMPD}_{\mathrm{null}}$$

where MPD is the mean pairwise phylogenetic distance among all species in the plot, $\mathrm{MPD}_{\mathrm{null}}$ is the MPD for that plot's 999 randomized null communities, and $\mathrm{sdMPD}_{\mathrm{null}}$ is the standard deviation of the MPD for those null communities. Null communities were generated using the independent swap algorithm, maintaining the species abundances and sample species richness, and was found to be the best among all models tested (Gotelli 2000). Positive NRI values indicate species occurring in a plot are more closely phylogenetically related than expected by chance, and NRI becomes negative with increasing phylogenetic overdispersion (distantly-related species co-occurring). An NRI score of zero indicates a community that is no different from random (Webb et al. 2002). The effects of year, vegetation type, mortality level, and all interactions were analyzed for plant abundance as well as each diversity metric using analysis of variance (ANOVA) in the R statistical platform (R Core Team 2014). Significance values for each test are displayed in Table 1.

RESULTS:

Community diversity responses

In support of our first hypothesis, we found that abundance and species richness were significantly higher in 2011 and in the juniper plots compared to the pinyon-juniper or ponderosa plots (Table 1, Fig. 4a,b). Species abundances had significant year, vegetation, and year x vegetation interaction effects, with higher abundances observed in 2011 and in the juniper plots (Fig. 4a). Tree mortality level did not influence species abundances in the understory, however, we found the high mortality plots had higher species richness compared to the low mortality plots, contrary to our expectation (Fig 4b). All explanatory variables except for the year x mortality interaction had significant effects on species richness (Table 1). Shannon's diversity significantly increased in 2011, but vegetation and mortality effects were non-significant (Fig. 4c).

Phylogenetic diversity metrics were calculated based on the plot-level phylogenies pruned from the regional phylogeny (Fig. 3) for each year surveyed. Phylogenetic diversity (PD) was significantly higher in 2011 compared to the drought years, and in the high mortality plots (Fig. 4d). It tracked species richness and Shannon's diversity well ($r = 0.92$ and 0.85, respectively), consistent with the high correlations between these metrics reported in Forest et al. (2007). Phylogenetic species variability (PSV) was significantly affected by year and vegetation type. The phylogenies for the pre-drought communities had the highest average PSV value of 0.76, indicating the lowest degree of relatedness, whereas the communities in 2004 and 2011 had the lowest PSV values, both with a mean of 0.70, suggesting these plant assemblages are becoming more closely related through time (Fig. 4e). In addition, PSV differed among forest types, with phylogenetic relatedness decreasing in pinyon-juniper and ponderosa sites compared to the low-elevation juniper sites. Consistent with PSV, the Net Relatedness Index showed higher phylogenetic clustering in 2011, indicative of more closely-related species co-occurring in a site. Again, the low-elevation juniper sites demonstrated the highest level of clustering (Fig. 4f). These results support our hypothesis of post-drought species assemblages that are more closely related. In summary, we found species abundance, richness, and diversity generally increased through time, in the high mortality plots, and in low-elevation juniper woodlands compared to pinyon-juniper or ponderosa-dominated forest plots. This was also consistent for phylogenetic diversity. The two measures of phylogenetic relatedness showed that communities became more closely-related through time. This pattern was amplified in the high mortality plots and in the lowest elevation juniper plots.

Community composition through time

The pre-drought communities and those surveyed during and immediately after the drought in 2002-2004 showed consistent dominance of woody species, followed by graminoids, and a paucity of herbaceous species (Fig. 5). This pattern was reversed in 2011, as forb abundance increased, becoming the most dominant group, with the relative abundance of woody species declining (year x habit: $P = 0.0276$).

Consistent with our hypothesis of individualistic responses to drought, non-metric multidimensional scaling (NMDS) ordinations show that plant communities became increasingly divergent from pre-drought communities through time (Fig. 6). This contrasts with the idea that lower elevation assemblages will become established at higher elevation sites where drought stress and tree mortality are high or that plant communities are returning to a pre-drought condition. Compositionally, all of the plant communities surveyed across years, vegetation zones, and mortality levels were statistically different from each other (Table 1). This pattern held when the dataset was sub-sampled to only include perennial species. The driving environmental variables of elevation, mortality, and year separate these communities (Fig. 6a). In order to decouple the vegetation zones that track elevation from the effect of drought, three separate NMDS ordinations are presented showing the 2002, 2004, and 2011 plant communities in the three vegetation zones (Fig. 6b-d). There is significant separation between 2002 and 2004 communities for each vegetation type, and in all three, this dissimilarity increases substantially in 2011, demonstrating the continued divergence of communities through time.

DISCUSSION

Drought as an environmental filter

The increase in species abundances, richness, and diversity in 2011 likely resulted from increased precipitation in 2011 relative to the "extreme drought" conditions in 2002-2004 (higher PDSI value in 2011, Fig. 1), leading to the increase in annual forbs, which were rare in earlier sampling efforts. This result is consistent with the finding of high species turnover following drought in an oak savannah in the Cedar Creek Reserve (Cavender-Bares and Reich 2012). Along with higher species richness, the co-occurring plant species became more closely related through time, as evidence by higher NRI and lower PSV scores in 2011 (Figs. 4d,e). This can be interpreted as a continued strengthening of habitat filters on the landscape since the drought began, as most years after the peak of the drought in 2002 showed PDSI values well below the -2 designation of "mild drought" (Fig. 1). The continued drought stress may have filtered out species not able to tolerate arid conditions, while still allowing an increase in the richness of drought adapted species. The transition from a phylogenetically overdispersed or randomly structured community to a clustered one is commonly thought to be determined by a shift in the influence of species interaction to one of environmental filtering (Cavender-Bares et al. 2004, Verdú and Pausas 2007, Cavender-Bares et al. 2009). Overdispersed communities can be organized through the competitive exclusion of closely related species to reduce niche overlap or the positive interaction of facilitation to increase distantly related species (Verdú and Pausas 2007), while clustered communities can indicate that environmental filters are selecting for certain adaptive traits that are shared among closely related species (Cavender-Bares et al. 2009). However, recent coexistence theory suggests that environmental filtering is difficult to distinguish from species interactions, and should only be considered the driving force in preventing species occurrence in the absence of biotic interactions (Kraft et al. 2015).

In 2011 the high mortality sites were more phylogenetically clustered compared to the low mortality sites within each respective vegetation zone. These results of higher clustering in the most disturbed sites agrees with the findings of increased phylogenetic clustering in sites experiencing frequent fires compared to plant communities in unburned woodlands (Cavender-Bares and Reich 2012). It is possible that in our high mortality sites, the abiotic environment exerted a stronger environmental filter and became more inhibiting to many species compared to the low mortality sites through time as overstory trees have not reestablished. Beneficial species interactions such as nurse plant associations or facilitation, which have been shown to promote the co-occurrence of distantly related species (Valiente-Banuet and Verdú 2007, 2008) especially in high-stress environments (Sthultz et al. 2007), might become less prevalent in high mortality sites that lost both the climatic and facilitative buffer of overstory trees. This is consistent with the finding of high environmental stress shifting the balance between facilitation and competition (Calloway and Walker 1997). In the low mortality sites, where environmental conditions were presumably less severe, environmental filtering may not have been as important relative to species interactions, whether competitive or facilitative, in structuring the community to include more diverse taxa.

In addition to increased water availability, the significant increase in species abundances, richness, and diversity in 2011 may be due to the substantial mortality of overstory trees providing increased micro-site variation and new habitat space for more species to co-exist without the negative effects of competition. Positive legacy effects of the recently dead trees can provide a pulse of nutrients and increased water retention into the otherwise nutrient and water limited cinder soils (Mueller et al. 2005, Kane et al. 2011) facilitating understory plant establishment. In reality, both processes of environmental filtering and species interactions are most likely happening, and the strength of each could be modified by annual changes in temperature and precipitation, as well as the concurrent species composition. Although species diversity showed marked increases, the breadth of different ecosystem functions that these communities can perform are likely shrinking, as evidenced by the trend toward increasing phylogenetic relatedness, if we take phylogeneic relatedness as a proxy for trait similarity (Helmus et al. 2010). An examination of the distribution of important functional traits across these community phylogenies would give more insight into the range of ecological strategies the species encompass and the mechanisms driving these phylogenetic shifts toward increasing relatedness. Clustered traits, such as those related to fire tolerance in a post-burn community (Verdú and Pausas 2007) could be important in understanding traits necessary to pass through an environmental filter (Cavender-Bares et al. 2004), or more general mechanisms for community assembly and co-existence.

Drought as an evolutionary event

In addition to affecting phylogenetic structure, several studies have shown that drought can be an evolutionary event that alters community structure. In this system, Sthultz et al. (2009) observed genetic variation in pinyon pine response to the 2002 drought, where differential mortality caused phenotypic frequencies to shift from insect-resistant dominated to equal frequency with insect-susceptible trees after the drought. Subsequent studies showed that the insect susceptible genotypes were more drought tolerant than the resistant trees, and that they supported different communities of mycorrhizal mutualists that performed better under drought conditions (Gehring et al. 2017). The drought therefore altered not only the genetic composition of pinyon pines, but the relative abundances of associated mycorrhizae on the landscape, assuming the fungi associated with the drought-intolerant trees died along with their hosts. As more extended community phenotypes are linked to the genotypic makeup of foundation tree species (Whitham et al. 2006), drought or other climate change events that non-randomly select against some genotypes will result in the subsequent reduction of these dependent organisms. Other studies have shown rapid evolution of multiple life-history traits, such as flowering time, in response to drought (Franks et al. 2007, Franks and Weis 2008), and recently multi-generational drought was shown to induce epigenetic mutations that are passed down through many generations, contributing to long-term drought adaptation and evolution in rice (Zheng et al. 2017). Thus, drought events can act as an agent of selection, affecting the genetic and epigenetic structure of foundation species, which may then result in altered species interactions and associated communities (Thompson 2013, Lamit et al. 2015, Gehring et al. 2017). In an area dominated by foundation tree species as in our study, a shift in tree density, its genotypic distribution, or the functional traits it expresses due to extreme climate events can have cascading effects on associated biotic communities (Ellison et al. 2005). These altered relationships between the dominant tree species and their dependent communities will further affect how the network of interacting organisms can respond to future environmental changes (Gilman et al. 2010).

Individualistic species response to drought

Similar to the individualistic responses to long-term warming that Brusca et al. (2013) found for plant species in the Santa Catalina Mountains of southern Arizona, we found plant communities as a whole are not replacing higher elevation assemblages in response to a hot drought event. Instead, novel communities are emerging as predicted with climate change (Hansen et al. 2001, Williams and Jackson 2007), with the potential for new species interactions (Gilman et al. 2010). Indeed, Whittaker and Niering (1965) perceived plant community-types as arbitrary, a convenient simplification for the field rather than a fixed ecological unit. Instead of seeing a low elevation community track the drought and replace a higher elevation assemblage, the plant communities are continuing to diverge through time. For example, with an increasingly arid climate resulting from prolonged drought conditions (Fig. 1) and the lack of overstory tree protection, one might expect the mid-elevation pinyon-juniper sites to be replaced by the lower-elevation juniper sites through time, or the ecotone between ponderosa forests and pinyon-juniper woodlands to shift upwards, as Allen and Breshears (1998) documented after a severe drought. Our analysis shows that this is not the case, with the plant communities in each forest type shifting away from the other communities through time (Fig. 6a). Within each of the three forest types, the communities in 2011 have diverged from the previous compositions (Figs. 6b-d), suggesting that none have returned to pre-drought compositions. Whether this community re-organization is still proceeding through a post-drought successional stage is unknown, but as hotter drought events increase (Garfin et al. 2013), the ability and time for post-mortality forest recovery is limited (Allen et al. 2015), allowing for further understory changes. As drought-stress and its associated disturbances such as pest outbreaks and more frequent and severe fires continues, many landscapes in the southwest will undergo vegetation conversions, shifting to more xeric compositions (Williams et al. 2012, McDowell and Allen 2015). Long-term, repeated community surveys will help define the time frame of both tree regeneration and community change, and enhance our understanding of how novel assemblages will form in the future.

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Table 1. Significance values for ANOVA and PERMANOVA tests. Bolded values indicate p-values < 0.05.

Table 1.

	Explanatory Variables						
Response variables	Year	Vegetation	Mortality	Year x Vegetation	Year x Mortality	Vegetation x Mortality	Year x Vegetation x Mortality
Total Abundance	**0.030**	**0.004**	0.375	**0.006**	0.688	0.277	0.467
Species Richness	**< 0.001**	**< 0.001**	**0.001**	0.066	**0.012**	**< 0.001**	**< 0.001**
Shannon's Diversity	**<0.001**	0.115	0.794	**0.045**	0.525	**< 0.001**	**0.014**
Phylogenetic Diversity	**<0.001**	0.102	**<0.001**	**0.028**	**0.008**	**<0.001**	**<0.001**
Phylogenetic Species Variability	**0.006**	**<0.001**	0.426	**0.005**	0.238	**<0.001**	0.118
Net Relatedness Index	**<0.001**	**<0.001**	0.775	0.081	**0.002**	**<0.001**	0.525
Composition: All species	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
Composition: Perennials only	**0.001**	**0.001**	**0.001**	0.134	0.082	**-**	**-**
Composition: Woody species only	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

LIST OF FIGURES

Figure 1. Average annual (black line) and August (red line) Palmer Drought Severity Index (PDSI) values for Northeastern Arizona (NOAA Climate division 2) from 1995-2014. Diamonds denote the years when plant communities were surveyed. The values from +2 to -2 are considered "mid-range", scaling from "incipient wet spell" to "mild drought", with zero considered no drought. This region experienced the "extreme drought" classification of -4 and below in 1996, 2002, 2003, 2004, 2006, and 2009, with the most pronounced value of -7.42 in August of 2002.

Figure 2. Photographs of the drought event in the Sunset Crater cinderfield. (a) Looking north into the juniper plots from above. Notice the yellowing of many of the junipers. Photo by K. Paxton and C. O'Brien taken in August, 2002. (b) Inside the juniper high mortality plot, looking south toward the San Francisco Peaks. Photo by H.F. Cooper taken in August, 2011.

Figure 3. Regional phylogenic tree comprising all species surveyed across years and sites. Branch lengths are estimated using Wikström et al. (2001) family-level divergence estimates. Two species traits, duration and habit, are coded by colored boxes. Perennial and annual species are colored as black and white, respectively. Growth habit is coded as woody (black), graminoid (yellow), or forb/herb (green).

Figure 4: Mean values (+/- 1 SE) for (a) Total Abundance (b) Species Richness, (c) Shannon's diversity index, (d) Phylogenetic Diversity, (e) Phylogenetic Species Variability, and (f) Net

Relatedness Index (NRI). Vegetation types are coded by color. Solid lines indicate high mortality plots and dotted lines indicate low mortality plots. Note that the x-axis is not continuous.

Figure 5. Relative abundances of forb (white), woody (light gray), and graminoid (dark gray) functional groups. Abundances are summed across all plots within a year.

Figure 6. Non-metric multidimensional scaling (NMDS) ordination of (a) all plant communities together surveyed in 2002, 2004, and 2011, (b) only the Juniper plots in 2002 and 2011, (c) only the pinyon-juniper plots in 2002 and 2011, and (d) only the ponderosa plots in 2002 and 2011. Symbols indicate year, and color indicates plot type (vegetation and mortality level).

Figure 1.

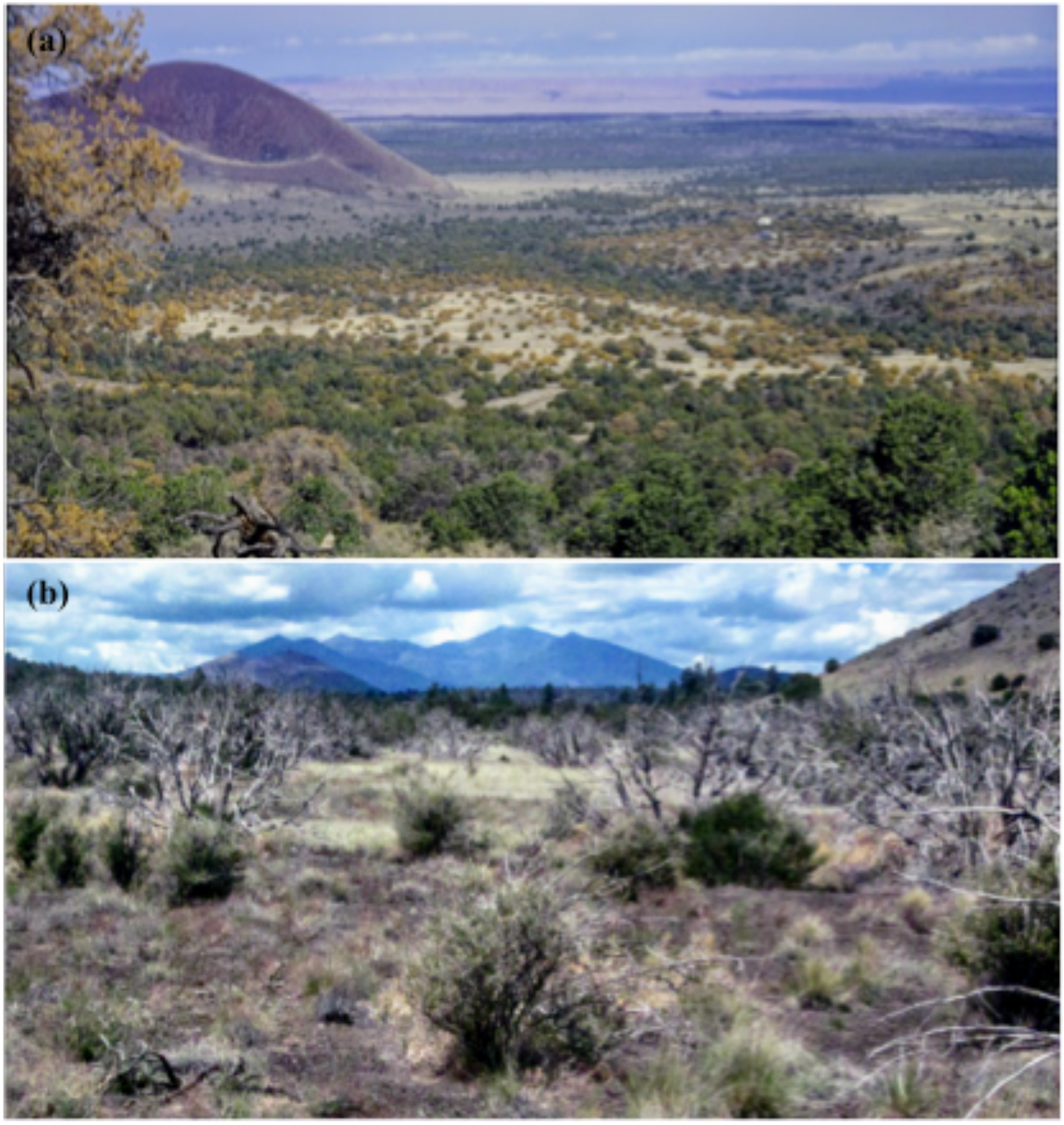


Figure 2.

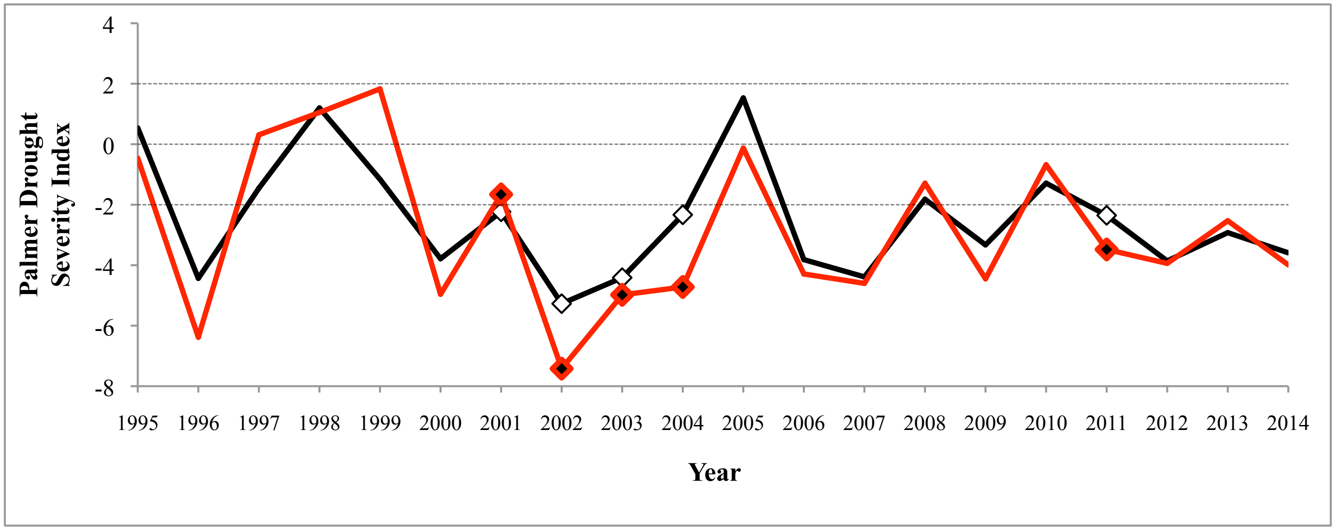

Palmer Drought Severity Index
4
2
0
-2
-4
-6
-8
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
Year

Figure 3.

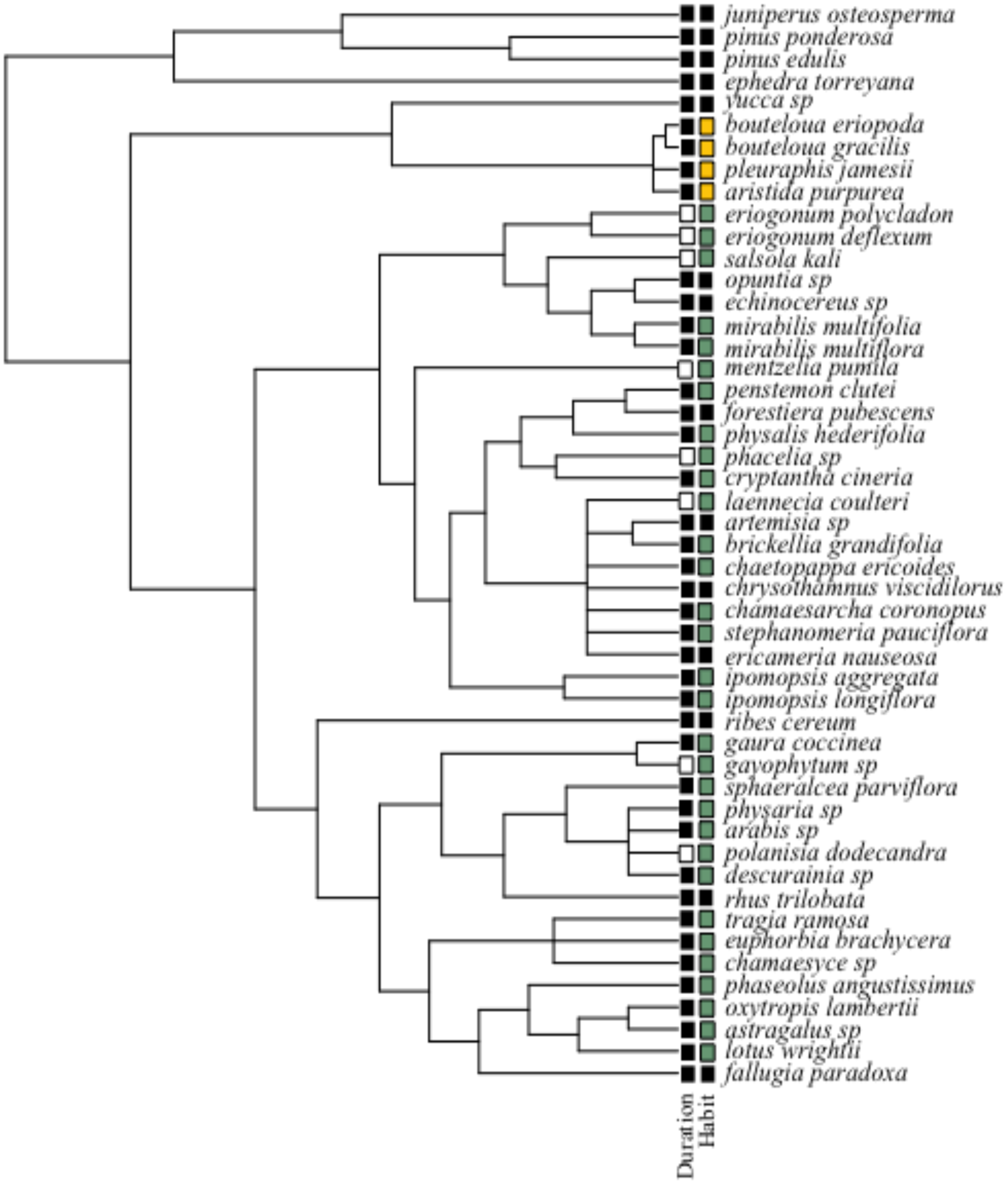

juniperus osteosperma
pinus ponderosa
pinus edulis
ephedra torreyana
yucca sp
bouteloua eriopoda
bouteloua gracilis
pleuraphis jamesii
aristida purpurea
eriogonum polycladon
eriogonum deflexum
salsola kali
opuntia sp
echinocereus sp
mirabilis multifolia
mirabilis multiflora
mentzelia pumila
penstemon clutei
forestiera pubescens
physalis hederifolia
phacelia sp
cryptantha cineria
laennecia coulteri
artemisia sp
brickellia grandifolia
chaetopappa ericoides
chrysothamnus viscidilorus
chamaesarcha coronopus
stephanomeria pauciflora
ericameria nauseosa
ipomopsis aggregata
ipomopsis longiflora
ribes cereum
gaura coccinea
gayophytum sp
sphaeralcea parviflora
physaria sp
arabis sp
polanisia dodecandra
descurainia sp
rhus trilobata
tragia ramosa
euphorbia brachycera
chamaesyce sp
phaseolus angustissimus
oxytropis lambertii
astragalus sp
lotus wrightii
fallugia paradoxa
Duration
Habit

Figure 4.

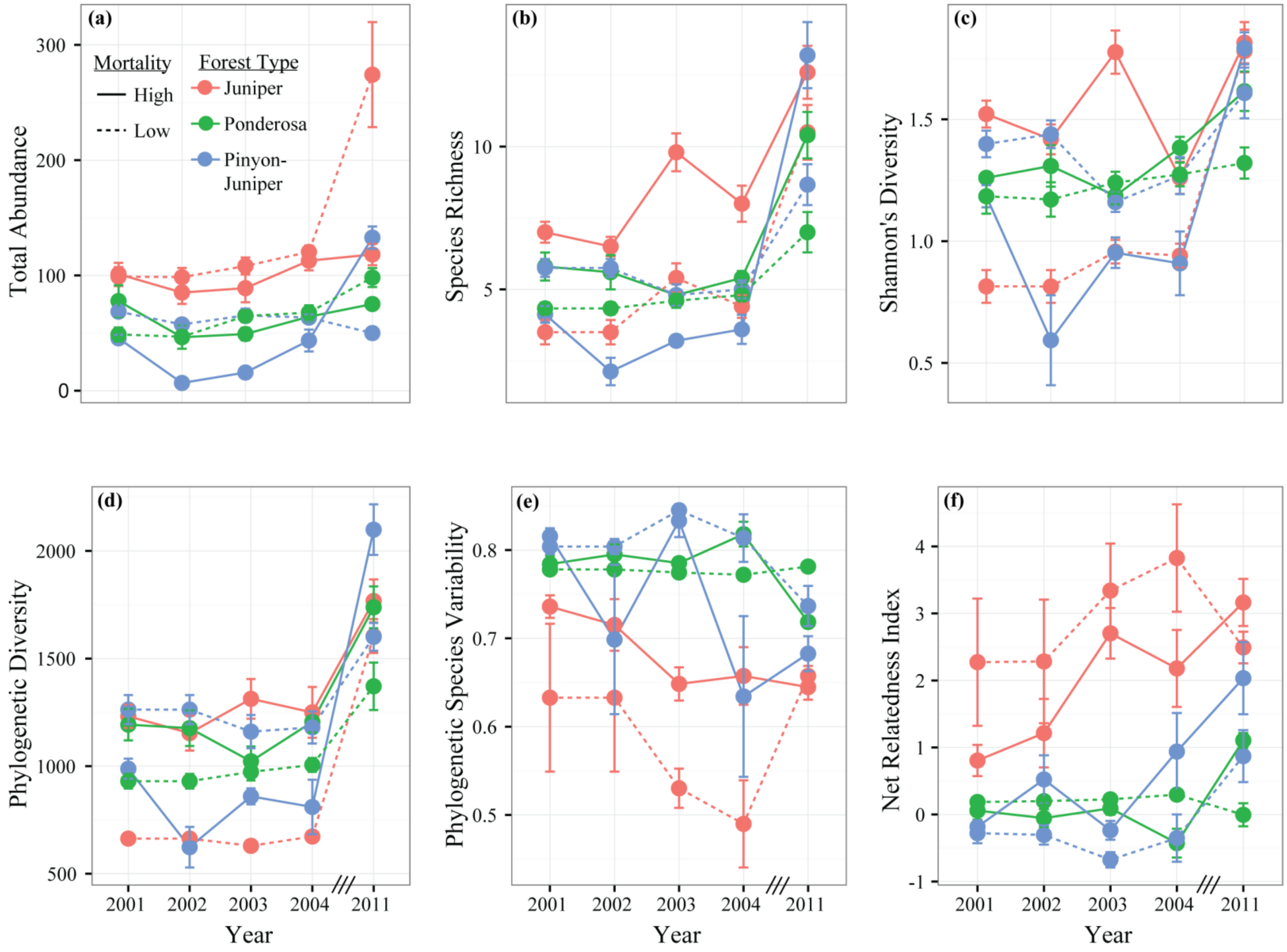
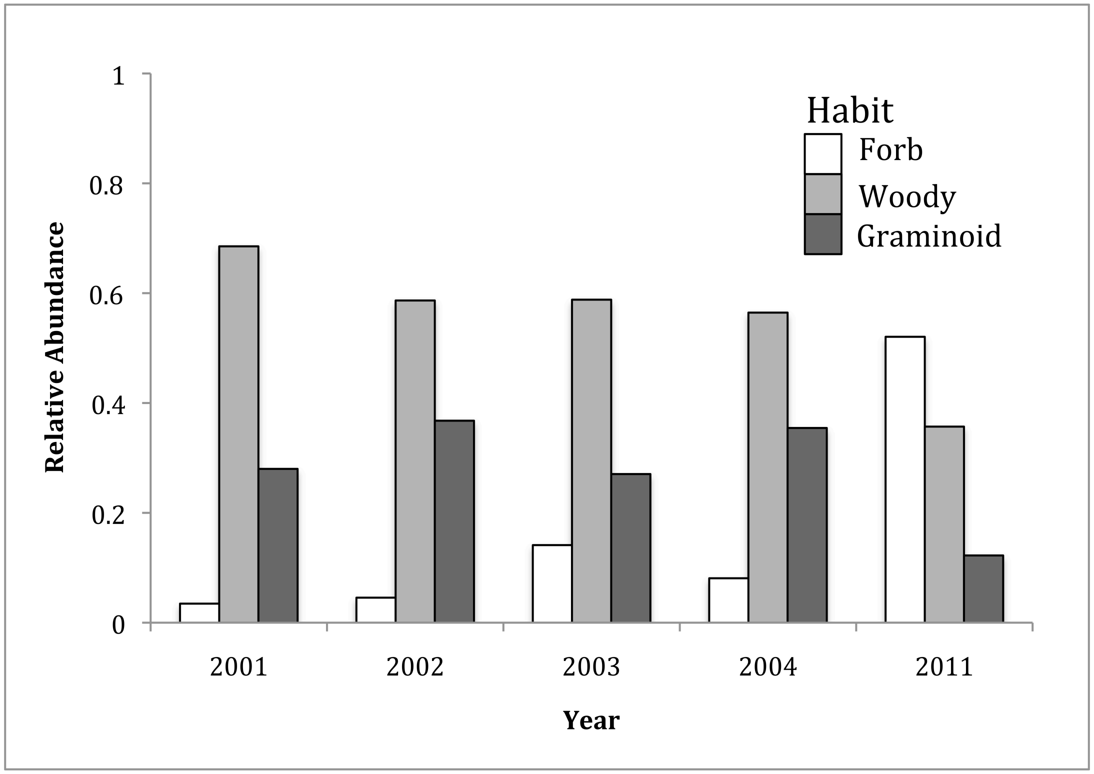
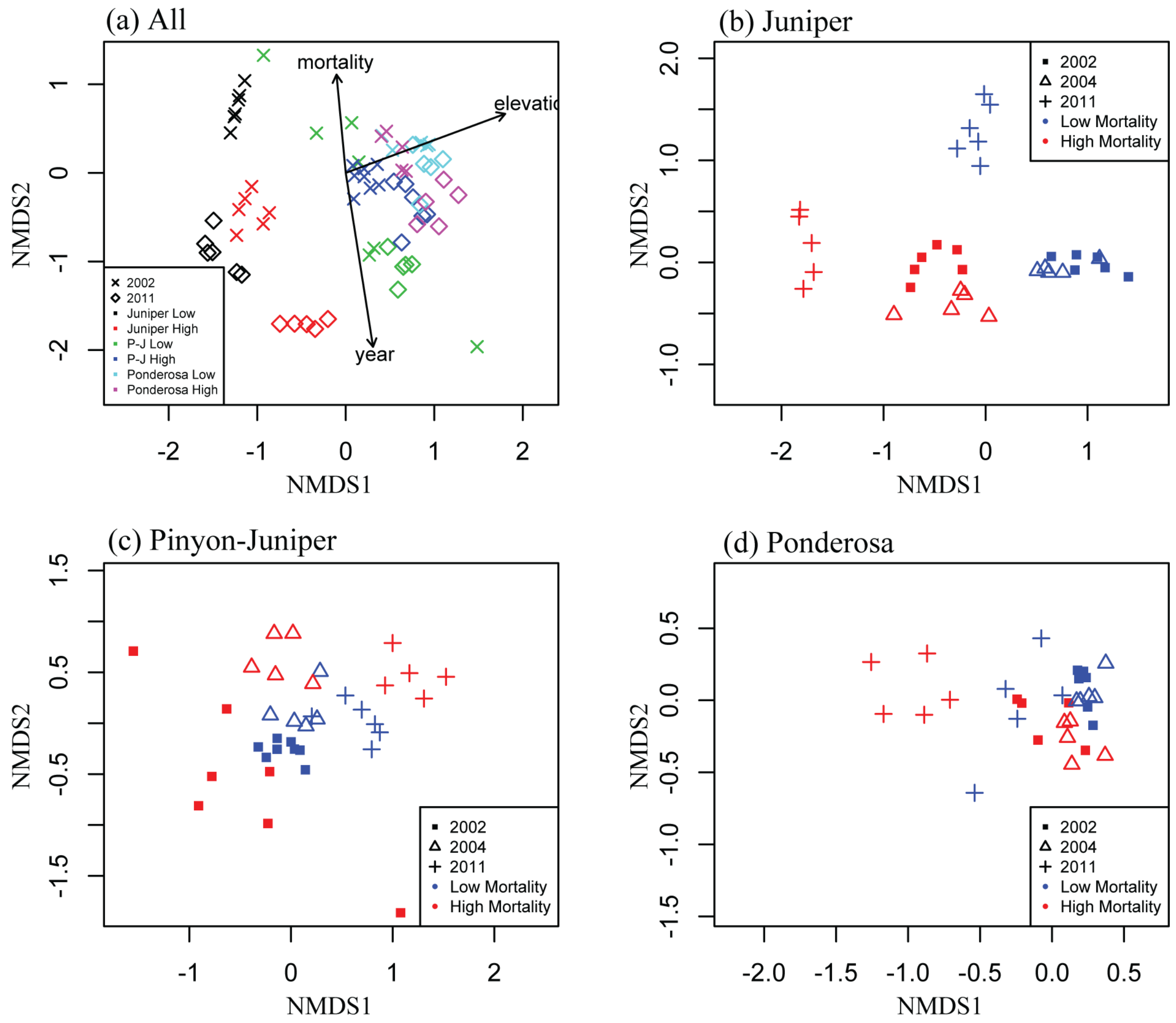

(a)
Total Abundance
0
100
200
300
Mortality
High
Low
Forest Type
Juniper
Ponderosa
Pinyon-Juniper
(b)
Species Richness
5
10
(c)
Shannon's Diversity
0.5
1.0
1.5
(d)
Phylogenetic Diversity
500
1000
1500
2000
(e)
Phylogenetic Species Variability
0.5
0.6
0.7
0.8
(f)
Net Relatedness Index
-1
0
1
2
3
4
2001
2002
2003
2004
2011
Year

Figure 5.



1
0.8
0.6
0.4
0.2
0
Relative Abundance
2001
2002
2003
2004
2011
Year
Habit
Forb
Woody
Graminoid

Figure 6.


(a) All
mortality
elevatio
year
NMDS1
NMDS2
-2
-1
0
1
2
2002
2011
Juniper Low
Juniper High
P-J Low
P-J High
Ponderosa Low
Ponderosa High
(b) Juniper
2002
2004
2011
Low Mortality
High Mortality
NMDS1
NMDS2
-2
-1
0
1
2.0
1.0
0.0
-1.0
(c) Pinyon-Juniper
2002
2004
2011
Low Mortality
High Mortality
NMDS1
NMDS2
-1
0
1
2
1.5
0.5
-0.5
-1.5
(d) Ponderosa
2002
2004
2011
Low Mortality
High Mortality
NMDS1
NMDS2
-2.0
-1.5
-1.0
-0.5
0.0
0.5
-1.5

CHAPTER 3: Genotypic variation in phenological plasticity: reciprocal common gardens reveal adaptive responses to warmer springs but not to fall frost

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KEY WORDS: phenotypic plasticity, phenology, *Populus fremontii*, climate change, common garden provenance trial, bud set, bud flush

ABSTRACT

Species faced with rapidly shifting environments must be able to move, adapt, or acclimate in order to survive. One mechanism to meet this challenge is phenotypic plasticity: altering one's phenotype in response to environmental change. Here, we investigated the magnitude, direction, and consequences of change in two key phenology traits (fall bud set and spring bud flush) in an important riparian tree species. Using replicated genotypes and reciprocal common gardens to investigate 16 populations of *Populus fremontii* from throughout Arizona, we identify four major findings: 1) There are significant genetic (G), environmental (E), and GxE components of variation for both traits across three common gardens; 2) The magnitude of phenotypic plasticity is correlated with provenance climate, where trees from hotter southern populations exhibited up to four times greater plasticity compared to the northern frost-adapted populations; 3) Phenological mismatches are correlated with higher mortality as the transfer distances increase between population and garden; and 4) The relationship between plasticity and mortality depends not only on the magnitude and direction of environmental transfer, but also on the type of environmental stress (i.e. heat or freezing) and how particular traits have evolved in response to that stress. Trees experiencing warming generally showed small to moderate shifts in an adaptive direction, a hopeful result for climate change. Trees experiencing cooler climates exhibited large, non-adaptive changes, suggesting the need for small transfer distances for assisted migration of stock from warmer provenances. This study is especially important as it deconstructs variation in traits affected by environmental cues that are rapidly changing (e.g. temperature and spring onset) and those that are fixed (photoperiod), and whose influence is variable across the species' range. Understanding the magnitude and adaptive nature of phenotypic plasticity of multiple

traits responding to multiple environmental variables is key in guiding pressing restoration decisions as climate continues to change.

INTRODUCTION

As climate change continues to push the limits of plant species' physiological tolerances, they will acclimate, adapt, migrate, or die out (Aitken *et al.,* 2008). For those species that cannot migrate quickly, such as long-lived trees (Davis & Shaw, 2001), persisting *in situ* through natural selection and/or phenotypic plasticity may be paramount to survival (Scheiner, 1993; Piglucci, 2005; Lande, 2009; Ghalambor, 2015). Given that the predicted rates of evolutionary responses are also much slower for long-lived species than the predicted rates of climate change (Etterson & Shaw, 2001), rapid plastic responses may be crucial for population persistence. Phenotypic plasticity, the ability of a genotype to produce distinct phenotypes when exposed to different environments, is increasingly found to be an important component of climate change responses (Nicotra *et al.,* 2010; Donelson *et al.,* 2011; Franks *et al.,* 2014; Merilä & Hendry, 2014), both within and across generations (i.e., transgenerational plasticity; Galloway & Etterson, 2007). When extreme climate events exceed historical levels of variation, locally adapted populations will experience environments for which current traits are poorly suited (Wang *et al.,* 2010; Kim & Donohue, 2013). These climatic changes can prompt plastic trait responses ranging from adaptive to maladaptive in direction, and small to large in magnitude (Ghalambor *et al.,* 2007), depending on the predictability of the environment (Lande, 2009; Hendry, 2016). Likewise, adaptive transgenerational plasticity is thought to be beneficial when parental environment reliably predicts offspring environment (Herman & Sultan, 2011), a condition that may diminish with climate change. Although there has been substantial research on plasticity resulting from climate warming (Kramer, 1995; Anderson *et al.,* 2012; Cleland *et al.,* 2012), increases in climate variability and extreme events (Garfin *et al.,* 2013) require the study of responses to both warming and freezing events, which may be best evaluated in replicated common gardens embedded in field sites with natural variation across multiple climate variables.

A major component of local adaptation in temperate and boreal trees is the evolution of precise phenological characteristics that match periods of plant activity, such as growth, reproduction, and dormancy, to suitable environmental conditions and cues (Kikuzawa, 1989; Körner & Basler, 2010; Harrington *et al.*, 2016). If species cannot shift their phenologies appropriately via evolution or plasticity, the cost-benefit balance of maximizing growing season length while avoiding frost damage will become disrupted and may result in lower fitness. For instance, more phenologically plastic species that tracked warming temperatures by advancing spring phenology increased their performance relative to less sensitive or canalized species (Cleland *et al.,* 2012). A phenological disruption could have cascading effects as associated species or entire dependent communities become mismatched with their hosts (Visser & Both, 2005).

Given the different environmental cues driving phenology in the spring versus fall seasons, the expectation for how plasticity in bud flush and bud set has evolved differs, which may produce different trait responses under climate change. Specifically, spring phenology is chiefly activated by the accumulation of days above a certain temperature following adequate chilling (Howe, 2003). Given increased warming trends, trees that are capable of plastic responses to temperature should shift to earlier bud flush dates (Franks *et al.,* 2014). In contrast, fall phenology is driven primarily by photoperiod, which is unaffected by climate change, so traits such as fall bud set are predicted to show limited plasticity (Evans *et al.,* 2016), however other environmental effects such as drought and temperature may affect these traits (Franks *et al.,* 2014). This distinction in how species have evolved spring and fall phenology responses could result in uneven plastic shifts for bud flush versus bud set as climate change differentially affects their environmental cues. This is especially important since climate change affects species differently across their distributions. Increased climate warming, for example, can affect populations at leading and trailing edges differently due to earlier spring initiation or increased drought, respectively (Vitasse *et al.,* 2010). This can become important as warmer fall and winter temperatures disrupt cold hardiness traits in populations that have evolved with freezing, or in extreme cold snaps that occur where populations have not evolved mechanisms to protect against freezing. Similar to the variable expectations of plasticity among phenology traits, the potential for plasticity is not constant across a species' distribution. Populations experiencing greater spatial and temporal environmental variation (Baythavong, 2011; Gianoli & González-Teuber, 2005), and those at range edge boundaries (Chevin & Lande, 2011) are expected to have evolved higher plasticity (Hendry, 2016). Together these complex interactions necessitate a detailed examination of the interplay among traits, evolutionary histories, and environmental change.

Fremont cottonwood (*Populus fremontii)* is an ideal system to address these numerous facets of plastic responses across multiple traits and a gradient of environmental changes. It is an important foundation species found along riparian corridors throughout the western U.S. (Ikeda *et al.,* 2017), but despite its widespread distribution, less than 3% of its historical range remains (Noss *et al.,* 1995; Lower Colorado River Multi-Species Conservation Program: LCR-MSCP, 2004). Our study region of the American southwest has experienced an average temperature increase of 0.9°C relative to the 1900-1960 average, with projections of up to 2°C by mid-century and 4.8°C by late-century (USGRP, 2017). In this study, we examined genetic and plastic variation in bud set and bud flush among 16 populations planted across three common gardens, which encompasses the thermal range of the species. This experimental design of replicating clonally propagated genotypes across multiple natural environments is considered one of the best ways to ascertain the impact and adaptive nature of phenotypic plasticity by substituting space for time as a climate change proxy (Franks *et al.,* 2014; Hendry, 2016). It also allows genotypes to experience much warmer and much colder temperatures compared to their source provenance to simulate both warming and cooling climates. We use this system to test four hypotheses. First, given previous evidence of genetic variation in functional traits in this species (Grady *et al.,* 2015; Evans *et al.,* 2016, Fischer *et al.,* 2017), coupled with the steep environmental gradient represented by the three gardens, we predict significant genetic (population and genotype (G)), environmental (E), and GxE effects on bud set and bud flush. We do not attempt to partition out the genetic component attributed to the maternal effect of transgenerational plasticity. Second, we predict that the GxE effects (variation in phenotypic plasticity among genotypes) will be related to the environmental conditions experienced in their home provenances. Third, we hypothesize that shifts in phenology will impact the performance (survival) of populations transplanted over a threshold climate transfer distance away from their home environment. We incorporate measures of phenotypic plasticity and bi-directional transfers (i.e., transfer to both warmer and cooler climates) to build on previous findings that populations transferred more than 3°C cooler compared to their home climate suffered performance declines (Grady *et al.,* 2015). Finally, using the definition of adaptive plasticity as a shift in phenotype toward the local trait optimum (Ghalambor *et al.,* 2007), we address whether plasticity appears to be adaptive or non-adaptive in the context of climate change and management using assisted migration techniques.

METHODS

Experimental Design and Trait Measurements

To assess the genetic and environmental contribution of population differentiation in functional traits, three common gardens were established in the fall of 2014. Cuttings from 16 populations of *Populus fremontii* from throughout Arizona (covering the climate range of the Sonoran Desert Ecotype (Ikeda *et al.,* 2017)), with 12 genotypes per population, were collected, rooted in the greenhouse for up to four months, and planted at each common garden site when saplings averaged 0.3m in height (Fig 1). Cuttings were collected from discrete genetic individuals with at least 20 m distance between individual trees. In prior research using similar populations, it was found that this sampling approach resulted in discrete genotypes and avoided clones that may result from branch senescence and resprouting (Grady *et al*., 2017). Each tree was tagged, and geographic coordinate position taken with a GPS.

The three common gardens span a wide elevation gradient of almost 2000 m and six degrees of latitude, encompassing the temperature and precipitation extremes experienced by *P. fremontii* (Table 1). The southernmost garden is located near Mittry Lake in Yuma, Arizona, and is maintained by the Bureau of Land Management. The central Arizona garden is located in Horseshoe Ranch adjacent to the Agua Fria River and is maintained by Arizona Game and Fish Department. The northernmost garden is located within Canyonlands National Park on Dugout Ranch land adjacent to the Colorado River, and is maintained by The Nature Conservancy's Canyonlands Research Center. Yuma is the hottest garden, with a mean annual temperature (MAT) of 22.8°C, Agua Fria represents the middle thermal range with a MAT of 17.2°C, and Canyonlands is the coldest garden with a MAT of 10.7°C. Yuma and Agua Fria gardens are flood irrigated, while the Canyonlands garden is drip irrigated. These gardens will be referred

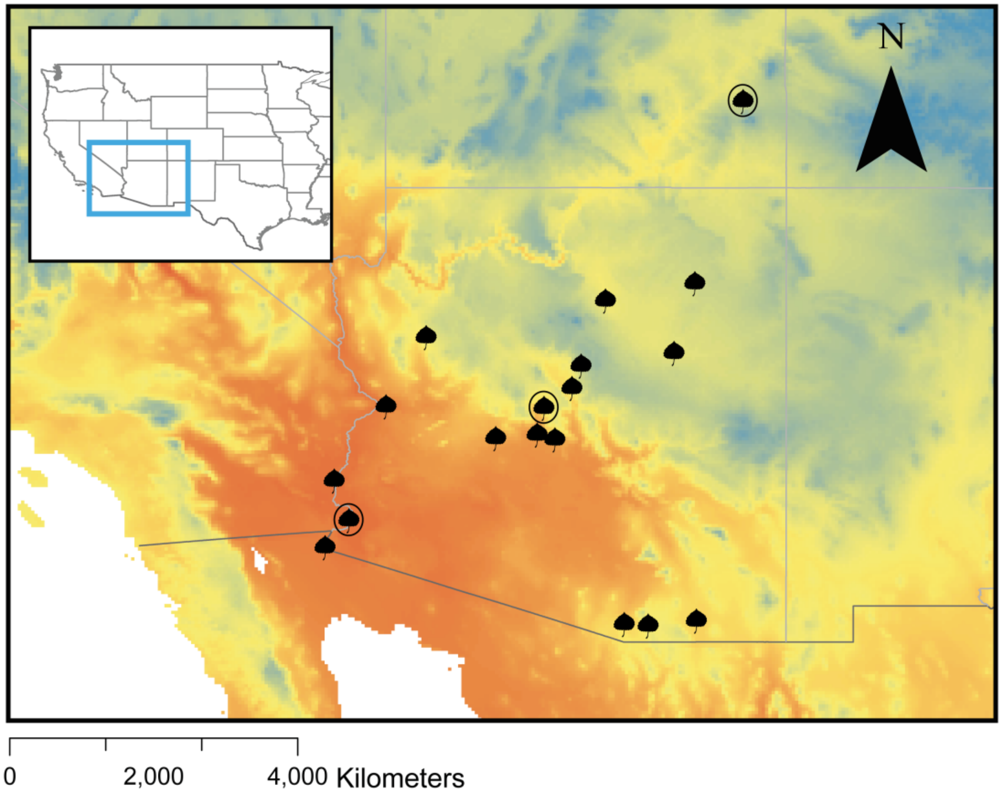

Figure 1. Location map of 16 provenance collection sites (leaf icon) of *Populus fremontii* and the three common garden locations (leaf with circle). The central garden is also a collection site. The shading corresponds to the degree-days above 5°C (DD5) throughout the region: red represents high DD5, blue low DD5.

to as hot (Yuma), mid (Agua Fria), and cold (Canyonlands). Each common garden consists of four replicated blocks of 16 population-level plots made up of 64 trees, for a total of 4,096 trees. Within each population plot, the 12 genotypes were replicated three to six times. Trees were spaced at 1.85 m in cardinal directions within each of the approximately 2 ha garden sites. Each garden was fenced with 2.5 m tall fencing to reduce potential for large ungulate grazing.

Survival was measured in the winters after the first and second growing seasons, when trees were dormant. Dead trees had lost all elasticity and were entirely dried out or absent from the plot. Timing of fall bud set was assessed at 6-10 day intervals from September through December of 2015 on three replicates of all 12 genotypes per population at each garden. We scored trees based on the bud stage exhibited by 50% or more of the apical meristems. This is a good approximation of whole plant progression towards dormancy as there was little within-plant variation in apical bud development. Bud set was recorded as the initiation of bud formation when internode elongation had ceased and the newly emerged, rolled up leaves were clustered at the same level on the stem and offset from the shoot axis (Frewen, 2000). Spring bud flush was recorded as the first sign of full leaf emergence on the tree. Bud flush was measured biweekly from February through the end of April in the Yuma and Agua Fria gardens, and through the end of May in the Canyonlands garden.

Climate analysis

We downloaded 21 abiotic variables from each of the population's home sites using ClimateWNA (Wang *et al.,* 2012; Table 1 and S1). To create a multivariate climatic trait representing environmental variation found throughout the 16 provenances, the abiotic ClimateWNA variables along with elevation, longitude, and latitude were analyzed in a principal component analysis (PCA) with the package *labdsv* (Roberts, 2007) and *vegan* (Oksanen *et al.,* 2016) in R (R Core Team, 2014).

Statistical analysis

The contribution of genetic (G), environment (E), and GxE effects on phenology were assessed for each population separately using mixed linear models fit by maximum likelihood with the *lme4* software package in R (R Core Team, 2014; Bates *et al.,* 2015). Individual phenology traits were modeled as response variables; environment (garden) was treated as a fixed effect with three levels, while genotype and the genotype-environment interaction (GxE) were random effects. Statistical significance for these variables was calculated using likelihood ratio tests for the random effects and analysis of variance (ANOVA) for the fixed garden effect in the package *lmerTest* (Kuznetsova *et al.,* 2015). A full model containing all populations was evaluated as well, with the genetic component represented at the population-level. Phenology dates were regressed against survival in each of the three common gardens to assess whether local populations outperformed foreign populations.

To test whether plasticity is related to source population environment, and whether plasticity is correlated with tree performance, we then calculated a plasticity score for each genotype. Plasticity can be visualized as a reaction norm of phenotypic change along an environmental axis (e.g. Fig. 2). If there are only two environments, then the plasticity of a genotype is either the slope of the reaction norm (Via *et al.,* 1985) or simply the mean difference in trait values between environments (Scheiner & Lyman, 1989). In our study design using three gardens, plasticity was calculated as the difference between the earliest and latest Julian date for each genotype mean across all environments. To test our hypothesis about the origin of plasticity, plasticity scores for both phenology traits were then regressed against the first axis of the environmental PCA to examine the climate-plasticity relationships using linear models in the *lme4* package.

Table 1. Common garden and source provenance environmental information. Climatic variables are derived from ClimateWNA (Wang *et al.,* 2012). 'Total genotypes' and 'Total trees' refers to total number of genotypes and trees in each garden. MAT = mean annual temperature, MWMT = mean warmest monthly temperature, MCMT = mean coldest monthly temperature, MAP = mean annual precipitation, AHM = annual heat-to-moisture index, DD>5 = degree-days above five.

Common Garden Sites	*Code*	*Latitude*	*Longitude*	*Elevation (m)*	*MAT (°C)*	*MWMT (°C)*	*MCMT (°C)*	*MAP (mm)*	*AHM*	*DD>5*	*Total Genotypes*	*Total Trees*
Mittry Lake, Yuma, Arizona	MYN-MLY	32.8498	114.4928	49	22.8	33.8	12.7	93	351.5	6496	192	4096
Agua Fria River, Horseshoe Ranch, Arizona	CAF-AUG	34.2567	112.0661	988	17.2	28.5	7.6	440	61.9	4512	192	4096
Canyonlands, Dugout Ranch, Utah	DIO-DUG	38.0925	109.5878	1581	10.7	24.6	-3.2	225	91.9	2798	192	4096
Provenance riparian system												
Clear Creek, Bullpen	BCE-BUL	34.5397	111.6966	1109	15.6	27.2	5.5	432	59.4	3971	12	256
Agua Fria, Horseshoe Ranch	CAF-AUG	34.2567	112.0661	988	17.2	28.5	7.6	440	61.9	4512	12	256
Cibola, CO River	CCR-COL	33.3621	114.6976	70	22.6	33.9	12.2	97	334.8	6429	12	256
Cave Creek	CCU-CAV	33.89	111.951	696	19.9	31.3	10	349	85.7	5439	12	256
Citadel Wash, Little CO River	CLF-LCR	35.6130	111.3190	1299	14.1	27.2	0.8	176	136.7	3635	12	256
Jack Rabbit, Little CO River	JLA-JAK	34.9600	110.4360	1507	12.3	25.3	-0.7	212	105.2	3140	12	256
Keams Canyon	KKH-OPI	35.8115	110.1695	1920	10.7	23	-1.3	258	80.1	2641	12	256
Willow Creek, Kingman	KWF-WIL	35.143	113.5428	1126	15	26.6	5	243	102.9	3760	12	256
Bill Williams, CO River	LBW-BIL	34.2760	114.0585	143	22.3	34.6	10.9	137	235.6	6308	12	256
Rattlesnake Canyon	MRN-RAT	34.7830	111.6137	1774	10.4	21.7	0.5	593	34.5	2454	12	256
New River, Phoenix	NVR-NEW	33.9476	112.1361	666	19.9	31.4	10	337	88.8	5460	12	256
Sonoita Creek, Patagonia	PSA-SON	31.5364	110.7631	1234	15.7	25.2	7.1	471	54.5	3957	12	256
San Luis, CO River	SCT-MEX	32.5270	114.8036	26	22.1	32.9	12.4	88	365.1	6246	12	256
Santa Cruz, Tumacacori	TSE-TUM	31.5647	111.0447	986	17.5	27.2	8.8	402	68.3	4584	12	256
San Pedro, Charleston	TSZ-SAN	31.6104	110.1668	1219	16.9	26.4	7.7	322	83.6	4373	12	256
Hassayampa, Wickenberg	WHY-HAS	33.9088	112.6764	575	19.6	31.4	9.4	284	104.3	5343	12	256

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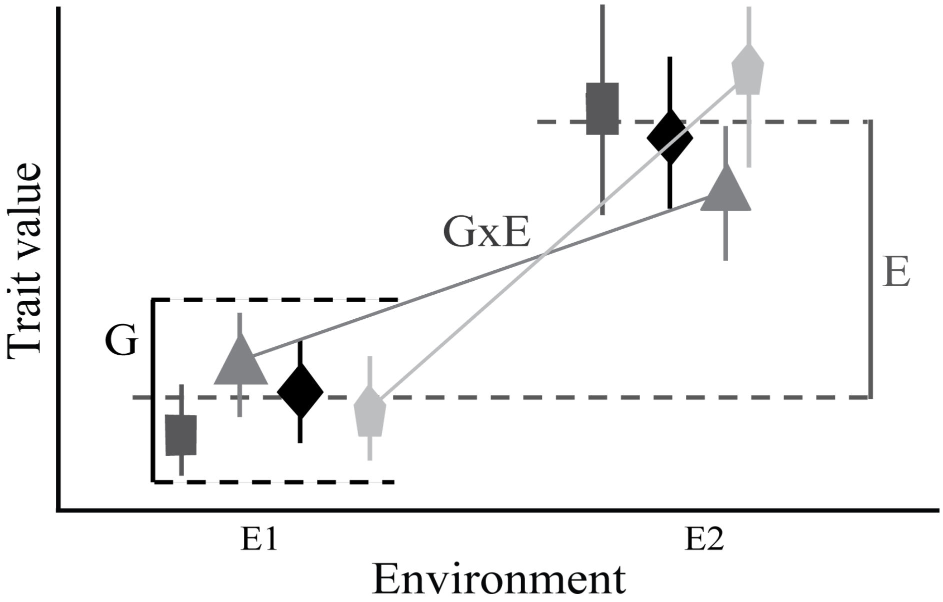

Figure 2. A two-environment reaction norm showing the components of phenotypic variation of four genotypes: G = trait variation due to population genetics within a single environment, E = trait variation due to change in environment (plasticity), GxE = the variation in plasticity among genotypes. Phenotypic variation (V_P) = $V_G + V_E + V_{GxE}$.

Finally, to test the adaptive nature of plasticity, each population was grouped into one of three major provenance zones using the primary axis of environmental variation to approximate each garden's regional climate. We use the definition of adaptive plasticity as a shift in phenotype in the direction of the local optimum trait value, while genotypes that exhibit a shift in the opposite direction are non-adaptive (Ghalambor *et al.,* 2007). "Adaptive" in this sense indicates that plasticity should increase population persistence and performance in the new environment, but does not claim anything about the evolutionary processes that have generated the plastic response. To examine the potential for plasticity to impact survival, we used regression analysis to test for a correlation between plasticity and the survival of genotypes when transplanted into the two common gardens outside of their home provenance zone. Tests for significance were conducted at the α=0.05 significance level.

RESULTS

Genetic, environmental, and GxE components of variation in phenology

Consistent with our first hypothesis, we found significant genetic and plastic variation in fall bud set and spring bud flush. Among all 16 populations planted across the three gardens, there were significant garden, population, and garden-by-population interaction effects (Table 2). Regardless of their growing environment, the northern, frost-adapted populations set their buds significantly earlier in the fall compared to the central and southern populations (Fig. 3a). Mean bud set dates were least variable among populations in the hot garden, with a difference of 14 days between first and last bud set, and most variable in the cold garden with a difference of ~36 days, more than doubling the duration of bud set timing. This suggests stronger environmental control on bud set in the hot garden, with larger genetic effects at the cold site.

Spring bud flush showed a similar pattern of relatively longer growing seasons for southern populations, as these flushed up to 70 days earlier than northern populations, and up to 14 days earlier than central populations, in the two warmer common gardens of Yuma and Agua Fria. However, in the cold Canyonlands garden, both the southern and central populations exhibited a delayed flush phenology – approaching the northern populations' late flush dates - compared to in the two hotter gardens. Unlike bud set phenology, which was most variable among populations in the cold garden, bud flush was most variable in the warmer gardens, suggesting a harder constraint on bud flush in areas with long, freezing winters. In Yuma (hot) and Agua Fria (mid), the duration of bud flush was 54 and 52 days, respectively, while Canyonlands (cold) produced a span of just over 22 days between the start and finish of flushing (Fig. 3).

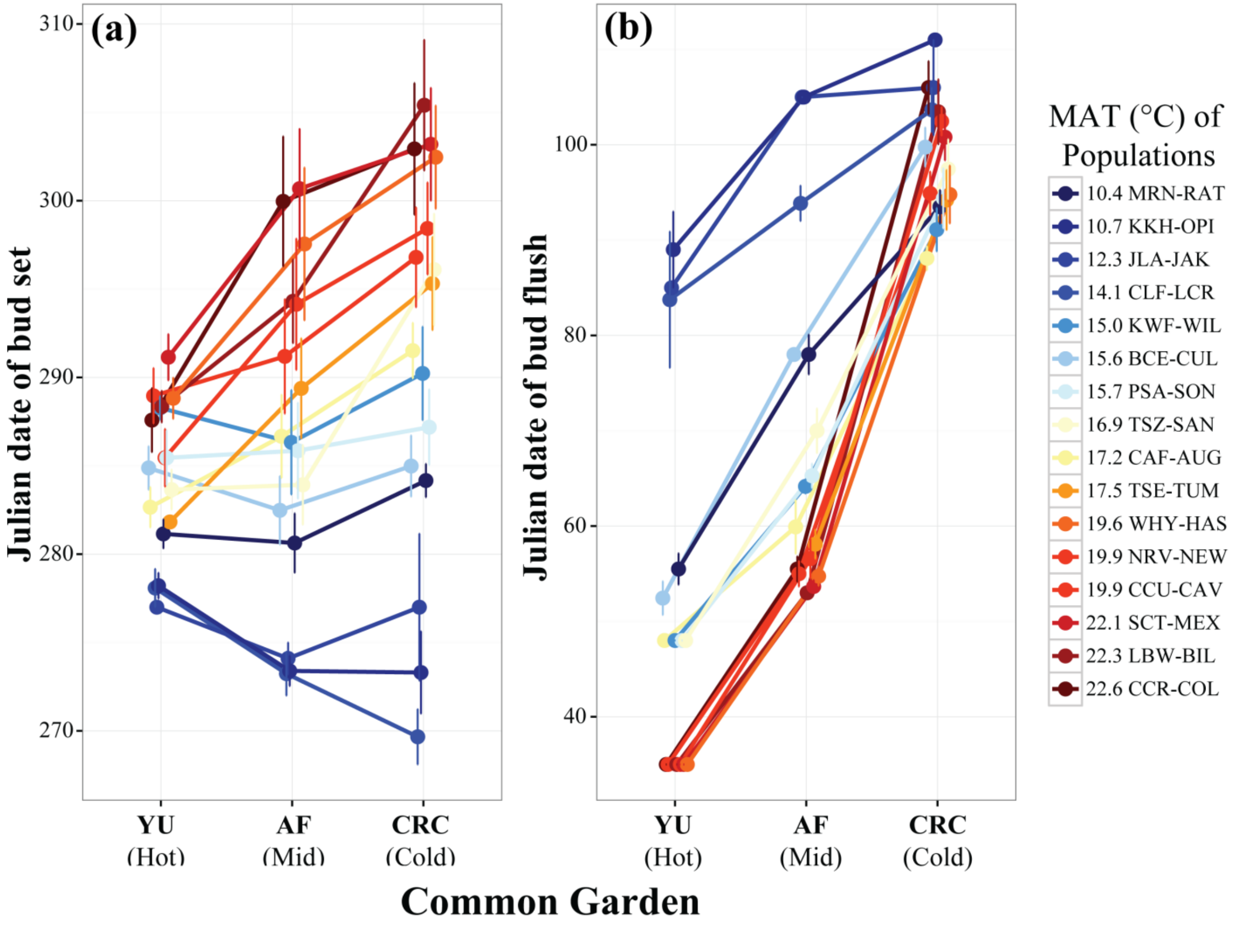

Figure 3. Population means (+/- 1SE) for (a) bud set and (b) bud flush across the three common gardens. Gardens are arranged from hottest to coldest: YU=Yuma, AF=Agua Fria, and CRC=Canyonlands Research Center. Populations are colored by the mean annual temperature (MAT °C) of their provenance.

In addition to clear population and population x environment effects, we also detected substantial variation within populations, especially for bud set. When analyzing each population separately, the bud set models showed seven of the 16 populations had significant genotype (G) differences, 11 had a significant garden (environmental, E) effect for bud set timing, and eight had significant GxE interaction effects (Table 2). The majority of populations with significant G and GxE effects were from warmer, low elevation provenances. Only two populations (MRN-RAT and JLA-JAK) showed neither environmental nor genetic effects on bud set; these two are among the highest elevation populations with the coldest temperatures and shortest growing seasons (Table 1). In contrast to bud set, bud flush results were far more consistent across populations. Aside from two populations (KKH-OPI and JLA-JAK) which did not have enough surviving genotype replicates for analysis, all populations showed significant garden effects, while only two populations exhibited significant genotype variation and just one had a significant GxE effect (Table 2). In summary, bud set showed significant environment, genotype, and GxE effects for many of the populations (11, 7, and 8 populations, respectively), while bud flush was governed predominantly by environment (all populations) and minimally by genetic or GxE effects (two and one population, respectively).

Table 2: Variance and P-values (α = 0.05) of the phenology models. Significant results are bolded. The bottom row shows how many populations out of the 16 for bud set and 14 for bud flush had significant results for each model variable.

		FALL BUD SET						SPRING BUD FLUSH					
		VARIANCE			P-VALUE			VARIANCE			P-VALUE		
	Among Populations	Popn	Popn x Garden	Residual	Popn	Popn x Garden	Garden	Popn	Popn x Garden	Residual	Popn	Popn x Garden	Garden
		63.1	16.01	202.34	**0.000**	**0.000**	**0.0003**	145.06	9.09	52.93	**0.000**	**0.000**	**0.000**
MAT (°C)	Within Populations	Genotype	Genotype x Garden	Residual	Genotype	Genotype x Garden	Garden	Genotype	Genotype x Garden	Residual	Genotype	Genotype x Garden	Garden
10.4	MRN-RAT	0.21	0.00	97.43	0.947	1.000	0.428	16.36	0.00	35.94	**0.033**	1.000	**0.000**
10.7	KKH-OPI	1.36	7.08	41.42	0.811	0.326	**0.033**						
12.3	JLA-JAK	5.42	35.78	16.65	0.867	0.208	0.647						
14.1	CLF-LCR	0.00	0.76	37.83	1.000	0.902	**0.003**	25.94	22.56	17.85	0.232	0.205	**0.002**
15.0	KWF-WIL	12.69	37.72	155.36	0.448	**0.004**	0.994	0.00	15.80	34.75	1.000	0.106	**0.000**
15.6	BCE-BUL	14.58	0.00	135.77	**0.028**	1.000	0.743	6.11	8.75	25.51	0.449	0.245	**0.000**
15.7	PSA-SON	40.84	0.00	103.62	**0.000**	1.000	0.502	0.00	19.76	15.38	1.000	0.110	**0.000**
16.9	TSZ-SAN	25.71	17.29	111.35	**0.025**	**0.046**	**0.000**	30.23	0.00	32.92	**0.034**	1.000	**0.000**
17.2	CAF-AUG	15.92	5.97	128.29	0.086	0.483	**0.010**	0.00	46.24	18.04	1.000	**0.000**	**0.000**
17.5	TSE-TUM	22.15	22.93	131.65	0.095	**0.018**	**0.001**	0.00	0.00	68.85	1.000	1.000	**0.000**
19.6	WHY-HAS	66.63	27.21	189.39	**0.010**	**0.041**	**0.002**	0.00	18.79	31.11	1.000	0.074	**0.000**
19.9	CCU-CAV	35.47	24.11	147.36	**0.036**	**0.017**	**0.046**	5.09	6.51	28.61	0.563	0.488	**0.000**
19.9	NRV-NEW	95.45	6.08	145.96	**0.001**	0.584	**0.005**	0.00	13.69	92.44	1.000	0.581	**0.000**
22.1	SCT-MEX	34.79	54.54	202.32	0.270	**0.003**	**0.043**	0.00	0.00	21.16	1.000	1.000	**0.000**
22.3	LBW-BIL	6.41	52.50	193.02	0.729	**0.001**	**0.001**	0.00	21.39	31.95	1.000	0.216	**0.000**
22.6	CCR-COL	77.04	29.96	211.00	**0.011**	**0.042**	**0.009**	7.21	0.00	44.60	0.353	1.000	**0.000**
# of Significant Populations					**7/16**	**8/16**	**11/16**				**2/14**	**1/14**	**14/14**

Magnitude of plasticity correlates to provenance environment

After combining all environmental variables in a PCA, the first principal components axis (PC1) represented 95.8% of the variation among the 16 provenances, with four environmental variables (degree-days above 5 °C, degree-days below 18 °C, degree-days above 18 °C, and summer heat-to-moisture index (Wang *et al.,* 2012)) plus elevation comprising the majority of this axis (see variable PCA loadings in Table S1). The provenance environment, represented by PC1, was significantly correlated with fall bud set plasticity ($R^2 = 0.22$; $P < 0.001$; $F_{1,156} = 45.6$) and spring bud flush plasticity ($R^2 = 0.63$; $P < 0.001$; $F_{1,41} = 73.1$; Fig. 4).

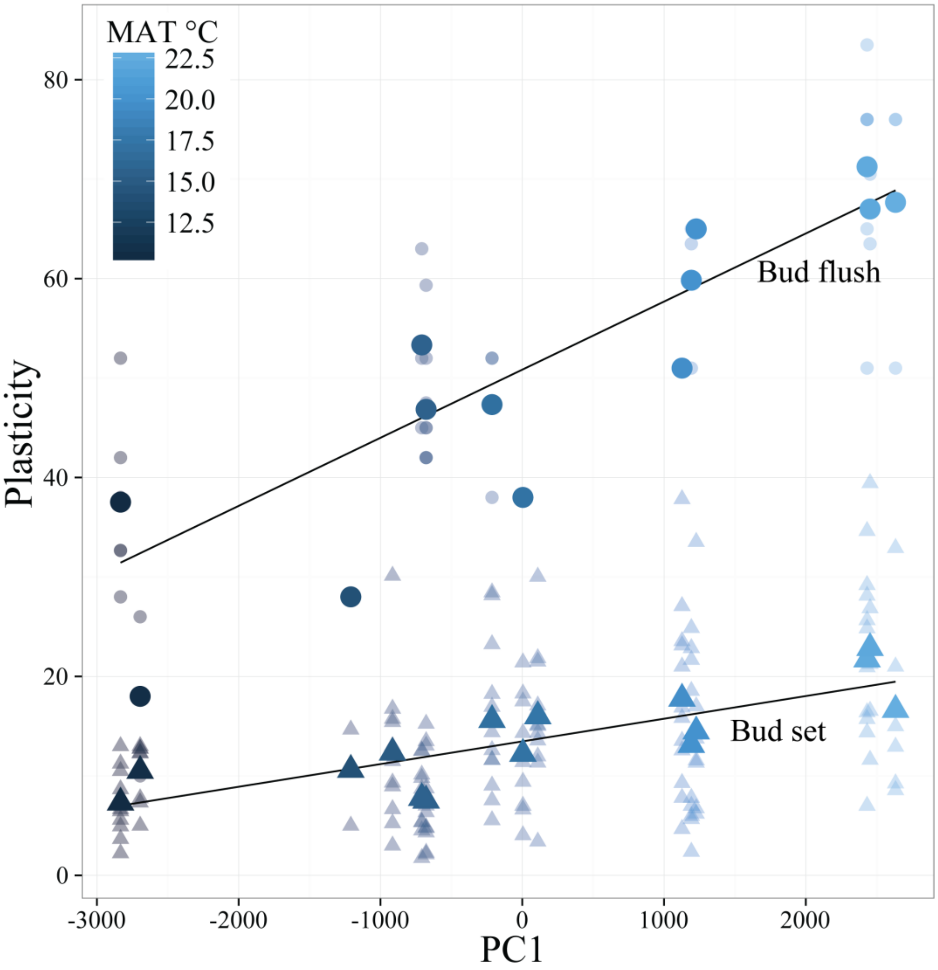

Figure 4. Bud flush and bud set plasticity are significantly positively correlated with axis 1 of the climate PCA. Hotter, drier, lower elevation source environments (positive PC1) correlate with higher plasticity scores. Smaller opaque symbols represent genotypes, while larger, solid

symbols indicate population means. Colors correspond to the mean annual temperature of the population.

This confirms our second hypothesis that environmental conditions might predict the magnitude of plasticity: greater plasticity in both traits was correlated with hotter and drier environments represented by more degree days above 5°C and 18°C, lower elevation, and greater summer heat to moisture ratio.

Mortality consequences of phenology-climate mismatches

Population-level survival was significantly related to bud set phenology in each common garden (Fig. 5), suggesting that the degree of mismatched phenology of foreign populations is one possible explanation for lower survival. This agrees with our third hypothesis that mortality increases with climate transfer distance between home and garden site. In the hot garden, survival was positively correlated with bud set phenology ($R^2 = 0.46$; $P = 0.003$), with populations exhibiting late bud set having the highest survivorship. In the middle garden, there was a quadratic relationship, with mid-season bud set dates related to highest survival ($R^2 = 0.44$; $P = 0.010$), and in the cold Canyonlands garden, trees with earliest bud set showed highest survival ($R^2 = 0.34$; $P = 0.011$). For bud flush, the only significant relationship appeared in the hot garden, where earlier flush dates correlated with higher survival (in Yuma: $P = 0.006$, $R^2 = -0.44$; in AF: $P = 0.807$; in CRC: $P = 0.991$). This provides additional evidence for local adaptation and genotype x environment interactions in Fremont cottonwood, with “local” outperforming “foreign” in each of the three common gardens (Kawecki & Ebert, 2004).

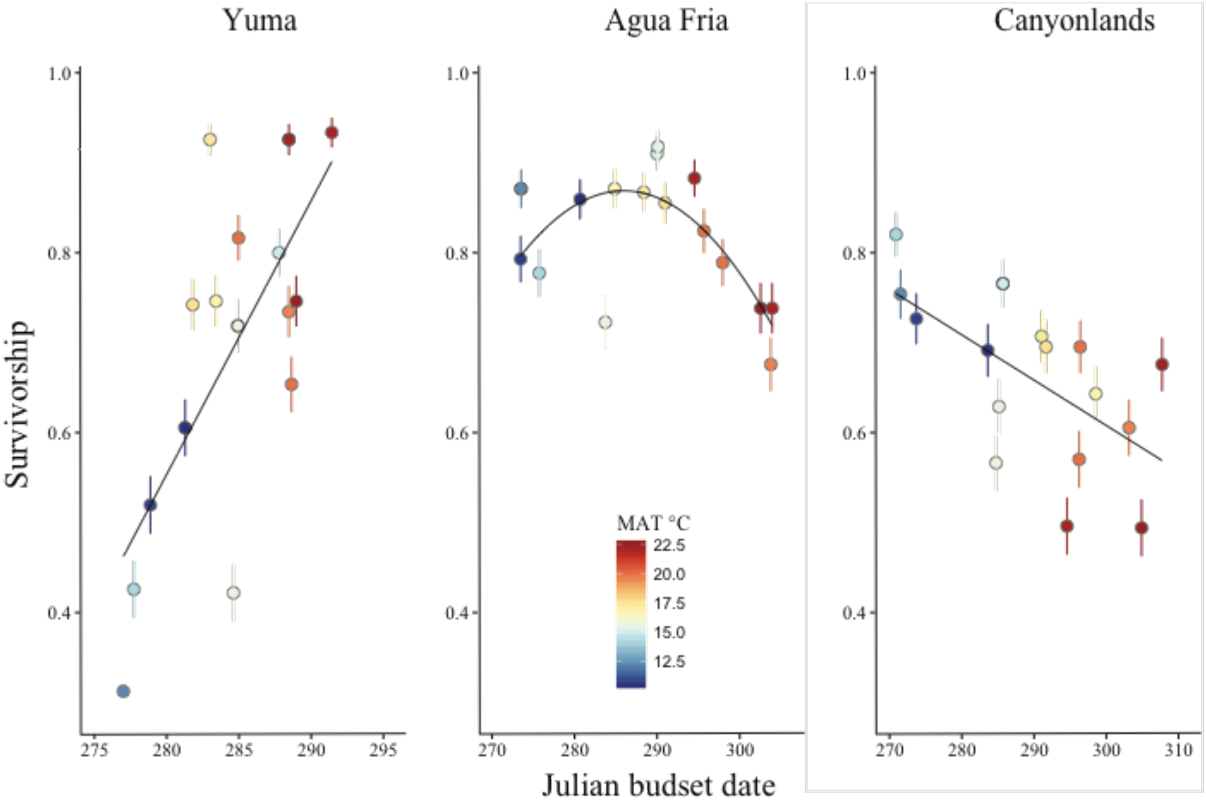

Figure 5. Population mean (+/- 1 SE) survival correlations with bud set date in each of the three common gardens. Populations are colored by the mean annual temperature (MAT °C) of their source provenance. In Yuma, survival is highest in the hotter source populations and is positively correlated with later bud set. The opposite is true in the coldest Canyonlands garden. A parabolic relationship was found at the mid garden of Agua Fria, where the highest survival correlated with mid-season bud set of the central Arizona populations.

We found that survival is a function of the transfer distance in growing season (measured as degree-days above 5°C, DD5; Wang *et al.,* 2010) between provenance and garden site. Survival is negatively correlated with DD5 transfer distance in the two extreme gardens, but not the middle garden (Yuma: *P* = 0.013; Agua Fria: *P* = 0.400; Canyonlands: *P* = 0.015), however there still is a negative trend in the middle garden (Fig. S1). A weaker pattern is expected in the middle garden since the maximum transfer distances are approximately half as great as those transferred between the hot and cold gardens.

Adaptive vs. non-adaptive plasticity

Using traits of populations from provenances near to each garden as an approximation of the local optima, we found shifts in phenology consistent with both adaptive and non-adaptive plasticity. Shifts in bud set demonstrated either adaptive or non-adaptive plasticity depending on the source population and the direction of transfer (e.g., from hot to cold). The transfer of southern and central populations to the cold northern garden resulted in non-adaptive plasticity. Instead of setting bud *earlier* in the fall to match the phenology of the local genotypes and avoid frost damage in the cold garden, these genotypes delayed their bud set *even later* (by up to 20 days) than in their native climates of Yuma and Agua Fria (Fig. 3a). On average, the northern populations showed much less plasticity in bud set, with some genotypes exhibiting as little as three days difference between mean bud set dates across the three gardens. However, the direction and magnitude of responses varied among genotypes. Some northern genotypes exhibited non-adaptive plasticity by advancing bud set in the two hotter gardens by ~five days earlier compared to in the cold garden, effectively shortening their growing season even more, while others displayed adaptive plasticity in the hot garden, delaying bud set later in the season to approach local populations' phenology (Fig. 3a).

Plasticity in spring bud flush was overall higher and showed a similar pattern of southern populations exhibiting higher plasticity compared to the northern populations across the three gardens (Fig. 4). The two hottest source populations showed a mean difference of 68 and 70 days between bud flush in the hot versus the cold garden, significantly altering the length of the growing season between these two locations. The northern, cold-adapted populations also exhibited significant, albeit lower, plasticity of around 20 days for this trait. In contrast to mixed results for bud set, the trends in bud flush plasticity were consistently in the adaptive direction.

The southern and central populations showed increasingly delayed flush timing from the hot to mid to cold gardens, while the northern populations showed progressively earlier flush phenology from the cold to mid to hot gardens (Fig. 3b). In every case, bud flush timing of the foreign trees shifted in the direction of the local phenology in each garden.

To separate the impacts of a population's climate of origin versus its plasticity on its performance when transferred to a new environment, we analyzed the correspondence between plasticity and survival for genotypes from each provenance zone separately. We found that this relationship differed among the three main provenance zones and between the two phenology traits. Genotypes transferred from the hot, southern provenance zone to the cold garden (Fig. 6 a,b), which tended to show non-adaptive plasticity in bud set relative to local trees, experienced lower survival in proportion to that plasticity ($R^2 = -0.32$; $P = 0.0133$). The relationship between bud flush plasticity and survival for this transfer is non-significant, perhaps partly due to the high over-winter mortality of southern populations in the northern garden, which resulted in lower sample size in the spring than the preceding fall ($R^2 = 0.11$; $P = 0.173$; Fig. 6b).

Genotypes from the cold, northern provenance zone transferred to the hot garden (Fig. 6 c,d) showed an unexpected, contradictory pattern. Here, genotypes that advanced bud set over 10 days earlier in the hot garden rather than delaying it to match local genotypes (a non-adaptive shift) had greater survival. In contrast, genotypes that did adaptively delay bud set had lower survival ($R^2 = -0.361$; $P = 0.0108$; Fig. 6c). This pattern reveals a contradiction in how adaptive plasticity is described. Northern genotypes that shift their phenology toward that of southern genotypes when planted in the south exhibit "adaptive plasticity" by the definition of Ghalambor et al. (2007), but this plasticity is not "adaptive" in the sense of increasing fitness (Dudley & Schmitt, 1996) and promoting population persistence in this new environment. In contrast, plasticity in bud flush for northern genotypes in the hot garden was adaptive in both senses - shifting phenology toward the new environment's local trait optimum and increasing survival (Fig. 6d). Those genotypes that were able to flush over 50 days earlier in the spring in the hot garden and up to 45 days in the middle garden, relative to in the cold garden, showed the highest survival rates compared to those which had minimal flush change (in Agua Fria: $R^2 = -0.65$, $P = 0.0001$; in Yuma: $R^2 = -0.27$, $P = 0.0722$). This was the only significant plasticity-survival relationship for an intermediate transfer involving the middle garden. These results suggest that plasticity in bud set can be adaptive or non-adaptive depending on the direction of the climate transfer, while spring bud flush plasticity is generally adaptive for populations experiencing warmer, drier climates.

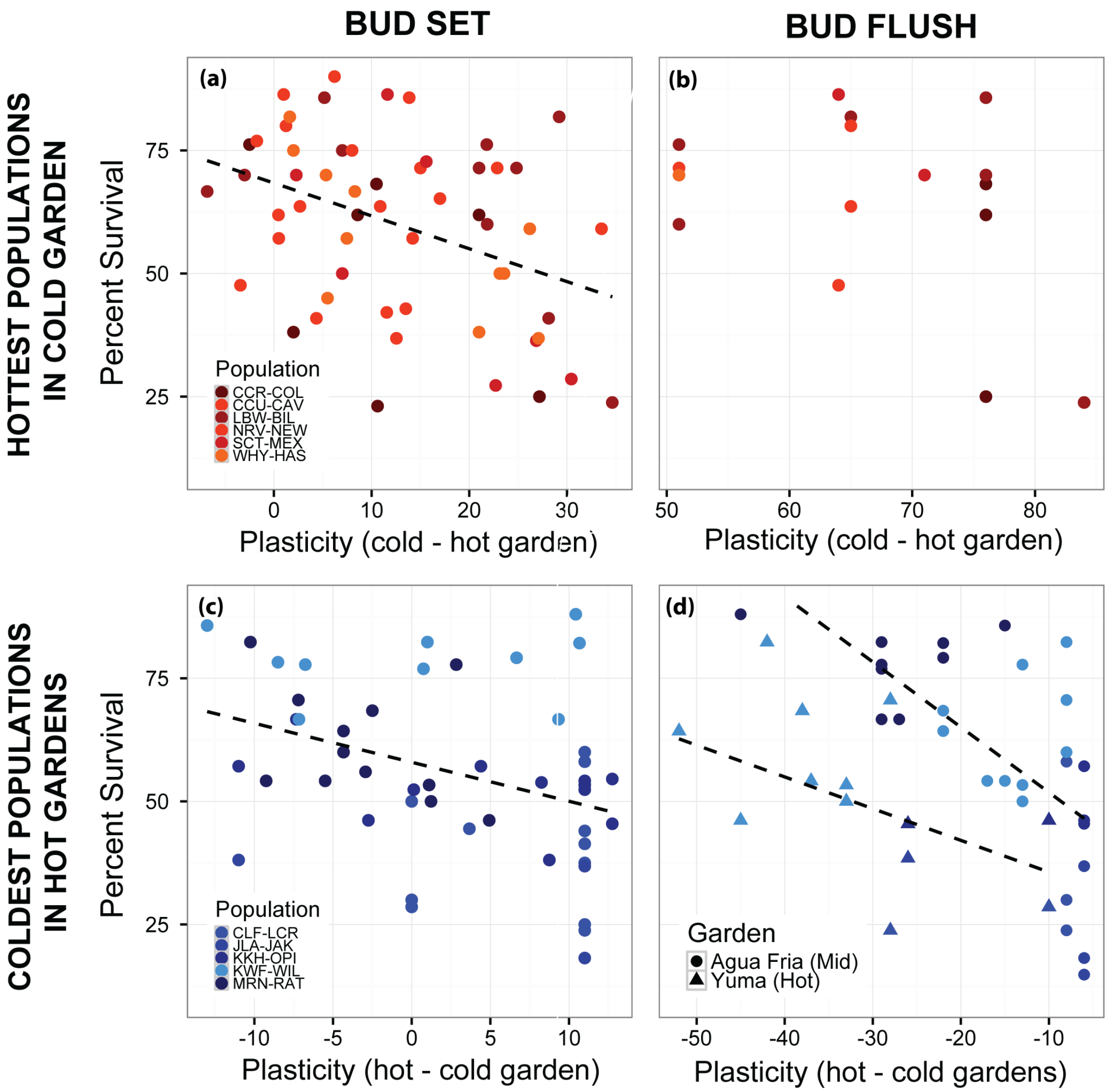

Figure 6. Survival-plasticity relationships for bud set (a, c) and bud flush (b, d). Plasticity is the difference in days between the two extreme gardens, except in (d) where plasticity is shown for both the hot and mid gardens compared to the cold garden. Negative plasticity scores indicate earlier season phenology events, while positive values indicate phenology events later in the year. Genotypes are colored using the same provenance mean annual temperature schematic as in Figure 3.

DISCUSSION

Patterns and drivers of phenological variation

The degree of genetic variation and the magnitude and direction of plastic responses will determine the extent to which populations can persist and adapt under climate change, and govern how far individual genotypes and populations can be moved for restoration and reforestation projects. Given that phenology shifts are one of the biggest climate change impacts reported thus far (Parmesan & Yohe, 2003; Westerling *et al.,* 2006; Thackeray *et al.,* 2010; Munguía *et al.,* 2011), there is a need to understand the responses of multiple traits to multiple phenological cues. This includes cues that are shifting under climate change such as temperature, and those, such as photoperiod, that are not. Here we have evaluated the variation in bud phenology traits in terms of genetic, environmental, and GxE variation using replicated common gardens which represent a more realistic climate change experiment by exposing genotypes to changes in numerous climatic variables rather than examining single variables in isolation.

Significant environmental effects on phenology were common across the populations for both traits. The majority of populations had significant environment effects for bud set, and all populations showed significant environmental effects for bud flush, highlighting the consequences of phenotypic changes in response to environmental change. The magnitude of plasticity in bud set and bud flush varied dramatically among populations and was positively correlated with increasing temperature of the provenance climate. This result contrasts with that of Vitasse *et al.* (2010), who showed no difference in the magnitude of plasticity for leaf phenology among oak and beech populations along a ~1500m elevation gradient spanning ~7 °C mean annual temperature, but agrees with the relatively high levels of plasticity in spring and fall phenological events observed in other woody species (Kramer, 1995; Vitasse *et al.,* 2010).

Variation in bud set showed a much higher influence of genotypic and GxE effects across the 16 populations compared to bud flush. Those with significant genotype and GxE variation in bud set tended to be the warmer, low elevation populations. Variation in bud flush, however, showed minimal genetic or GxE effects, These results corroborate the findings of Evans *et al.*

(2016), who found among-provenance and provenance x garden variance terms were larger than among-garden variance for bud set, emphasizing genetic effects over environment. In our study, population-level differences in bud set are related to increasing mortality as populations were transferred increasing climatic distances between their home and garden sites.

Understanding genetic variation within populations is critical to understanding the potential for adaptation to climate change in the absence of assisted migration (Kelly *et al.*, 2011). The two basic conditions for the evolution of plasticity are that genetic variation in plasticity is present (significant GxE interactions) and that correlations exist between plasticity and fitness (Crispo *et al.,* 2010). In our study, these conditions have been met in some populations, especially for bud set. This could indicate potential for further adaptation; if plasticity increases fitness in a new environment, increased levels of plasticity would be expected to evolve. In contrast, decreased plasticity might evolve when maladaptive plasticity leads to fitness declines (Crispo *et al.,* 2010).

Adaptive and non-adaptive phenotypic plasticity with global change

In general, our study shows that northern, cold-adapted populations exhibit phenotypic plasticity for bud set and bud flush in the right direction when moved into hotter climates, whereas southern populations exhibit plasticity in the wrong direction for bud set when moved into colder climates. Despite lower levels of plasticity compared to the central and southern populations, the northern populations extended their growing season via earlier flush and later bud set, providing evidence that populations will be able to partially adjust their phenology as the climate warms in the future. This trend of adaptive plasticity of northern populations toward the local trait optima when moved to hotter, drier climates is not a perfect solution to warming.

Despite the most plastic genotypes exhibiting higher survival than the canalized genotypes in the hot garden (Fig. 6), much like Cleland *et al.* (2012)'s findings on phenology shifts, these northern trees exhibited >30% higher mortality rates compared to the local populations. Southern populations, however, displayed much higher plasticity for both traits, but in the wrong direction for bud set when planted into colder climates. In the context of a warming Southwest, this finding that southern populations are unable to anticipate freezing temperatures is less important than our finding that northern populations appear fundamentally constrained in their response to a warming climate. The northern populations transferred to the middle garden experienced an increase in mean annual temperature of ~2-7°C, and a 10-12°C increase when transplanted into the hot Yuma garden. The central populations transferred to the hot garden experienced a change of ~3-6°C. The warming increases associated with these intermediate garden transfers (cold to mid, and mid to hot) are more consistent with model projections for the end of the century, especially when taking extreme heat wave predictions into account (Garfin *et al.,* 2013). As temperatures continue to warm and the threat of freezing temperatures diminishes, trees that can maximize their growing season through phenological plasticity will likely become the most productive, and may outcompete trees insensitive to warming (Cleland *et al.,* 2012). In general, the northern populations in the middle garden set bud later and flushed earlier, advancing and extending their growing season. The central populations transferred to the hot garden, however, had minimal bud set change or shifted toward earlier bud set, shortening their growing season. This non-adaptive plasticity in bud set of the central populations is offset by adaptive plasticity in bud flush, shifting these populations to an earlier growing season, but not extending growing season length. This result supports Kramer's (1995) finding of advanced growing season with warming temperatures due to equal shifts earlier in fall and spring phenology. The ability to shift phenology earlier and extend the growing season, trends that we observed for populations experiencing the intermediate warming garden transfer, will become increasingly important as rising temperatures force an earlier spring (Westerling *et al.,* 2006; Thackeray *et al.,* 2010).

The large differences in adaptive and non-adaptive plastic responses among populations moved from cold to hot climates versus hot to cold climates may be a result of different evolutionary histories in phenology cues relating to cold hardiness. The onset of growth in the spring is regulated mainly by temperature, while fall growth cessation and the development of buds are initiated by photoperiod and chilling requirements (Howe *et al.,* 1995; Chiune *et al.,* 2001; Howe, 2003). In our study, southern populations naïve to freezing temperatures may be photoperiod-insensitive and initiate bud set using only temperature cues, since it is advantageous to have the longest growing season possible in southern latitudes where temperatures rarely dip below freezing. Alternately, northern populations that have evolved with freezing temperatures may be sensitive to photoperiod, setting bud using day length cues, so as to avoid frost damage by tracking temperatures at the "wrong" time of year (Körner & Basler, 2010). This is consistent with our finding that the hottest, southern populations set bud latest in all sites, while the northern, cold-adapted populations exhibited consistent early-season bud set. In the cold Canyonlands garden, the southern populations waited until the first freeze date on November 1st (Julian date 305, Utah Climate Center; Figure 3) to set bud, resulting in frost damage and likely contributed to their high mortality in this cold garden. Interestingly, a similar pattern of delayed spring phenology in a northernmost common garden was also recorded for an annual European aster (Lustenhower *et al.,* 2017). Consistent early bud set timing across the three gardens supports the importance of day length cues for northern populations. The four coldest populations all set their buds in early October, at average Julian dates of 276 in Canyonlands and

Agua Fria, and 279 in Yuma; at these dates, the three locations had nearly identical day lengths (≤ 5 minutes difference; NOAA Solar Calculator).

The reduced plasticity in phenological traits of the northern populations may be linked to physiological adaptations to freezing temperatures. In this case, adaptations to large seasonal shifts in growth and dormancy (i.e., when winters predictably involve periods of freezing) may represent a trade-off against adaptations or plastic responses to climate shifts within growing seasons that stay above freezing temperatures. In some ways, this is opposite to the evidence that more variable environments facilitate the evolution of greater plasticity when environmental cues are predictable (Via & Lande, 1985; van Tienderen, 1991; Hendry, 2016). Indeed, we found the greatest plasticity expressed in populations experiencing unpredicted winter freezing. However, early theory papers point to the degree of cold hardiness of a species as related to the degree of the seasonal variation it experiences (Allee *et al.,* 1949), and the probability of surviving climatic extremes increasing with greater seasonal climatic deviations from the mean (Janzen, 1967). It follows then, that populations that do not experience predictable freezing winters would not shift phenology to survive freezing temperatures. This plasticity pattern, however, may be limited to specific phenology traits such as bud set, which is partially determined by fixed cues like photoperiod, as well as within species that span frost-tolerance thresholds.

Management implications for assisted migration

Although our results suggest that adaptive phenotypic plasticity may allow plants to cope with hotter environments with climate change to some degree, it is important to evaluate its limits. Prior to a critical mortality or productivity threshold being reached, alternative strategies such as assisted gene flow may become important in areas where once locally adapted populations are becoming maladapted to their new environment as a result of climate change (Aitken & Whitlock, 2013). Thus, a growing interest in assisted migration as a way to mitigate the effects of climate change has led to the development of seed transfer zone guidelines where warmer seed sources are being planted northward or at higher elevation (Wang *et al.,* 2010; Rehfeldt *et al.,* 2014). Increases in the upper elevation boundary of seed transfer standards have been recommended for numerous commercial tree species in British Columbia (O'Neill *et al.,* 2008) to maintain forest resiliency and productivity by planting genotypes pre-adapted to warmer climates (O'Neill *et al.,* 2017). Our results showing non-adaptive plastic responses in southern populations moved up in latitude and elevation to a colder common garden points to caution in moving genotypes for assisted migration. This may be especially important in managing those species whose populations cross frost-tolerance thresholds. This non-adaptive bud set shift constrains the climate transfer distance for *P. fremontii* in the context of assisted migration and reinforces the idea of small, step-wise transfers over shorter time periods to keep pace with warming (O'Neill *et al.,* 2008, Grady *et al.,* 2015). However, as climate change pushes the frost-line north, planting genotypes from lower elevations or latitudes adapted for long growing seasons might be the best restoration practice, especially in areas that do not experience freezing winters.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest, commercial, financial, or otherwise, associated with this publication.

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Table 1. Common garden and source provenance environmental information. Climatic variables are derived from ClimateWNA (Wang *et al.,* 2012). 'Total genotypes' and 'Total trees' refers to total number of genotypes and trees in each garden. MAT = mean annual temperature, MWMT = mean warmest monthly temperature, MCMT = mean coldest monthly temperature, MAP = mean annual precipitation, AHM = annual heat-to-moisture index, DD>5 = degree days above five °C.

Table 2. Variance and P-values of the phenology models. Significant results are bolded. The bottom row shows how many populations out of the 16 had significant results for each model variable.

Supplementary Table. Principal Components Analysis (PCA) loadings scores for climate variables. Bolded values indicate the five highest loadings scores for that PC axis.

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Figure 1. Location map of 16 provenance collection sites (leaf icon) of *Populus fremontii* and the three common garden locations (leaf with circle). The central garden is also a collection site. The shading corresponds to the degree-days above 5°C (DD5) throughout the region: red represents high DD5, blue low DD5.

Figure 2. A two-environment reaction norm showing the components of phenotypic variation of four genotypes: G = trait variation due to population genetics within a single environment, E = trait variation due to change in environment (plasticity), GxE = the variation in plasticity among genotypes. Phenotypic variation (V_P) = $V_G + V_E + V_{GxE}$.

Figure 3. Population means (+/- 1SE) for (a) bud set and (b) bud flush across the three common gardens. Gardens are arranged from hottest to coldest: YU=Yuma, AF=Agua Fria, and CRC=Canyonlands Research Center. Populations are colored by the mean annual temperature (MAT °C) of their provenance.

Figure 4. Bud flush and bud set plasticity are significantly positively correlated with axis 1 of the climate PCA. Hotter, drier, lower elevation source environments (positive PC1) correlate with higher plasticity scores. Smaller opaque symbols represent genotypes, while larger, solid symbols indicate population means. Colors correspond to the mean annual temperature of the population.

Figure 5. Population level mean (+/- 1 SE) survival correlations with bud set date in each of the three common gardens. Populations are colored by the mean annual temperature (MAT °C) of their source provenance. In Yuma, survival is highest in the hotter source populations and is positively correlated with later bud set. The opposite is true in the coldest Canyonlands garden. A parabolic relationship was found at the mid garden of Agua Fria, where the highest survival correlated with mid-season bud set of the central Arizona populations.

Figure 6. Survival-plasticity relationships for bud set (a, c) and bud flush (b, d). Plasticity is the difference in days between the two extreme gardens, except in (d) where plasticity is shown for both the hot and mid gardens compared to the cold garden. Negative plasticity scores indicate earlier season phenology events, while positive values indicate phenology events later in the year. Genotypes are colored using the same provenance mean annual temperature schematic as in Figs. 3-5.

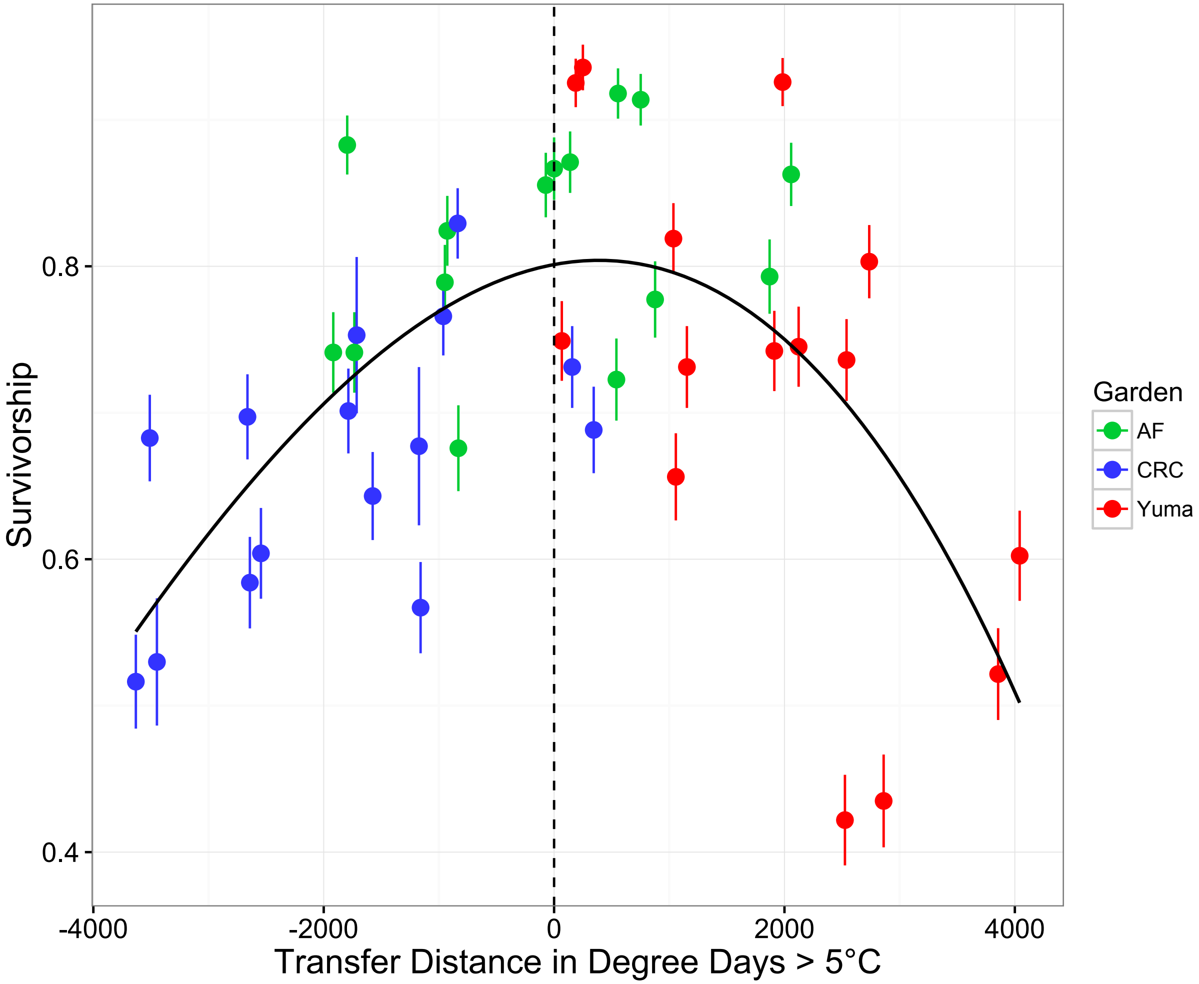
Supplementary Figure.

Populations survival as a function of growing season transfer distance, approximated by degree-days above 5 (DD5) between garden and source climate. Survival is highest for transfers where the climate distance between source and garden is small, and decreases as populations are transferred increasing climate distance from their source climate. Extreme mortality declines occur at the hot (Yuma) and cold (CRC = Canyonlands) gardens, with smaller declines at the middle garden (AF = Agua Fria).

Supplementary Table.

Climate variable	**Abbreviation**	**PC1**	**PC2**	**PC3**
Latitude	Lat	0.000	0.003	-0.004
Longitude	Lon	0.001	0.002	0.003
Elevation	Elev	**-0.321**	-0.168	-0.431
Mean annual temperature	MAT	0.002	-0.001	0.000
Mean warmest monthly temperature	MWMT	0.002	0.002	-0.004
Mean coldest monthly temperature	MCMT	0.002	-0.004	0.003
Continentality (MWMT-MCMT)	TD	0.000	0.006	-0.007
Mean annual precipitation	MAP	-0.042	**-0.294**	-0.148
Mean summer precipitation	MSP	-0.020	-0.176	0.088
Annual heat-to-moisture index	AHM	0.040	**0.190**	**0.250**
Summer heat-to-moisture index	SHM	**0.123**	**0.572**	**0.425**
Degree-days below zero	DD<0	-0.036	0.111	-0.099
Degree-days above five	DD>5	**0.726**	0.022	**-0.329**
Degree-days below 18	DD<18	**-0.454**	**0.599**	**-0.338**
Degree-days above 18	DD>18	**0.364**	**0.332**	**-0.420**
Number of frost-free days	NFFD	0.030	-0.031	-0.027
Beginning of FFP	bFFP	-0.021	-0.003	0.029
Ending of FFP	eFFP	0.013	-0.002	-0.020
Frost-free period	FFP	0.034	0.001	-0.049
Proportion of precipitation as snow	PAS	-0.004	0.010	-0.012
Extreme minimum temperature over a 30-year normal	EMT	0.005	-0.004	-0.002
Extreme maximum temperature over a 30-year normal	EXT	0.002	0.003	0.001
Mean annual solar radiation	MAR	0.000	0.000	0.004
Reference atmospheric evaporative demand	Eref	0.093	-0.089	**0.358**

Supplementary Figure



CHAPTER 4: Divergent selection on growth, leaf, and phenology traits in *Populus fremontii* across a climate gradient shows high local adaptation and the potential for maladaptation under future climate change

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ABSTRACT

Widespread tree species encounter large climatic gradients, which often lead to high levels of local adaptation and phenotypic divergence of populations across their range. To evaluate the extent of population differentiation and relative roles of selection and drift on phenotypic traits, we compared molecular and quantitative genetic variation of 16 populations of Fremont cottonwood (*Populus fremontii*) planted reciprocally across three common gardens using Q_{ST}-F_{ST} analysis. We present three major findings: 1) There is significant within- and among-population variation for functional traits in each of the three common gardens. 2) We found evidence of strong divergent selection ($Q_{ST} > F_{ST}$) for spring and fall phenology traits. We also show selection acting on tree height and specific leaf area, but variation in trunk basal diameter could not be distinguished from the neutral expectation of drift ($Q_{ST} \approx F_{ST}$). 3) There are strong relationships between population traits and climate of origin, with the strongest relationships showing the highest levels of differentiation among populations (Q_{ST}), providing evidence for local adaptation and climate-driven selection. We conclude that climatic gradients have contributed to significant phenotypic differences and local adaptation in growth and phenology traits among populations in this species. These results are important because as the climate continues to change, traits such as phenology that are finely-tuned to local conditions, may become increasingly maladaptive, with negative consequences for tree productivity and fitness, as well as the dependent communities they support.

INTRODUCTION

Understanding the processes shaping the phenotypic diversification found in species is a major objective of ecology and evolutionary biology (Schluter 2000). Trait variation within widespread species can be extensive due to historic demographic processes and spatially and temporally heterogeneous landscapes exerting different selection pressures across their range (Whitlock 2008). Over time, subpopulations will become genetically and phenotypically differentiated due to neutral processes, such as drift, gene flow, and mutation, as well as the adaptive process of natural selection (Wright 1931; Spitze 1993; Holsinger & Weir 2009; Leinonen *et al.* 2013). The relative importance of these stochastic versus selective forces is still debated and of great interest for understanding the basis of phenotypic diversification (O'Hara 2005; Hangartner *et al.* 2012; Leinonen *et al.* 2013). There is much evidence for significant genetic differences and a high degree of local adaptation in forest trees (Savolainen *et al.* 2007; O'Neill *et al.* 2008; Leimu *et al.* 2008; Hereford 2009), including the *Populus* study system (Whitham *et al.* 2006; Grady *et al.* 2011; Grady *et al.* 2013; Evans *et al.* 2016; Fischer *et al.* 2017). These intraspecific genetic differences can be as great or greater than those observed among species (Des Roches *et al.* 2017). Together, these factors make widespread forest trees ideal for evaluating climate-trait relationships and the basis of adaptation, which may be crucial in developing genetics-based restoration strategies to mitigate the impacts of climate change (Evans *et al.* 2016).

Evidence of significant phenotypic differences among populations is not enough to invoke the role of natural selection in shaping those differences (Lande 1977; Lynch & Hill 1986; Spitze 1993), as genetic differentiation can arise through drift. An appropriate null model to test for evidence of selection is by comparing Q_{ST}, the variation in quantitative traits, to F_{ST}, the variation in neutral genes (Wright 1951; Lande 1992; Spitze 1993). Q_{ST} is the quantitative genetic analog to F_{ST}, and measures the proportion of additive genetic variance in a trait attributed to among population differences. If Q_{ST} exceeds the neutral expectation of F_{ST}, there is evidence that directional selection is responsible for population-level phenotypic differentiation.

If $Q_{ST} \approx F_{ST}$, the null model that population differences are due to genetic drift alone cannot be rejected. Finally, if Q_{ST} is lower than F_{ST}, this suggests uniform or stabilizing selection acting to constrain among-population divergence (Spitze 1993). Q_{ST}-F_{ST} comparisons have been primarily used to detect selection, and evaluate the degree of local adaptation among populations, but have increasingly been used as a management and conservation tool (Leinonen *et al*. 2013). For example, Q_{ST} has been used to designate populations as separate conservation units (Leinonen *et al.* 2008), to assess the adaptive potential of invasive species, measure the rates of evolution in different environments, and look at the constraints on adaptation due to increased habitat fragmentation (Leinonen *et al.* 2013). An exponential increase in both experimental and theoretical studies comparing molecular and quantitative genetic variation and are revealing a major role of natural selection in shaping intraspecific variation in quantitative traits (McKay & Latta 2002; Leinonen *et al.* 2008; Leinonen *et al.* 2013), with approximately 70% of all studies showing $Q_{ST} > F_{ST}$ (Leinonen *et al.* 2008). Q_{ST} studies are often used as an exploratory analysis to first see the selective patterns across a suite of traits, and then target those traits with the highest levels of differentiation to examine their genetic architecture under selection more closely (Leinonen *et al.* 2008; Whitlock 2008). In assessing population differences across multiple environments, Q_{ST}-F_{ST} comparisons can also be used to elucidate which traits are under stronger selection and how the phenotypic differences are manifested across a climatically heterogeneous gradient.

Climate gradients are a major force structuring phenotypic differences within species. For example, phenology traits are closely linked to temperature and photoperiod, both of which follow strong latitudinal clines (Howe *et al.* 2003; Savoleinen *et al.* 2007; Evans *et al.* 2016). Within *Populus*, growth and phenology traits are genetically based (Frewen *et al.* 2000; Howe *et* *al.* 2000; Fischer *et al.* 2017), with evidence of clinal variation and adaptive differences among populations (Grady *et al.* 2011; McKown *et al.* 2014; Evans *et al.* 2014). *Populus fremontii* is highly adapted to temperature clines, with large population differences in mortality, productivity, and phenology traits expressed in common garden experiments at both the cold and hot edge of the species' tolerance (Grady *et al.* 2011; 2013; 2015). Population structure in *P. fremontii* has also been attributed to differences in spring and winter precipitation, which can affect flowering phenology and therefore gene flow, across its range (Cushman *et al*. 2014; Ikeda *et al.* 2017). The extent to which the differences among populations can be attributed to selective forces, and how these differences are expressed across environmental gradients however is unknown.

Experimental common gardens are well suited for evaluating the processes shaping phenotypic divergence across populations. They are considered the most reliable method of ensuring that among-population variance components reflect genetic differences and are not inflated by environmental effects (Leinonen *et al.* 2013). Experimental gardens can also indicate whether populations are locally adapted to past or current environments, and when individuals are reciprocally planted in multiple environments, they can reveal traits that vary across environmental gradients (Franks *et al.* 2014). Common gardens can reveal components of climate that contribute to trait divergence and associated phenotypic plasticity (Franks *et al.* 2014). Furthermore, replicated gardens or reciprocal transplant experiments can quantify the intensity of selection across space and the effects of plasticity on the expression of traits (Whitlock 2008).

In this study, we transplanted cloned cuttings from 16 populations of *Populus fremontii* collected throughout Arizona into three common gardens. Both the collection and garden sites span an elevation gradient of almost 2000 m, consistent with the species' range and including a difference of 12°C mean annual temperature and > 500 mm in mean annual precipitation. In order to address whether natural selection is an agent of phenotypic diversification across these populations we first assessed the amount of genetic variation present in each of the three common gardens. Based on previous studies in this species (Grady *et al.* 2011, 2013; Fischer *et al.* 2017), we hypothesized that there would be significant among and within population variation in all traits measured. Secondly, we hypothesized that $Q_{ST} > F_{ST}$, suggesting selection has plays a larger role than genetic drift in shaping trait divergences. By planting the same genotypes from multiple populations across the common gardens, we can test whether how this drift-selection balance is manifested across the landscape and among traits. We hypothesized that phenology traits would be the most differentiated (highest Q_{ST}) based on evidence of strong clinal variation in phenology (Savoleinen *et al*. 2007) and a high degree of differentiation found in other *Populus* species (Hall *et al.* 2007; Evans *et al.* 2016). We also predicted that the environmental conditions in the two extreme gardens would elicit stronger trait differences among populations compared to the middle garden. Lastly, we hypothesized that trait variation would show strong associations with population climate of origin, and this relationship would be correlated with Q_{ST}. In other words, the most locally adapted traits are also under the strongest climate-driven selection.

MATERIALS AND METHODS

Collection sites and common gardens

Sixteen populations of *P. fremontii* were collected throughout Arizona, encompassing the environmental variation experienced by the Sonoran Desert Ecotype (Ikeda *et al.* 2017) of this species (Table 1). Cuttings were from individuals collected over 20 m distance from each other to insure independent genotype sampling. Clonal replicates of 12 genotypes per population were planted in the summer and fall of 2014 in the three common garden sites after rooting in the greenhouse for ~4 months. Using this design, within and among population variation for phenotypic traits could be assessed.

The three experimental common gardens span wide environmental gradients (Table 1), resulting in extreme climatic transfers for some populations. The northernmost garden is located adjacent to Canyonlands National Park, Utah and is maintained by The Nature Conservancy's Dugout Ranch. The middle Arizona garden is located adjacent to the Agua Fria River in Agua Fria National Monument and is maintained by the Arizona Game and Fish Department. The southernmost garden is located in Yuma, Arizona near Mittry Lake, and is maintained by the Bureau of Land Management. Each common garden is composed of four blocks, with each block made up of 16 randomized population plots. Each population plot has 64 trees composed of replicates of 12 genotypes, arranged in a randomized 8 x 8 grid, with trees spaced 1.85m in each cardinal direction.

We downloaded 21 abiotic climate variables (1961-1990 means) for the 16 provenance sites and the three common gardens using the program ClimateWNA (Wang *et al.* 2012). To create a multivariate climatic trait representing the environmental variation found throughout the 16 provenances, the ClimateWNA variables plus elevation, latitude, and longitude, were analyzed in a principal component analysis (PCA) using *labdsv* (Roberts 2007) and *vegan* (Oksanen *et al.* 2016) packages in the R statistical language (R Core Team 2014).

Trait analysis

We analyzed five traits for phenotypic differentiation: height, specific leaf area (SLA), basal diameter, fall bud set and spring bud flush. Height and SLA were assessed the first year of growth in the summer of 2015 for every genotype in the common gardens. Specific leaf area was measured using the average of three fully expanded leaves that were free of herbivory damage when possible, collected from the southern aspect of each tree. Leaves were scanned and leaf area was measured using ImageJ software (Schneider *et al*. 2012). After the area scans, leaves were dried in an oven at 70°C for at least 72 hours, following protocol recommendations in Perez-Harguindegay *et al*.'s (2013) updated trait handbook.

Basal diameter was measured during the second summer of growth as the diameter at root collar, ~10cm from the soil, on every tree in the gardens. Diameter at root collar was used instead of diameter at breast height due to the small nature of the stems. Mortality surveys of the gardens were also made at this time.

Leaf phenology of bud set and bud flush were measured in the fall of 2015 and the spring of 2016, respectively. Bud set was recorded as the initiation of bud formation, where internode elongation had ceased and the newly emerged, bundled leaves were clustered at the same level on the stem, offset from the shoot axis (Frewen 2000). It was measured at 6-10 day intervals from September through December of 2015 on three replicates of all 12 genotypes per population in each garden. We scored trees based on the bud stage exhibited by 50% or more of the apical meristems. This is a good approximation of plant progression towards dormancy as there was little within-plant variation in apical bud development. Spring bud flush was recorded as the first sign of full leaf emergence on the tree. Bud flush was measured biweekly from February through the end of April in the Yuma and Agua Fria gardens, and through the end of May in the Canyonlands garden.

Genetic analysis

Genomic DNA was extracted from ~0.2g silica-dried leaf tissue from all 192 genotypes (12 genotypes per population, 16 populations) using the Thermo Scientific MagJET Genomic DNA Kit (Thermo Scientific). Double-digest restriction-associated DNA (ddRAD) libraries were prepared using 2-5ng/µL concentrations of DNA per sample following a modified Peterson *et al.* (2012) protocol. Briefly, restriction and ligation was carried out simultaneously in 20µL reactions using restriction enzymes MspI and EcoRI and universal adapter sequences for indexing PCR. Ligation products were amplified following 25 cycles of PCR. After indexing products were checked on an agarose gel and purified, samples were pooled and size selected for fragments between 200 and 350bp using a Pippin Prep (Sage Science, Inc., Beverly, MA). The size-selected pool was quantified by qPCR and sequenced on an Illumina MiSeq Desktop Sequencer (Illumina, Inc. San Diego, CA) in 2x75 mode. Sequence reads were processed using a modified Stacks pipeline (Catchen *et al.* 2013, Andrews 2018). Potential chloroplast and mitochondrial sequences were filtered from the dataset by comparing them to other *Populus* sequences downloaded from GenBank. Specifically, we removed sequences that matched chloroplast sequences from *P. fremontii* and mitochondrial sequences from *P. tremula* x *P. alba.* Following the Stacks protocol (Catchen *et al.* 2013), sequences of each individual were clustered into similar stacks. The resulting stack was summarized into consensus sequences, which were then clustered among individuals. Parameter values for clustering were based on tests following parameterization recommendations in Mastretta-Yanes *et al.* (2015). The minimum stack depth for each individual was three and the minimum number of individuals per locus cluster was three.

To ensure we measured F_{ST} on only neutral loci free from selective forces, all SNPs included in the measure of F_{ST} adhered to Hardy-Weinberg equilibrium. Testing marker neutrality is the first step in any Q_{ST}-F_{ST} study (Leinonen *et al.* 2013). F_{ST} was calculated for each pair of populations at each variable site in the populations programs in Stacks. Q_{ST} must be compared to the distribution range of F_{ST} estimates, not the mean (Whitlock 2008; Leinonen *et al.* 2013). In order to obtain this distribution around F_{ST}, the standard deviation of all 120 pairwise F_{ST} values was quantified.

Statistical Analyses

To investigate the within and among population variation in phenotypic traits, each garden was modeled separately using linear mixed models fit by maximum likelihood using the *lme4* package in R (R Core Team 2014; Bates *et al.* 2015). The plant traits were modeled as response variables, while population and genotype were random effects. Garden plot was included as a random variable to help account for within garden environmental variance. Statistical significance was calculated using likelihood ratio tests for the random effects using the package *lmerTest* (Kuznetsova *et al.* 2015).

We compared the quantitative trait variation (Q_{ST}) with genetic variance at neutral loci (F_{ST}) in each garden. For quantitative traits, the ratio of variances can be described as

$$Q_{ST} = \sigma_P / (\sigma_P + 2\sigma_G)$$

where σ_p is the additive genetic variance among populations and σ_G is the additive within-population variance (Spitze 1993; McKay & Latta 2002). Each trait was analyzed using the model described above, and population and genotype variances were extracted to calculate Q_{ST}. Parametric bootstrap and Bayesian estimation are considered the best methods to obtain a precision estimate around Q_{ST} (O'Hara & Merilä 2005). We performed parametric bootstrapping to obtain a 95% confidence interval for Q_{ST} by resampling the 16 populations, with replacement 1000 times, allowing the number of genotypes per population to vary, and estimating Q_{ST} for each bootstrapped data set. Resampling over the highest level in a hierarchical experimental design (here the population) is considered best practice (O'Hara & Merilä 2005). Variance in Q_{ST} becomes quite large as the number of populations decreases (< 20), especially if populations are highly differentiated (O'Hara & Merilä 2005; Goudet & Büchi 2006). Goudet & Büchi (2006) recommend sampling many populations relative to the number of families. Our design of 16 populations with 12 genotypes per population comes close to their recommended sampling design of upwards of 20 populations with 10 families (O'Hara & Merilä 2005; Goudet & Büchi 2006). We directly compared the confidence intervals for F_{ST} and Q_{ST} to ascertain significance. In using clonally replicated genotypes, our estimate of σ_G includes both additive and non-additive genetic effects. Non-additive effects such as dominance have been shown to lower Q_{ST} estimates making this a conservative test of $Q_{ST} > F_{ST}$ (Goudet & Büchi 2006, Whitlock et al. 2008), however in some instances gene interactions may increase estimates of Q_{ST} (López-Fanjul et al. 2007).

In order to test whether traits showed strong climatic relationships, we calculated the Pearson's product moment correlation coefficient in R (R Core Team 2014) between population trait means and the first principal component (PC1) from the environmental PCA. Systematic differences among populations seen in these trait-climate correlations are another test to rule out genetic drift (Whitlock 2008). To then test whether those traits showing strong clinal patterns also showed evidence of stronger selection, we fit a linear model of Q_{ST} to the absolute value of the trait-climate correlation coefficient (|r|) using the *lmer* package (Bates *et al.* 2015), following Evans *et al*. (2016).

RESULTS

Neutral Genetic Variation

Our dataset of 192 genotypes analyzed with RADseq yielded ~3,000 SNP loci for genetic structure. The 16 Arizona populations are moderately differentiated with an average pairwise F_{ST} = 0.10 (standard deviation of 0.095). This result is consistent with the larger population structure (F_{ST} =0.221) found among populations collected throughout the entire range of *P. fremontii*, obtained with microsatellite loci (Cushman et al. 2014). It is within the range of other *Populus* species, from another strongly differentiated species, *P. angustifolia* with a F_{ST} = 0.21 (Evans *et al.* 2014), to very low genetic structure (F_{ST} = 0.01) found in *P. tremula* (Hall *et al.* 2007).

Phenotypic Variation

Significant within and among population variation for traits was found at each of the three common gardens (Fig. 1, Table 2), supporting our first hypothesis. Among-population variation was strong for both phenology traits, explaining ~90% of the variation in bud flush in Yuma and Agua Fria, while within-population variation was not as important in structuring phenology differences. In the Yuma garden, four out of the five traits showed a stronger population effect compared to genotype (SLA had a higher genotype variance). In Agua Fria and Canyonlands, population and genotype contributions were relatively equal among traits.

Q_{ST} - F_{ST}: Comparison of quantitative trait differentiation to neutral genetic expectation

We found evidence of divergent selection ($Q_{ST}>F_{ST}$) driving phenotype differences in most traits (Fig. 2, Table 3). Overall, the average Q_{ST} value across all traits and all gardens (0.48) was above the F_{ST} confidence interval (CI), indicating directional selection has shaped local adaptation across these collections. Phenology traits exhibited some of the highest levels of population differentiation, with mean Q_{ST} values for bud flush between 0.53-0.90 and budset between 0.42-0.79. In all but one case (budset in Agua Fria), the confidence intervals for these Q_{ST} values lie outside the confidence interval for F_{ST}. Tree height showed significant divergent selection ($Q_{ST}>F_{ST}$) occurring at the hottest common garden in Yuma, Arizona, but was not statistically different from F_{ST} in the two cooler gardens of Agua Fria and Canyonlands. Specific leaf area showed the opposite pattern to growth traits, with divergent selection apparent in the mid and cold gardens of Agua Fria and Canyonlands, but not in the hot Yuma garden.

Climate analysis

In each garden, the phenotypic expression of most traits was significantly correlated to their population's provenance climate (Table 4). The multivariate climate axis (PC1) explained 95.8% of the variation in provenance environment and was influenced primarily by four temperature-related climate variables (degree-days above 5 °C, degree-days below 18 °C, degree-days above 18 °C, and summer heat-to-moisture index (Wang *et al.* 2012)), plus elevation. Positive correlations with PC1 indicate that trait values are higher in populations from locations with higher temperatures, lower precipitation, lower elevation, and longer growing seasons. Phenology traits showed the strongest correlations with provenance climate while growth traits showed more moderate correlations. In general, tree height and basal diameter showed evidence of local adaptation, with southern populations growing larger in the hottest

Arizona garden, and northern populations growing larger in the coldest Utah. Only two traits (specific leaf area and basal diameter) measured at the middle garden of Agua Fria showed no relationship with climate. Furthermore, across all three gardens and traits measured, there is a significant correlation between the strength of Q_{ST} and the trait-climate correlation (Fig. 3, $P = 0.00123$, $R^2 = 0.57$, $F_{(1,13)} = 16.88$). This shows that traits with the highest amount of differentiation across populations are those where that trait variation is most strongly correlated with the climate.

DISCUSSION

Divergent selection shapes population differences

We found evidence for divergent selection acting on all traits measured across the Arizona collections. This result is supported by reviews showing that the majority of studies find Q_{ST} values are generally larger than F_{ST} (McKay & Latta 2002; Savoleinen *et al.* 2007; Leinonen *et al.* 2013). The largest Q_{ST} values were found for spring bud flush, followed by fall bud set, consistent with other studies showing high phenology divergence across latitudinal clines (Hurme 1999; Howe *et al.* 2003; Hall *et al.* 2007; Evans *et al.* 2016). Spring bud flush is highly differentiated among *P. fremontii* populations, with a difference of up to eight weeks observed in flush timing in three separate common garden studies (Grady *et al.* 2015; Cooper *et al.*, in preparation (see Chapter 3)). We also found large population differences in budset timing of ~2-5 weeks across three common gardens. Bud flush and budset also showed evidence of divergent selection ($Q_{ST}>F_{ST}$) in the congeneric species, *P. angustifolia* (Evans *et al.* 2016), as well as in *P. tremula* (Hall *et al*. 2007). In contrast to these studies, however, we found bud flush had a higher degree of population differentiation compared to budset (Fig. 2). This is intriguing, since spring bud flush is thought to be governed by the accumulation of degree-days above a specific temperature, while fall budset is often linked to precise day length periods (Howe *et al*. 2003), an environmental cue that is entirely linked to latitude, while temperature can vary each year. Our result of less differentiated budset timing may be due to the fact that our collection of populations spans the winter frost line. The southern populations in our study do not experience predictable freezing winters, and may not have adapted to the cue of shorter day lengths indicating oncoming freezing events. When these populations were transplanted to the northernmost Utah garden, they failed to set bud early in the fall, delaying budset much later than the local northern populations (Fig. 1).

Within the same study system, variability in Q_{ST} values can show that the relative roles of selection and drift are different for individual traits (McKay & Latta 2002). In our study, we found the phenology traits had the highest degree of differentiation, while growth traits were less differentiated. This suggests that the neutral processes of gene flow and drift may affect growth and leaf traits such as SLA more than it does for phenology, where stronger selection can overcome the effects of gene flow (Saint-Laurent et al. 2007; Leinonen *et al*. 2013). Likewise, variation in the Q_{ST} value of the same trait across the three gardens (Fig. 2) suggests that trait variation observed and the detection of natural selection is dependent on the environment in which traits are measured. For example, we found a large Q_{ST} value indicating directional selection acting on tree height in the hot Yuma garden, but not in the central or northern gardens. Similarly, Evans *et al.* (2016) found that annual environmental fluctuations can reveal or hide past selection patterns on traits, as $Q_{ST} > F_{ST}$ for tree height in one year but not the next. Long-term common garden experiments have the ability to demonstrate how population differences are expressed across variable environments through time, an important research area as intensification of extreme events and climate variability is expected to increase in the future (Jentsch *et al.* 2007; Ganguly *et al.* 2009; Garfin *et al.* 2013).

Climate – trait relationships

Significant population and genotype variation was found for all functional traits measured across the common garden gradient. In general, phenology traits showed strong population differentiation, with lower genotype effects. Populations from the warmer, drier provenances in southern latitudes set buds later in the fall where hard frosts are rare compared to more northern populations adapted for regular fall frosts, regardless of common garden environment (Fig. 1). Earlier budset in the populations from colder source locations is expected, since these trees are adapted to critical day lengths that signal predictable freezing events in the fall (Howe *et al*. 2003). The southern populations flushed their leaves significantly earlier in spring compared to the northern populations in the two warmer sites (Fig. 1). This result indicates that populations from warmer source locations may be able to track spring temperatures better than those from colder locations and time their leaf emergence with earlier springs. Bud flush has been hypothesized to be a more plastic trait, given its reliance on temperature cues over photoperiod (Franks *et al.* 2014), however, we found that the northern, cold-adapted populations flushed consistently late in the spring across the common garden sites. Together, these traits emphasize the large latitude/elevation difference in growing season length experienced by populations across Arizona. As the climate continues to warm in the Southwest (Seager *et al.* 2007; Garfin *et al.* 2013), delaying fall frosts and advancing spring onset (Parmesan 2006), the populations that can track warming temperatures by shifting their phenology stand to outcompete populations with traits that are more canalized (Cleland *et al.* 2012).

Growth traits also showed significant correlations with climate of origin, and declined as populations were transferred increasing climate distance away from their home sites. In Yuma, trees from hotter source locations had significantly larger trunk diameters and were taller than trees from colder provenances (Fig 1., Table 4). In the middle and northern gardens, the reverse was true, where trees from cooler provenances grew significantly taller than those from the warmer sites. Declines in growth as the climate transfer increases point to local adaptation, with the highest productivity observed in populations whose source climate best matches that of the garden climate. Based on the above studies and our results, we can expect decreases in growth and productivity and the potential for maladaptation as local conditions become increasingly arid. The traits with the highest climate correlations were also the most strongly differentiated among populations (highest Q_{ST} values).

Management implications

Given the ubiquity of local adaptation among forest trees (Savoleinen *et al*. 2007) and that variation in climate explains the majority of population-level phenotypic differentiation (Alberto *et al.* 2013), populations stand to become less optimally adapted to their environments as the climate continues to change (Shaw & Etterson 2012; Franks *et al.* 2014). Provenance trials have shown this response experimentally with tree productivity declining as the climate distance transferred between home site and garden site increases (O'Neill *et al.* 2008; Evans *et al.* 2014; Grady *et al.* 2015). Transfer functions can help determine how far a population can be moved before growth declines below a specified level; this tool combined with climate change forecasts are one of the best ways to implement assisted migration in order to manage for future forest health and productivity. They have been used to recommend seed transfer zones and distances for many commercial conifer species in British Columbia (O'Neill *et al.* 2017), and to propose moving genetic stock at a reasonable, step-wise pace to track climate change rather than moving trees too far too fast (Grady *et al.* 2015).

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Table 2: Model variance and p-values for each trait measured in each garden, showing the population, genotype, and plot-level effects on trait variation. * = p<0.05, ** = p<0.01, ***=p<0.001.

Table 3. Q_{ST} and broad-sense heritability, H^2 (+ 95% Confidence Interval).

Table 4. Pearson's product moment correlation coefficient (r) between traits and provenance climate, (PC1 score), at each common garden. Statistical significance is represented by * = p<0.05 and ** = p<0.001.

Table 1.

Gardens	Latitude	Longitude	Elevation (m)	MAT	MWMT	MCMT	TD	MAP	MSP	AHM	SHM	DD<0
Canyonlands	38.093	109.588	1581	10.7	24.6	-3.2	27.8	225	104	91.9	236.5	309
Agua Fria	34.257	112.066	988	17.2	28.5	7.6	20.9	440	173	61.9	165.4	27
Yuma	32.850	114.493	49	22.8	33.8	12.7	21.1	93	35	351.5	973.8	6
Collections												
BCE-BUL	34.540	111.697	1109	15.6	27.2	5.5	21.6	432	172	59.4	157.9	47
CAF-AUG	34.257	112.066	988	17.2	28.5	7.6	20.9	440	173	61.9	165.4	27
CCR-COL	33.362	114.698	70	22.6	33.9	12.2	21.8	97	36	334.8	947.1	7
CCU-CAV	33.890	111.951	696	19.9	31.3	10	21.3	349	110	85.7	285.6	14
CLF-LCR	35.613	111.319	1299	14.1	27.2	0.8	26.3	176	89	136.7	306.1	129
JLA-JAK	34.960	110.436	1507	12.3	25.3	-0.7	26	212	106	105.2	237.6	185
KKH-OPI	35.812	110.170	1920	10.7	23	-1.3	24.4	258	105	80.1	218.6	230
KWF-WIL	35.143	113.543	1126	15	26.6	5	21.6	243	102	102.9	259.8	55
LBW-BIL	34.276	114.059	143	22.3	34.6	10.9	23.7	137	46	235.6	755.6	10
MRN-RAT	34.783	111.614	1774	10.4	21.7	0.5	21.2	593	208	34.5	104.3	174
NRV-NEW	33.948	112.136	666	19.9	31.4	10	21.4	337	109	88.8	289.4	14
PSA-SON	31.536	110.763	1234	15.7	25.2	7.1	18.1	471	272	54.5	92.6	32
SCT-MEX	32.527	114.804	26	22.1	32.9	12.4	20.4	88	34	365.1	966.2	7
TSE-TUM	31.565	111.045	986	17.5	27.2	8.8	18.4	402	249	68.3	109.3	20
TSZ-SAN	31.610	110.167	1219	16.9	26.4	7.7	18.7	322	211	83.6	125.5	26
WHY-HAS	33.9088	112.676	575	19.6	31.4	9.4	22	284	110	104.3	284.5	16

Gardens	**DD>5**	**DD<18**	**DD>18**	**NFFD**	**bFFP**	**eFFP**	**FFP**	**PAS**	**EMT**	**EXT**	**MAR**	**Eref**
Canyonlands	2798	3171	526	214	123	282	160	18	-33.2	41	16.8	1155
Agua Fria	4512	1447	1157	316	81	324	242	1	-15.5	43.3	18.7	1496
Yuma	6496	593	2329	360	28	354	326	0	-4.8	49	19.8	1744
Collections												
BCE-BUL	3971	1810	932	279	103	309	205	3	-21.2	42.4	19.3	1493
CAF-AUG	4512	1447	1157	316	81	324	242	1	-15.5	43.3	18.7	1496
CCR-COL	6429	635	2305	355	34	350	316	0	-7.2	49.2	19.6	1769
CCU-CAV	5439	1001	1667	344	50	341	291	0	-10.5	45	18.9	1587
CLF-LCR	3635	2328	887	247	111	299	188	4	-28.1	42.4	18	1451
JLA-JAK	3140	2710	640	221	125	291	166	9	-31.6	41.4	18.7	1350
KKH-OPI	2641	3086	410	204	140	286	146	19	-32.7	39.5	18.3	1202
KWF-WIL	3760	1952	838	264	113	303	190	2	-22.6	43	20.3	1472
LBW-BIL	6308	758	2309	350	42	347	305	0	-9.1	49.3	19.3	1715
MRN-RAT	2454	3052	301	203	143	283	140	38	-30.3	38.4	19.1	1258
NRV-NEW	5460	996	1683	343	51	340	289	0	-10.7	45	18.8	1591
PSA-SON	3957	1636	780	274	110	306	196	1	-21.3	40.8	19.6	1579
SCT-MEX	6246	650	2136	355	32	349	317	0	-6.8	49.2	21	1803
TSE-TUM	4584	1293	1080	297	95	316	220	1	-18.2	42.7	19	1690
TSZ-SAN	4373	1432	1007	301	91	316	225	1	-18.2	41.7	19.6	1560
WHY-HAS	5343	1076	1637	327	69	330	261	0	-14.3	45.8	18.7	1682

Table 2.

TRAIT	GARDEN	VARAIBLE	VARIANCE	P-VALUE
Height	CRC	Population	407.5	0.004**
		Genotype	567.3	<0.001***
		Plot	213.1	<0.001***
	AF	Population	33.82	0.5
		Genotype	104.67	<0.001***
		Plot	183.56	<0.001***
	Yuma	Population	64.99	<0.001***
		Genotype	41.12	<0.001***
		Plot	33.13	<0.001***
Specific Leaf Area (SLA)	CRC	Population	1.339	<0.001***
		Genotype	0.207	<0.001***
		Plot	0.326	<0.001***
	AF	Population	0.323	0.011*
		Genotype	0.208	0.001**
		Plot	0.236	<0.001***
	Yuma	Population	0.307	<0.001***
		Genotype	0.386	<0.001***
		Plot	0.080	0.001**
Diameter at Root Collar	CRC	Population	0	1.0
		Genotype	36.83	<0.001***
		Plot	38.35	<0.001***
	AF	Population	3.051	0.6
		Genotype	8.871	<0.001***
		Plot	44.059	<0.001***
	Yuma	Population	5.194	<0.001***
		Genotype	1.331	<0.001***
		Plot	4.093	<0.001***
Bud set initiation	CRC	Population	104.74	<0.001***
		Genotype	58.76	<0.001***
		Plot	7.913	0.003**
	AF	Population	54.1	<0.001***
		Genotype	76.3	<0.001***
		Plot	23.9	<0.001***
	Yuma	Population	14.78	<0.001***
		Genotype	6.42	<0.001***
		Plot	4.13	<0.001***
Bud flush initiation	CRC	Population	30.641	<0.001***
		Genotype	13.508	0.05
		Plot	8.104	0.07
	AF	Population	314.864	<0.001***
		Genotype	18.108	<0.001***
		Plot	2.987	<0.001***
	Yuma	Population	365.241	<0.001***
		Genotype	18.704	<0.001***
		Plot	0	1.0

Table 3.

Trait	Garden	H^2	Q_{ST}
Bud set initiation	Yuma	0.07 (0.05-0.26)	0.79 (0.40-0.86)
	Agua Fria	0.23 (0.24-0.35)	0.42 (0.19-0.46)
	Canyonlands	0.24 (0.20-0.25)	0.47 (0.46-0.57)
Bud Flush initiation	Yuma	0.83 (0.58-0.99)	0.88 (0.80-0.97)
	Agua Fria	0.52 (0.24-0.89)	0.90 (0.67-0.97)
	Canyonlands	0.17 (0.04-0.33)	0.53 (0.22-0.87)
Specific Leaf Area	Yuma	0.21 (0.17-0.36)	0.32 (0.10-0.47)
	Agua Fria	0.13 (0.07-0.18)	0.32 (0.26-0.61)
	Canyonlands	0.15 (0.05-0.24)	0.76 (0.43-0.92)
Height	Yuma	0.12 (0.04-0.19)	0.45 (0.26-0.70)
	Agua Fria	0.15 (0.05-0.21)	0.14 (0.00-0.62)
	Canyonlands	0.31 (0.21-0.35)	0.21 (0.08-0.42)
Diameter at Root Collar	Yuma	0.06 (0.06-0.11)	0.66 (0.14-0.74)
	Agua Fria	0.04 (0.03-0.06)	0.16 (0 - 0.53)
	Canyonlands	0.22 (0.13-0.73)	0.03 (0.00 - 0.09)

Table 4.

Trait	Garden	Correlation with PC1
Bud set initiation	Yuma	0.42 **
	Agua Fria	0.40 **
	Canyonlands	0.47 **
Bud Flush initiation	Yuma	- 0.77 **
	Agua Fria	- 0.75 **
	Canyonlands	0.12 *
Specific Leaf Area	Yuma	0.21 **
	Agua Fria	- 0.06
	Canyonlands	0.31 **
Height	Yuma	0.15 **
	Agua Fria	- 0.20 **
	Canyonlands	- 0.35 **
Diameter at Root Collar	Yuma	0.34 **
	Agua Fria	0.02
	Canyonlands	- 0.08 **

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Figure 1. Population means (+/- 1SE) for each functional trait measured in each garden. Populations are arranged in order of increasing principal component axis 1 (PC1) values and colored by mean annual temperature (MAT °C). Note the y-axis varies in scale among the three gardens.

Figure 2. Q_{ST} means and 95% confidence intervals (point and vertical lines, respectively) for the phenotypic traits measured at each of the three gardens. The average pairwise F_{ST} value + 95% confidence interval (0.10 +/- 0.095) is shown as the grey band. Common gardens are abbreviated as Y = Yuma, A = Agua Fria, and C = Canyonlands.

Figure 3. Positive relationship between the level of population trait differentiation (Q_{ST}) and the strength of correlation of each trait with its provenance climate. Traits with the strongest correlations to provenance climate (r) are also the most differentiated (largest Q_{ST}). Gardens are coded by color and traits by symbol.

Figure 1.

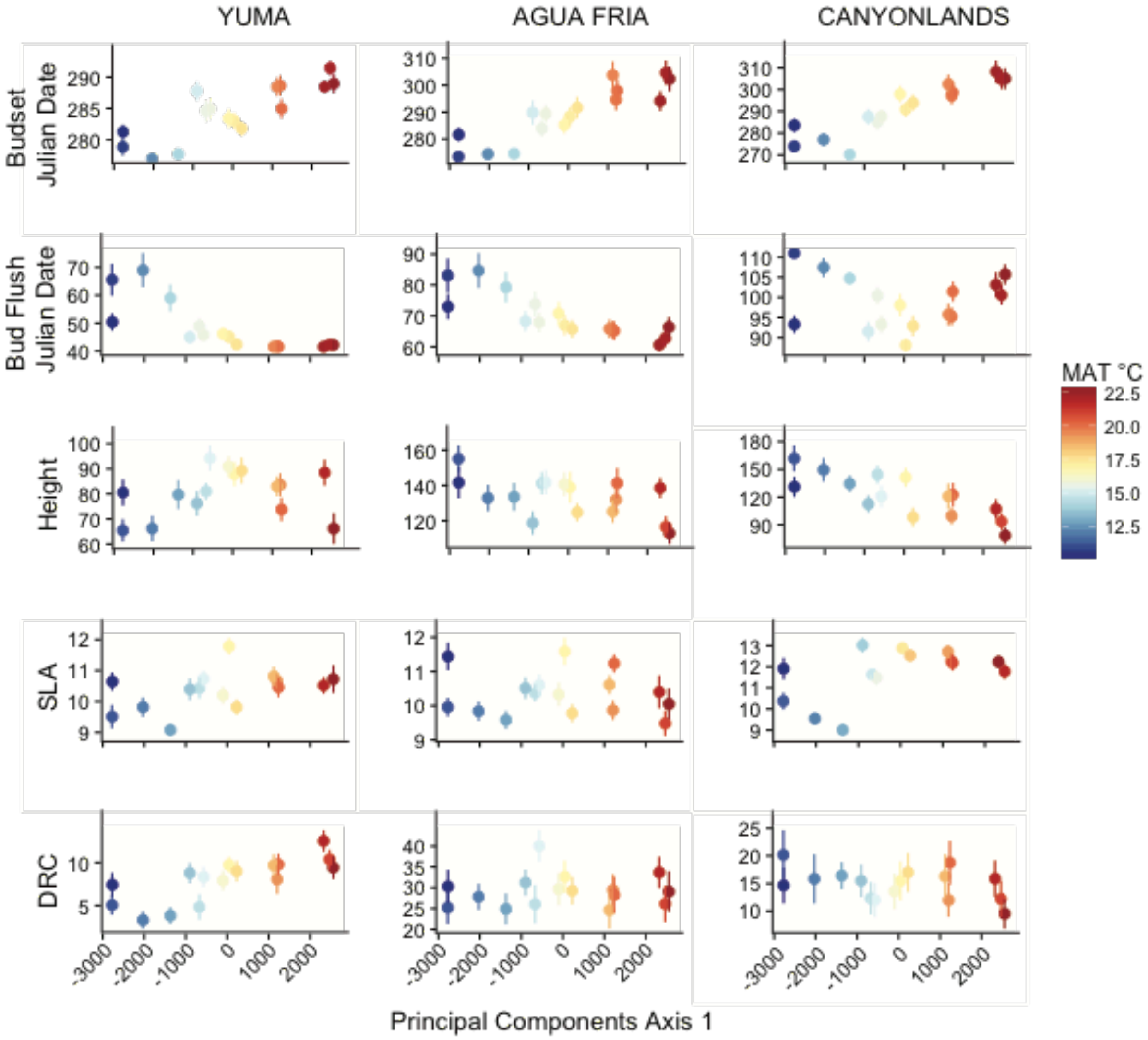
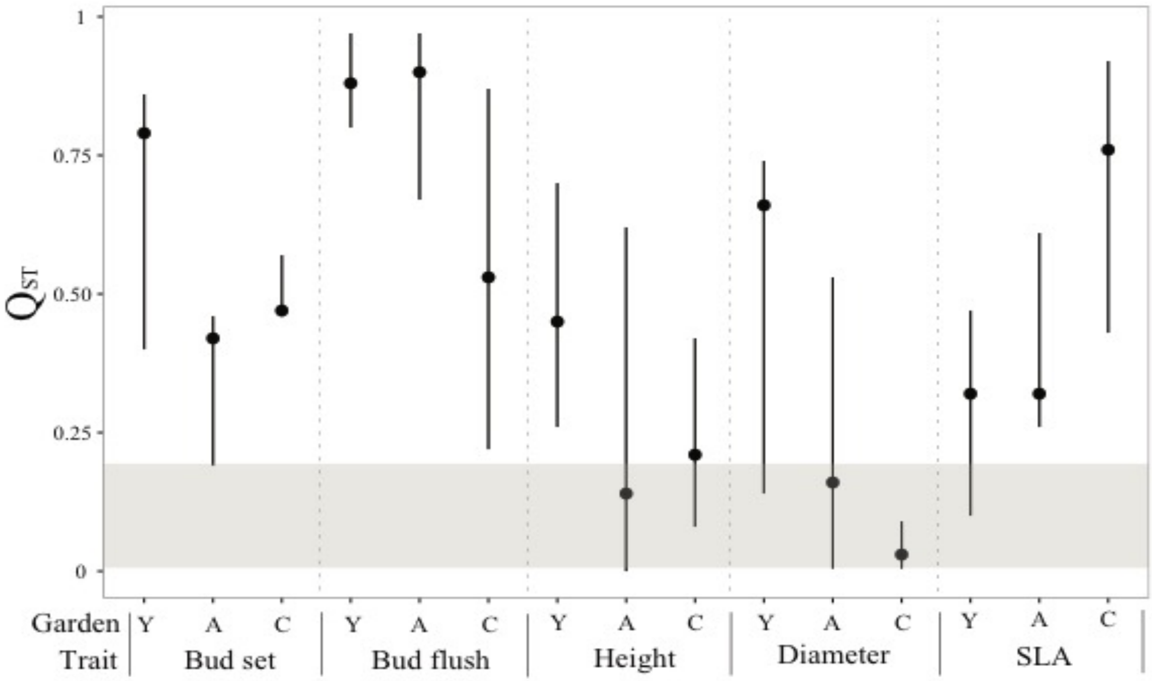
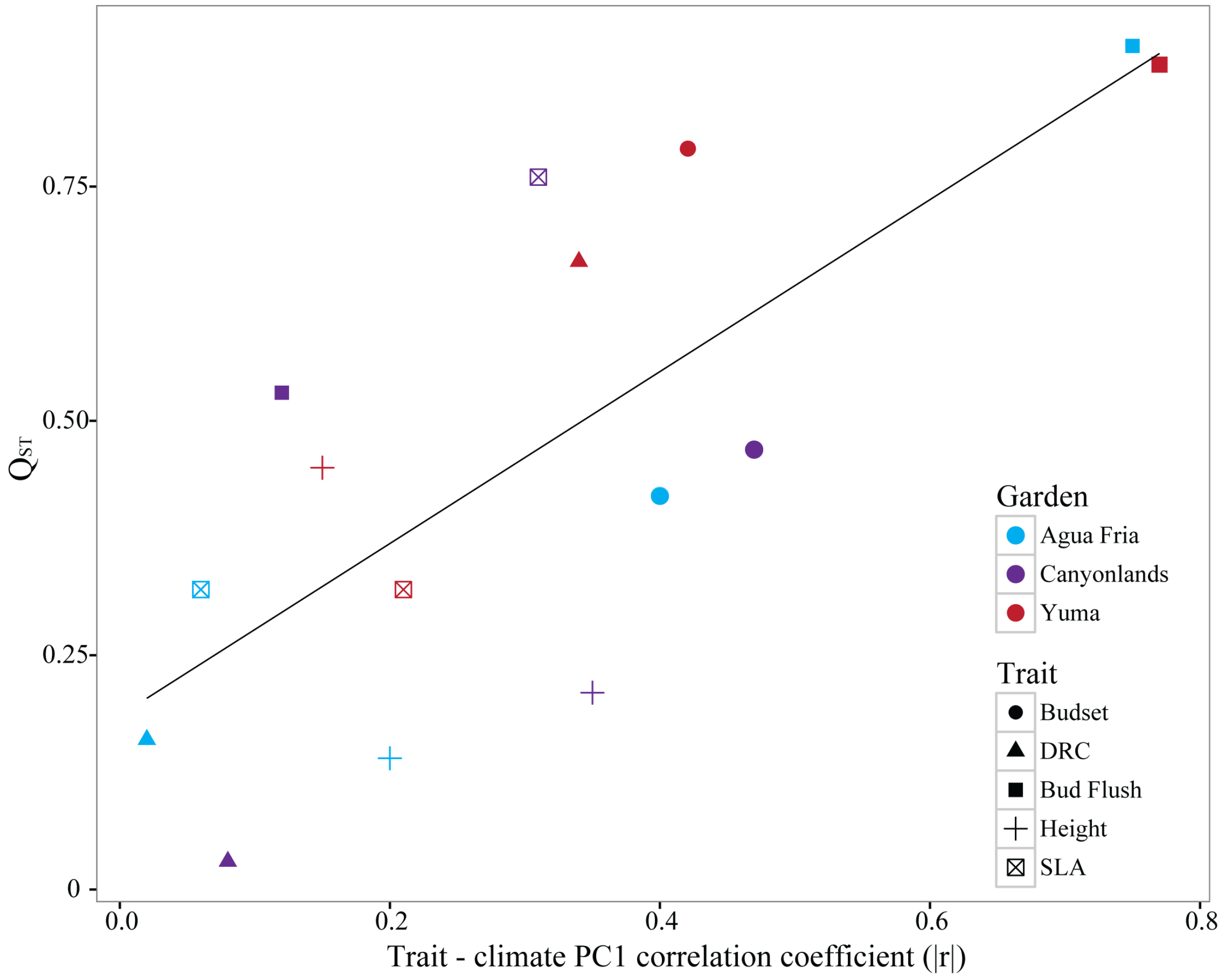

YUMA
AGUA FRIA
CANYONLANDS
Budset Julian Date
Bud Flush Julian Date
Height
SLA
DRC
MAT °C
22.5
20.0
17.5
15.0
12.5
Principal Components Axis 1
-3000
-2000
-1000
0
1000
2000

Figure 2.



QST
1
0.75
0.50
0.25
0
Garden
Trait
Y
A
C
Bud set
Bud flush
Height
Diameter
SLA

Figure 3.


0.75
0.50
0.25
0
Q_{ST}
0.0
0.2
0.4
0.6
0.8
Trait - climate PC1 correlation coefficient (|r|)
Garden
● Agua Fria
● Canyonlands
● Yuma
Trait
● Budset
▲ DRC
■ Bud Flush
+ Height
☒ SLA

DISCUSSION OF RESULTS AND CONCLUSIONS

The research presented in this dissertation illustrates some of the ways that key foundation species as well as entire plant communities native to the southwest can respond to climate change. The results of these studies will hopefully be disseminated out of the academic sphere and into land management practices, a crucial step in addressing the complex consequences of global change on the ground, especially for southwestern ecosystems that have been particularly impacted and are predicted to experience increasing climate shifts in the near future (Garfin *et al.* 2013, USGCRP 2017). Through the use of long-term studies, the influence of annual climate fluctuations and extreme events on ecosystems can be captured. By repeating plant community surveys a decade after a major drought event, I found that these communities are still diverging and have not returned to pre-drought assemblages. Furthermore the most current communities sampled show that closely related species are coexisting more than expected by chance, suggesting a shrinking in the range of plant traits and function. Continued monitoring is necessary to understand if these ecosystems have passed a threshold where more frequent, severe droughts will delay forest recovery to the point where dominant trees are eliminated from the area, permanently altering community composition and function (Allen *et al.* 2015).

The experimental common gardens I used to research the impact of climate transfers on cottonwood populations can also benefit from long-term monitoring to capture climate fluctuations and assess the degree that plants can change through time. This is especially important as responses to climate change can vary along spatial and temporal scales (Sandel *et al.* 2010), such as from juvenile to mature developmental stages, or when an extreme climate event occurs (Sthultz *et al.* 2007). These common gardens, although not as biotically complex as the full communities I analyzed in Chapter 1, have the benefit of eliminating environmental variation among populations, so that trait differences are explained by the underlying genetic (and epigenetic) variance. I was able to add to the robust literature of genetic differences found in and among cottonwood populations (Whitham *et al.* 2006, Grady *et al*. 2011, Evans *et al.* 2016, Fischer *et al.* 2017) for important functional traits such as phenology and growth. In addition to the single garden estimates of genetic variance, the three garden array allowed me to extend these findings across environmental gradients to look at the influence of phenotypic plasticity in shaping trait differences when populations were transferred to both colder and warmer environments. I found that the influence of environment (plasticity) can be much greater than genetic variation in shaping trait differences. Furthermore, this plasticity did not always move the trait means in an adaptive direction. For instance, large plastic responses were correlated with increased mortality of southern, hot-adapted populations planted in the coldest Utah garden. These findings are critical in determining which individual genotypes or populations to move in order to mitigate the effects of climate change through assisted migration techniques. Similar to other cottonwood studies that transplanted gentoypes into colder locations (Grady *et al.* 2015, Evans *et al.* 2016), my results caution against moving warmer-adapted genotypes too far north, where they do not adjust phenology timing for earlier fall frosts. In contrast, I found the northern populations adjusted their traits in an adaptive, although limited, manner when planted into warmer sites. Those that could track the warming climate best had greater survival than the genotypes or populations with more fixed traits, a finding consistent with phenological plasticity increasing fitness under climate change (Cleland *et al.* 2012). These results suggest that cold-adapted populations could withstand limited temperature increase *in situ* or be moved down in elevation or latitude into environments that still undergo freezing, where populations from hotter climates may not survive even rare chilling events. In comparing these population differences in phenology, growth, and leaf traits to both the multivariate climate of their source locations (trait-climate regression) and to the variance in neutral genes (Q_{ST}-F_{ST} comparison), I found evidence for a high degree of local adaptation among the Arizona populations, and showed climate-driven natural selection is operating to differentiate important functional traits. The high levels of local adaptation to their past and current climates suggest there is potential for maladaptation as climate change continues to alter the environment where these populations exist (Franks *et al.* 2014). Although we have seen plasticity in some traits, this may not be enough to withstand large environmental changes and intervention via assisted migration may be needed to ensure healthy and productive cottonwood stands remain throughout the west. These types of complex experiments where populations are transferred to multiple environments and subject to high fluctuations in climate through time are essential for successful land management as the southwest is predicted to become not only hotter, but experience increasing variability in climate, manifested in extreme heatwave, drought, flooding, freezing, and fire events (USGCR 2017). In order to expand on the work presented in this dissertation, continued surveys of both natural events such as community changes in the wild after drought or fire, as well as within experimental gardens are needed. The effects of large genetic and plastic variation seen among Arizona cottonwoods should be examined on associated communities scaling up in tropic levels (e.g. soil, root, and leaf biota, arthropods, birds, etc.) to understand climate change impacts in more realistic, complex systems. This is important not only to gauge how other species can withstand global change, but also because foundation species interactions and networks affect how climate change impacts are experienced within the community (Gilman *et al.* 2010), and in turn these interactions can be directly affected by climate change (Tylianakis *et al.* 2008, Gehring *et al.* 2017).

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