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Santa Barbara

Tradeoffs and Trait Variation in Tamarisk (*Tamarix* spp.) Across Environmental Gradients

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Ecology, Evolution, and Marine Biology

by

Randall William Long

Committee in charge:

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June 2020

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June 2020

Tradeoffs and Trait Variation in Tamarisk (*Tamarix* spp.) Across Environmental Gradients

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Randall William Long

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Vita of Randall William Long
March 2020

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- **Long R**., S.E. Bush, K.C. Grady, D.S. Smith, D.L. Potts, C.M. D’Antonio, T.L. Dudley, S.D. Fehlberg, J.F. Gaskin, E.P. Glenn, K.R. Hultine (2017) Can local adaptation explain varying patterns of herbivory tolerance in a recently introduced woody plant in North America? Conservation physiology.
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ABSTRACT

Tradeoffs and Trait Variation in Tamarisk (*Tamarix* spp.) Across Environmental Gradients

by

Randall William Long

Environmental conditions are generally the best predictors of terrestrial communities, and have long been used to define patterns of plant species distributions. Both extreme temperatures, whether hot or cold, and water availability have been shown to select for certain trait correlates that result in the increased fitness of a plant species. In this dissertation I conducted several experiments across large and small landscapes to determine tradeoffs and trait variation exhibited in *Tamarix* when collected across a range of temperature and water availability.

Tamarix provided a unique opportunity to study how rapidly these trait variations become established in woody plants, due to it being an introduced plant. Although it was already established at many sites in North America by the late 1800's, it is a perennial woody plant that resprouts from underground tissues after fire and flood disturbances and stand replacement does not occur often. Because of these traits most stands are likely only separated from the founding originals by only a few generations.

Using populations sourced from across a broad thermal range in Arizona I was able to find evidence that there was variation in phenology, allocation to storage, and reproductive output across the different populations of *Tamarix*. The plants were grown in a common garden in Yuma, AZ and I witnessed that plants from colder sites had delayed bud break and

flowering in the spring and allocated greater amounts of carbon to storage, at the expense of growth and reproduction. Delayed phenology protected the plants from frost events that occur in the spring at the colder sites, and the increased allocation to carbon could serve as a reserve to regrow plant tissues in the spring after late frost events.

To investigate trait variation at a smaller scale I used plants sourced from two sites with different salinities. The sites were located at the Cibola National Wildlife Refuge on the Lower Colorado River in southeast California. The sites were only separated by 1.5 km, but the salinity increased six fold across that distance. Increasing salinity is analogous to decreasing water availability as high solute concentrations reduces the plant available water. Using both *in situ* and greenhouse experiments I found that plants from high salinity sites exhibited more conservative water use traits than those from low salinity sites. I also found that plants grew best at salinities closest to that of the source site. Increased salinity reduced net photosynthesis and growth in the low salinity populations, while high salinity populations grew best when solute concentrations were high.

To determine how these tradeoffs would interact with an additional stressor we used the tamarisk leaf beetle (*Diorabda carinulata)* in a salinity x herbivory experiment. We found that the plants from the high salinity sites were most impacted by the beetle herbivory. The more conservative traits exhibited by the high salinity populations resulted in lower biomass accumulation between herbivory events and the plants were unable to recover from repeated defoliation events. Taken together these three studies show that trait variation exists across *Tamarix* populations and that there are apparent tradeoffs between the traits that reduce the fitness of the individuals when exposed to additional stress.

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I. Introduction: Can local adaptation explain varying patterns of herbivory tolerance in a recently introduced woody plant in North America?

Abstract

Patterns of woody-plant mortality have been linked to global-scale environmental changes, such as extreme drought, heat stress, more frequent and intense fires, and episodic outbreaks of insects and pathogens. Although many studies have focused on survival and mortality in response to specific physiological stresses, little attention has been paid to the role of genetic heritability of traits and local adaptation in influencing patterns of plant mortality, especially in non-native species. *Tamarix* spp. is a dominant, non-native riparian tree in western North America that is experiencing dieback in some areas of its range due to episodic herbivory by the recently introduced northern tamarisk leaf beetle (*Diorhabda carinulata*). We propose that genotype x environment interactions largely underpin current and future patterns of *Tamarix* mortality. We anticipate that 1) despite its recent introduction, and the potential for significant gene flow, *Tamarix* in western North America is generally adapted to local environmental conditions across its current range in part due to hybridization of two species; 2) local adaptation to specific climate, soil and resource availability will yield predictable responses to episodic herbivory; and 3) the ability to cope with a combination of episodic herbivory and increased aridity associated with climate change will be largely based on functional tradeoffs in resource allocation. This review

focuses on the potential heritability of plant carbon allocation patterns in *Tamarix*, focusing on the relative contribution of acquired carbon to non-structural carbohydrate (NSC) pools versus other sinks as the basis for surviving episodic disturbance. Where high aridity and/or poor edaphic position lead to chronic stress, NSC pools may fall below a minimum threshold because of an imbalance between the supply of carbon and its demand by various sinks. Identifying patterns of local adaptation of traits related to resource allocation will improve forecasting of *Tamarix* population susceptibility to episodic herbivory.

Introduction

Plant ecologists have recently paid considerable attention to woody plant mortality because of continental-scale die-offs of woody plants across the globe (Allen et al. 2010). Rapid increases in mortality rates have been largely attributed to global environmental changes that have resulted in extreme droughts, heat waves, increased episodic insect and pathogen outbreaks and a measurable increase in forest fire frequency and intensity (Allen et al. 2010; Van Der Werf et al. 2010; Carnicer et al. 2011). Recent research has addressed the combined impacts of warming temperatures and water deficits on plant survival and there is now a wealth of data on the physiological mechanisms that underpin mortality surges in many regions (Mcdowell et al. 2008; Plaut et al. 2012; Sevanto et al. 2014). However, a clear genetic basis underlying reductions in plant fitness is still lacking for the expression of traits such as phenology, resource allocation or susceptibility to cavitation that are related to plant tolerance and resistance to environmental change. Understanding genetic variation in response to environmental change will improve predictions of future patterns of mortality across broad spatial scales.

A primary hurdle for addressing genetic versus environmental contributions to trait expression in plant mortality studies is that heritability is often difficult to measure in field settings. Common gardens provide excellent opportunities for testing hypotheses about traits that are favored under specific environmental conditions. Numerous studies using common gardens of various species that incorporate genotypes from several source populations have yielded a broad range of information on genotype- and population-level patterns of net primary productivity (NPP), biomass allocation, water use efficiency, nutrient fertilization impacts on NPP, and hydrologic processes (Zhang et al. 1993; Powers and Reynolds 1999; Savolainen et al. 2007; Grady et al. 2011; Gray et al. 2016). Some of the strongest evidence that specific phenotypic traits are locally adapted to environmental conditions has emerged from common garden studies (Clausen et al. 1941; Savolainen et al. 2007; Grady et al. 2011). However, the relationship between local adaptation to a given stress and patterns of whole plant mortality and canopy dieback under changing environmental conditions is largely unstudied (but see Yanchuk et al. 2008; Williams et al. 2014). This is particularly true for non-native plant taxa where few experimental common gardens have been established to address the potential for rapid selection on recently established populations (but see Alexander et al. 2012; Liao et al. 2016) .

The genus *Tamarix* comprises a group of riparian woody species and their hybrids from Eurasia introduced to, and now distributed broadly across arid-and semi-arid regions of western North America. As with native woody plants in these regions, *Tamarix* spp. are experiencing moderate to extreme drought, and in addition exhibit dieback from episodic defoliation by a foliage-feeding beetle, *Diorhabda* spp. (Chrysomelidae), released for biological control of this genus (Bean et al. 2012). The northern tamarisk leaf beetle, *Diorhabda carinulata*, also native to Eurasia, was released more than a decade ago and has since affected thousands of hectares across the southwestern U.S. The beetle produces two or more generations in a season and can develop high population densities that can completely defoliate *Tamarix* stands in less than two weeks. Repeated defoliation events eventually result in significant stand dieback and mortality (Pattison et al. 2011a), but individual susceptibility to dieback can vary dramatically. Recent surveys across *Tamarix* populations have found that dieback can range from near 0% to more than 80%, after two to five years of repeated herbivory (Hultine et al. 2015; Kennard et al. 2016). These disparate responses to repeated herbivory events invite many questions such as, whether some *Tamarix* populations express phenotypic traits that make them more tolerant to episodic canopy disturbances than other populations. If so, two important follow up questions are, (1) are there fundamental plant tradeoffs in carbon allocation patterns associated with herbivory tolerance?, and (2) is herbivory tolerance / sensitivity tied to adaptation to local environmental conditions? Although considerable research has been undertaken on *Tamarix* invasion into arid riparian ecosystems of North America, including identification of the hybrid nature of the genus in the western U.S. (Gaskin and Kazmer 2009), information is lacking on the potential adaptive evolution of this highly successful non-native plant. Nevertheless, as beetle populations continue to disperse into broader geographic locations, clues are beginning to emerge on the extent to which *Tamarix* genotypes vary in their ability to cope with episodic foliage herbivory.

This paper synthesizes ongoing research on the patterns and mechanisms of *Tamarix* canopy dieback and mortality in response to intense episodic herbivory by *D. carinulata*. We focus on *Tamarix / Diorhabda* interactions as a model system to test hypotheses related to plant resource allocation, local adaptation, and the impacts of multiple stressors on plant mortality and fitness. We present *Tamarix / Diorhabda* as a model system because of the intense episodic patterns of foliage herbivory by *Diorhabda*, coupled with the wide geographic distribution of *Tamarix* across broad environmental gradients and potential stressors. Together these provide a system to investigate variation in traits associated with survival in response to defoliation events under a wide range of stressors. First, we provide a brief overview of the history of *Tamarix* in North America, followed by a review of recent research on the genetic diversity and evidence of local adaptation of *Tamarix* in its novel environment. We then propose potential tradeoffs associated with physiological traits related to carbon allocation. Here, we specifically address cases in which the expression of a given trait leads to resistance to one stress mechanism at the cost of reduced resistance to another stress mechanism, including stressors that are introduced or occur episodically, such as defoliation by *D. carinulata*. We pay special attention to the impacts that changes in mortality pressures can have on directional selection resulting in reduced genetic and phenotypic diversity, and potentially reduced tolerance of other stressors or competition. Finally, we summarize experiments that we believe are critical to merge studies of *Tamarix* mortality with those that focus on patterns of local adaptation, including the construction of common gardens and reciprocal transplant experiments. The specific hypotheses that are advanced here include: 1) despite its recent introduction, and the potential for significant gene flow, *Tamarix* in western North America is generally adapted to local environmental conditions across its current range in part due to hybridization of two species, 2) local adaptation to specific climate, soil and resource availability conditions will yield predictable responses to episodic herbivory, and 3) the ability to cope with a combination of episodic herbivory and increased aridity associated with climate change will be largely based on functional tradeoffs in resource allocation that fall along a predictable trait spectrum.

History and Ecology of *Tamarix*

Tamarix has become one of the most successful non-native woody plants in the western U.S., covering nearly 500,000 hectares (Friedman et al. 2005; Nagler et al. 2011), with a range that spans much of North America. Trees in this genus were introduced to the western states in the mid 19th century as ornamentals and for erosion control by governmental agencies due to their ability to thrive in xeric and saline environments (Horton 1977). The genus was identified as a threat to native ecosystems in the 1930s (Robinson 1965). Previously, the two most widely distributed species in North America, *T. ramosissima* and *T. chinensis,* were treated as either two separate taxa (Baum 1978; Gaskin 2003)or as a single aggregate species (Allred 2002). These conflicting classifications are partly explained by results from molecular analyses that revealed that as much as 85% of *Tamarix* sampled from populations in the United States were a mosaic of hybrids between *T. ramosissima* and *T. chinensis* (Gaskin and Kazmer 2009). While some hybridization of *Tamarix* species had been recognized in previous studies (Gaskin and Schaal 2002; Gaskin and Shafroth 2005), these two species and their related hybrids (hereafter referred to as *Tamarix*) are now recognized to dominate desert riparian habitats (Gaskin and Schaal 2002; Sher 2013). In addition to *T. ramosissima* and *T. Chinensis* there are six other species of *Tamarix* in North America, and some of these hybridize with *T. ramosissima* and *T. Chinensis* (i.e. *T. gallica, T. canariensis, T. aphylla*), but these are less common species and even rarer as parents of hybrids (Gaskin and Schaal 2002; Gaskin and Shafroth 2005). *Tamarix* has had substantial impacts on hydrological function, the occurrence of fire, and food web structure in riparian ecosystems in the southwestern U.S. and northern Mexico, in part due to the initial widespread planting of diverse *Tamarix* species (Friedman et al. 2005; Sher 2013), as well as a suite of traits that allow *Tamarix* to be a rapid post-disturbance colonizer, a strong competitor, and/or capable of tolerating considerable stress (Hultine et al. 2013).

Tamarix has been targeted for large-scale removal projects in attempts to conserve water and maintain flows in arid regions based on assumptions that replacement of native taxa by *Tamarix* resulted in an overall increase of transpiration across riparian land areas (Shafroth et al. 2005). However *Tamarix* removal from invaded systems has proved difficult, and control efforts using fire, mechanical removal at ground level, and herbicide treatments have mostly been proven to be ineffective or unsustainable (Gaskin 2003). *Tamarix* resprouts from underground storage tissues following these eradication techniques, necessitating repeated treatments and sometimes soil reclamation to establish native species. The cost of removal alone is often prohibitive, around USD 1500-1700 ha^{-1} (Shafroth *et al.*, 2005), and for successful eradication of established stands where revegetation and restoration efforts have been undertaken the cost can be upwards of USD 12000 ha^{-1} (Zavaleta 2000).

The USDA Agricultural Research Service began investigating the use of a biological control for *Tamarix* in the 1960s (DeLoach et al. 2003). *Tamarix* seemed to be an ideal candidate for a biocontrol program, since there are no native congeners of *Tamarix* in North America (Gaskin, 2003). Three specialist insects from the *Tamarix* native range were approved by regulatory agencies for release, and beginning in 1998 controlled field trials began for the northern tamarisk leaf beetle (*Diorhabda carinulata*) to determine its suitability as a biocontrol agent (DeLoach *et al.*, 2003). Open field releases followed successful cage trials in Colorado, Nevada, and Utah, that resulted in establishment and associated defoliation of *Tamarix* stands by *D. carinulata* (Pattison et al. 2011a; Dudley and Bean 2012); three other species of *Diorhabda* were subsequently released, primarily in Texas (Knutson et al. 2012). Multiple defoliation events typically occur over a single season, with up to three events at warmer sites (with longer growing season), and tree mortality has been documented after multiple years of defoliation (Bean *et al.*, 2012). Some populations appear to be more tolerant to defoliation. For example sites along the Humboldt River in Nevada tolerated three defoliation events per year for at least three years before any mortality was observed (Pattison et al. 2011). Likewise, sites along the Virgin River, a tributary of the Colorado River, have also exhibited low mortality rates after as many as seven defoliation events (Hultine et al. 2015). By contrast, mortality of approximately half of individual plants has been documented at some sites along the Colorado River near Moab, Utah three years after the first observations of defoliation associated with *Diorhabda* feeding (Hultine et al. 2013). To date, the range of *D. carinulata* continues to expand southward, despite initial projections that physiological constraints would inhibit establishment south of the 38th parallel (Bean *et al.*, 2012).

Hybridization and the potential for local adaptation

Early investigators (Baker 1974) suggested that invaders were successful because they had a “general purpose genotype” that would perform well across a range of environmental conditions (Dlugosch and Parker 2008). This hypothesis was grounded in the idea that founder effects reduce genetic variation in a species’ introduced range compared to its native range, and that populations of non-native species that become established would be comprised of highly plastic individuals that could be successful across a range of environmental conditions (Baker 1974). However, even early supporters of the "general purpose genotype" hypothesis recognized that it could be offset by multiple introductions, or by plants with putative adaptive traits that are specialized to specific environments. It is believed that after initial colonization by a non-native species, local adaptation may play an important role in continued existence (Baker 1974; Liao et al. 2016). There is building evidence that the hybridization of *Tamarix* may provide variation in traits that could promote local adaptation.

The hybridization of *T. ramosissima* with *T. chinensis* is widespread in North America (and southern Africa where the two species have also been introduced), but not in Eurasia in part because of the allopatric distributions in their native ranges (Gaskin and Schaal 2002; Mayonde et al. 2016). Hybridization can also provide novel gene combinations that may promote adaptations to specific ecological problems in the new range that were not expressed by either parental lineage, as well as help overcome genetic bottlenecks that arise from founder effects (Dlugosch and Parker 2008). Furthermore, studies have shown that most naturalized *Tamarix* are more closely related to other wild naturalized *Tamarix* than they are to nearby ornamental populations, suggesting that most recent recruitment has occurred from naturalized individuals, despite potential gene flow between ornamental and naturalized populations (Gaskin and Kazmer 2009). This suggests that naturalized genetic sources may be better adapted for successful invasion into these ecosystems than cultivated genetic lineages. Since *Tamarix* plants are fairly long lived (> 70 years), and there has been extensive backcrossing, it is likely that hybridization occurred soon after introductions into western North America (Gaskin et al. 2012). Two pieces of evidence support the early hybridization hypothesis: 1) most individuals that have been sampled for molecular analysis from naturalized populations are the product of multiple generations of hybridization, as exhibited by varying levels of introgression (Gaskin and Kazmer 2009); and 2) studies of populations in southern Utah showed that the plants established there in the 1930s were *T. ramosissima* X *chinensis* hybrids (Gaskin et al. 2012). Nevertheless, information is generally lacking on whether breeding efforts by nurseries played a significant role in the observed patterns of hybridization in North America.

Molecular studies conducted across a latitudinal gradient from Texas to Montana showed that plants from lower latitudes were most representative of genetic material from the *T. chinensis* parental strain and that the higher latitude plants were most representative of *T. ramosissima* parents (Friedman et al. 2008; Gaskin and Kazmer 2009). The climates in the extreme northern and southern ranges of the study reflect some of the differences between the climates of *T. ramosissima* and *T. chinensis* in their respective native ranges. Specifically, *T. ramosissima* occurs in the Eurasian interior where minimum temperatures are typically much lower than the thermal range of *T. chinensis* (Baum 1978; Friedman et al. 2011). A common garden investigation of cold hardiness in *Tamarix* across this latitudinal gradient showed that plants from Montana were able to survive temperatures 21°C lower than the genotypes from Texas, and that there was a correlation between the latitude of origin and overwinter survival (Friedman et al. 2008). These data suggest that there has been local adaptation to climatic pressures (described in greater detail in the next section), and that hybridization of the two parental species may play a role in these adaptive traits as increased introgression towards *T. ramosissima* increased the cold hardiness of plants.

A recent genetic survey using microsatellites as described by Gaskin et al. (2006) and Friedman et al. (2008) was completed in 2016 from 18 Tamarix populations sampled across an elevation gradient in Arizona and southern Utah and showed that intra-population diversity was higher than inter-population diversity (Fehlberg, unpublished data). The amount of introgression towards *T. ramosissima* in these Arizona / southern Utah populations matched the levels of hybridization reported earlier from other locations in western North America (Gaskin and Schaal 2002; Gaskin and Kazmer 2009) and supports evidence that the majority of naturalized plants are hybrids of the two species. The novel genotypes created in such hybrid swarms could increase the capacity for emergence of a variety of genotype x environment combinations. Taken together, results from these genetic studies indicate that hybridization may explain the wide distribution and high abundance of *Tamarix* in the United States. Intermediate traits between the two parental species and novel gene combinations could provide the material for adaptation to local climatic and edaphic conditions or increase plastic expression of traits that allow for acclimatization to a range of sites by individuals. Variation in tolerance to cold, salinity, and herbivory all indicate that hybridization could play an important role in the propensity of *Tamarix* to dominate riparian habitats.

Evidence for local adaptation in *Tamarix* common garden studies

Local adaptation is driven by divergent natural selection on genotypes for traits that favor fitness in any given genotype x environment combination and should result in local populations that are more fit in their local habitat, defined by the suite of environmental factors, compared to populations from other habitats (Williams 1966; Kawecki and Ebert 2004). Gene flow, lack of genetic variation, extinction, and environmental variability all have the potential to slow the emergence of local adaptation. Evidence has shown that gene flow can be maladaptive for populations, specifically away from the core of a species distribution range (Kirkpatrick and Barton 1997), yet in small populations, increases in genetic variation resulting from gene flow can favor local adaptations (Moore and Hendry 2009). Even in areas with high rates of gene flow, local adaptation may arise when there is strong selection due to high spatial heterogeneity in the environment (Macnair 1991; Kawecki and Ebert 2004) Multiple introductions may promote local adaptation by increasing the genetic diversity of a species in it's introduced range, even potentially increasing diversity beyond that found in native ranges (Kolbe et al. 2004). This is especially true when introductions occur from across a large geographical distribution where plants may have low genetic diversity within a population but high diversity across populations (Dlugosch and Parker 2008).

Native species have been shown to inherit traits linked to variation in cold hardiness and senescence periods, where selection over long periods of time have resulted in local populations that are more fit than conspecific individuals from other habitats in response to different photoperiods across latitudinal gradients and minimum winter temperatures in local climates (Howe et al. 1995). Similarly, introduced species can also express significant levels of local adaptation in their novel ranges (Rice et al. 1992; Dlugosch and Parker 2008; Oduor et al. 2016). It has been shown that local adaptation can sometimes be as important as phenotypic plasticity in the capacity of some invasive plants to occupy a broad range of environments (Liao et al. 2016; Oduor et al. 2016). The bulk of literature available regarding local adaptation of non-native plants however, has been based on annuals or herbaceous perennial species, and a paucity of work has been conducted with invasive perennial woody species.

Common garden studies involving *Tamarix* from populations exhibiting varying amounts of *T. ramosissima* introgression show that there is a range of traits that are expressed across a climate gradient (Friedman et al. 2008; Williams et al. 2014). A common garden study in Ft. Collins, Colorado compared characteristics of a native riparian tree species, *Populus deltoides* (Plains cottonwood), and *Tamarix* across a latitudinal gradient to investigate variations in cold hardiness and phenology (Friedman et al. 2008). As expected the northern *P. deltoides* populations entered dormancy earlier and had higher rates of survival over the winter when compared to the southern populations. *Tamarix* populations showed similar results, with fewer individuals from the southern populations surviving through the winter (Friedman et al. 2008). Likewise, the date of leaf senescence was correlated with latitude for both *Tamarix* and *P. deltoides* genotypes in the common garden (Friedman et al. 2011). Studies have also shown that northern populations of *Tamarix* have a larger root to shoot ratio, indicating that northern populations allocate more biomass to belowground tissues than southern populations (Sexton et al. 2002; Williams et al. 2014). For populations experiencing higher rates of freeze-induced dieback, it may be adaptive to have larger pools of carbon in the form of belowground biomass for replacement growth of dead tissues when the growing season resumes. Similar trends of increased cold hardiness and early leaf senescence in *Tamarix* when compared to *P. deltoides* suggest that there may be rapid acclimatization of the introduced species to local environments.

Preliminary work from a *Tamarix* common garden study in Yuma, AZ also supports evidence of variation in phenology and foliar gas-exchange rates across populations.

Phenology and leaf-level gas exchange patterns tend to correlate with minimum winter and maximum July/August temperatures of source populations. The common garden study conducted at the University of Arizona Mesa Farm (lat. 32.6151°N, long. -114.6365°W, elev. 60 m) involves 18 populations derived from an elevation gradient from 15 to 1940 meters and a latitudinal range of 32.0° to 37.3° N. Beginning in January 2016, bimonthly phenological observations of floral characteristics were made on at least 36 individual plants from each population. Each individual was assigned a score for foliage greenness on a scale from 0-5, with a score of zero indicating that the plants were still dormant, and a five indicating that the leaves were fully flushed out. Low elevation populations broke dormancy and were fully leafed out earlier than those that originated from mid- and high elevation sites (Figure 1). Likewise, leaf gas exchange measurements conducted in mid June 2016 revealed that genotypes collected from low elevations sites with similar climate means as the common garden location in Yuma exhibited higher rates of midday net photosynthesis (A_{net}) than genotypes collected from higher elevations with cooler climate means than in Yuma (upper panel of Figure 2). The low-elevation populations also had higher rates of stomatal conductance (g_s) when compared to high-elevation populations (lower panel of Figure 2). Although the physiological mechanism that allows genotypes originating from warmer climate regions to maintain high levels of leaf-level photosynthetic rates is not known, the fact that phenology and physiology varied among provenances provides evidence that low elevation genotypes may be adapted to the extreme heat that typifies low deserts of North America. Consequently, locally adapted Tamarix plants could become locally maladapted under projected future climate conditions.

Fine-tuning carbon allocation to cope with multiple stressors

Plants face many challenges for avoiding mortality and maximizing fitness including competition, drought, disease, and episodic disturbance from herbivory, fire and flooding. Compounding these challenges are changes in climate that are predicted to bring warmer temperatures, increased water deficits, alterations of fire regime, and insect outbreaks, particularly in arid regions over the next century (Seager et al. 2007). For trees, and other perennial plants, mortality is often not caused by a single stressor, but by multiple stressors interacting to reduce resource uptake and metabolic function below a minimum threshold for survival. For example, surges in forest mortality have been attributed to climate-induced stress coupled with insect outbreaks and fire (Kurz et al. 2008; Allen et al. 2010; Carnicer et al. 2011; Anderegg et al. 2015). For many plants, the interaction between chronic drought and episodic disturbance leads to a cumulative reduction in resource supply relative to demand, particularly if already exposed to a long-term stressor such as poor edaphic conditions (Manion 1991; Levanič et al. 2011). Therefore, persistence of individual plants and populations may depend on prior adaptation to stress, acclimation to edaphic and topographic location, as well as exposure to episodic disturbance events.

Regardless of edaphic location, disturbance and life history stage, plants must find ways to optimize carbon uptake and allocation by acquiring limited resources - mainly water, nutrients sunlight - under varying environmental conditions (Bloom et al. 1985). When resources are available above a minimum threshold, plants acquire carbon through photosynthesis and the products of photosynthesis (i.e. sugars) are used to maximize plant fitness - defined here as fecundity multiplied by life span. Maximizing fitness requires sugars to be allocated into a complex suite of carbon sinks, including tissue growth, reproduction, cellular respiration, defense chemistry (i.e. secondary metabolites and phenolics) and the storage of non-structural carbohydrates (NSC) for subsequent utilization. Most fundamentally, osmotically active NSC pools maintain cell turgor and serve as a reservoir to buffer imbalances between carbon supply and demand (Chapin et al. 1990). Recently, particular attention has been given to the role NSC storage plays in overall plant function, fitness and capacity to withstand stress. These storage pools generally increase with plant size, which may serve as a major benefit since larger plants require larger buffers to cope with carbon imbalances in response to stress or episodic disturbance (Hoch et al. 2002; Sala and Hoch 2009; Woodruff and Meinzer 2011). Likewise, NSC pools are critical for vascular function (phloem and xylem) in response to varying environmental conditions. In fact, several studies have documented that NSC pools can play a primary role in maintaining plant water balance and long-distance water transport in the xylem by osmotically repairing xylem conduits following either drought-induced (Bucci et al. 2003; Nardini et al. 2011) or freeze-thaw-induced embolism (Woodruff and Meinzer 2011). Given the importance of NSC pools for both carbon and water balance of long-lived woody plants, maintaining a minimum pool size may be a key trait for surviving resource limitations across various temporal scales.

Traditionally, NSC storage was viewed as a consequence of weakening carbon sinks from reduced growth and respiration near the conclusion of the growing season when there was a surplus of carbon being acquired (Chapin et al. 1990). Recent evidence however, suggests that NSC storage may be highly regulated and is often a competing sink for recently acquired carbon throughout the growing season (Figure 3) (Hoch et al. 2003; Sala et al. 2012). Active regulation of carbon allocation may be coordinated by a complex genetic linkage among traits related to photosynthesis, growth, storage and other carbon movement (Smith and Stitt 2007). Therefore, carbon allocation strategies could be highly variable within and among plant populations, depending on selective pressures from competition, resource limitation and disturbance. For example, if total carbon pools are equal between two plants (i.e. similar rates of total carbon assimilation via photosynthesis), then one plant demonstrating greater tissue synthesis and subsequent growth will do so at the expense of having less photosynthate to allocate to other sinks including NSC storage (Figure 3). In locations where competition for sunlight is high and episodic disturbance is low (e.g. tropical rainforests), natural selection should favor individuals who maximize growth at the expense of NSC storage. Conversely, where competition is low, but disturbance or stress is high, or resource availability fluctuates, natural selection should favor individuals who allocate more of their photosynthate to NSC storage. However, plants that grow in riparian ecosystems, including *Tamarix*, are often exposed to competition, stress and episodic disturbance, requiring a high level of functional diversity in physiological traits. Therefore, riparian plant species will likely express a range of carbon allocation strategies, particularly in locations where there is strong gene flow among populations.

The biocontrol agent, *D. carinulata* and other recently released *Diorhabda* species are exerting intense herbivory pressure on *Tamarix* throughout the southwestern US, resulting in a potentially significant shift in what could be optimal plant carbon allocation. *Tamarix* has evolved under intense pressure from herbivory: in its home range, *Tamarix* is attacked by more than 320 species of insects and mites from 88 genera (Kovalev 1995). This plant-insect co-evolution has likely contributed to a diverse set of strategies to cope with damaging insects. Among these strategies could be one in which *Tamarix* plants reserve relatively large pools of NSC with which to replace damaged tissues following herbivory (Hultine et al. 2015), although these pools can be rapidly depleted following recurrent episodic herbivory (Hudgeons et al. 2007; Figure 3a). As mentioned above, replenishing NSC pools comes at the cost of reduced allocation to other sinks (Figure 3b), especially if canopy-scale photosynthetic capacity is reduced as a consequence of foliage herbivory (Strauss and Agrawal 1999; Pattison et al. 2011b). Herbivore pressure by *Diorhabda* is, nonetheless, a new phenomenon in North America where previously there had been little selection for herbivory tolerance in *Tamarix* plants. This shift in selective pressures could result in a potentially significant change in optimal plant carbon allocation. However, desert riparian settings require physiological traits to best cope with a combination of competition, temperature extremes, salinity and stochastic recruitment opportunities (Table 1). These selective pressures will, at least in part, favor allocation to growth, respiration (growth and maintenance respiration), reproduction and solute synthesis (Box 1), all at the potential expense of reduced allocation to NSC storage. Thus, plants that exhibit allocation to traits associated with success in the most taxing desert riparian settings (e.g. high salinity with extreme temperatures) may be maladapted to deal with herbivory-caused reduced NSC storage compared to individuals growing in low stress sites.

Across the geographic range of *Tamarix,* allocation to NSC storage may be selected for in populations that experience freezing temperatures during the growing season. A recent common garden experiment indicated that *Tamarix* genotypes from higher latitudes, where freezing temperatures during the growing season are common, showed greater tolerance to herbivory by *D. carinulata* (i.e. expressed higher canopy regrowth following herbivory) than lower latitude genotypes (Williams et al. 2014). One explanation for the observed pattern is that high-latitude genotypes maintained higher NSC storage due to a reduction in sink strength as plant growth ceases at the conclusion of the growing season (Hoch et al. 2002). In other words, NSC pools could increase as a consequence of imbalances between carbon supply and demand as growth and respiration decline (Chapin et al. 1990; Sala et al. 2012). Alternatively, high-latitude genotypes may have been selected to actively maintain high NSC pools. The same common garden experiment revealed evidence for natural selection and subsequent adaptation to local environment conditions as high-latitude genotypes expressed earlier leaf senescence, lower biomass production and higher root-to-shoot ratios compared to genotypes from lower latitudes which exhibited increased aboveground biomass accumulation (Friedman et al. 2011; Williams et al. 2014). The latter trait is important because the largest NSC pool is presumably stored belowground (Canham et al. 1999). Given that high-latitude populations experience occasional canopy dieback associated with freezing temperatures during the growing season, natural selection may favor genotypes that allocate more biomass to belowground NSC storage presumably for rebuilding frost-damaged tissues. As a by-product of selection to cope with low temperatures, high latitude genotypes may be better adapted to tolerate episodic disturbance. The relative strength of carbon allocation tradeoffs within and among *Tamarix* populations is an open question, but recent evidence suggests that these tradeoffs might be profound. Measurements of radial growth from annual tree rings showed that surviving trees within populations grew slower in years prior to the arrival of *D. carinulata* beetles compared to trees that ultimately succumbed to repeated herbivory events (Hultine et al. 2013). These patterns were presumably a function of surviving trees allocating a higher proportion of their carbon pool to NSC storage at the expense of slower annual growth rates (Hultine et al.

2013). If plants selected for faster growth are killed by beetles at a higher rate than slower growing plants, then population mean productivity of individuals may decline in response to episodic herbivory. This pattern of directional selection could in turn, impact population fitness by altering competitive interactions with other species. This is particularly true given evidence that *Tamarix* already has lower growth rates relative to native riparian tree species *Salix* spp. and *Populus* spp. at germination (Sher and Marshall 2003).

Implications for conservation and future research directions

This review highlights three main points. The first is that despite its recent introduction into North America, *Tamarix* expresses several functional trait variations consistent with adaptation to regional climate conditions, which may be in part a result of extensive hybridization. This result is consistent with previous studies showing local adaptation occurs in invasive plant species (Liao et al. 2016; Oduor et al. 2016). The second is that *Tamarix* genotypes adapted to warmer climates may be maladapted to the effects of episodic herbivory due to allocating a larger proportion of their total carbon pool to growth and respiration at the expense of lower overall storage of nonstructural carbohydrates. Finally, both of these patterns suggest that the combination of herbivory and increased aridity driven by climate change could synergistically reduce growth, fitness and overall dominance of *Tamarix* (Figure 4). As the distribution of *D. carinulata* and related species continue to expand their range into warmer and more arid regions in North America, rates of *Tamarix* mortality will likely increase compared to current mortality rates in more northern or higher elevation locations. Likewise, *Tamarix* growing under conditions of high soil and / or groundwater salinity may be especially sensitive to episodic herbivory. Plants growing in high salinity will not only be exposed to chronic stress from lower soil water potentials, but will potentially require greater allocation of carbon to solute synthesis in order to osmotically facilitate salt exudation from leaf tissues (Box 1). To better understand how the combination of climate and episodic disturbance (i.e. herbivory) will impact *Tamarix* plants in the southwestern US, we suggest that future research projects take place on four inter-related thrusts: 1) the construction and maintenance of common gardens, such as the one described earlier and currently in operation in Yuma, Arizona; 2) expanded efforts to quantify plant carbon budgets over time, particularly better seasonal accounting of changes in whole-plant NSC pools across genotypes and populations; 3) genomics and epigenetics research to link phenotypic traits such as resource allocation, tolerance to salinity and drought to specific molecular markers and gene expression, and; 4) ground and remote-sensing-based monitoring of *Diorhabda* spp. distributions and subsequent impact on *Tamarix* fitness, recruitment and survival.

Information acquired from current research activities, and research approaches advocated here would provide critical information for targeted restoration and conservation of valued riparian ecosystems. Data on plant genetics and phenotypic trait expression could potentially guide restoration ecologists and land managers on how to best use limited resources for riparian restoration by improving predictive ability based on phenotype/genotype screening on when and where climate change by defoliation interactions will most impact *Tamarix*. These projects could target a wide range of specific objectives depending on the projected impacts of foliage herbivory on *Tamarix* including fire prevention, native plant restoration and habitat restoration. For example, *Tamarix* populations that are predicted to experience the highest levels of mortality and canopy dieback could be areas that are given the highest priority for restoration efforts. Knowing these patterns may be particularly important for guiding habitat restoration of threatened and endangered avian species such as the southwestern willow flycatcher (*Empidomax traillii extimus*) that under some circumstances, rely on *Tamarix* canopies for nesting (Bateman et al. 2010). It is currently unclear to what extent biological control of *Tamarix* will impact riparian habitat for *E. trallii extimus*. However, the combination of common garden studies of plant resource allocation and stress tolerance with technological advances in molecular genomics will provide critical information on how *Tamarix* populations will likely respond to multiple stressors going into the future.

Box 1 – Mechanisms and costs of salinity tolerance in *Tamarix*

Halophytes, such as *Tamarix* spp. are defined as plants that have a high tolerance to salt. Generally speaking, there are three distinct ways that plants tolerate high salt concentrations (Munns and Tester 2008): 1) tolerance to osmotic stress; 2) salt exclusion by plant roots so that salts do not accumulate in the leaves; and 3) compartmentalization of salts to avoid toxic concentrations in the cytoplasm, especiall;y the cytoplasm of mesophyll cells in the leaf. Compared to most riparian plants, *Tamarix* has a fairly high tolerance to osmotic stress (Cui et al. 2010; Ding et al. 2010) and therefore can maintain gas exchange at lower leaf water potentials than most co-occurring species such as willows (*Salix* spp.) or cottonwoods (*Populus* spp.) (Pockman and Sperry 2000; Hultine and Bush 2011). More importantly, *Tamarix* avoids long-term salt toxicity in leaf tissues by compartmentalizing and excreting salts through specialized salt glands (Storey and Thomson 1994; Glenn et al. 2012). Compartmentalization takes place by synthesizing compatible organic solutes, such as sucrose and other compounds, at high enough concentrations in the cytosol and organelles of leaf cells to balance the osmotic pressure of ions in the cell vacuole (Flowers 1977; Munns and Tester 2008). However, compatible solute synthesis and subsequent removal of salts once they have entered the leaf comes with a considerable metabolic cost resulting in a potential reduction in carbon allocation to other sinks (Figure 3). To be more precise, about seven moles of ATP are required to accumulate one mole of Na^+ as an osmoticum, whereas it takes approximately 52 moles of ATP to synthesize one mole of sucrose (Raven 1985). The high metabolic cost of solute synthesis may allow plants to survive the presence of high external concentrations of salt, but at the expense of higher vulnerability to other stressors. For example, *Tamarix* dieback and mortality in response to episodic herbivory by *D.*

carinulata increased along a soil salinity gradient in a Mojave Desert river watershed (Hultine et al. 2015). Therefore, soil salinity may exert a combination of high osmotic stress (resulting in lower net photosynthesis) and increased metabolic costs (resulting in lower carbon allocation to NSC storage) that may synergistically predispose *Tamarix* to the negative effects of episodic foliage herbivory and other stressors.

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Figure Captions

Figure 1 – The correlation of percent green foliage versus population source elevation for *Tamarix* branches in January 2016 at a common garden in Yuma, AZ. Eighteen populations were represented at the common garden, collected across an elevation gradient from Arizona and Southern Utah. Populations originating from sites of lower elevation and correspondingly higher minimum winter temperature showed earlier leaf out relative to high elevation populations

Figure 2 – A comparison of mean ± SE net photosynthesis (A_{net}, µmol CO_2 m^{-2} s^{-1}; upper panel) and mean ± SE stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}; lower panel) of four populations at a progeny study of *Tamarix* in Yuma, AZ. The common garden is located at an elevation of 56 m. Populations that were from nearby sites exhibited higher rates of photosynthesis and conductance compared to populations that were from higher elevations, showing some evidence for local adaptation based on differences in expressed traits in local and foreign populations.

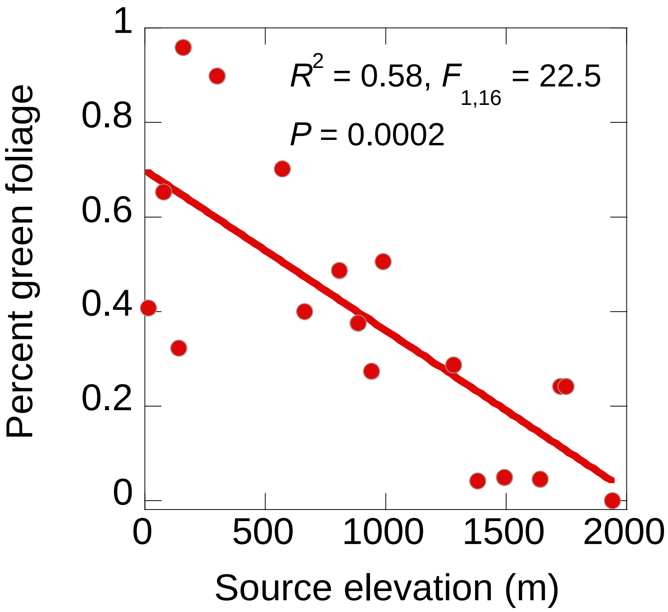
Figure 3 – Figure 3a. A conceptualized carbon budget of woody plants showing the source of carbon coming from photosynthesis and the major carbon sinks. The conceptual diagram highlights non-structural carbohydrate storage (NSC; purple box on the left) as a competing sink with all other sinks (shown on the right) including tissue growth, respiration (mitochondrial plus photo-respiration), reproduction, defense and solute synthesis. In *Tamarix* plants the various carbon sinks that compete with NSC storage can be strong at different periods of the year given its typical environmental niche, trait expression and herbivory pressure. Figure 3b (Redrawn from Hudgeons *et al*., 2007): Mean percentage of NSC storage measured in the root crown of mature *Tamarix* trees in northern Nevada. The trees had been exposed to a range of 0 to 4 years of episodic herbivory by the northern *Tamarix* leaf beetle (*Diorhabda carinulata*). Error bars represent the standard error of the means. Figure 3c (Redrawn from Hultine *et al*., 2015b): The seasonal pattern of NSC concentrations in the twigs of mature *Tamarix* trees occurring in southeastern Utah (n = 20 trees). The patterns show a reduction in NSC storage during the growing season, revealing competing sinks between growth and storage as shown in the schematic on the left of Figure 3.

Figure 4 – The predicted relationship between growing season aridity (i.e. vapor pressure deficit) above the annual mean and the productivity and fitness of *Tamarix* genotypes with and without the presence of the northern *Tamarix* leaf beetle (*Diorhabda carinulata*).

Table 1 - Common environmental conditions *Tamarix* plants face during their life history and the carbon allocation strategies required to maximize fitness and survival under specific conditions.

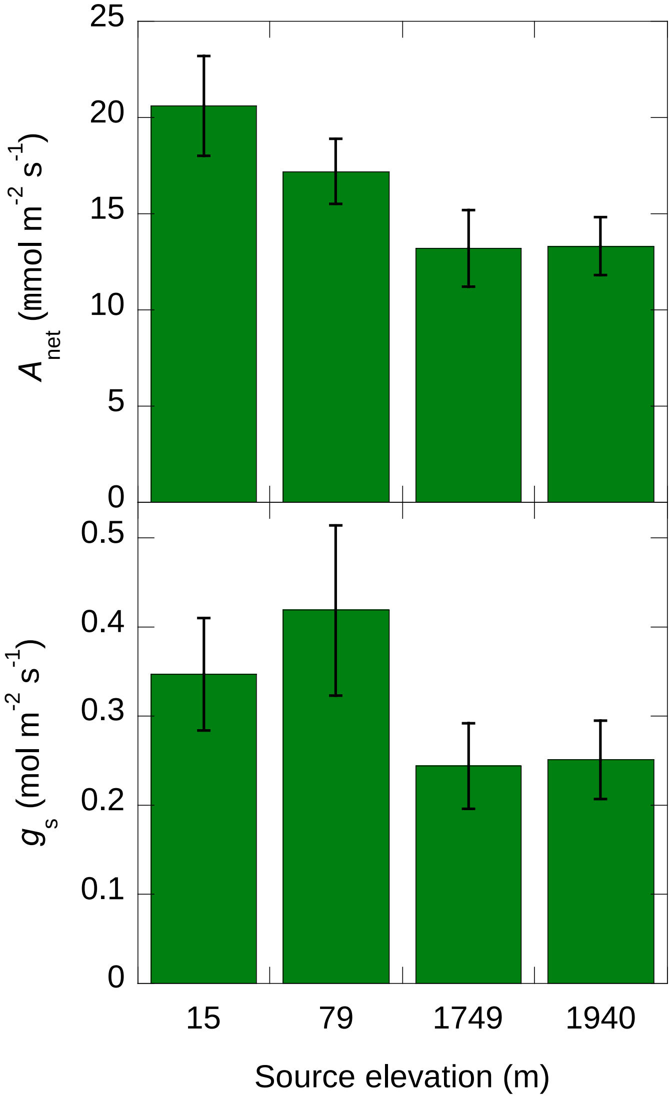
Environmental condition	Carbon cost
Insect / pathogen infestation	Resistance from defensive chemistry / secondary metabolite production
High soil / groundwater salinity	Solute synthesis to osmotically exude salts from leaves
Increasing depth to groundwater	Tissue construction for rapid root growth
Intra - Inter specific competition for sunlight	Tissue construction for rapid canopy growth rates
High growing season temperature	High mitochondrial respiration rates
Stochastic recruitment opportunities	Continuous flower and seed production to cope with unpredictable soil moisture conditions
Potential growing season freezing events	High NSC concentrations for tissue growth following frost-induced dieback
High fire frequency	High NSC concentrations for resprouting following episodic fire
High episodic foliage herbivory	High NSC concentrations to construct new foliage following herbivory events

Figure 1



1
0.8
0.6
0.4
0.2
0
0
500
1000
1500
2000
Percent green foliage
Source elevation (m)
R^2 = 0.58, $F_{1,16}$ = 22.5
P = 0.0002

Figure 2



25
20
15
10
5
0
A_{net} (mmol m^{-2} s^{-1})
0.5
0.4
0.3
0.2
0.1
0
g_s (mol m^{-2} s^{-1})
15
79
1749
1940
Source elevation (m)

Figure 3

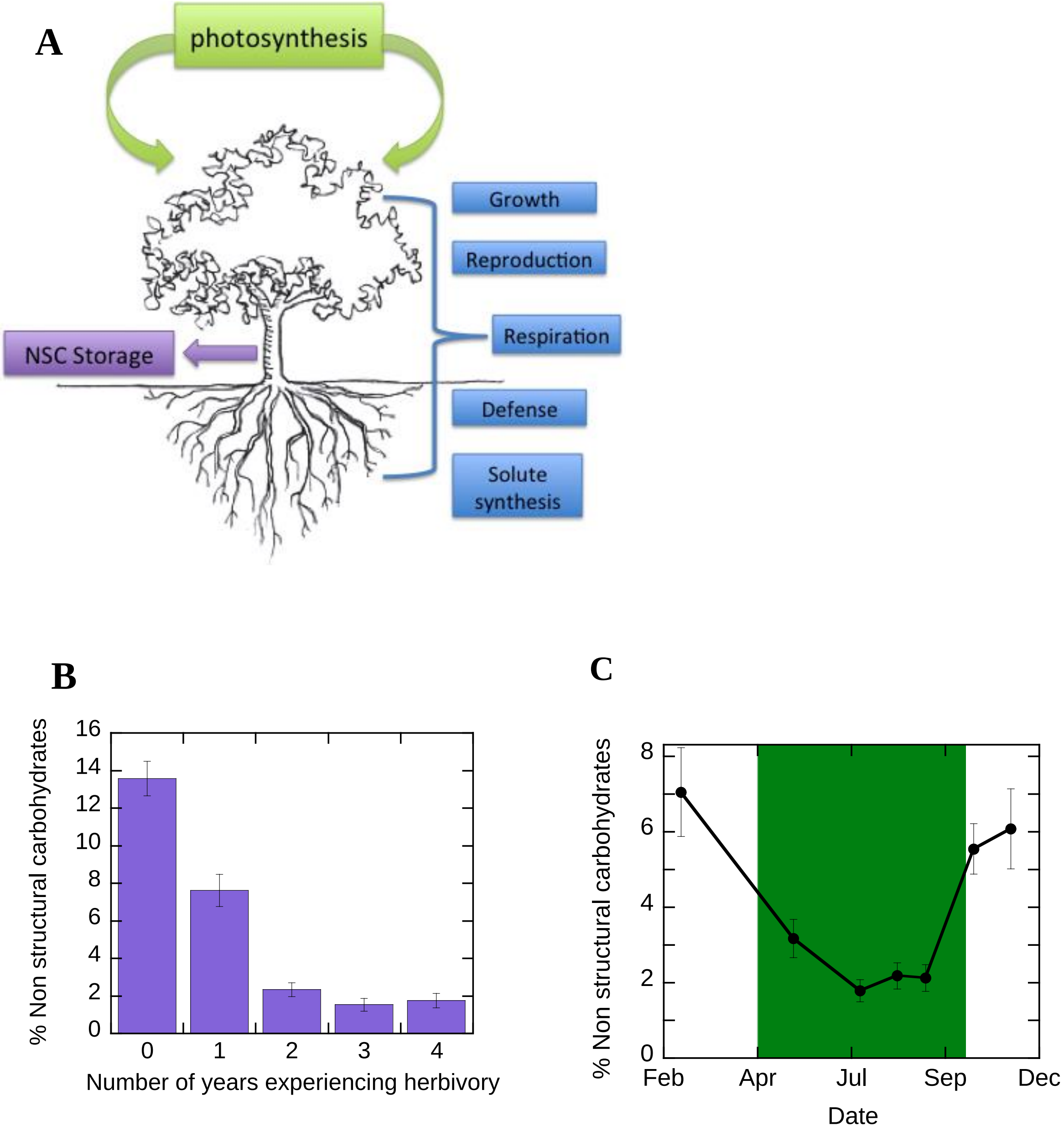
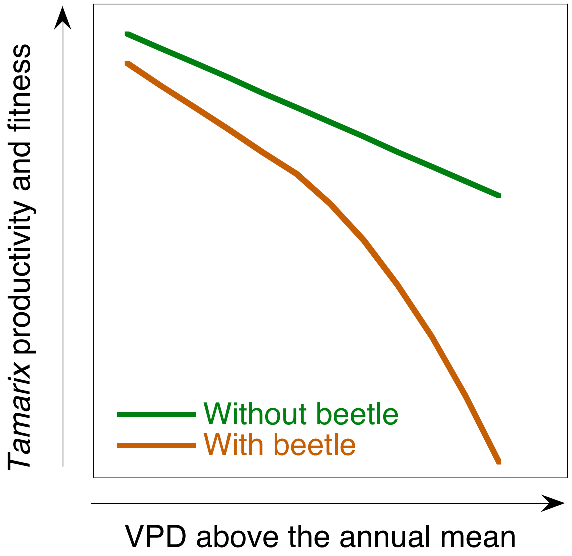

A
photosynthesis
Growth
Reproduction
Respiration
Defense
Solute synthesis
NSC Storage
B
% Non structural carbohydrates
16
14
12
10
8
6
4
2
0
0
1
2
3
4
Number of years experiencing herbivory
C
% Non structural carbohydrates
8
6
4
2
0
Feb
Apr
Jul
Sep
Dec
Date

Figure 4



Tamarix productivity and fitness
VPD above the annual mean
Without beetle
With beetle

II. Spenders versus savers: climate-induced carbon allocation tradeoffs in a recently introduced woody plant

Summary

1) Non-structural carbohydrate (NSC) storage may be under strong selection in woody plants that occur across environmental gradients. We, therefore investigated whether carbon allocation strategies vary in a widely distributed, introduced woody plant due to exposure / non-exposure to freezing temperatures. We predicted genotypes from cold climates would have elevated NSC concentrations but with the tradeoff of reduced growth and reproduction relative to warm-adapted genotypes.
2) We established an experimental common garden using genotypes of *Tamarix spp.*, sourced across a broad thermal gradient. We measured seasonal NSC storage patterns in coarse roots and stems, flower production and above-ground growth.
3) NSC concentrations were 50% higher in genotypes from sites with spring freeze events compared to genotypes from warmer sites. Cold-adapted genotypes also had a 2.3-fold higher starch to soluble sugar ratio than warm-adapted genotypes. Conversely, warm-adapted genotypes had significantly higher growth and reproduction than cold-adapted genotypes.
4) Results suggest that *Tamarix* from colder locations cope with spring freeze events by maintaining large storage pools to support tissue regrowth, but with the tradeoff of reduced growth and reproduction. Our results provide strong evidence of rapid selection in carbon allocation strategies in response to climate in recently introduced woody species.

Keywords: Rapid evolution, *Tamarix chinensis X ramosissima* (Saltcedar), non-structural carbohydrates, frost tolerance, plant starch concentrations, experimental common garden

Introduction

Plant economic theory predicts that there should be strong selection for plants to maximize individual fitness by allocating limited resources to competing pools of growth, reproduction, and storage (Bloom et al. 1985; Hinman and Fridley 2018). If so, introduced plant populations that are exposed to frequent and predictable resource limitations should be more likely to exhibit a bet hedging strategy by actively allocating a higher percentage of their overall carbon gain (C) to non-structural carbohydrate (NSC) storage, rather than competing pools, relative to populations that are exposed to less frequent resource limitation (Iwasa and Kubo 1997; Long et al. 2017). Cyclical disturbances that remove live biomass are likely to result in intraspecific variation in C allocation strategies as reserves in perennial woody plant species can act as a 'bank account' available for recovery/compensation (Kozlowski 1992; Hudgeons et al. 2007; Clarke et al. 2013). Common garden experiments using populations sourced across broad environmental gradients can be used to evaluate patterns of local adaptation, and in some cases rapid evolution of recently introduced species that result in variable C allocation patterns in response to climate.

Populations exposed to late-winter or spring cold temperatures are at risk from two different, potentially lethal, disturbance phenomena. The first risk is cell death in newly grown tissues due to near freezing temperatures shortly after leaf flush, and the second is the risk of freeze-thaw cavitation in xylem tissue (Sperry and Sullivan 1992; Pockman and Sperry 1997; Rodrigo 2000). These stressors should select for genotypes that are active C savers, rather than spenders. A high percentage of photosynthate allocated to C storage relative to other C pools could be highly adaptive because 1) plants can mobilize NSC from storage pools in roots or woody stems for regrowth following dieback of sensitive aboveground tissues (Clarke et al. 2013), 2) soluble sugars in xylem sap reduce the risk of cavitation by lowering the freezing point (Regier et al. 2009) and 3) NSC could be used in reversing cavitation from freeze-thaw events through phloem loading of sugars or for growth of new xylem after cavitation (Nardini et al. 2011; Secchi and Zwieniecki 2012; Lintunen et al. 2016). Conversely, populations from more arid sites where freeze events are rare or absent could accumulate NSC, specifically as soluble sugars to maintain high osmotic pressure to prevent turgor loss during drought (Bartlett et al. 2014; O'Brien et al. 2014).

Regardless of potential constraint on carbon uptake, increased C allocation to labile storage should result in fitness tradeoffs because C that is stored is not readily available for other immediate uses such as growth, reproduction, or defense (Baker 1974; Bloom et al. 1985; Chapin et al. 1990; Long et al. 2017). There is however, some debate as to whether plants actively allocate C to storage for later use or if accumulation of NSC is a passive process (Bloom et al. 1985; Chapin et al. 1990; Hoch et al. 2002; Korner 2003; Hartmann and Trumbore 2016). Recent reviews and syntheses have indicated that the regulation of C storage processes is not well understood (Sala et al. 2012; Furze et al. 2018; Hartmann et al. 2018), but some studies have categorized species as either carbon 'savers' or 'spenders' (Hinman and Fridley 2018). 'Spender' species are those exhibiting higher than average growth and/or reproduction and lower total NSC concentrations, while 'savers' preferentially allocate carbon to storage (NSC) at the expense of the other uses. Studies on the variation in C allocation strategies among perennial populations within individual species are rare (but see Oleksyn et al. 2000) potentially due to the logistical difficulties and high expense of establishing trials for long lived woody plant taxa (Wiley and Helliker 2012).

The *Tamarix* species complex in North America, comprised of five species and their hybrids, has spread rapidly since its introduction in the 1800s and now occupies a wide range of environments throughout riverine systems and wetlands in the American west. This invasion is predominately by *T. chinensis* and *T. ramosissima*, their F1 hybrids and subsequent backcrosses (Gaskin and Schaal 2002). Hybrid individuals dominate low elevation desert riparian environments that experience extreme mid-summer heat and aridity as well as high elevation riparian areas where populations are exposed to harsh winters and late-spring freeze events (Friedman et al. 2008). This broad climate distribution provides a compelling model system for studying evolution in C allocation strategies across environmental gradients, since many recently introduced plant species with large geographic extents often exhibit high local genetic variation and/or phenotypic plasticity (Liao et al. 2016; Oduor et al. 2016). Given the large variation in climate conditions across the range of *Tamarix*, we established a common garden experiment to test for interpopulation variation related to C allocation strategies in the *T. chinensis* X *T. ramosissima* hybrid complex (hereafter referred to as *Tamarix*). Heritable variation in plant traits that vary predictably across an environmental gradient would provide strong evidence of rapid evolution in this dominate, widely distributed taxon.

In this study, we addressed the following questions: (1) is there evidence for interpopulation variation in NSC storage? (2) does this variation among populations correlate with tradeoffs in allocation to growth or reproduction? and (3) is this variation related to climatic conditions? We tested the hypothesis that labile carbon storage would increase with environmental stress potential (freezing or thermal stress) of source populations, but with the tradeoff of reduced allocation to growth and reproduction. Our basic hypothesis gives rise to three inter-related predictions: 1) strong tradeoffs will be detected in C allocation strategies such that plants with high above ground growth rates and reproductive yields (i.e. flower production) will consequently have seasonally lower concentrations of NSC, 2) genotypes sourced from cold climates that are regularly exposed to early growing season freeze events will display higher starch storage in their roots for tissue construction following episodic frost dieback, and 3) genotypes from sites with extreme aridity (low elevations) will allocate a higher percentage of their labile carbon pool to soluble sugar, as opposed to osmotically neutral starch molecules, to maintain cell turgor through osmotic regulation during extreme heat waves. Results from this study provide insight into intraspecific variation in C allocation strategies that can rapidly evolve in long-lived woody plants in response to climatic stress.

Materials and Methods

Common garden site and provenances

A *Tamarix* common garden was established April 2015 in Yuma, AZ (lat. 32.6151°N, long. -114.6365°W, elev. 60 m) at the University of Arizona Mesa Facility, near its most southern and arid distribution. The site is a former agricultural field that was fallow for several years prior to the study. The field was tilled and levelled prior to planting to facilitate evenly applied flood irrigation. Soils are classified by the National Resource Conservation Service as superstition sand comprised of approximately 95% sand, and 5% clay. The climate is typically hot, with dry summers interrupted by rare monsoonal precipitation, and cool wetter winters. Climate means for the garden, as well as population source (hereafter, provenance sites) locations, are provided in Table 1. *Tamarix* cuttings were collected from provenance sites throughout Arizona and SE Utah (Fig 1). Sites were selected to represent an elevational gradient (45 – 1791 m), which was a proxy for both minimum (range: -6.4 - 6.3 °C), and maximum temperatures (range; 32.4 - 42.4 °C; Table 1). Precipitation at the collection sites varied from 90 mm to 449 mm (Table 1). However, because *Tamarix* is associated with riverine areas and wetlands in the western US, all of the collected genotypes were located within riparian terraces and presumably had continuous access to groundwater.

We collected cuttings, each approximately 15-20 cm long and 2-4 cm in diameter, from 16 individuals from each of nine populations in fall 2014. Cuttings were treated with root hormone and grown in a vermiculite/perlite potting mix in 328 cm^3 pots at the Northern Arizona University greenhouse facility in Flagstaff, Arizona prior to out-planting. The potted individuals were planted at the University of Arizona Mesa Farm in Yuma, Arizona in April of 2015. Populations were planted in a randomized block design, with each population being represented once in eight blocks for a total of eight replications. For each population replication, each of the 16 individuals were assigned a random location in a 4 x 4 planting arrangement with 2 m spacing between plants.

These populations hereafter P_g (garden populations), were selected to represent an elevation / climate gradient, with three populations each from low, mid and high elevations. We believe this represented a testable range for measuring foliage and flowering phenology, labile C storage, and biomass accumulation. A subset, hereafter P_f (focal populations), included three populations that were selected from the P_g for more intensive monitoring, including one population each from a high-, mid- and low-elevation site: the latter being from the nearest provenance site to the experimental garden.

Carbon saving: non-structural carbohydrates

We sampled stem and root tissues to assess seasonal variation in NSC concentrations across populations. Collection dates for P_f were May 26th (Spring), July 22nd (Summer) and October 21st (Fall) in 2016 and February 26th (Winter) in 2017. P_g were sampled on May 26th and October 21st, 2016. Temperature ranges for the collection dates were as follows: Spring 16-31°C, Summer 31-45°C, Fall 21-36°C, Winter 12-21°C. Between these collections periods, maximum temperature at the garden location reached 48.1 °C on July 4th, 2016, and a low of 4.0 °C on January 26th, 2017, while vapor pressure deficit reached a maximum of 10.8 kPa on July 4th, 2016 (Supplemental Fig 1). Mature second year stem samples (7-12 mm diam) were sampled from breast height (1.37 m above soil surface) and coarse root tissue samples (5-10 mm diam), from a soil depth range of 10-15 cm. Six genotypes were randomly selected for NSC analysis and were sampled from three of the blocks (n=18 for each population). For the NSC analysis, we used the methods described as best practices in Quentin *et al.* (2015). Samples were placed on dry ice immediately after excising from the tree and microwaved within 24 hours to prevent enzymatic degradation. Samples were then oven dried at 60 °C for 48 hours and kept in a freezer (-20 °C) until they were prepared for extractions. Samples were first coarse ground with a #20 mesh Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ), and then fine ground with a dental amalgamator (Wig-L-Bug, Dentsply Rinn, Charlotte, NC). A sub-sample of 50 mg (± 1.5 mg) was used for extractions with the final weight recorded. All water-soluble sugars (low molecular weight sugars) were extracted using 80% ethanol in an 85 °C water bath. After being centrifuged, the supernatant was removed and saved for later analysis; this process was repeated three times. Total water-soluble sugars were quantified using a phenol- sulfuric acid reaction to determine the relative sugar concentration (Chow and Landhäusser 2004). Any remaining ethanol was evaporated and an enzymatic digestion using Alpha amylase and amyglucosidase was used to degrade starches into glucose. The percent glucose was quantified using the PGO enzyme method at 490 nm (Chow and Landhäusser 2004). The percent glucose by biomass was equivalent to the starch concentration in the sample.

Relative risk of freeze-thaw events

We used linear regressions to analyze the risk of freeze events as predictors of carbon allocation. We defined freeze events as any day between January 1st and June 1st when the minimum temperature was below 0 °C and the maximum temperature was above 0 °C. To determine the number of events where temperatures would rise above, and then drop below the freezing point, we interpolated daily maximum and minimum temperatures from 1992-2012 for all P_g sites using data from the PRISM Climate Group, Oregon State University (http://prism.oregonstate.edu, created 10 Jan 2018). The freeze-thaw risk of each site was calculated as the proportion of total freeze events at a given site divided by the mean number of events at a site with the highest number (42.2 events).

Carbon spending: reproduction and growth

Flower phenology and output

Tamarix has small (<2 mm) flowers that are borne on secondary racemes, each supporting an average of 50-60 flowers (Andersen and Nelson 2013). Tamarix plants may support as many as 550,000 flowers, each producing small amounts of sucrose rich nectar to attract pollinators ((Andersen and Nelson 2013). Bimonthly phenological observations were made From February 2016 until June 2016 on 12 individual plants from three different blocks from each of the P_g. Only twelve individuals were used of the original 16 due to some mortality during planting that reduced the total replication for those genotypes, and three of the eight blocks were selected randomly. Reproductive output was evaluated by estimating the number of flowering racemes on each individual during each measurement period. Measurements were calibrated during each sampling event for each population by estimating the area that would represent one hundred racemes and then counting each raceme within that area for six different individuals. If there was a discrepancy of greater than ± 10% between the estimation and actual number of racemes then the area was recalibrated.

Basal area and canopy volume

The basal area on six individuals from the P_g was measured in three different blocks (n = 18 for each population) to determine mean growth increments of each population during the growing season. Initial measurements were taken in late spring (May 25th 2016) and again in the fall (October 25th 2016). All stem diameters were measured with a caliper at 10 cm above the soil surface to determine the total basal area (A_b) of the trees. Due to irregular stem shapes, two measurements were taken at orthogonal directions from each other and area was calculated as an oval where r is stem radius:

$$A_b = \frac{\pi r_1^2 + \pi r_2^2}{2} \tag{1}$$

Three representative stems were selected for repeat measurements and marked with paint pens at ten cm above the ground surface so that they could be re-measured at the same points. Mean basal area increment was calculated for each population from the measurements at each of the three stems. Basal area increments (BAI; mm d^{-1}) were calculated according to (Lambers et al. 2008) where A_{bf} is the final measured basal area, A_{bi} is the initial basal area and *d* is the number of days between A_{bf} and A_{bi}:

$$BAI = \frac{A_{bf} - A_{bi}}{d} \qquad (2)$$

Canopy volume was measured on May 25th, 2016 for the P_g by measuring the width of the trees at their widest point and the corresponding orthogonal width. The canopy volume (Vol_{can}; m^3) was calculated using the radii of two widths (W_1, W_2) and half the max height (H) of the tree and the formula for the volume of ellipse

$$Vol_{can} = \frac{(4\pi)(H)(W_1)(W_2)}{3} \qquad (3)$$

Statistical Analyses

Correlations were analyzed using linear regressions, and all comparisons were done with one-way ANOVA and post hoc analysis were completed using Tukey's Honest Significant Differences using the 'stat' package in R (R Core Team 2017). The spring phenology was analyzed using a repeated measures MANOVA using the 'nlme' package in R (Pinheiro J et al. 2017) to account for measurements completed on the same individuals during the sampling period. Version 1.1.423 of RStudio was used for all analyses.

Tradeoff modeling

Additionally, to aggregate the two different components of spending we used the scale function in the 'stats' package (R Core Team 2017) to determine z scores of individual plants compared to the garden mean for reproduction (total racemes), growth (canopy volume), and storage (total NSC in fall stems). Z scores of reproduction and growth were added together to determine an aggregate spending score.

Results

Carbon saving

Among the three P_f, NSC levels were dynamic throughout the year, but there were significant differences among populations (ANOVA $F_{6, 104} = 5.221$, $p < 0.001$, Fig 2), and a post hoc analysis revealed that the high elevation population had greater concentrations of NSC than the low elevation population during the fall ($p < 0.0001$) and winter ($p = 0.0095$) sampling periods. In the high and mid elevation populations NSC were more abundant in root tissue than in stems throughout the year, and high elevation fall root concentrations (176 ± 6.35 mg g^{-1}) were nearly twice as high as those found in low elevation fall roots (90.14 ± 11.49 mg g^{-1}).

The highest risk site based on the PRISM climate data had an average of 42.2 freeze-thaw days between January and June when the minimum temperature dropped below 0 °C at night after being above 0 °C during the day. For all the populations measured we observed that the risk of freeze events at the provenance site was positively correlated with increased NSC storage (Fig 3), both as total NSC (Total NSC = 103.64 + 24.78*relative risk, $r^2 = 0.48$, $F_{1,7} = 6.50$, $p = 0.038$) and starch (Total starch = 64.75 + 21.47*relative risk, $r^2 = 0.61$, $F_{1,7} = 10.75$, $p = 0.014$). None of the low elevation sites were at risk of exposure to freeze-thaw events, while mid elevations had a low risk (0.07 - 0.19) and high elevation sites had a high risk (0.64 -1.00).

Ratios of water-soluble sugars to starches in the fall were also found to differ across the three P_f (ANOVA $F_{2,80} = 3.85$, $p = 0.025$, Fig 4). The high elevation population had a significantly higher ratio of starch to soluble sugars in both roots and stems in the fall compared to the low elevation population (Tukey's HSD $p < 0.0001$ and $p = 0.022$

respectively). However, the mid elevation population was intermediate between the high and low elevation populations in both roots and stems, with no significant differences detected (Fig 4).

Carbon spending

Flowering phenology and output

Flowering differed considerably over the spring among P_g source elevations ($F_{18, 135}$ = 3.8, $p < 0.0001$, Fig 5a). Most notably, the average peak number of racemes of the low elevation populations was substantially higher than the other elevation groups ($p < 0.0001$) but not between mid and high elevations ($p = > 0.99$). Peak flowering dates were March 5th, April 2nd, and March 19th respectively for low, mid and high elevations. At their respective peak flowering dates low elevation populations (922 ± 175) had more than four times the number of racemes than the high elevation populations (200 ± 30.7). There was also a significant difference between elevation groups in total racemes produced per individual over the full monitoring period from February 4th to June 11th, 2016 (ANOVA $F_{2, 356}$ = 22.04, $p < 0.001$). Unlike NSC accumulation, there was no significant relationship between number of racemes produced and the risk of freeze events at the provenance site (Fig 5b; number of racemes = 201 – relative risk*201, $r^2 = 0.12$, $F_{1,7} = 0.97$, $p = 0.36$).

Basal area and Canopy Volume

Although there was a negative trend with some growth measurements and risk of exposure to freeze events, none was found to be a significant predictor among populations (Fig 6). Specifically, there was a non-significant relationship between risk of freeze events and both spring basal area (total basal area = 0.39 m^2 – relative risk*0.0064 m^2, $F_{1,7} = 1.18$,

$r^2 = 0.14$, $p = 0.31$) and canopy volume (canopy volume = 6.6 m^3 – relative risk*1.47 m^3. $r^2 = 0.14$, $F_{1,7} = 1.097$, $p = 0.33$). Basal area increments (BAI) also showed no relationship with relative risk (BAI = 0.00077 cm^2 cm^{-2} day^{-1} - relative risk * 0.00024. $r^2 = 0.041$, $F_{1,7} = 0.3$, $p = 0.60$) (Fig 6).

Tradeoffs in carbon allocation

To visualize the tradeoffs between saving and spending of carbon we developed a comparison of normalized scores encompassing growth, reproduction, and labile carbon storage. Although our method of using normalized scores does not consider the relative carbon costs of the different pools it allowed us to evaluate how allocation was prioritized by comparing individual scores to the average values of all the populations. Since we only studied *Tamarix*, the carbon costs of specific tissues (e.g. flowers) should be similar across all genotypes. The strategy exhibited by the different populations can be described by which quadrant they occupy in a cartesian plane (Fig 7). Populations occupying quadrant II are defined as spenders since they have positive spending scores, and negative saver scores; while those in quadrant IV are classified as savers since they exhibit the opposite scores. Populations in quadrant III could still be considered spenders since they had less than average saver scores, assuming there is some spender category that we did not account for in our analysis [e.g. herbivore defense (Herms and Mattson, Jr. 1992)]. Quadrant I could only be occupied by populations that had higher than average spending and saving rates, none of our population means existed in this space, but a few individual genotypes did. In our study system, high elevation populations all occupied the saver space of the coordinate plane, while mid and low populations were found in spender quadrants.

Discussion

We found evidence for rapid evolution of carbon allocation strategies in an invasive tree/shrub in response to a broad temperature gradient. Using a common garden design, we detected considerable differences among genotypes in allocation to labile carbon storage in the form of non-structural carbohydrates that can be predicted by source population's exposure to freeze risk. Specifically, populations from colder environments allocated more carbon to storage, but with the potential cost of reduced growth and reproductive output. The variation in the degree of 'bet-hedging', allocating carbon to storage instead of growth or reproduction, may explain the wide-spread success of *Tamarix*, and other woody deciduous species, across broad climactic gradients as populations adapt to local stressors (Gleason and Ares 2004; Alexander 2013). More broadly, these results shed new light on the importance of active NSC accumulation in long-lived woody taxa.

Carbon saving: non-structural carbohydrates

Tamarix genotypes showed fall accumulation of NSC that is consistent with patterns found in many winter deciduous species that accumulate or translocate carbohydrates in storage organs prior to leaf fall (Hoch et al. 2003; Hultine et al. 2015; Martinez-Vilalta et al. 2016; Furze et al. 2018). For all populations, we observed at our common garden site that the risk of freeze events at the provenance site was positively correlated with increased NSC storage in the roots in the fall (Fig 3). While we did not investigate the specific adaptive response of increased NSC (e.g. regrowth, tissue repair, or osmotic regulation), higher storage may decrease mortality from disturbance regimes and/or facilitate rapid regrowth following tissue damage (Dietze et al. 2014; Long et al. 2017). Although it is unclear which response or mechanism may be driving selection in our study system, the differences in the ratio of starch to simple sugars across the provenance sites may highlight the different adaptive uses of NSC for regrowth versus osmotic regulation in relation to seasonal temperature variations. High and medium risk sites had a higher starch to sugar ratio in roots when compared to low risk sites (Fig 4). This was consistent with our prediction that genotypes from cold environments would maintain higher NSC concentrations, primarily as starch. These patterns indicate that cold-adapted plants are actively storing carbon, Increased carbon storage in the roots is an adaptive response to cold or other related canopy dieback events (Hudgeons et al. 2007; Charrier and Ameglio 2011; Clarke et al. 2013; Pratt et al. 2014). Likewise, populations of *Tamarix* from colder northern latitudes maintain higher root to shoot ratios than those from southern locations (Sexton et al. 2002; Williams et al. 2014), potentially amplifying the already considerable per-dry mass differences in NSC concentrations. Another study using the same genotypes (P_f) from the common garden in this study found that high elevation plants also allocated more biomass to roots than low elevation populations (Bush et al. unpublished data).

Although reduced growth in the fall with continued carbon sequestration can result in higher NSC concentrations in deciduous trees, the concentrations were not significantly different between summer and fall sampling in the high elevation population; which we would expect to see if cooler fall temperatures were limiting growth. Greater storage of NSC in the stems as well as roots could be adaptive in high-elevation genotypes to minimize freeze-thaw cavitation events, despite the risk of loss of stored NSC due to stem breakage during the winter. The localized storage of NSC could provide the osmotic potentials necessary to repair embolism, specifically when starch stored in xylem associated tissue (parenchyma or phloem) is degraded to simple sugars and loaded into the embolized xylem

(Brodersen et al. 2010; Nardini et al. 2011; Hartmann and Trumbore 2016). As predicted, we detected lower ratios of starch to sugars at our common garden in genotypes from the more arid low risk sites, where osmotically active NSC (i.e. water-soluble sugars) play an important role in osmotic regulation and drought tolerance (Regier et al. 2009). These results do not conflict with the observed increase in total allocation to NSC, specifically as starch which is osmotically neutral, in genotypes exposed to low-temperature-related disturbances or stressors in their source environments.

No differences in carbon storage were detected among populations during the spring collection period in May. However, the results in spring could be have been masked by variation in phenology among the populations, with net carbon sequestration occurring only after early season use of stored NSC to support spring growth of photosynthetic tissues (Richardson et al. 2013; Furze et al. 2018). Compared to mid and high elevation populations, leaf flush and flower production occurred earlier in the spring in low elevation populations, triggering earlier photosynthetic carbon gain and greater sink strength. Low elevation populations were fully foliated by late march, two months before the spring NSC collection period. Those populations were no longer depleting their NSC reserves, but rather were actively acquiring carbon which may explain the convergence of NSC concentrations among populations in May. Furthermore, some genotypes from the low elevation sites never fully entered dormancy and instead maintained green photosynthetic tissues throughout the entire year, as opposed to the mid and high elevation genotypes that had all fully senesced during the winter (R. Long, unpublished data). This seasonal pattern is likely why reductions in NSC concentrations were smaller from the fall to winter in the low elevation populations, since they were actively acquiring carbon, than those from colder provenance sites.

Carbon spending

Flowering phenology, basal area and canopy volume

Spring flowering differed among the low, mid and high elevation populations, but unlike NSC accumulation, there was no significant relationship between number of racemes produced and the risk of freeze events at a given provenance site (Fig 4). Similarly, we did not find significant relationships between source population freeze risk and the growth metrics measured in the common garden. These results may have been due to an apparent tradeoff between competing active carbon sinks such as growth and reproduction. For example, mean canopy volume for the low "frost" risk sites ranged from 4.1 to 8.9 m^3 and the reproductive output of those same populations ranged from 206 to 726 racemes per individual, but the population with the highest reproductive output also had the smallest canopy volume. We expected to find the strongest tradeoff between storage and growth/reproduction as carbon is allocated to the various competing pools (Chapin et al. 1990; Kozlowski 1992; Dietze et al. 2014; Hartmann and Trumbore 2016), but tradeoffs have also been shown to occur between growth and reproduction (Obeso 2002; Banuelos and Obeso 2004; Climent et al. 2008; Capelli et al. 2016). One plausible explanation for why growth and phenology were not correlated at the population level is that strong tradeoffs were present between growth and reproduction, and genotypes from low elevations tended to have a relatively high growth rates or reproductive output, but rarely both.

Tradeoffs between labile carbon storage, growth and reproduction

Saver populations preferentially allocated carbon to storage at the cost of growth and reproduction (Fig 7). Broadly, selection for savers could be a result of any increased disturbance that removes aboveground tissue where having a "bank" of labile carbon to regrow would be advantageous (Iwasa and Kubo 1997). Alternatively, high concentrations of NSC could be associated with osmotic regulation or freeze tolerance in some genotypes of species (Greer et al. 2000). In our study system, high elevation populations all occupied the saver space of the coordinate plane, while mid and low populations were found in spender quadrants (Fig 7). This supports the hypothesis that populations from sites exposed to more frequent disturbances would preferentially store carbon (Bloom et al. 1985; Dietze et al. 2014; Long et al. 2017; Hinman and Fridley 2018). Specifically, we found that more frequent exposure to freeze events at the source environments increased the amount of labile carbon storage with reduced commitment to growth and/or reproduction.

Conclusions

We found considerable evidence for local adaptation in *Tamarix,* with distinct "saver" and "spender" populations due to remarkably strong tradeoff between carbon storage and growth/reproduction; specifically genotypes exposed to higher frequency of freeze events maintained higher stores of NSC than those from less frequent freeze events when grown in our common garden. The bet hedging strategy of allocating greater resources to storage has been witnessed among communities at large taxonomic scales (Hinman and Fridley 2018), but our study reveals that these patterns are predictable within species that span broad environmental gradients and can evolve over a small number of generations. The introduction of multiple *Tamarix* species and subsequent hybridization, along with high rates of gene flow, has produced a massive hybrid swarm in North America with high genetic and apparent phenotypic diversity (Gaskin and Schaal 2002; Lee et al. 2017; Long et al. 2017). These factors combined with strong gradients in climate, late freeze disturbances, and large reproduction events (more 500,00 flowers per individual during peak reproduction events [Andersen and Nelson 2013]) appears to have resulted in rapid population differentiation in expressed physiological traits related to carbon allocation. Indeed, *Tamarix* appears to show similar levels of local adaptation as dominant co-occurring native woody species in expressed traits such as cold hardiness and foliage phenology (Friedman et al. 2008). The extent that individual *Tamarix* genotypes are "savers" and "spenders", may affect their resilience to increased disturbances such as fire and herbivory in the future. However, this increased resilience is likely to co-occur with decreased allocation to growth and/or reproduction as a result of predicted tradeoffs between competing pools. Although caution should be taken when applying an adaptive significance to results collected from a single common garden with no reciprocal plantings in other climates, these results add to the growing body of evidence that carbon allocation strategies are under strong selection.

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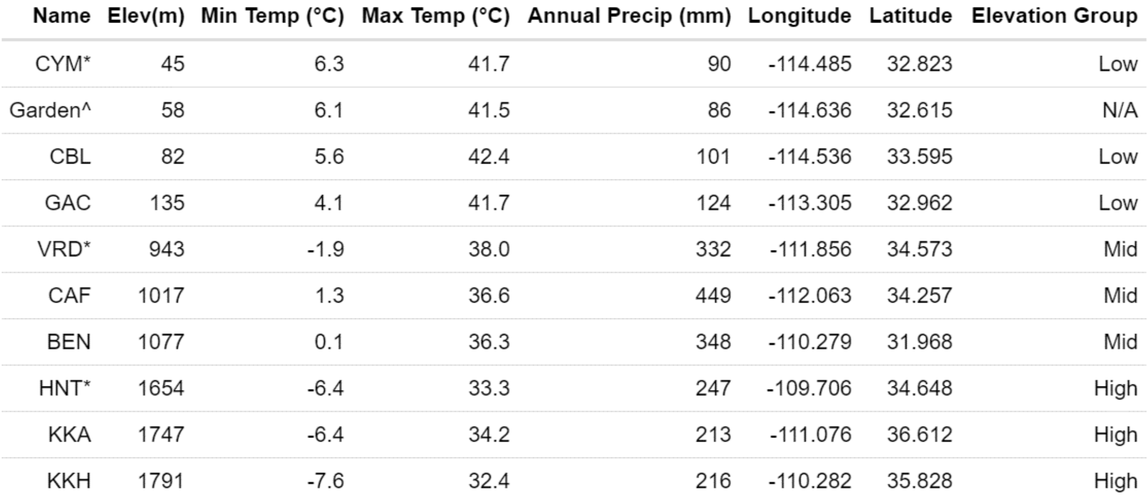
Table

Table 1. Origin of source *Tamarix* populations for the common garden in Yuma, AZ. Source population sites are ordered from high-to-low elevation. Weather data was collected from the PRISM Climate Group, Oregon State University (http://prism.oregonstate.edu, created 10 Jan 2018)



Name	Elev(m)	Min Temp (°C)	Max Temp (°C)	Annual Precip (mm)	Longitude	Latitude	Elevation Group
CYM*	45	6.3	41.7	90	-114.485	32.823	Low
Garden^	58	6.1	41.5	86	-114.636	32.615	N/A
CBL	82	5.6	42.4	101	-114.536	33.595	Low
GAC	135	4.1	41.7	124	-113.305	32.962	Low
VRD*	943	-1.9	38.0	332	-111.856	34.573	Mid
CAF	1017	1.3	36.6	449	-112.063	34.257	Mid
BEN	1077	0.1	36.3	348	-110.279	31.968	Mid
HNT*	1654	-6.4	33.3	247	-109.706	34.648	High
KKA	1747	-6.4	34.2	213	-111.076	36.612	High
KKH	1791	-7.6	32.4	216	-110.282	35.828	High

^Common garden site *Focal Populations (P_f)

Figure Captions

Fig 1. Distribution of populations sources used in the *Tamarix* common garden study. Common garden was in Yuma, AZ, indicated by the star. Map colors represent mean annual temperature variation across Arizona. Source: Esri. "USA Mean Temperature" [basemap]. "USA Mean Temperature". September 20, 2018. https://landscape3.arcgis.com/arcgis/rest/services/USA_Mean_Temperature/ImageServer. (November 18, 2019).

Fig 2. Seasonal variation of non-structural carbohydrate (NSC) concentrations in 3 populations of *Tamarix* occurring in an experimental common garden. Concentrations of NSC were measured as total water-soluble sugars (Glucose, Fructose and Galactose) and starch in roots (**A**) and stems (**B**) from P_f representing low, mid and high elevations (n=10 for each tissue x date x elevation source). Error bars represent the ± standard error of the means. Differences between populations during different sampling periods is denoted by ** ($p < 0.01$) or *** ($p < 0.0001$)

Fig 3. Relationship between nonstructural carbohydrates and the risk of freeze events in *Tamarix* genotypes sourced across a broad climactic gradient. Nonstructural carbohydrates were measured as total starch and water-soluble sugars in the fall in stems of nine populations and compared to the relative risk of freeze-thaw events. Relative risk of freeze-thaw was defined as the frequency of freeze-thaw events divided by the greatest number of events experienced at the coldest site. Points are the means of samples, which were pooled samples of six individual genotypes from three different blocks (n = 18) within the common garden, plotted with the linear regression and 95% confidence interval.

Fig 4. Ratio of starch to sugar of fall root and stem tissue (n=24 for each tissue x elevation source) in *Tamarix* genotypes sourced across a broad climate gradient. Higher starch to sugar ratios indicates increased allocation to storage. Error bars are ± standard errors.

Fig 5. Reproductive output of *Tamarix* populations from different elevation groups. **A)** Seasonal dynamics of reproductive output for low, mid, and high elevation groups. Each point represents the mean of all individual genotypes observed for the three different populations per elevation groups (n = 144 per elevation). Error bars represent the ± standard error of the means. The same individuals were measured at each of the time points. **B)** The log transformed total reproductive output from February 4th to June 11th 2016 for all nine populations. Colors are the elevations of provenance sites and shapes are elevation groups. Points are the means of samples, which were pooled samples of 12 individual genotypes from three different blocks (n = 36) within the common garden, plotted with the linear regression and 95% confidence interval.

Fig 6. Carbon investments as plant growth compared to relative risk for freeze events. Points are the means of samples, which were pooled samples of 12 individual *Tamarix* genotypes from three different blocks (n = 36) within the common garden, plotted with the linear regression and 95% confidence interval. Populations are used to compare investments in growth across three parameters. A) Basal area (cm^2) at 10 cm above the soil for one-year old plants collected May 25 2016. B) Canopy volume (m^3) calculated as the area of an ellipse using orthogonal widths at the widest point and height. C) Basal area increase as cm^2 cm^{-2} day^{-1} from May 25th to October 25th 2016.

Fig 7. Individual measurements for saving and spending were scaled by assigning z scores compared to the global mean of *Tamarix* genotypes sourced across a broad climate gradient. Colors are the elevations of provenance sites and shapes are elevation groups. Spender score was derived from the total NSC in fall stems, an indication of winter storage pool, while spender score is the sum of z scores derived from the total numbers of racemes produced from February to June 2016 and the canopy volume measured May 25th 2016. Populations in quadrant II represent spender populations (negative storage z score and positive spender z score) and populations in quadrant IV are considered saver populations. Error bars represent the ± standard error of the means (n=18 per population).

Fig 1.

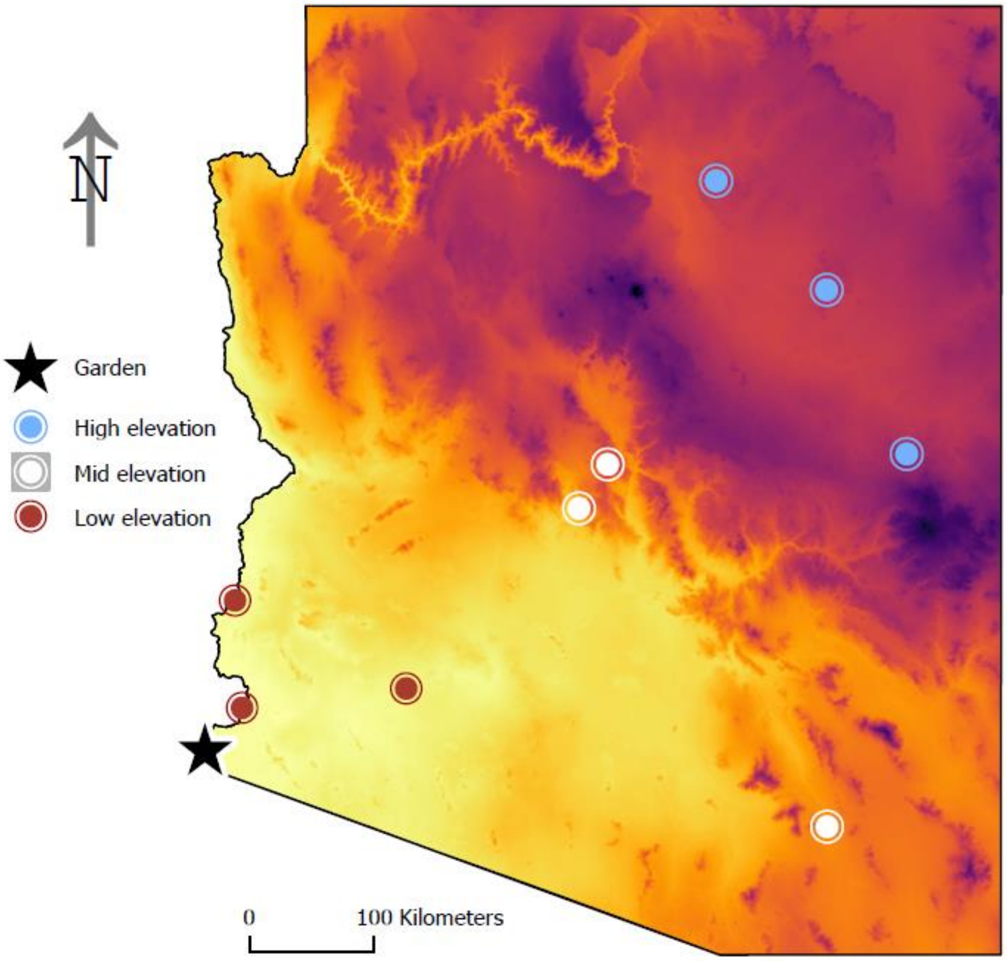

N
Garden
High elevation
Mid elevation
Low elevation
0
100 Kilometers

Fig 2.

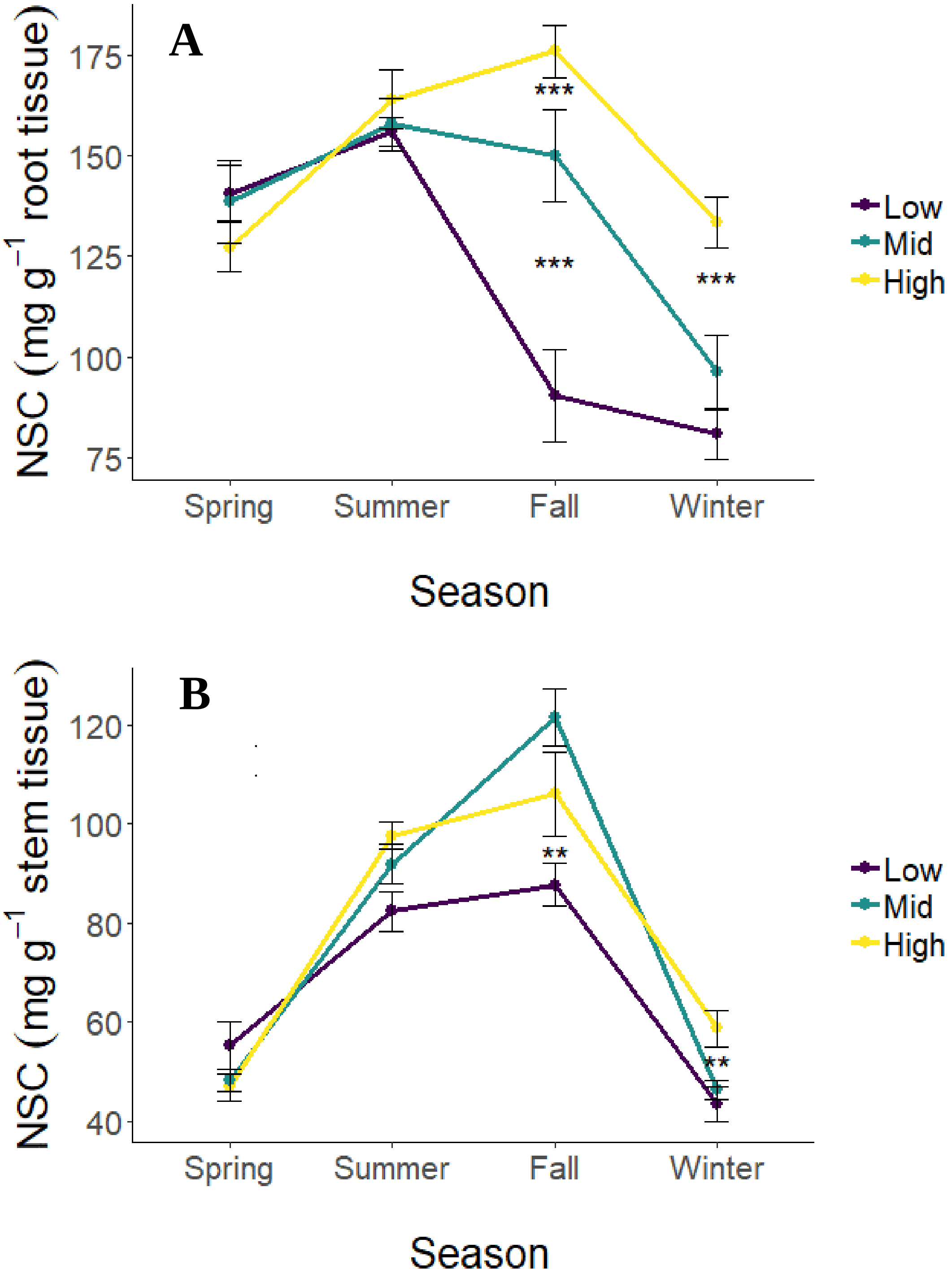

A
NSC (mg g^{-1} root tissue)
175
150
125
100
75
***
***
***
Spring
Summer
Fall
Winter
Season
Low
Mid
High
B
NSC (mg g^{-1} stem tissue)
120
100
80
60
40
**
**
Spring
Summer
Fall
Winter
Season
Low
Mid
High

Fig 3.

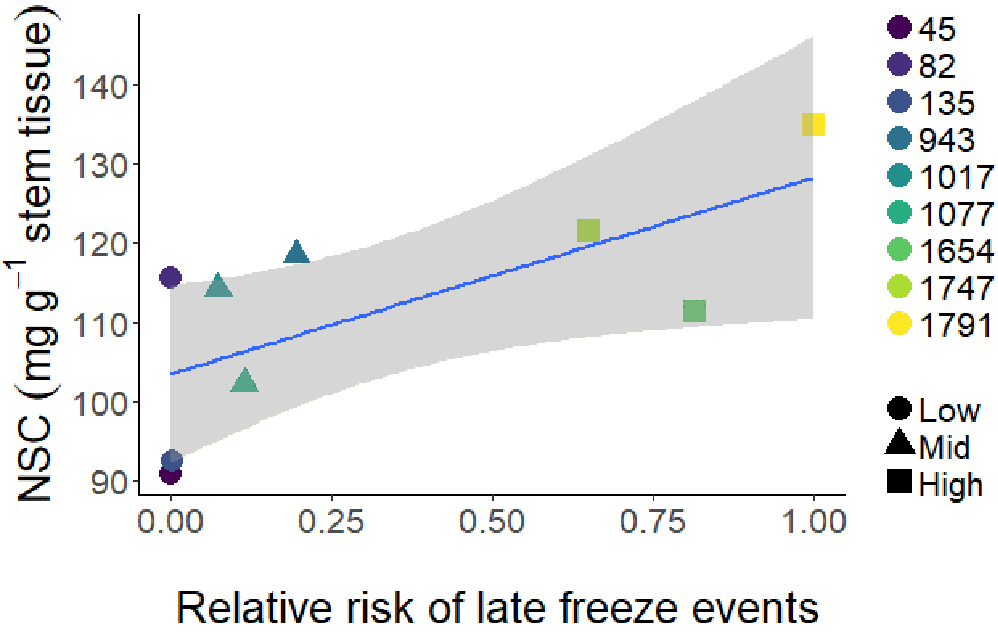

NSC (mg g^{-1} stem tissue)
140
130
120
110
100
90
0.00
0.25
0.50
0.75
1.00
Relative risk of late freeze events
● 45
● 82
● 135
● 943
● 1017
● 1077
● 1654
● 1747
● 1791
● Low
▲ Mid
■ High

Fig 4.

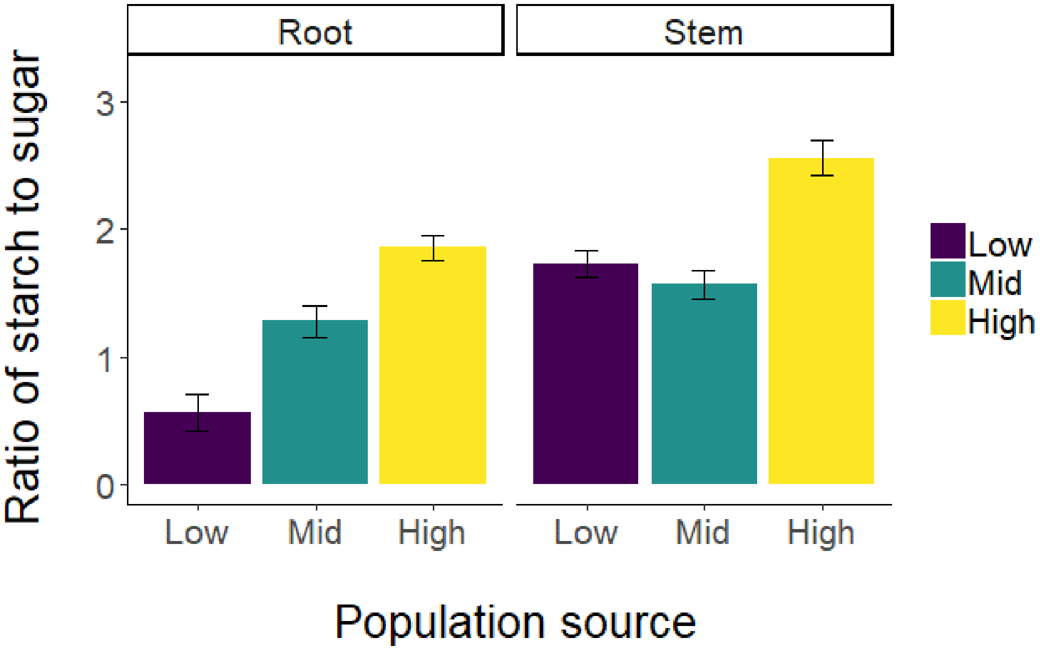

Root
Stem
Ratio of starch to sugar
3
2
1
0
Low
Mid
High
Low
Mid
High
Population source
Low
Mid
High

Fig 5.

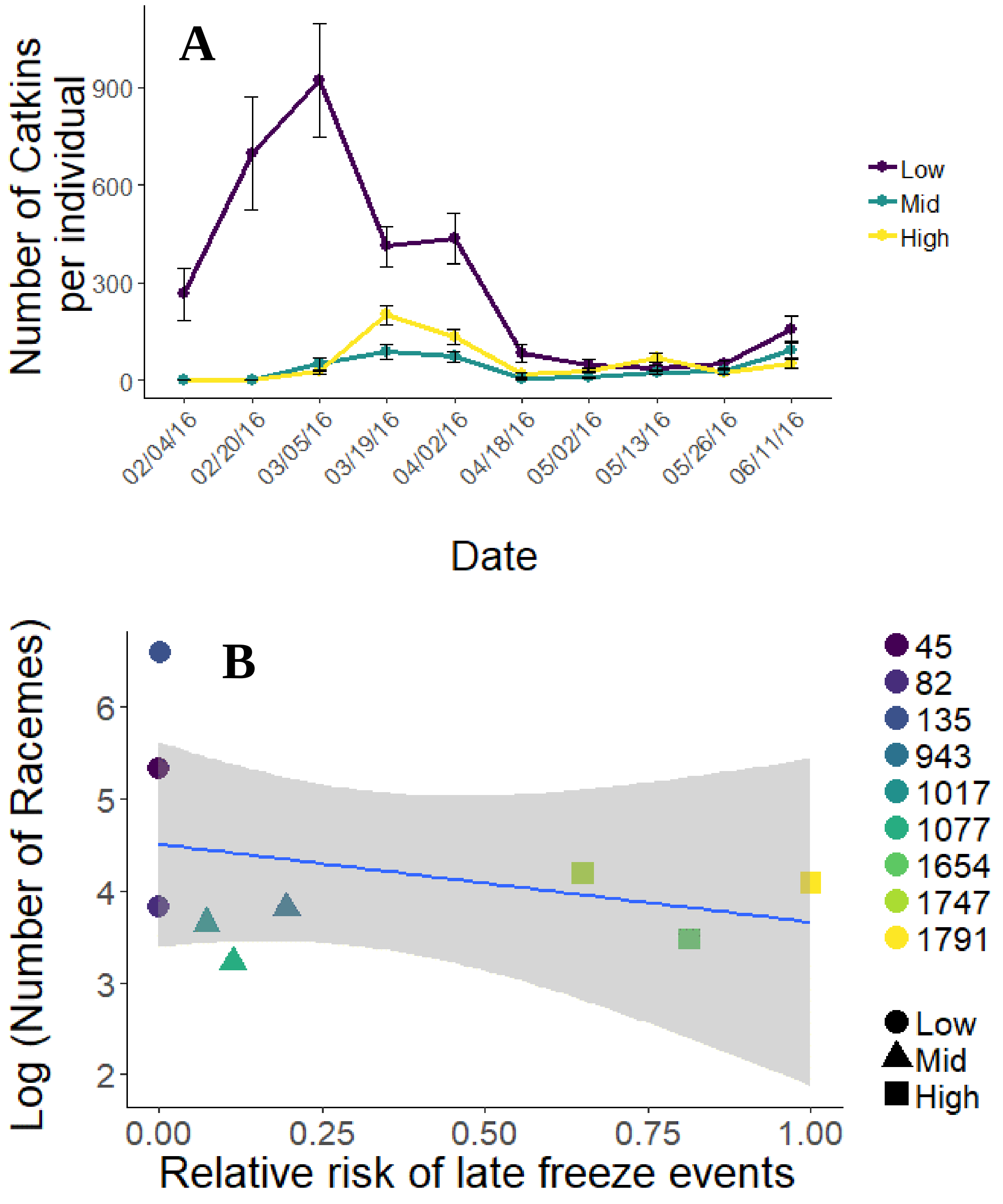

A
Number of Catkins
per individual
900
600
300
0
02/04/16
02/20/16
03/05/16
03/19/16
04/02/16
04/18/16
05/02/16
05/13/16
05/26/16
06/11/16
Date
Low
Mid
High
B
Log (Number of Racemes)
6
5
4
3
2
0.00
0.25
0.50
0.75
1.00
Relative risk of late freeze events
45
82
135
943
1017
1077
1654
1747
1791
Low
Mid
High

Fig 6.

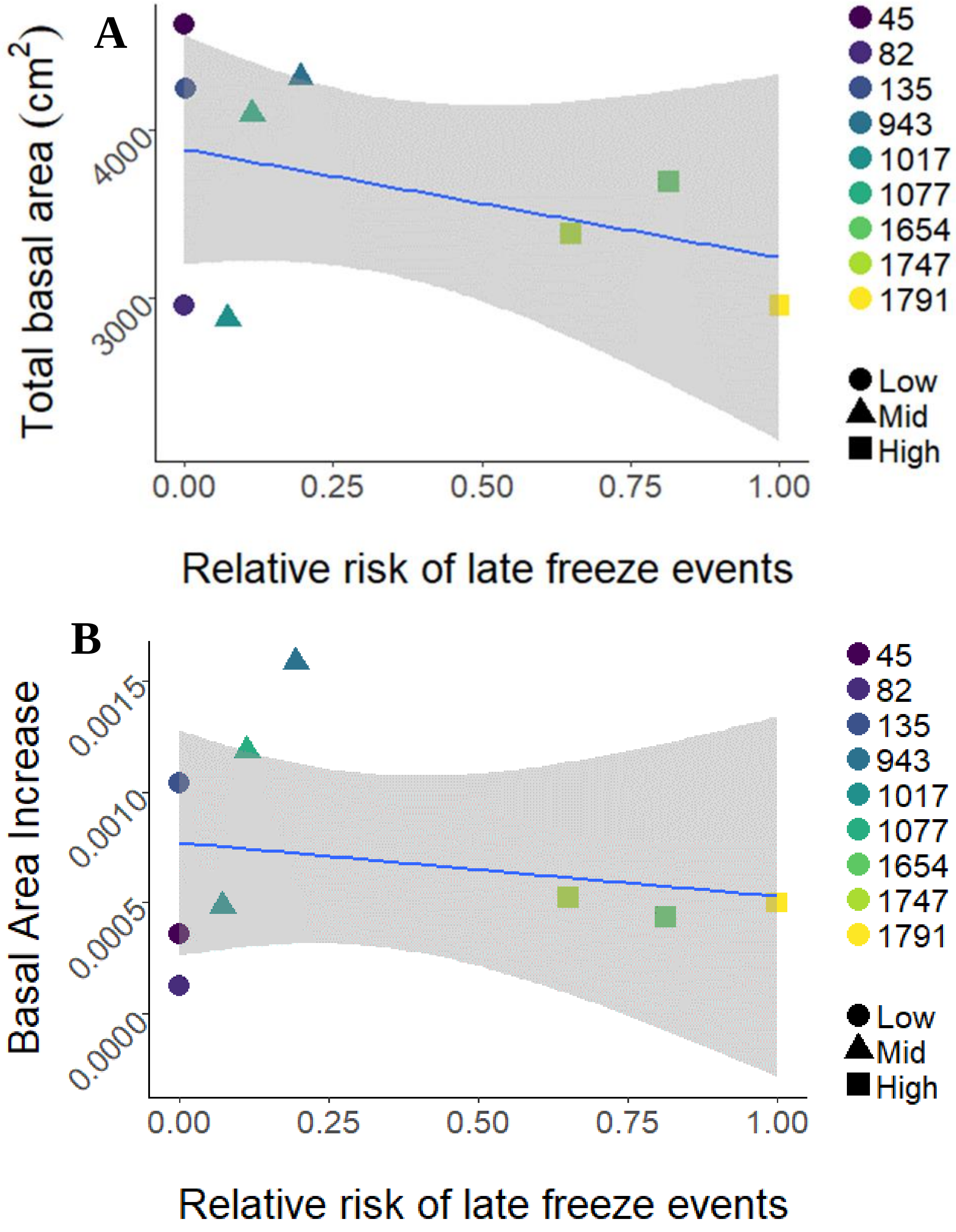
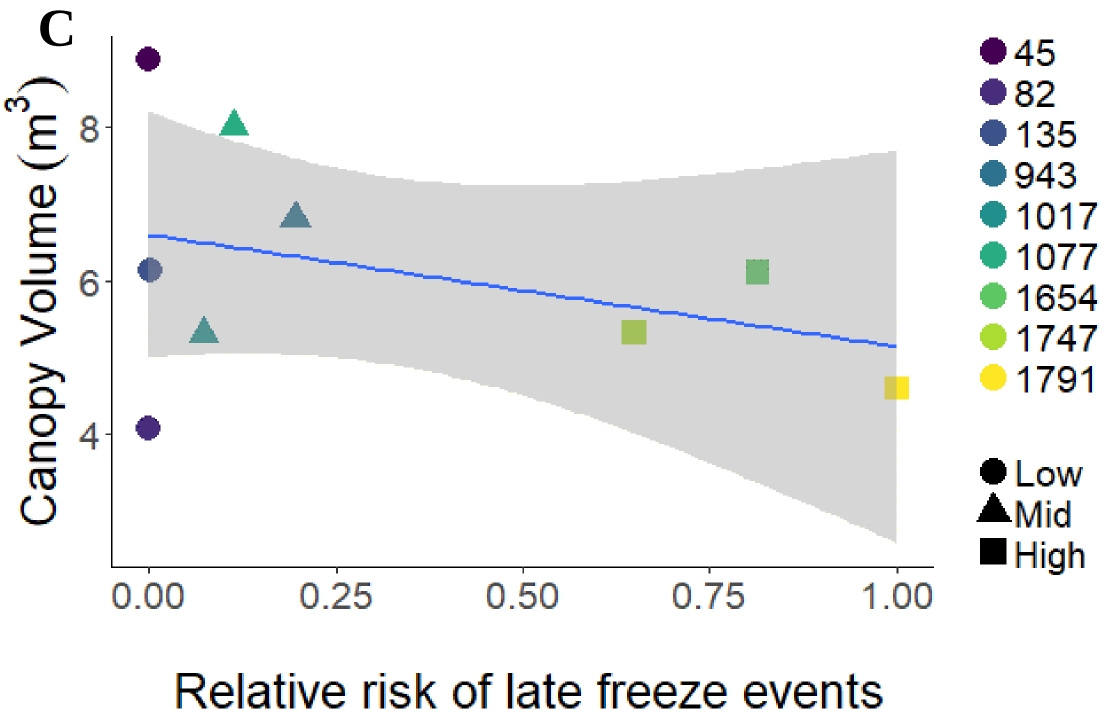

A
Total basal area (cm^2)
3000
4000
0.00
0.25
0.50
0.75
1.00
Relative risk of late freeze events
45
82
135
943
1017
1077
1654
1747
1791
Low
Mid
High
B
Basal Area Increase
0.0000
0.0005
0.0010
0.0015
0.00
0.25
0.50
0.75
1.00
Relative risk of late freeze events
45
82
135
943
1017
1077
1654
1747
1791
Low
Mid
High

Fig 6 (cont).


C
Canopy Volume (m3)
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0.00
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Relative risk of late freeze events
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Mid
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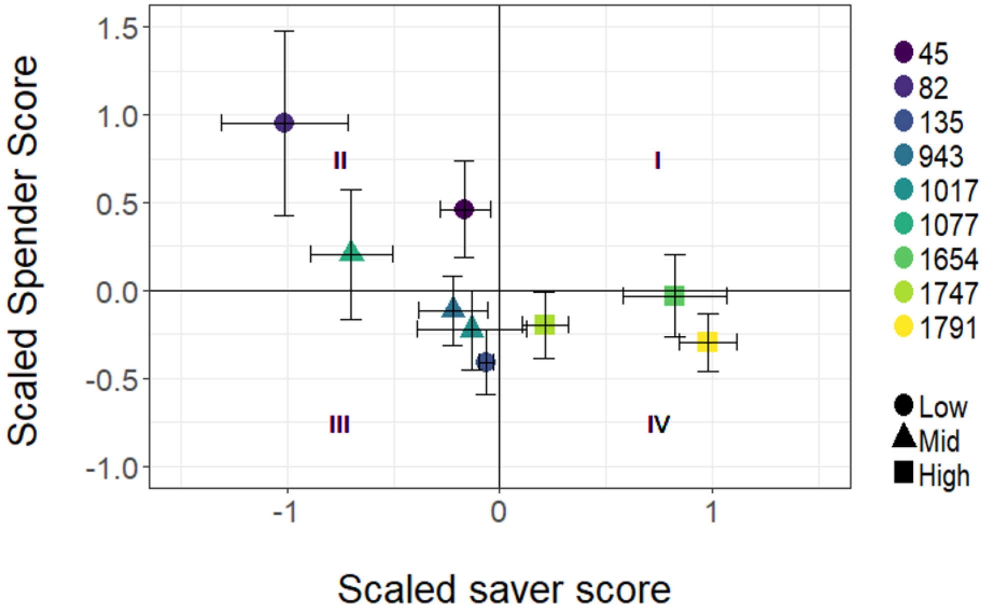
Fig 7.


1.5
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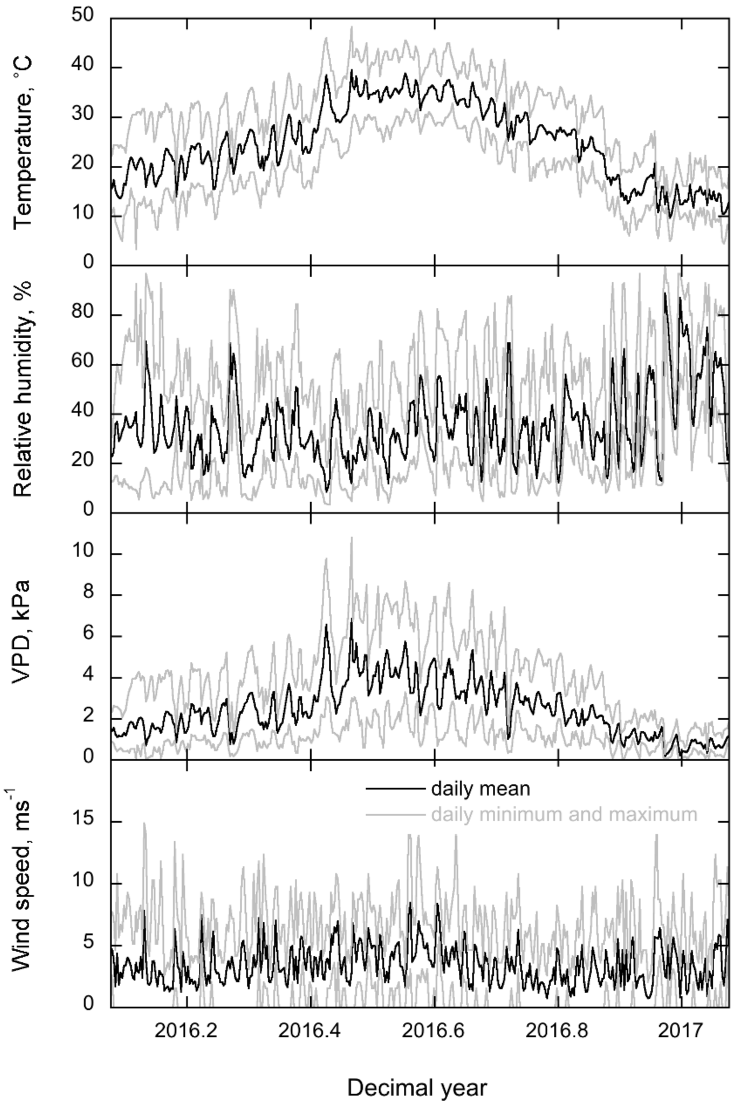

Supplementary Fig 1: Meteorological data from February 2016 to February 2017 for Yuma International Airport (KNYL), approximately two kilometers from the common garden site.

III. The high cost of salinity tolerance: water use strategy tradeoffs in a recently introduced halophyte

Introduction

Water availability is a primary agent of selection in phenotypic traits related to plant hydraulic architecture, xylem structure and function, stomatal behavior and habitat suitability. Consequently, water availability is second only to temperature for explaining plant species distributions in terrestrial ecosystems (Elith and Leathwick 2009, Whittaker 1970), and is further correlated with ecosystem productivity and biodiversity (Bai et al. 2008). Plants in environments with high water availability maximize xylem hydraulic efficiency, stomatal canopy conductance and carbon uptake at the expense of increased sensitivity to water limitation (Pittermann and Sperry 2003; Martínez-Vilalta et al. 2012; Skelton et al. 2015; Gleason et al. 2016). Plants in environments with low water availability exhibit adaptations that are associated with maintaining water conductivity at low water potentials or foraging for water in deep soil layers (Schulze et al. 1987; Dudley 1996; Reich et al. 2003; Mcdowell et al. 2008). Areas with high soil salinity are analogous to areas with low water availability, even when water appears to be abundant, as increased solute concentrations create more negative water potentials in the soil water column (Munns and Tester 2008). For example, high-order rivers and streams are often characterized by highly saline water tables and soils as a function of leaching, and in arid regions, such as the southwestern United States, salinity is often elevated due to high soil evapotranspiration rates (Jolly et al. 2008). This chronic water limitation, as opposed to acute or intermittent drought, should select for similar plant traits to those observed in environments with low water availability (Touchette et al. 2007; Flowers and Colmer 2008). Thus, halophytic (i.e. salt-tolerant) plants that are adapted to high saline conditions may be expected to exhibit a suite of traits that maximize hydraulic safety over efficiency (Reich et al. 2003; Munns and Tester 2008).

Halophytic plants also require mechanisms to protect tissues from high solute concentrations in soil water and ion toxicity, most notably from sodium (Na^+) and/or chlorine (Cl^-), which can lead to necrosis or death (Munns 1993). There are several mechanisms related to salt tolerance, including salt exclusion, compartmentation, or tolerance (Munns and Tester 2008). The most salt tolerant plants are salt exuders (Lambers et al. 2008), a specialized type of compartmentation. They have developed specialized cells that remove salt from leaf tissue, either through trichomes (*Atriplex patula*) or specialized salt glands (*Tamarix* spp., *Avicennia germinas*) (Gonçalves-Alvim et al. 2001; Rand 2002; Lambers et al. 2008). However, because ATP is required to move ions across a semi permeable membrane or to synthesize compatible osmoticum (Tyerman et al. 2019), there is a metabolic cost with both compartmentation and salt exudation. This cost may result in a tradeoff resulting in decreased growth, reproduction, labile carbon storage and/or allocation to defense compounds. As a consequence of the high metabolic cost of salt exudation, the expression of traits related to salt exclusion are in some cases highly plastic and undergo rapid modification if plant salinity exposure fluctuates (Richards et al. 2010; Rewald et al., 2012). Nevertheless, it remains an open question how rapidly woody halophytic phenotypes occurring along riverine systems can adjust metabolic functions in response to dynamic changes in salinity exposure.

In addition to tradeoffs between salinity tolerance and growth, halophytic plants should show more conservative trait correlates with increasing salinity; similar to other plants with diminishing water availability. More conservative trait correlates include those that maximize safety against hydraulic failure by cavitation over high rates of conductance, such as smaller xylem diameters/pit pore size (Pittermann and Sperry 2003; Martínez-Vilalta et al. 2012), increased stomatal sensitivity (Skelton et al. 2015), and/or changes in anatomy that increase leaf water use efficiency (Brodribb et al. 2009). Plants may also be adapted to maintain cellular functions at more negative water potentials and continue photosynthesis and growth when water availability is low (Flexas et al. 2006). If the water potential of a living cell drops below a critical threshold – i.e. turgor loss point - critical functions are inhibited as the cell becomes flaccid (Bartlett and Scoffoni 2012). Although halophytes are adapted to high salinity, we should expect to see variation in water use strategies because of the expected trade-off between hydraulic safety and conductance rates (Reich et al. 2003; Munns and Tester 2008; Ambrose et al. 2009; Gleason et al. 2016); with plants growing in high salinity sites exhibiting more conservative traits than populations in low salinity sites.

Tamarix spp. are introduced halophytic woody plants that occupy a wide range of salinities and climates throughout arid riverine systems and wetlands in the American west. The *Tamarix* species complex in North America has spread rapidly since its introduction in the 1800s. This invasion is predominantly comprised of *T. chinensis* and *T. ramosissima,* their F1 hybrids and subsequent backcrosses which have produced a hybrid swarm in North America with high genetic and apparent phenotypic diversity (Gaskin and Schaal 2002; Lee et al. 2017; Long et al. 2017). These factors combined with strong abiotic gradients and large episodic recruitment events (Andersen and Nelson 2013) appears to have resulted in rapid population differentiation in expressed physiological traits (Friedman et al. 2008; Long et al. 2017). Hybrid individuals dominate low elevation desert riparian environments that experience extreme mid-summer heat and aridity as well as site salinities that vary across river terraces (Friedman et al. 2008; Nagler et al. 2011); including at Cibola National Wildlife Refuge (CNWR) along the lower Colorado River where this study occurred. This location was selected because of previous work that documented *Tamarix* populations growing across a gradient of sites with increasing groundwater salinity as distance to the river increases, but depth to groundwater remains relatively constant at approximately 3.4 m (Nagler et al. 2008; Zhu et al. 2011; Glenn et al. 2013). Salinity near the river (200 m away) is approximately 2 parts per thousand (ppt), while ground water salinity 1.5 km from the river is 12 ppt (more than one third of mean seawater salinity) due to high evapotranspiration rates and low fresh water inputs (Nagler et al. 2008). Given the large variation in salinity at Cibola, we made *in situ* measurements and established a greenhouse experiment to evaluate trait variation related to salinity tolerance in the *T. chinensis* X *T. ramosissima* hybrid complex (hereafter referred to as *Tamarix*).

In this study, we specifically asked how Tamarix water use related traits varied as a function of the salinity at the site of origin. We tested two interrelated hypotheses: (1) *Tamarix* growing at field sites with higher salinities would exhibit more conservative traits relative to phenotypes originating from sites with low salinity, and (2) phenotypes originating from sites with high salinity lack the requisite plasticity to adjust phenotypic expression of traits when grown in reciprocal greenhouse experiments. Specifically, we tested the following predictions: (1) phenotypes from lower salinities would exhibit highest growth and survival rates at low salinities compared to high salinities, (2) phenotypes from low salinities will have larger xylem vessels and higher gas exchange rates, and (3) high salinity phenotypes will maintain turgor pressure at more negative water potentials. Variation in plant traits across small-scale salinity gradients would provide strong evidence of rapid acclimation to groundwater salinity and reveal tradeoffs between efficiency and hydraulic safety related to water use in this rapidly evolving invasive tree.

Methods

Site Characteristics and *in situ* measurements

Cibola National Wildlife Refuge (CNWR) is located on the border of California and Arizona on the lower Colorado River, and our specific sites were north of the Three Fingers Lake area on the California side of the refuge (Low salinity site: 33.2748, -114.6851 and high salinity site: 33.2661, -1146992). This site was chosen for its use in previous work examining the effect of salinity on *Tamarix* evapotranspiration (Nagler et al. 2008). The two sites representing the highest and lowest salinity from that study were selected for further investigation here. The sites are both dominated by *Tamarix*, with no other woody plants and a paucity of forbs and grasses growing in the understory. Sixteen individuals at the two sites were physically tagged and identified with GPS coordinates for tracking throughout the study. The refuge is characterized by mean annual temperature of 23.2°C (5.4°C minimum and 42°C maximum) and mean precipitation of 91.9 mm (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 14 Dec 2019). Ground water and soil salinity at the sites had been characterized in a previous study, with the lower salinity site groundwater salinity being 2.25 ppt and the high salinity site being 12 ppt (Swamp and Diablo sites respectively from Nagler et al. 2008). Soil texture was slightly different between sites, changing from a loamy sand near the river to a silt loam at the high salinity site, however depth to groundwater was similar at the two sites (2.7-3.4 m and 3.4-3.7 m (Nagler et al. 2008)), and *Tamarix* is known to be a phreatophyte accessing groundwater at depths > 4 m (Brock 1994).

Site water availability

Diurnal leaf water potentials were measured on June 15th, 2017 to estimate water stress at the sites. Three terminal stems from south facing branches with diameters ranging from 7-10 mm were collected from all 16 individuals at each of the sites for predawn and midday measurements (between 4:30 and 5:30H and 12:00 and 13:00H respectively, Fig 1). Branches were placed into plastic bags with moist paper towels and put into coolers with ice to reduce transpiration prior to measurements of leaf water potentials using a Scholander type pressure chambers (PMS instruments, 600 model series, Albany, OR).

Xylem measurements

On Nov 18th, 2017 stem segments were collected from both sites to examine the *in situ* xylem anatomy of the two populations. Samples were placed in a solution of glycerol and water (1:1) and kept on ice until stored at 4 °C. Freehand cross sections of stems were made and then images were captured using a confocal microscope at 50x magnification (Leica DMI600, Leica Microsytems Buffalo Grove, IL). For each sample four different areas were selected, one in each cardinal direction and halfway from the center of the stem to the bark. Percent lumen area of the heartwood and mean vessel area were calculated using ImageJ from each of the sampled areas (National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/), from this the mean hydraulic diameter (D_h) was calculated using the area (A) and wetted perimeter (P) of xylem vessels in each sampled area (Lewis 1992):

$$D_h = \frac{(4A)}{(P)} \qquad (1)$$

Greenhouse salinity experiments

In January 2016 stem cuttings were collected from all sixteen individuals at both the high and low salinity sites. Stem cuttings were between 40 and 50 cm in length and diameters of 10 to 15 mm at the base. The cuttings were rooted in tap water and then planted in a soilless peat mix in the greenhouses at the University of California, Santa Barbara. In order to reduce maternal effects these cuttings were grown in a common environment for four months and cuttings (10-12 cm in length, 7-12 mm in diameter) were then taken from them to start new plants. These second cuttings were propagated using rooting hormones at a 5X dilution (Dip ‘N Grow, Athens, GA) on a mist bench in 100% perlite. This process was repeated twice so that the plants ultimately used in the greenhouse study had been grown under a greenhouse setting for one year and had been clonally propagated three times. Greenhouse conditions were set to maintain daytime temperatures between 25 and 35 °C and nighttime temperatures between 25 and 30 °C. Artificial lighting was used to supplemental natural light up to 1600 PAR and 16 hours of daylight year-round (6:00h – 22:00h). The experimental plants were grown in pure sand, and bottom watered with a fertilizer using a water soluble, all-purpose fertilizer (Peter's 20-20-20, ICL Specialty Fertilizer's Summerville, SC). To avoid salt build up plants were flushed every other week with the same diluted fertilizer.

Salinity Treatments

Once plants had been grown from the final cuttings for two months, they were separated into different salinity treatments in January 2017. Forty plants from each population source were divided into five treatments, (0, 4, 8, 16, and 32 ppt NaCl); representing a range of salinity below and above the salinity of source sites. Sodium chloride was selected to adjust the salinity because Na^+ and Cl^- are the ions that contribute the most to the excess salinity in the lower Colorado River system (Ohmart et al. 1988), and because they are the ions most often associated with growth reduction in plants (Greenway and Munns 1980). Cuttings were planted in 0.6 ga treepots (Stuewe & Sons, Tangent, OR) in river sand and bottom watered with the salinity treatment made with rock salt (99.9% NaCl, Diamond Crystal, Savannah, GA) and a weak nutrient solution of 200 ppm Nitrogen (Peter's 20-20-20, ICL Specialty Fertilizer's Summerville, SC). Plants were flushed with the same salt/nutrient solution once per week to leach out any accumulating salts.

Salinity water potentials

On February 2nd 2017, three weeks after the salinity treatments had been implemented, predawn water potentials were taken on 3 or 4 individuals for all treatments. A fourth measurement was taken if there was large variation (> 1 MPa) between any of the initial three measurements. Measurements were taken between 4:00 and 5:30 am; three stems per individual were excised and the stem water potential was measured immediately using Scholander type pressure chambers (PMS instruments, 600 model series, Albany, OR). All plants were well watered at the time of the measurements.

Biomass collections

After four months of growing in the salinity treatments described, aboveground and belowground biomass was collected. Belowground biomass collection was facilitated by the plants being grown in pure sand. The sand and root systems were removed from the pots and soaked in water for four hours, during which time the denser sand sank to the bottom of the containers and the roots floated to the top. The water solution was agitated halfway through the soaking period to help release fine roots from the sand particles. All plant material was dried in an oven for 48 hours at 60 °C prior to obtaining dry weights. Root mass ratio was calculated as follows:

$$RMR \quad = \frac{(Dry\ mass\ of\ roots)}{(Total\ dry\ mass\ of\ plant)} \tag{1}$$

Gas exchange

We used a Licor 6400XT (Licor industries Lincoln, NE) to evaluate how the salinity treatments affected the water use and gas exchange of the two different populations. Weekly spot gas exchange measurements were taken between February 15th and March 9th 2017. Measurements were taken from three different terminal stem segments (including green photosynthetic stem tissue and leaves) between 13:00h and 15:00h and chamber conditions were set to match the ambient conditions in the greenhouse; 25 °C, 400 ppm CO_2, 1600 PAR. Due to the irregular nature of *Tamarix* leaves the leaf material in the chamber was collected and then leaf area was calculated post hoc. Leaves were scanned on a flatbed scanner and then leaf area was calculated using ImageJ (National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/).

Dry down experiment and turgor loss point measurements

We examined the turgor loss point and stomatal responses of the different populations. Measurements were taken on a different set of plants from those used in the salinity treatment experiment but from the same greenhouse grown populations. Six plants from each population were grown for six months in a pure sand media using the same technique and fertilizer treatments described above, except that they were grown in one-gallon nursery pots. First the turgor loss point was determined using the bench top dry down method; where excised stems were weighed, and their water potential was measured with a pressure chamber (Tyree and Hammel 1972). This was repeated for ten time points over eight hours to develop a response curve with the inflection point being the turgor loss point (Koide et al. 1989). Next, the plants were then subjected to a dry down experiment, where no additional water was added to the bottom watering trays. Each day, predawn (4:00h – 5:00h) and midday (1:00h - 2:00h) water potentials were taken to estimate the soil and minimum water potentials, respectively. At the same time as the midday measurements, stomatal conductance was measured to determine responses to drying soils. A Licor 6400XT (Licor industries Lincoln, NE) was used to measure stomatal conductance and chamber conditions were set to imitate the ambient conditions in the greenhouse; 25 °C, 400 ppm CO_2, 1600 PAR. The dry down experiment started on July 30th 2018 and lasted until August 3rd when all the leaves on the plants had fully desiccated and there was no change in stomatal conductance or water potential from the day before.

Statistical Analysis

All analyses were completed using the 'stat' package in R (R Core Team 2017). Correlations between water potentials and salinity, stomatal conductance, and gas exchange were analyzed using linear regressions. Comparisons between two groups were completed using one sided Welch's t-tests for the *in situ* water potential, xylem measurements, and turgor loss points. Effects and interactions for the salinity trials were completed with two-way ANOVAs, and post hoc analyses were completed with Tukey's Honest Significant Differences. For the dry down experiment an ANCOVA was performed, to account for covariation between populations and water availability. Version 1.1.423 of RStudio was used for all analyses.

Results

In situ measurements

Water potentials were found to be significantly different at the two sites (ANOVA $F_{1,60} = 49.78$, $p < 0.001$, Fig 1), and between midday and predawn (ANOVA $F_{1,60} = 8.20$, $p = 0.0058$). The high salinity site had the most negative water potentials during midday (Fig 1) and had lower potentials at predawn compared to the midday water potentials for the low salinity site. The least negative water potentials were recorded during the predawn measurements for the low salinity site. The mean xylem lumen area from the high salinity site was found to be significantly smaller than that measured at the low salinity site (Welch two sample t-test, t = 1.76, df = 124.95, p = 0.04, Fig 2a). The total percentage of wood that was xylem lumen area however, was not significantly different among sites (Welch two sample t-test, t = -1.12, df = 105.31, p = 0.26 Fig 2b).

Greenhouse measurements

Salinity water potentials

Predawn water potentials in well-watered greenhouse plants were found to be correlated with salinity treatments in both low salinity (MPa = -1.09 -0.056*PPT, $r^2 = 0.64$, $F_{1,17} = 30.29$, $p << 0.001$, Fig 3) and high salinity (MPa = -87 -0.078*PPT, $r^2 = 0.86$, $F_{1,15} = 91.74$, $p << 0.001$, Fig 3) phenotypes. Salinity treatment was the only significant effect on the predawn water potentials (ANOVA $F_{1,32} = 97.056$, $p < 0.001$): population had no effect (ANOVA $F_{1,32} = 0.083$, p = 0.78) and there was no interaction of treatment and population (ANOVA $F_{1,32} = 2.77$, p = 0.11).

Biomass

Each population had a salinity at which it performed the best. Across salinities, the population from the low salinity site had the greatest oven dried biomass at 4 ppt. This was significantly larger than what the high salinity origin plants were able to achieve (Welch two sample t-test, t = -3.26, df = 12.41, p-value = 0.0033, Fig 4). Similarly, genotypes from the high salinity source population had their greatest biomass at 16 ppt which was significantly larger than what the low salinity origin plants were able to achieve (Welch two sample t-test, t = 1.81, df = 9.44, p-value = 0.051) (Fig 4). There were no significant differences between the two populations at 0, 8, or 32 ppt. We found a significant effect of population and treatment on RMR, the interaction was disordinal and differences were only found at 0 ppt, driven by a high RMR in the high salinity population (ANOVA F $F_{4,108} = 2.96$, p = 0.023 Supplementary Fig 1)

Gas exchange measurements

Net carbon assimilation rates varied significantly between populations (ANOVA $F_{1,138} = 14.13$, $p < 0.001$), and the interaction of population and treatment was also significant (ANOVA F $F_{4,138} = 2.71$, $p = 0.033$ Fig 5). Low salinity phenotypes had the highest photosynthetic rates at 0 and 4 ppt, while there were no significant differences between any of the treatments for the high salinity phenotypes. Using a Tukey's HSD post hoc analysis it was apparent that all of the significant differences were driven by interactions between the low salinity populations under 0 and 4 ppt treatments and the high salinity populations at all but the 16 ppt salinity treatment (Table 1).

Turgor loss point and dry down experiment

Turgor loss points of the high salinity populations were found to be significantly more negative than those of the low salinity populations ($t = -5.53$, $df = 5.7$, p-value = 0.0008, Fig 6). On the fifth day without water the high salinity plants had reached an average negative water potential of -3.74 MPa, while the low salinity plants had reached an average of -5.86 MPa and there was a significant interaction between populations and time since watering (ANCOVA F $F_{1,43} = 3.96$, $p < 0.001$ Fig 7). High salinity plants reduced stomatal conductance (Fig 8a) and net assimilation (Fig 8b) at a less negative water potential than those from low salinities. There was a significant interaction between populations and water availability for stomatal conductance (ANCOVA $F_{1,38} = 7.43$, $p = 0.0098$) but not for net assimilation (ANCOVA $F_{1,38} = 1.056$, p 0.31)

Discussion

We found evidence for the expression of different water use strategies in two populations of the invasive woody plant *Tamarix* spp., specifically there was support for our predictions that each population would perform best at a specific salinity and exhibit traits adapted to those salinities. Both *in situ* measurements and results from the reciprocal greenhouse experiments indicate that despite the short distance between the two sites (<1.5 km); plants were adapted to the local salinities. In the greenhouse, plants performed best when grown at salinities closest to their source site and we found differences in traits suggesting that plants from high salinities exhibited more conservative traits than those from low salinities. Specifically, they had smaller mean hydraulic diameters and showed greater stomatal control in response to soil drying. These traits should decrease the risk of cavitation at more negative water potentials (Brodribb et al. 2009; Martínez-Vilalta et al. 2012) that would be experienced under high salinity conditions (Munns 2002). They also exhibited lower turgor loss points which would allow them to maintain cellular function at lower water potentials (Bartlett and Scoffoni 2012). This variation in traits adapted to localized gradients may play a role in the invasive success of *Tamarix* and other nonnative species (Alexander et al. 2012).

Evidence for local phenotypic differences

We found significant differences in growth rates between the different populations when grown in a greenhouse setting using reciprocal treatments. This type of experiment is the strongest evidence for local adaptation (Clausen et al. 1947; Kawecki and Ebert 2004); and supports our hypothesis that *Tamarix* exhibits traits adapted to different site salinities, despite the close proximity of sites. We found the highest growth rates for plants grown under similar soil salinities as those found at their source sites. Our maximum growth rates for the low salinity populations matched the treatment salinity where the A_{net} of those populations were the highest. The depression of growth rates in the low salinity plants as salinity increased was expected, as higher levels the Na^+ and Cl^- ions can become toxic and inhibit plant growth, even in halophytes (Glenn and Brown 1998; Munns 2002). The lower growth rates we observed in the no salt treatment (except those found in the fertilizer) has also been seen in other experiments with halophytes (Glenn et al. 1998). In our high salinity plants, the highest growth rates did not correspond with the highest A_{net}, but still matched that of the salinity closest to the source site. Some evidence for tradeoffs between growth and salinity tolerance have been witnessed in arid riparian systems with elevated salinity; with plants performing best at specific salinities. For instance, Qi *et al.* found that among three dominant species growing across a salinity gradient at their site that each species performed best at different salinities, with biomass accumulation highest at salinities near their source salinity (Qi et al. 2018); Glenn et al.(1998) found that a halophyte, *Allenrolfea occidentalis*, had higher biomass accumulation at elevated salinities than in control treatments..

One explanation may be the lower turgor loss point of the high salinity population plants compared to the low salinity plants. If a plant cell drops below its turgor loss point critical metabolic functions are inhibited as the cell becomes flaccid (Flexas et al. 2006; Bartlett and Scoffoni 2012). Salt tolerant plants have been shown to allocate Na^+ ions in the transpiration stream to vacuoles, thus increasing the solute concentration in the leaves (Guerrier 1996; Ueda et al. 2003). Higher solute concentrations in cells contribute to lower turgor loss points and has been shown to maintain higher gas exchange and growth rates at lower water potentials (Brodribb et al. 2003; Mitchell et al. 2008; Bartlett et al. 2012). The lower salt concentration treatments may have not had enough solutes in the soil water to maintain the optimum turgor pressure for plants from high salinities, possibly causing an osmotic imbalance within the cells.

Conservative water use traits

Consistent with our initial predictions, we also found that plants sourced from the high salinity site exhibited more conservative traits related to conductance and hydraulic safety. This included smaller xylem lumen *in situ* and a higher regulation of stomatal conductance during dry the down experiment. Smaller xylem lumen are more resistant to cavitation as a function of both smaller diameters and fewer/smaller interconduit pores, yet they are less efficient at water transport (Sperry and Sullivan 1992; Gleason et al. 2016). We saw that the plants growing at the high salinity site, which experienced more negative water potentials, had smaller xylem than those from the low salinity sites; indicating a more conservative strategy by the plants from the high salinity site. Another conservative water use strategy is to have a higher stomatal regulation to prevent cavitation in drying soils (isohydric), as opposed to those that continue to maintain high rates of gas exchange and transpiration as soil water is depleted (Skelton et al. 2015). We found that during our dry down experiments, the high salinity population had higher stomatal control when exposed to water potentials greater than those *in situ* during the middle of summer. The stomatal conductance of this population approached zero as the water potentials approached -4 MPa, which is similar to the highest measured water potential *in situ.* The low salinity populations maintained stomatal conductance well below the water potentials at their source site, and mortality in the plants was witnessed after just a few days of exposure to soil drying.

Conclusions

We found strong evidence for variation in water use strategies in *Tamarix* across a strong salinity gradient over a small spatial scale; specifically, we found that populations from higher salinity sites exhibited more conservative water use strategies when grown in a common garden setting with plants from a low salinity site. Our data support the hypothesis that water stress at high salinity sites has selected plants with smaller xylem, greater stomatal control, and lower turgor loss points. These traits result in greater hydraulic safety at lower water potentials, but with a tradeoff in lower hydraulic efficiency (Skelton et al. 2015; Gleason et al. 2016). Populations from low salinity sites had larger (more efficient) xylem and lower stomatal control. We witnessed the effect that these two different strategies had on growth rates in a reciprocal greenhouse experiment; with populations having the highest biomass accumulation at the salinity nearest to that of their source site, after controlling for maternal effects. This variation to support rapid adaptation of *Tamarix* to local conditions in North America is likely due to the introduction of multiple, previously isolated, species and subsequent hybridization that has produced a hybrid swarm with high genetic and apparent phenotypic diversity (Gaskin and Schaal 2002; Lee et al. 2017; Long et al. 2017). These factors combined with the strong salinity gradient at CNWR appears to have resulted in the observed rapid divergence in water use strategies. Future genetic research may be able to identify the exact genetic controls that shape these changes.

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Figure captions

Fig 1. Diurnal water potential measurements taken at the Cibola National Wildlife Refuge on June 15th 2017 from the low and high salinity populations (n = 16 for each population and sampling period). High salinity populations always had lower water potentials than the low salinity populations at both timepoints ($F_{1,60} = 49.80$, $p < 0.001$). There were also significant differences between predawn and midday water potentials for either population ($F_{1,60} = 8.20$, $p = 0.0058$). Horizontal line is the median, boxes and whiskers are each one quartile.

Fig 2. *In situ* measurements of xylem lumen traits at a high salinity and low salinity site at Cibola National Wildlife Refuge. Plants at the higher salinity site had smaller mean xylem lumen areas than those at the low salinity site (A), but no differences were found in total xylem lumen area (B). Error bars represent ± SE of the mean. Asterisk indicates signicant difference in the Welch's t-test between the two populations.

Fig 3. Predawn water potentials of well-watered plants from two field populations grown in five different salinities in a greenhouse. Water potentials were correlated with salinity treatment due to increased solute potential. There was no effect of population. Points are averages of three measurements from different stems on the same individual (n = 18 for low salinity and n = 18 for high salinity populations), plotted with the linear regression and 95% confidence interval for all individuals.

Fig 4. Total aboveground biomass accumulation varied in response to the salinity treatments depending on the source population in the greenhouse. Populations were found to have the highest total biomass at salinities closest to those at their sources site. Error bars represent +/- SE of the mean, letters are differences from a Tukey's HSD post hoc analysis.

Fig 5. Photosynthetic activity varied in response to salinity treatments depending on the source (N = 12 for each treatment x population). Error bars represent +/- SE of the mean. Letters are significant groupings from a Tukey's HSD.

Fig 6. Turgor loss points for plants grown in the greenhouse from two different source populations (n = 8 for each population). Plants were grown at two different salinities (4 ppt and 16 ppt), but turgor loss points were pooled for both salinities because they were not significantly different. High salinity populations had significantly lower turgor loss points, letters are results from a Tukey's HSD posthoc analysis and error bars represent +/- SE of the mean.

Fig 7. Midday water potentials of plants during dry down experiment. Points are individual plants that were sampled over five days. All individuals were showed a negative relationship between water potentials and days since watering. There was also a significant interaction between population source and days since watering. Initially five plants from each population were used, but on day four one of the plants from the low salinity population had died and we were unable to obtain a water potential, on day five another plant from the low salinity group had died. Leaf mortality was witnessed in high salinity plants, but water potentials did not go below -4 MPa. Shading is the 90% confidence interval.

Fig 8. Gas exchange measurements from a dry down experiment. Water potentials, (A) stomatal conductance (B) and net assimilation rates were taken on five plants from two populations from different site salinities (n=5 for each) over the period of five days, at which point permanent wilting was reached in all plants. Both stomatal conductance and net assimilation decreased with lower water potentials. There was a significant interaction between population and water potential on stomatal conductance, but not on net assimilation rates. Shading is the 90% confidence interval.

Supplementary Fig 1. Root mass ratio varied in response to salinity treatments depending on the source (N = 12 for each treatment x population). Error bars represent +/- SE of the mean. Letters are significant groupings from a Tukey’s HSD.

Fig 1.

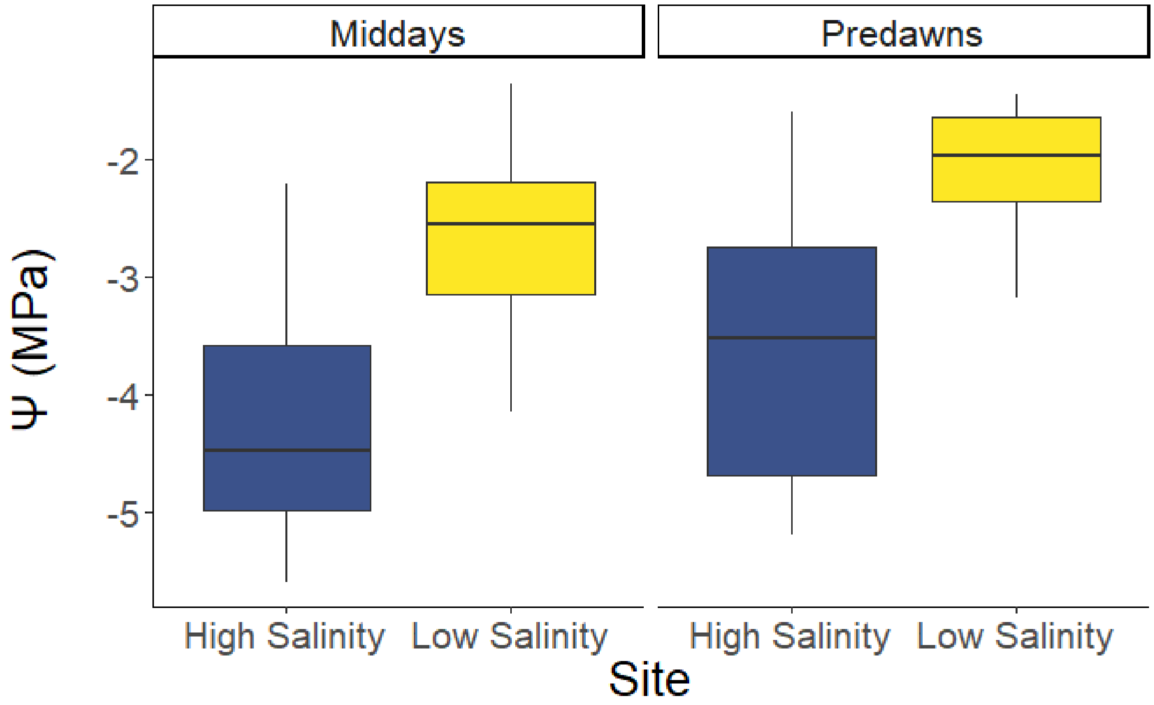

Middays
Predawns
Ψ (MPa)
-2
-3
-4
-5
High Salinity
Low Salinity
High Salinity
Low Salinity
Site

Fig 2.

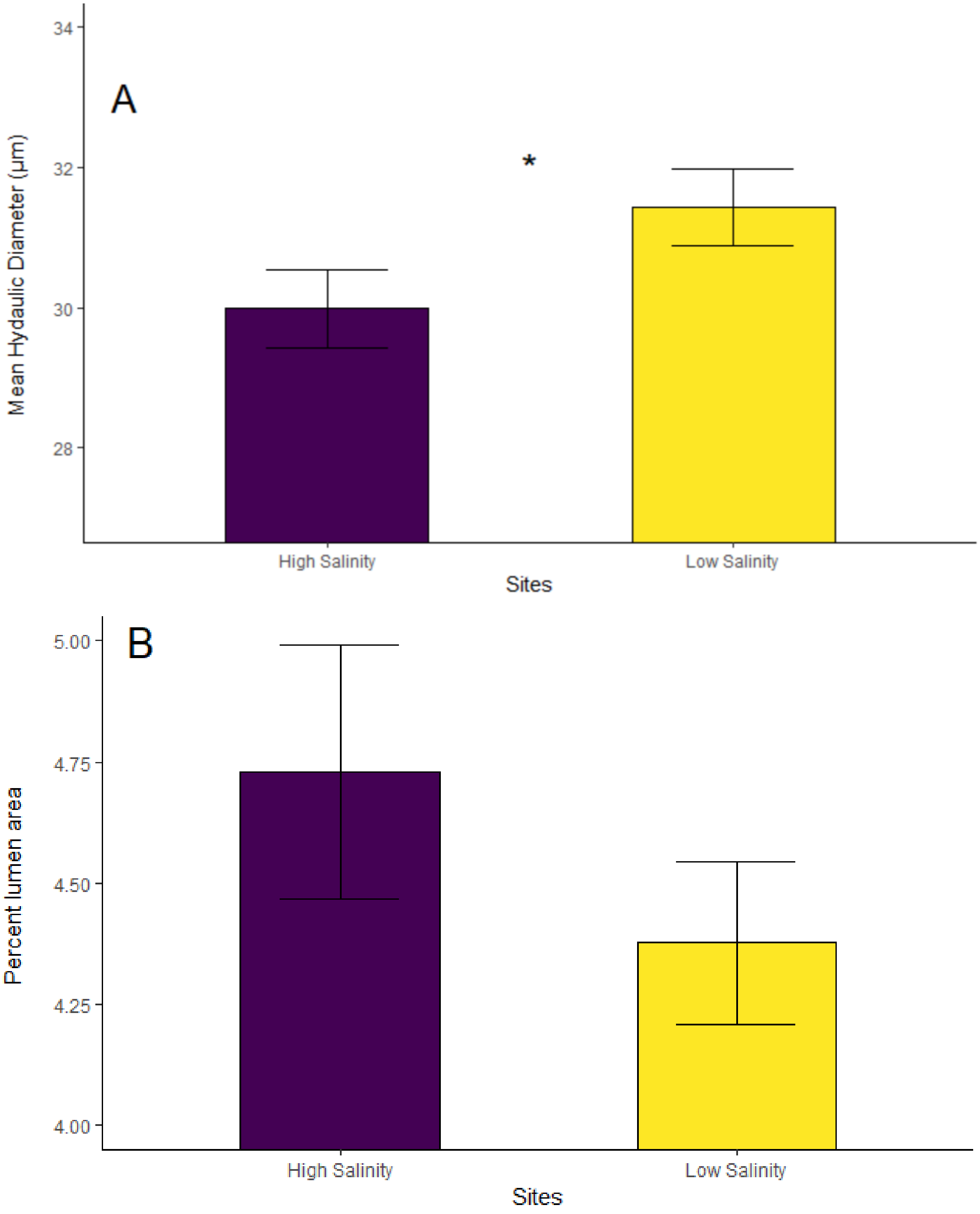
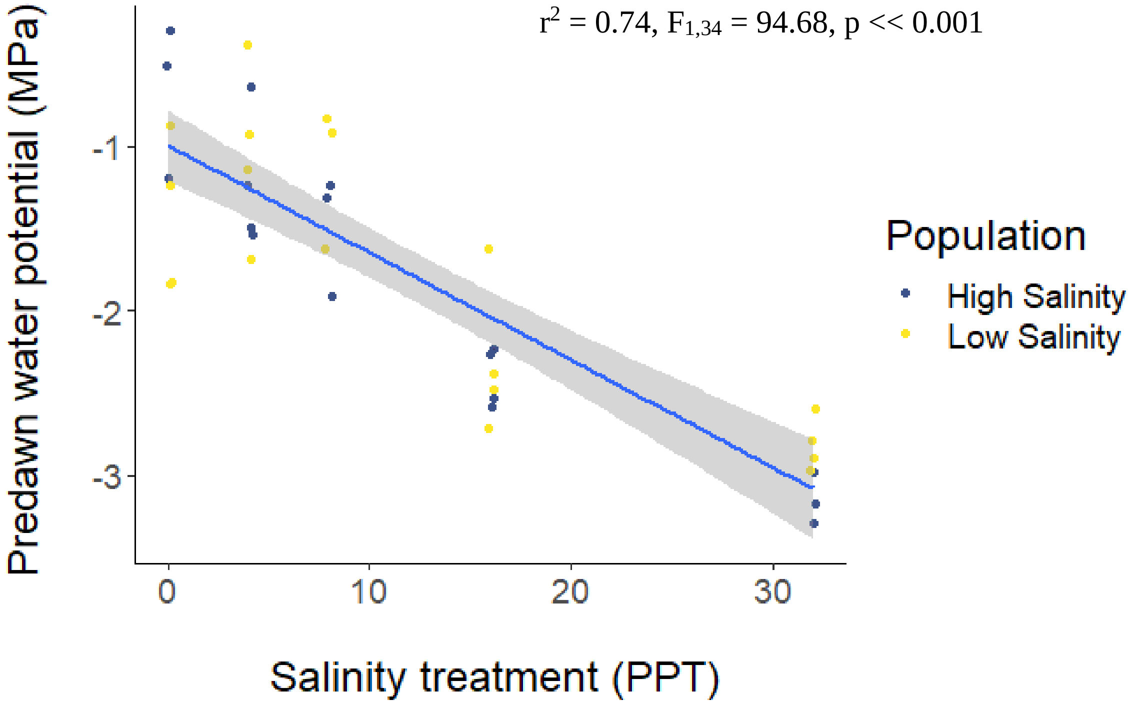
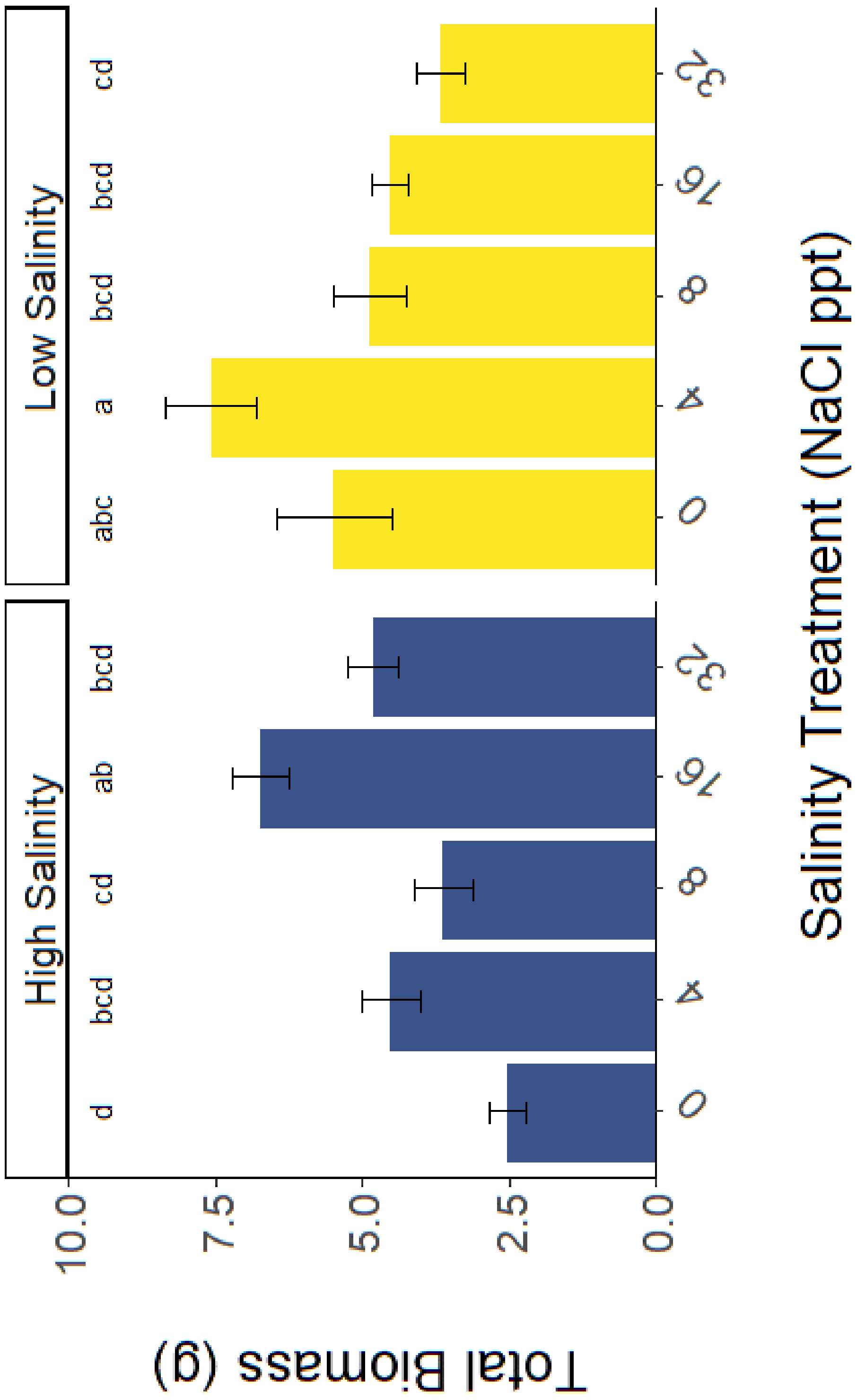

A
Mean Hydaulic Diameter (µm)
34
32
30
28
*
High Salinity
Low Salinity
Sites
B
Percent lumen area
5.00
4.75
4.50
4.25
4.00
High Salinity
Low Salinity
Sites

Fig 3.



$r^2 = 0.74$, $F_{1,34} = 94.68$, $p << 0.001$

Fig 4.



High Salinity
Low Salinity
d
bcd
cd
ab
bcd
abc
a
bcd
bcd
cd
Total Biomass (g)
10.0
7.5
5.0
2.5
0.0
0
4
8
16
32
0
4
8
16
32
Salinity Treatment (NaCl ppt)

Fig 5.

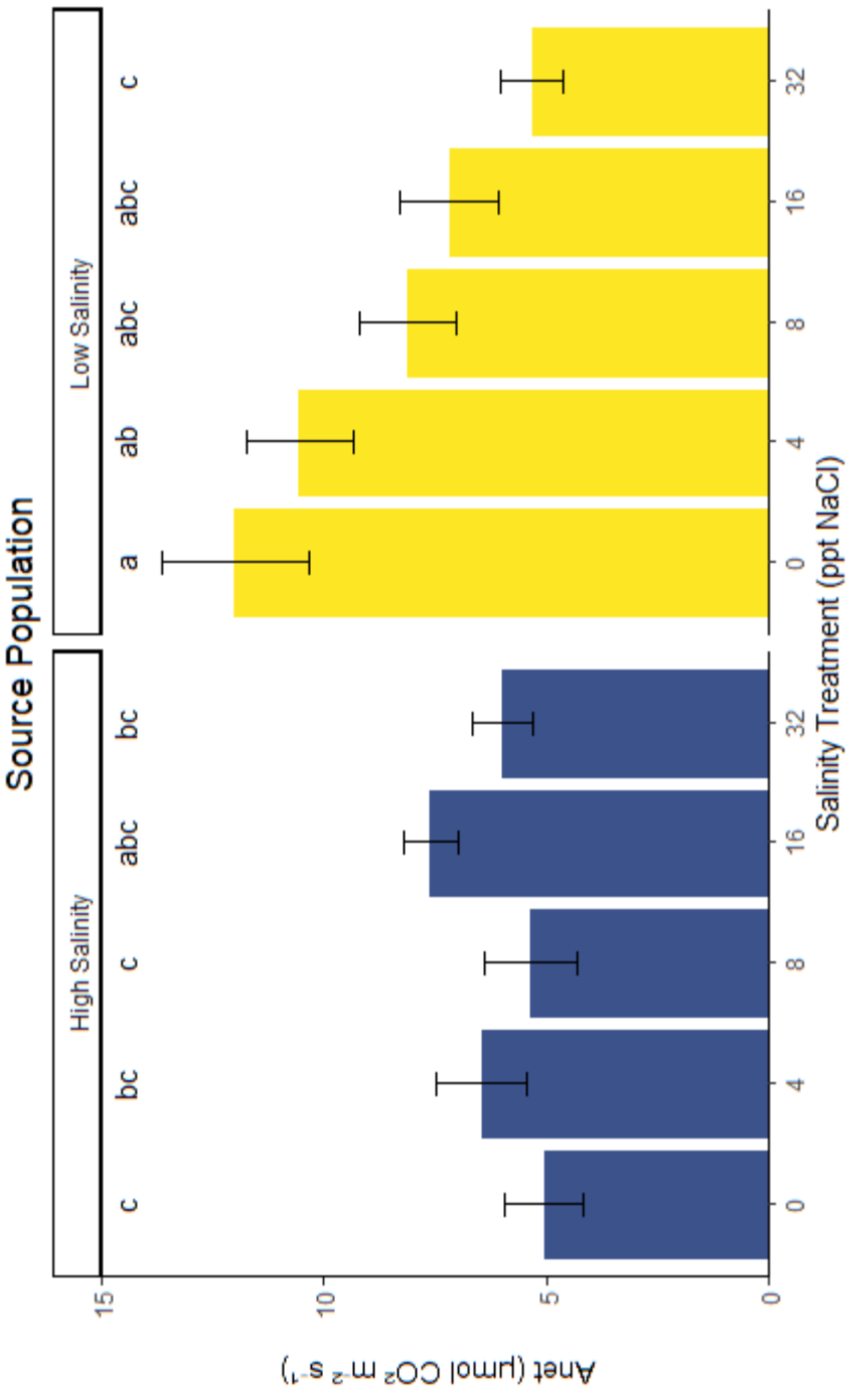

Source Population
High Salinity
Low Salinity
c
bc
c
abc
bc
a
ab
abc
abc
c
Anet (µmol CO2 m-2 s-1)
15
10
5
0
0
4
8
16
32
0
4
8
16
32
Salinity Treatment (ppt NaCl)

Fig 6.

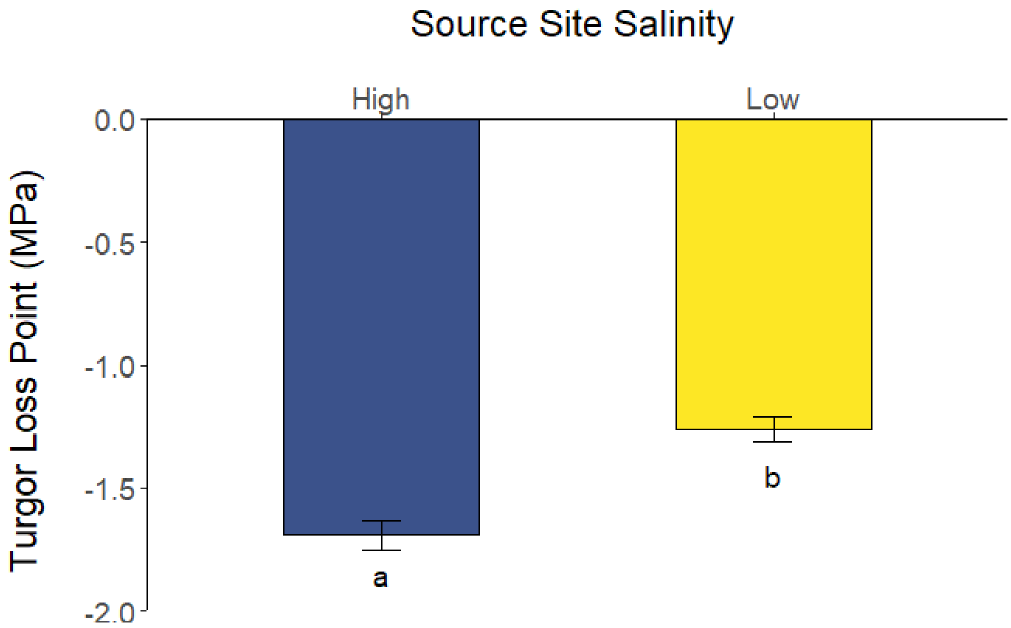

Source Site Salinity
High
Low
0.0
-0.5
-1.0
-1.5
-2.0
Turgor Loss Point (MPa)
a
b

Fig 7.

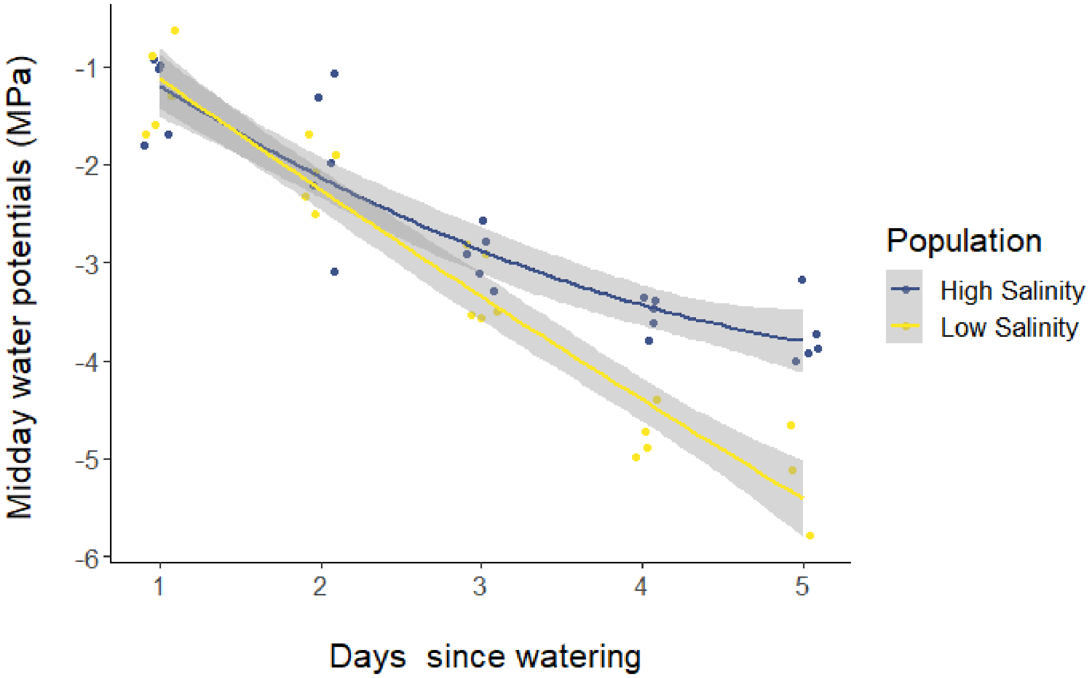
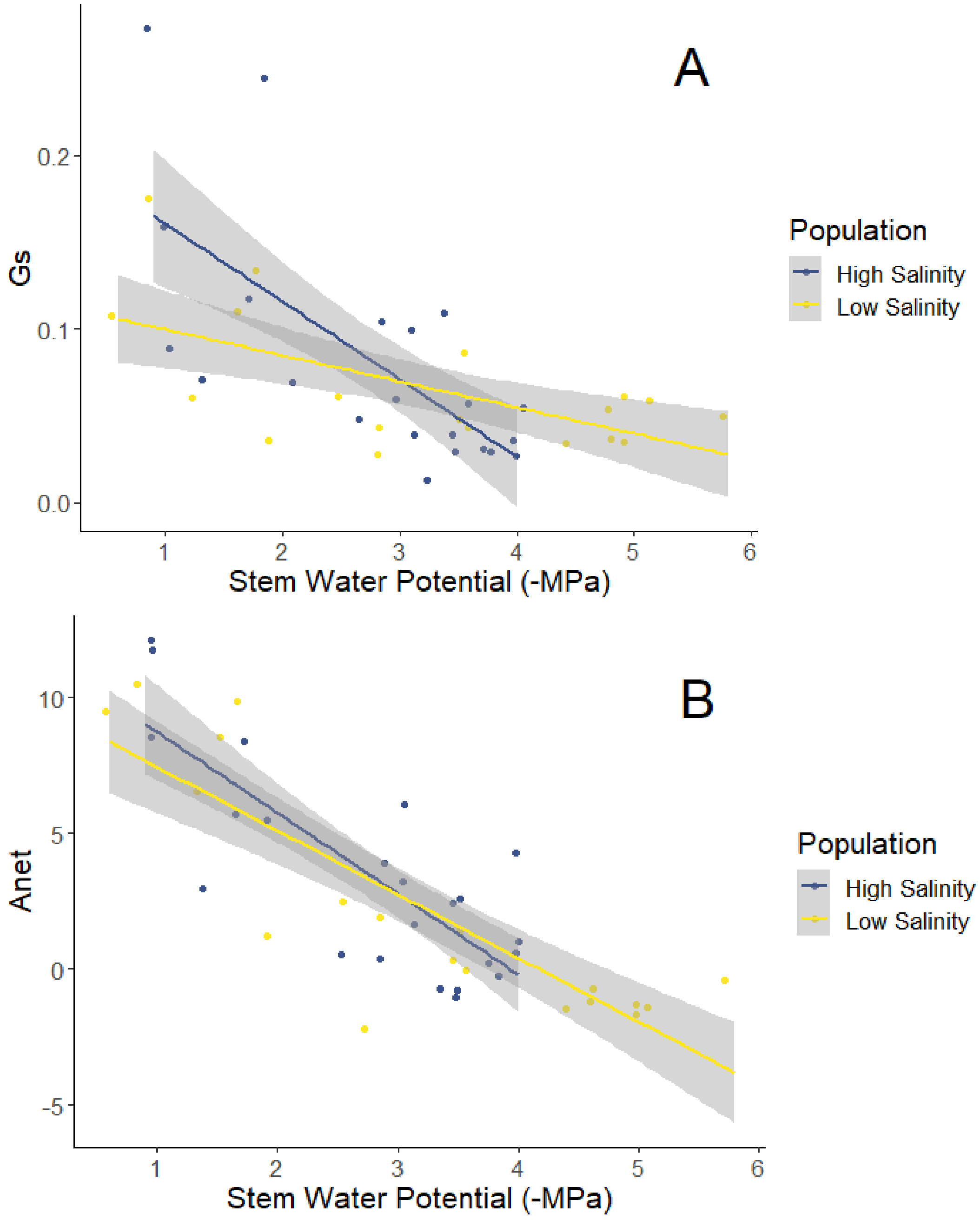
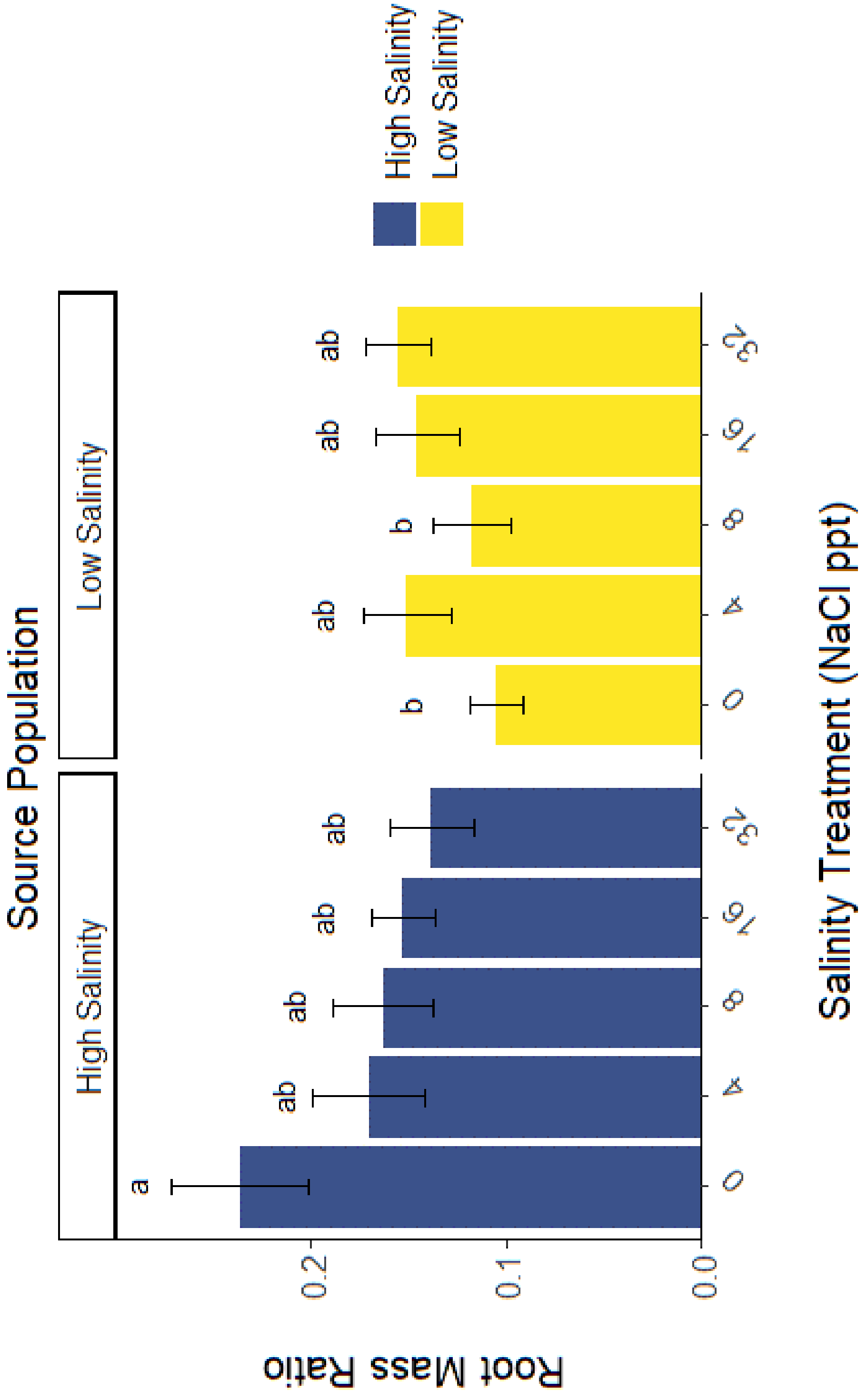

Midday water potentials (MPa)
-1
-2
-3
-4
-5
-6
1
2
3
4
5
Days since watering
Population
High Salinity
Low Salinity

Fig 8.


A
Gs
0.0
0.1
0.2
1
2
3
4
5
6
Stem Water Potential (-MPa)
Population
High Salinity
Low Salinity
B
Anet
-5
0
5
10
1
2
3
4
5
6
Stem Water Potential (-MPa)
Population
High Salinity
Low Salinity

Supplementary fig 1.



IV. Salinity driven interactions between plant growth and preference by a biological control agent

Abstract

Abiotic conditions can influence the effect that herbivores have on plant growth. Such biotic and abiotic interactions are of special interest in plant biological control programs because the goal of herbivore suppression of the target weed may not be reached in some abiotic settings. Because target invasive plants typically occur across large landscapes, there is likely some degree of local adaptation to specific conditions creating phenotypic variation to which control agents may respond. Here we used *Tamarix,* an invasive plant, and it's associated biological control agent, *Diorhabda carinulata,* to investigate how local variation in salinity and host phenotype influence interactions between the two species. To test if *Tamarix* was adapted to local conditions we collected plants from a low and a high salinity site and grew them through multiple clone generations and treated them with reciprocal salinity levels. We found the plants that accumulated the most biomass were those grown at salinities of their origin site, and that the agent, *D. carinulata* preferred those plants when given a choice against plants grown in the reciprocal salinity treatment. We further found that plants compensated for herbivory by regrowing foliage over three defoliation events, but they concomitantly had a reduction in basal area and 62% lower root biomass compared to the controls. Thus herbivory causes a shift in plant allocation and reduces root and stem investment. Overall, we saw that *D. carinulata* caused a 65% reduction in biomass in the plants sourced from the high salinity sites when grown at those same salinities. Plants from low salinity populations only exhibited a 32% reduction in

biomass when grown at their source salinity. Thus, the *Tamarix* biological program should focus on control in high salinity areas where the interaction between these two species and the abiotic environment are amplified.

Introduction

The effectiveness of weed biological control depends on the interactions between the invasive species, the imported herbivore, and the abiotic environment into which it is released (Heard and Winterton 2000). Biological control programs may be more successful locally when they act with other proximal stressors to influence plant growth or recovery from defoliation (Manion 1981; Cox and McEvoy 1983; Allen et al. 2010). Imposing stress from herbivory on top of environmental factors can lead to either additive or synergistic effects through any number of mechanisms (Cox and McEvoy 1983; Stamp 2003; Eyles et al. 2009). Yet, responses to stress may also increase resistance or tolerance to insect herbivory if synthesis of defense or storage compounds increases (Herms and Mattson, Jr. 1992). Thus, better predictions of plant responses to a biological control agent may be achieved by integrating variation in plant stress tolerance across populations, which may arise from local adaptation, with variation in insect herbivory.

Tamarix, a non-native woody plant, and its biological control agent, (*Diorhabda carinulata*) provide an ideal system to investigate population level variation in these biotic-abiotic interactions. *Tamarix* has been shown to be adapted to local conditions and express different phenotypic traits when grown in common garden experiments. Depending on the source site, phenotypes may have a higher freeze tolerance, allocate more biomass to belowground tissues, or break dormancy earlier in the spring (Sexton et al. 2002; Friedman et al. 2008; Long et al. 2017). Given these widespread differences in traits, it is likely that phenotypes adapted to high salinity also exist, in which plants from lower salinities have decreased growth and survival at sites with higher salinities. Plants from higher salinities may invest more in salt tolerant traits (e.g. more salt glands or vacuoules), but at the cost of reduced growth (Munns and Tester 2008; Tyerman et al. 2019). Conversely, without the high investment costs of increased salt tolerance low salinity phenotypes should exhibit higher growth rates when not influenced by salinity. It is unclear how these different phenotypes would interact with defoliation by *D. carinulata.*

D. carinulata (tamarisk leaf beetle) is a specialist on *Tamarix*, and was introduced by the United States Department of Agriculture (DeLoach et al. 2003). Since its release it has proven effective in extensive defoliation of *Tamarix* stands in many areas of the Western U.S. (Bean et al. 2012). Although *D. carinulata* will defoliate individual plants two or three times in a season, plant dieback and mortality varies among locations (Kennard et al. 2016), depending on variation in abiotic conditions, such as soil salinity (Hultine et al. 2015). Yet it is not known if *Tamarix* exhibits persistent population level variation in growth characteristics indicative of local adaptation to stressors.

To evaluate the interactions of salinity, plant phenotype, and beetle defoliation, we ask the following questions: 1) What preferences do *D. carinulata* show for *Tamarix* plants grown at different salinities?; 2) How do the impacts of herbivory on plant biomass vary for *Tamarix* plants grown under different salinities?; and 3) How do plant phenotype and salinity interact to affect herbivory impacts of *D. carinulata* on *Tamarix*? We developed a greenhouse experiment using *Tamarix* plants collected from a low and a high salinity site at the Cibola National Wildlife Refuge (CNWR) along the lower Colorado River in California where the salinity increases six-fold across 1.5 km. Plants collected from the two sites received reciprocal salinity treatments and then were subjected to either *D. carinulata* preference tests or to a repeated defoliation experiment. We tested the hypothesis that *D. carinulata* would prefer plants grown in lower salinities, and that herbivore effects on biomass will increase with salinity because high salinity should reduce the ability of plants to recover from defoliation. We also hypothesized that high salinity phenotypes would be less affected by herbivory at high salinities than those from low salinity sites. We predicted that 1) *D. carinulata* would preferentially feed on plants grown at low salinities, regardless of the source site; 2) plants in the herbivory treatments would have accumulated less total biomass, including regrowth foliage, at the end of the experiment compared to non-defoliated plants; and 3) plants from low salinity sites would show the greatest effect of herbivory on biomass in the high salinity treatments compared to all other herbivory x salinity combinations.

Materials and Methods

Source material

In March 2017, stem cuttings were collected from individuals at the Cibola National Wildlife Refuge (CNWR) in southeastern California, along the Colorado River. Two different populations were selected for use in this study based on previous work done at CNWR that identified a high and low salinity site (Nagler et al. 2008). Salinity near the river (200 m away) is approximately 2 ppt, while ground water salinity 1.5 km from the river is 12 ppt due to high evapotranspiration rates and low fresh water inputs (Nagler et al. 2008). To create stock plants for the herbivory experiments we collected stem cuttings from eight individuals per site, each between 40 and 50 cm in length and with a diameter of 15 to 20 mm at the base. The cuttings were rooted in tap water and then planted in a soilless peat mix. To minimize maternal site effects, cuttings were grown for four months and cuttings (10-12 cm in length, 7-12 mm in diameter) were then taken from these new plants to start a establish another round of new plants. Cuttings were treated with rooting hormones at a 5X dilution (Dip ‘N Grow, Athens, GA) and propagated in 100% perlite on a mist bench. This process was repeated twice so that the stock plants that provided the material for the greenhouse experiments had been grown in a greenhouse setting for over one year and been clonally propagated three times. Experimental plants were grown in pure sand, and bottom watered with a water soluble, all-purpose fertilizer (Peter’s 20-20-20, ICL Specialty Fertilizer’s Summerville, SC). To avoid salt accumulation in pots the plants were flushed by removing the pots from the reservoirs and adding enough of the same diluted fertilizer to achieve a steady flow of leachate from the bottoms of the pots. Greenhouse conditions were set to maintain daytime temperatures between 25 and 35 °C and nighttime temperatures between 25 and 30 °C. Artificial lighting was used to provide supplemental light up to 1600 PAR and 16 hours of daylight during the experiment year-round (6:00h – 22:00h)

D. carinulata adults were collected from just north of Needles, CA along the Lower Colorado River. At the time of collection in August of 2018, they were the closest population to where the plant material was sourced. The collected adults were kept in secure environmental chambers at the University of California, Santa Barbara (UCSB), where they laid eggs and we were able to maintain a viable population over multiple generations for herbivory experiments and choice trials outlined below. Beetles were kept in 57 L plastic tubs with a metal mesh lid and sand at the bottom for 3 months. Cages were cleaned weekly and beetles were fed *Tamarix* locally collected from the Santa Clara River in Santa Paula, California.

Salinity treatments

In March 2018, cuttings were taken from the stock plants in the greenhouse and after being grown for six months with only fertilizer and tap water they were divided into either a low (4 ppt) or high (16 ppt) salinity treatment. Sodium chloride was selected as the sole addition to adjust the salinity because Na^+ and Cl^- are the ions that contribute the most to the excess salinity in the lower Colorado River system (Ohmart et al. 1988). They are also the ions most often associated with growth reduction in plants (Greenway and Munns 1980). Cuttings were planted in 2.25 L treepots (Stuewe & Sons, Tangent, OR) in sand and bottom watered with the salinity treatment made with rock salt (99.9% NaCl, Diamond Crystal, Savannah, GA) and a balanced nutrient solution at a rate of 200 ppm Nitrogen (Peter's 20-20-20, ICL Specialty Fertilizer's Summerville, SC). Plants were flushed with the same salt/nutrient solution once per week to leach out any accumulating salts.

No choice and preference experiments

We first used a no choice trial to test whether salinity or phenotype alone would affect beetle behavior on a plant. One plant was placed inside a 1 m x 1 m x 1 m mesh cage with lid, and one beetle was placed on the foliage of the plant. This was repeated a total of 16 times for each phenotype x salinity treatment for a total of 64 trials, using a new beetle for each trial. Each beetle was observed for a total of 30 minutes while recording its location (on or off the plant) at intervals of 1, 2, 3, 6.5, 10, 15, 20, 25, and 30 minutes.

Based on the preliminary results from the no choice test indicating no behavioral differences (Fig 1a) on individual plants, we conducted preference trials to determine if beetle behavior would change when given two different phenotypes treated with the same salinity. Beetles were again observed for 30 minutes, and their location was recorded at the end of the period. For this experiment, two plants were placed in opposite diagonal corners within the mesh cage and one beetle was placed on the foliage where the two plants touched. The two plants within the cage were always a plant from a high salinity population and one from a low salinity population, both plants in the cage had been given the same low or high salinity treatment. Eighteen trials were completed both treatment levels.

Herbivory experiments

To examine the interaction of salinity, herbivory and population source we used *D. carinulata* beetles to defoliate greenhouse grown *Tamarix* plants. For the herbivory experiments we grew a different set of plants than those used in the preferences trials, but from the same stock plants collected from CNWR. This set had been grown under the low (4 ppt) or high (16 ppt) salinity treatments outlined above and then distributed to control and herbivory treatments. One individual was used from each of the original eight plants collected from CNWR in each herbivory x salinity x population source combination. The first beetle defoliation event occurred on 31 August 2018, in which 25 adults and 25 larvae were placed into mesh bags enclosing individual plants. Mesh bags with no beetles were placed on the control plants to maintain similar ambient conditions. The mesh bags were kept in place for two weeks to allow for full defoliation of the plants. After two weeks the mesh bags were removed, and any foliage litter that accumulated in the bags was separated from beetle carcasses and collected. The plants were then allowed to grow for 5 weeks before another herbivory treatment was applied. This was repeated one more time for a total of three defoliation events (Defoliation events occurred from 1 August -14 September 2018, 26 October - 9 November 2018, and 14-28 December 2018). We used the foliage that was collected in the mesh bags as an approximation for total foliage biomass that was accumulated before the beetles were introduced to the plants. We felt this method approximated the dry biomass that was produced due to the feeding method of *D. carinulata.* Instead of consuming the entire tissue they often scrape away part of the epidermis and cause rapid desiccation which results in foliage senescence and large amounts of litter accumulation. On 28 December the plants were harvested, including belowground biomass. The sand and root systems were removed from the pots and soaked in water for four hours, during which time the sand sank to the bottom of the containers and the roots floated to the top. The water solution was agitated halfway through the soaking period to help release fine roots from the sand particles. All plant material was dried for 48 hours at 60° C prior to obtaining dry weights. Root mass ratio (RMR), which indicates the degree of resource allocation to roots (Lambers et al. 2008), was calculated as:

$$RMR \quad = \frac{(Dry\ mass\ of\ roots)}{(Total\ mass\ of\ plant)} \tag{1}$$

Basal diameter was recorded at the beginning of the experiment, before the first defoliation event (14 September 2017) and after the last defoliation event (28 December 2017). Given that *Tamarix* stems tend to be oblong, the basal diameter was measured by taking the average of two orthogonal measurements using calipers 5 cm above the soil line. Basal area increments (BAI; mm d^{-1}) were calculated according to (Lambers et al. 2008) where A_{bf} is the final measured basal area, A_{bi} is the initial basal area and d is the number of days between A_{bf} and A_{bi}:

$$BAI = \frac{A_{bf} - A_{bi}}{d} \tag{2}$$

Statistical Analyses

Statistical analysis was completed using the program RStudio (Version 1.2.5033, R Core Team 2019) using the ‘stat’ package (R Core Team 2019), except for the MANOVA which used the ‘dplyr’ package (), and the post hoc analyses which were completed with the ‘agricolae’ package (De Mendiburu 2009). Differences in the no choice trials were completed using a two-way ANOVA, comparing the amount of time spent on plants in low versus high salinity treatments, the low versus high salinity phenotypes, and the interaction between them. The preference trials were analyzed using a chi-squared test on a contingency table of observed versus expected values assuming no difference based on our no choice trials. A repeated measures analysis was completed using an ANOVA with time as an error factor. This was completed to account for observations over multiple time periods.

Biomass data from the herbivory experiments were first analyzed with a MANOVA on the effects and interactions of salinity, herbivory, and phenotype on total biomass and then on the three different plant organs separately (foliage, stems, and roots), investigating the effects and interactions of the same variables. When interactions were present interaction plots were used to determine if the interactions were ordinal or disordinal. Interactions were considered disordinal when the group with the highest mean changed over the treatment levels. Post hoc analysis of the tsignificant interactions was conducted using Tukey’s Honest Significant Differences from the ‘agricolae’ package (De Mendiburu 2009). For ordinal interactions post hoc analysis was completed on the main effects, when disordinal interactions were present analysis was only completed on the interactions.

Results

No choice and preference trials

In no choice trials, we found no significant differences between the time that beetles spent on plants regardless of salinity treatment ($F_{1, 58} = 0.66$, $p = 0.42$, Fig 1a) or plant phenotype ($F_{1, 58} = 1.61$, $p = 0.21$, Fig 1a). However, when given the choice between plant phenotypes that were treated with low or high salinity, we found that beetles did show nonrandom preferences ($\chi2(2) = 20.67$, $p << 0.001$, $\alpha = 0.05$, Fig 1b). After time was accounted for in the preference trial using a repeated measures ANOVA, treatment and phenotype (pop. source) still both had significant effects on the beetle choice (Treatment: $F_{1, 346} = 19.34$, $p < 0.001$, Phenotype: $F_{1, 346} = 12.54$, $p < 0.001$) A posthoc analysis of the standardized residuals from the chi-squared revealed that the beetles preferred foliage from low salinity phenotypes grown in low salinity. In the low salinity treatment, beetles were 74% more likely to be on the plants from the low salinity site compared to those originating from the high salinity site. For plants grown under high salinity, the beetles were 58% more likely to be on plants collected from the high salinity site than from the low salinity site. In summary, beetles preferred plants grown in the salinity treatment most like the site where the plant population had originated. They did not consistently prefer low or high salinity phenotypes: their preference depended on the interaction between phenotype and salinity treatment.

Herbivory experiments

We found that for total dry biomass, which included the foliage that senesced due to beetle feeding damage, there was a three-way interaction that reflected an uneven influence of beetles on plant biomass depending on both salinity and population source. Plants sourced from the high salinity site had the greatest reduction from herbivory when grown in the high salinity treatment ($F_{1, 56} = 7.21$, $p = 0.0095$, Table 1, Fig 2a). The total biomass was unchanged by defoliation for that same population when grown at low salinity, but they were also the smallest plants without defoliation. Beetle herbivory had a significant main effect on total biomass with controls yielding 74% more total biomass compared to defoliated plants ($F_{1, 56} = 20.34$, $p < 0.0001$, Fig 2a). Without defoliation, plants performed best in salinities similar to their source site; with the high salinity phenotypes being the largest in the high salinity treatment and the smallest in the low salinity treatment, but this pattern was reversed for the low salinity source population ($F_{1,56} = 27.16$, $p < 0.001$, Fig 2a). Considering only salinity treatment and not plant phenotype, defoliation resulted in a reduction of 57% in total biomass of plants grown in high salinity, versus a reduction of only 21% in plants grown at low salinity ($F_{1,56} = 7.16$, $p = 0.0098$, Fig 2a).

Allocation to the three measured plant structural components was affected by the defoliation and salinity treatments (Table 1). Defoliation caused a significant reduction of total stem biomass ($F_{1,56} = 12.94$, $p < 0.001$, Fig 2b) as well as a 62% reduction in roots compared to the controls ($F_{1,56} = 7.42$, $p = 0.0086$, Fig 2d). Foliage production, which included regrowth between treatments, was increased by the beetles, with the defoliation treatments producing on average 18% more foliage compared to the controls ($F_{1,56} = 3.70$, $p = 0.06$, Fig 2c). This increase happened in both high and low salinity phenotypes but was mainly driven by plants of either type grown in the low salinity treatments where we saw an increase of 68% in foliage production. All three structural components were similarly affected by the interaction of salinity treatment and source site, showing the same pattern of increased biomass at the salinity of the origin site (Stems, $F_{1,56} = 12.49$, $p < 0.001$, Fig 2b;

Foliage, $F_{1,56} = 46.50$, $p < 0.001$, Fig 2c; Roots, $F_{1,56} = 8.11$, $p = 0.0062$, Fig 2d). We also found that there was a significant shift in root mass ratio (RMR) with beetle defoliation ($t = 6.65$, df = 59.27, p-value < 0.001), with plants in the control group having 50% higher RMR than those in the defoliation treatments. There was no effect of treatment or population on RMR. Basal area increment (BAI; mm d^{-1}) was also negatively affected by beetle defoliation ($t = 2.38$, df = 60.5, p-value = 0.01, Fig 4), with the control group having a positive BAI and the defoliation group having a negative BAI.

Discussion

Herbivory experiments

As expected, we found that beetles caused a reduction in total plant biomass, despite regrowth of foliage in between defoliation events. Thus, plants cannot continue to full compensate when they are repeatedly defoliated: after three complete defoliations, the control plants had accumulated 74% more total dry biomass than the defoliated plants. While we found that source population had a strong influence on total biomass when in combination with salinity, results were opposite of our expectations. We had predicted that low salinity phenotypes would be the most impacted by herbivory at high salinities, yet we found that the high salinity plants were most susceptible in that treatment, mainly witnessed as decreases in root and stem biomass. In our low salinity treatments these decreases were concomitant with an increase in production of foliage, with plants producing more total foliage over the experiment.

This increase in foliage production after injury is known as a compensatory response, where plants adjust their resource allocation to growth or reproduction from other carbon demands to maintain (or increase) individual fitness compared to controls (Belsky 1986; Trumble et al. 1993; Hawkes and Sullivan 2001). These types of responses are predicted to exist along a continuum from partial-, to exact-, to overcompensation (*sensu* Belsky and Hawkes**).** Overcompensation occurs when the cumulative total dry weight, including the regrowth between defoliations, is greater in herbivore treatments than in the controls. In our low salinity treatments we saw evidence for this, but in our high salinity treatments we saw either exact or partial compensation. Our results support the continuum of responses model (Maschinski and Whitham 1989) that predicts overcompensation to occur in areas of higher resources, with our low salinity treatments having higher water availability. There has been mixed support for this hypothesis, with the results of a meta-analysis showing monocots generally overcompensating in high resource environments, but woody plants overcompensating only 42% of the time in high resource conditions (Hawkes and Sullivan 2001). Evidence for overcompensation with high resources in the *Tamarix-D. carinulata* system may be due to some key differences between the physiology of *Tamarix* compared to many woody species used in the meta-analysis. *Tamarix* is known to have high resprouting abilities and maintains levels of carbohydrate storage to support regrowth after fires or other disturbances, including herbivory (Hudgeons et al. 2007; Drus et al. 2014). Woody plants with higher resource levels are expected to more rapidly replenish those carbohydrate stores following defoliation events (Dietze et al. 2014), which could lead to sufficient in plant resources to support regrowth. However, after our three defoliation events we saw that root biomass had decreased in all of our plants so it is unclear if overcompensation in above ground growth would have continued in our low salinity populations with more defoliation events.

We did find different plant responses to salinity between the two populations, with there being an interaction between phenotype and salinity treatments. Without defoliation, plants grown at salinities most consistent with their source site had increased biomass accumulation compared to those under the reciprocal treatments. *Tamarix*, like other halophtyes (salt tolerant species), express a set of diverse adaptations that allow them to survive and outcompete other species in the conditions (Flowers and Colmer 2008). These adaptations can generally be categorized as salt exclusion, compartmentation, or exudation; and each adaptation has unique costs or tradeoffs (Munns and Tester 2008). Exclusion prevents salts from being transported into the xylem stream but is only efficient in low to moderate salinity environments with freshwater inputs, whereby the salts excluded from the plant will be flushed away from the roots. Compartmentation allows salts to enter the xylem stream and then storing the salts in specialized vacuoles within leaf tissue to prevent toxicity to surrounding cells; eventually the leaves are shed and the salt is removed from the plant. Exudation, which is witnessed in true halophytes have specialized cells that transfer salt to the outside of leaf tissues, either via trichomes (*Atriplex patula*) or specialized salt glands (*Tamarix spp., Avicennia germinas*) (Gonçalves-Alvim et al. 2001; Rand 2002; Lambers et al. 2008). There is a metabolic cost with both compartmentation and salt glands as ATP is required to move ions across a semi permeable membrane (Tyerman et al. 2019). This cost may result in decreased growth, reproduction, and/or allocation to defense compounds (Glenn et al. 1998; Qi et al. 2018). However, salt accumulation in and on the exterior of plants can serve a defensive function through increased toxic levels of Na and Cl, and salt crust on leaves may also may indirectly act as a deterrent to herbivores if more palatable plants or plant parts are readily available (Larsson 1989; Renault et al. 2016).

Herbivore preferences

We had hypothesized that salt exudation might provide a possible defense and thus had predicted that beetles would always prefer plants that were grown at low salinities. Previous work on native halophytes with specialist herbivores supported this prediction, with either a reduction in number of gall making insects (Moon and Stiling 2002; Albarracin and Stiling 2006) or leaf miners (Hemminga and Vansoelen 1992; Schile and Mopper 2006) when plants are grown under increased salinity. By contrast, our results indicate that when beetles were given a choice between *Tamarix* grown under different conditions, they displayed a preference for plants that were grown under conditions similar to their home site; that is, they favored saline-origin plants grown under high salinity. Historically, environment was believed to be more important than plant phenoptype in herbivore performance (Davidson and Andrewartha 1948; White 1978; Björkman et al. 1991), but similar results to ours have been witnessed in other studies that found that herbivores performed best on local genotypes grown in common gardens (Fritz 1990; Strauss 1990; Stiling and Rossi 1996; Barker et al. 2019). In one system, which used the halophyte *Borrichia frutescens,* increased gall frequency was found on "better quality" plants, those with higher growth rates and increased nitrogen concentrations (Rossi and Stiling 1998). In our choice experiments the plants that beetles preferred were the largest plants in our herbivory experiment. As compared to other genotype by environment studies on herbivore performance, ours and the *B. frutescens* work is unusual in examining halophyte-herbivore interactions. Despite showing preference for the correct phenotype-salinity match, we found in our no choice experiment that beetles would still feed on plants grown in suboptimal conditions when no other food source was available. This indicates that the beetles are likely to feed on any *Tamarix* plants if population densities are high enough, despite the finding of preference for certain genotypes in lab settings (Williams et al. 2014). *In situ* observations of beetle defoliation show similar behavior, with beetles feeding on all available *Tamarix* (Pattison et al. 2011, pers. obs.), and causing massive defoliation across populations in "swarm" events even though they may show some preference when densities are low (Moran et al. 2009).

Conclusions

The complex interaction between salinity, phenotype, and herbivory may explain the variability in regrowth and mortality witnessed across *Tamarix* populations where *D. carinulata* has been introduced (Hultine et al. 2015; Kennard et al. 2016). *Tamarix* appears to express adaptive traits specific to site salinity, with phenotypes performing best at the salinity most similar to their collection sites. Despite being adapted to local conditions, the high salinity phenotypes in the high salinity treatment were most affected by defoliation. This is consistent with the observation *in situ* that the greatest dieback has occurred at sites with high soil salinity (Hultine et al. 2015). The reduced allocation to root biomass in the defoliated plants is likely to have even greater impacts in areas with reduced water availability due to high salinization or dropping water tables with increasing droughts in the southwest (Capon et al. 2013).

Our results have two implications for land managers and the biological control program for *Tamarix*. First, plant populations appear to be adapted to local salinities and perform poorly when grown under reciprocal salinity conditions. Reducing soil salinity in saline sites should reduce the growth of *Tamarix*, however this task would require large amounts of fresh water input and diversion from human uses (Erwin 2009). A second, and more practical, implication is that eradication and restoration efforts should focus on high salinity sites since plants from those sites were most impacted in our herbivory experiments, showing a 65% reduction is total biomass. Without active restoration plans in place at these sites secondary invasion by other noxious weeds, such as *Acroptilon repens* or *Bromus tectorum* are likely to occur concurrently with increased canopy dieback (González et al. 2017). Plants in high salinity sites have already shown higher amounts of dieback at sites where beetle defoliation has begun to occur (Hultine et al. 2015). Low salinity sites should be given lower priority since the defoliation treatments reduced their biomass by only 32%. Our study reveals that although plants are regrowing after defoliation, they are doing so at the cost of reduced allocation to stem and belowground growth. Thus, biological control in high salinity sites may be more likely to achieve the desired outcome of reduction of *Tamarix* more rapidly than at lower salinity sites.

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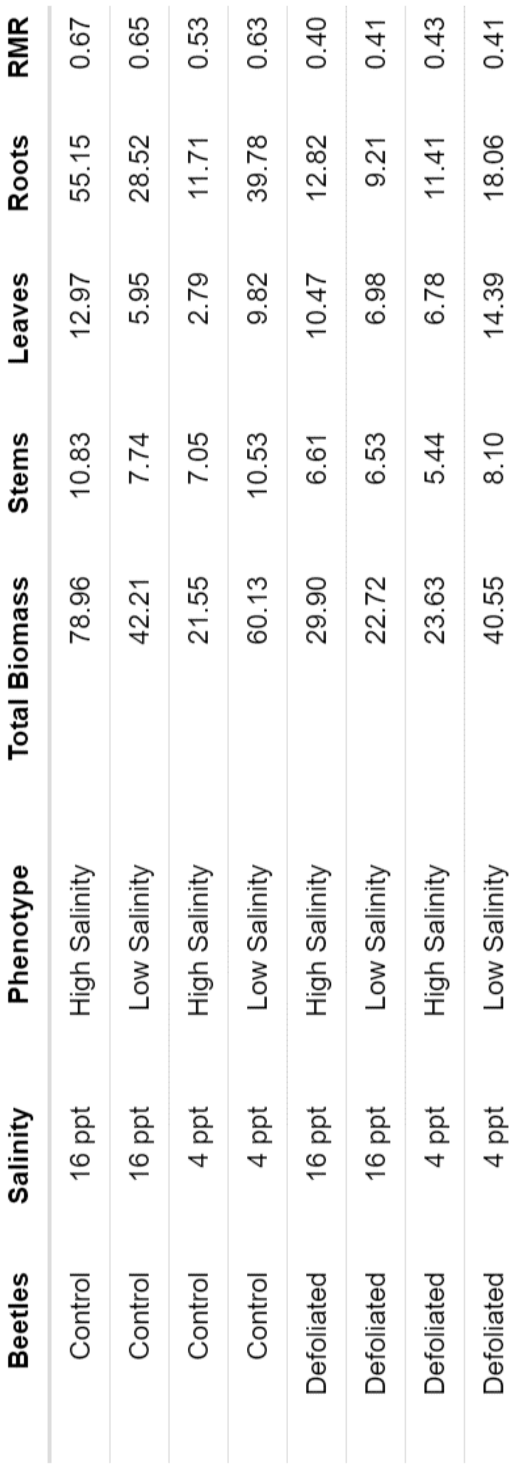
Table

Table 1. Mean dry biomass (g) and root mass ratio (RMR) results from the defoliation experiment on populations sourced from two different salinities grown under reciprocal salinity treatments



Beetles	Salinity	Phenotype	Total Biomass	Stems	Leaves	Roots	RMR
Control	16 ppt	High Salinity	78.96	10.83	12.97	55.15	0.67
Control	16 ppt	Low Salinity	42.21	7.74	5.95	28.52	0.65
Control	4 ppt	High Salinity	21.55	7.05	2.79	11.71	0.53
Control	4 ppt	Low Salinity	60.13	10.53	9.82	39.78	0.63
Defoliated	16 ppt	High Salinity	29.90	6.61	10.47	12.82	0.40
Defoliated	16 ppt	Low Salinity	22.72	6.53	6.98	9.21	0.41
Defoliated	4 ppt	High Salinity	23.63	5.44	6.78	11.41	0.43
Defoliated	4 ppt	Low Salinity	40.55	8.10	14.39	18.06	0.41

Figure captions

Fig 1. Beetle preference in two different behavior experiments. A) Beetles showed no difference in the time spent on plants when given only one plant to feed on in a cage. Plants from each phenotype were treated with salinity like that found at their source site and the reciprocal salinity (n = 16 for each combination). Neither salinity treatment nor phenotype influenced beetle behavior. Bars are +/- standard error of the mean. B) Location of beetles when given a choice between two different phenotypes treated with the same salinity. Beetles were recorded as either on one of the two phenotypes or as neither if they left the plants. Beetles showed a nonrandom choice to select plants grown in a salinity most like the source site (n = 18 for each treatment level).

Fig 2. Dry biomass accumulation from *D. carinulata* defoliation x salinity treatments. Plants from high and low salinity sites were grown in a greenhouse at either 4 or 16 ppt and subjected to three defoliation events by *D. carinulata*, and allowed to grow for five weeks after each event (n = 8 for each salinity x phenotype x defoliation combination) A) The total dry biomass of stems, live foliage tissue, collected foliage litter, and roots. Defoliation resulted in a decrease in total biomass, except in the high salinity phenotype grown at 4 ppt. In the control treatments, plants grew best when the phenotype matched the salinity treatment. B) The total stem biomass decreased with defoliation in all of the salinity x phenotype combinations. C) The total collected foliage biomass, which included the foliage litter collected in mesh bags after each defoliation event plus the biomass at the end of the experiment. Foliage biomass decreased with defoliation in the high salinity treatments but was higher in the low salinity treatments. D) Total root biomass was obtained after separating roots from sand substrate they were grown in for the experiment. There was an overall decrease in root biomass, except in the high salinity phenotype grown at 4 ppt. Error bars are +/- standard errors of the mean, different letters are results from a posthoc Tukeys HSD of $p = 0.05$.

Figure 3. Root mass ratio of plants in defoliation experiments, high and low salinity phenotypes were combined (n = 16 for each treatment). Control plants had larger root masses for the same amount of stem and foliage tissue. Horizontal line is the median, boxes and whiskers are each one quartile. Significant difference of $p < 0.001$ between treatments is denoted by ***.

Figure 4. Basal area increments in defoliation experiments, high and low salinity phenotypes were combined (n = 16 for each treatment). Control plants basal areas increased over the experiment, while the defoliated plants saw a reduction in basal area. Significant difference of $p < 0.01$ between treatments is denoted by **.

Fig 1.

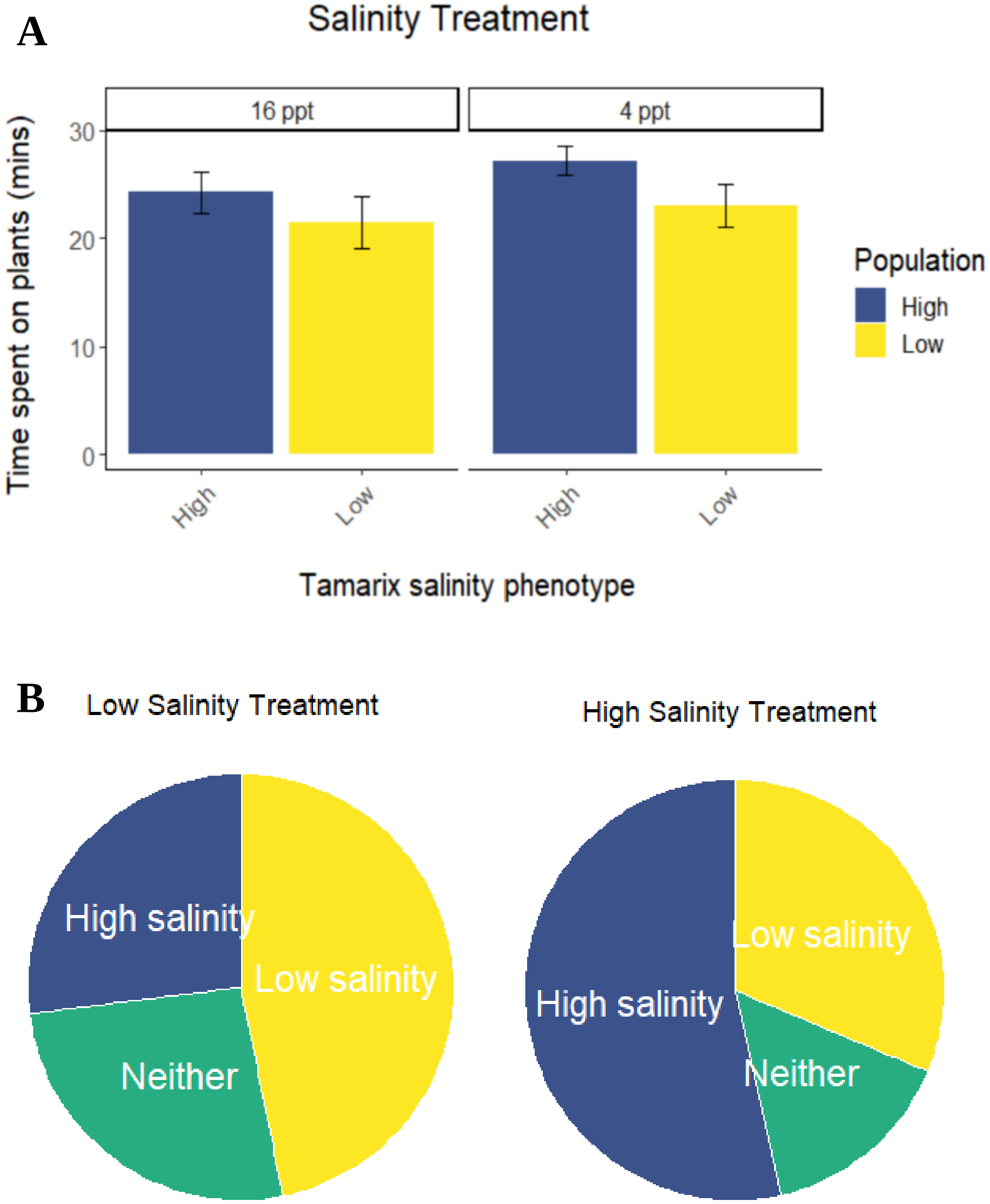

A
Salinity Treatment
16 ppt
4 ppt
Time spent on plants (mins)
0
10
20
30
High
Low
High
Low
Tamarix salinity phenotype
Population
High
Low
B
Low Salinity Treatment
High salinity
Low salinity
Neither
High Salinity Treatment
Low salinity
High salinity
Neither

Fig 2.

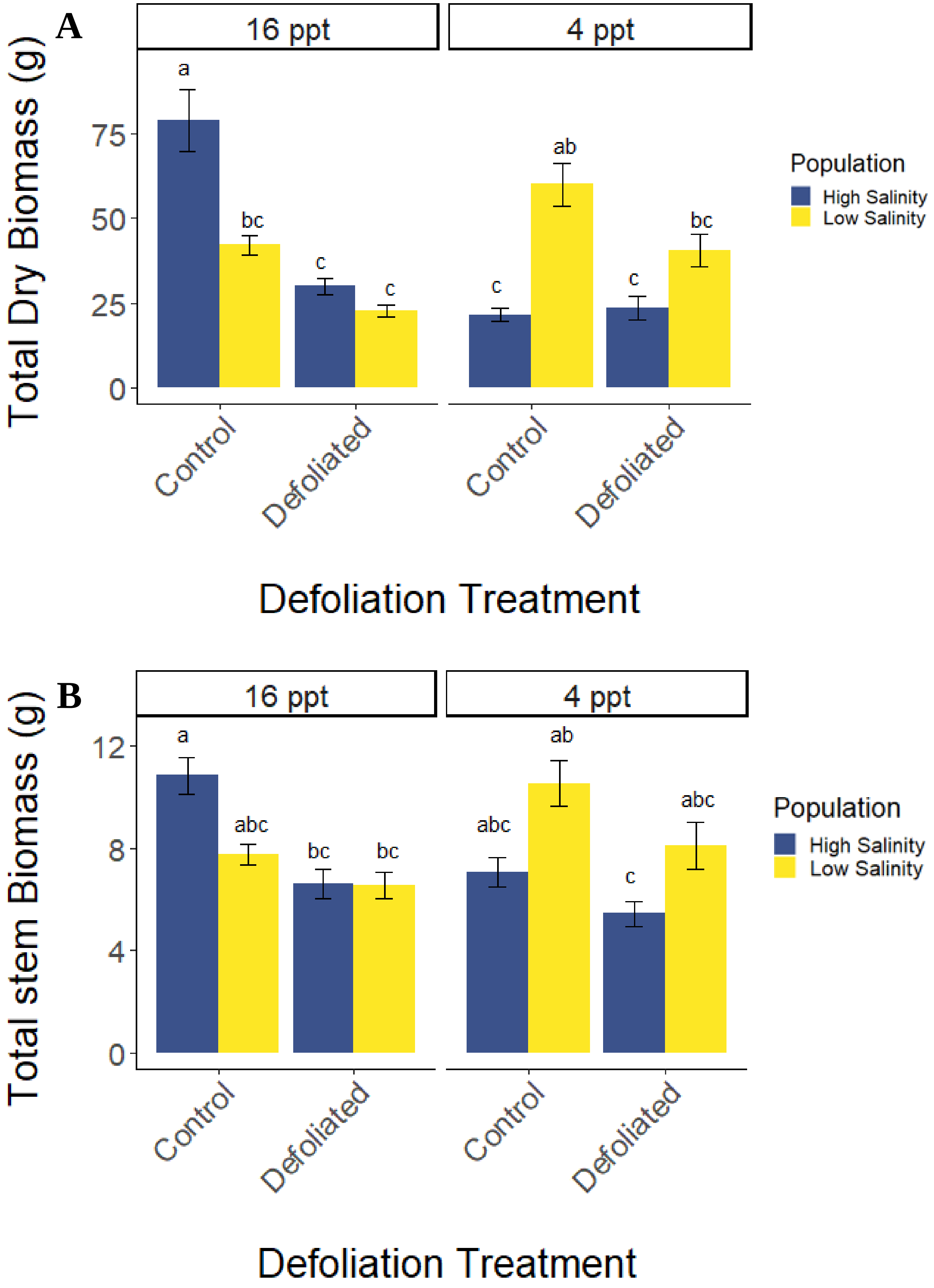

A
16 ppt
4 ppt
Total Dry Biomass (g)
0
25
50
75
a
bc
c
c
c
ab
c
bc
Control
Defoliated
Control
Defoliated
Defoliation Treatment
Population
🟦 High Salinity
🟨 Low Salinity
B
16 ppt
4 ppt
Total stem Biomass (g)
0
4
8
12
a
abc
bc
bc
abc
ab
c
abc
Control
Defoliated
Control
Defoliated
Defoliation Treatment
Population
🟦 High Salinity
🟨 Low Salinity

Fig 2(cont).

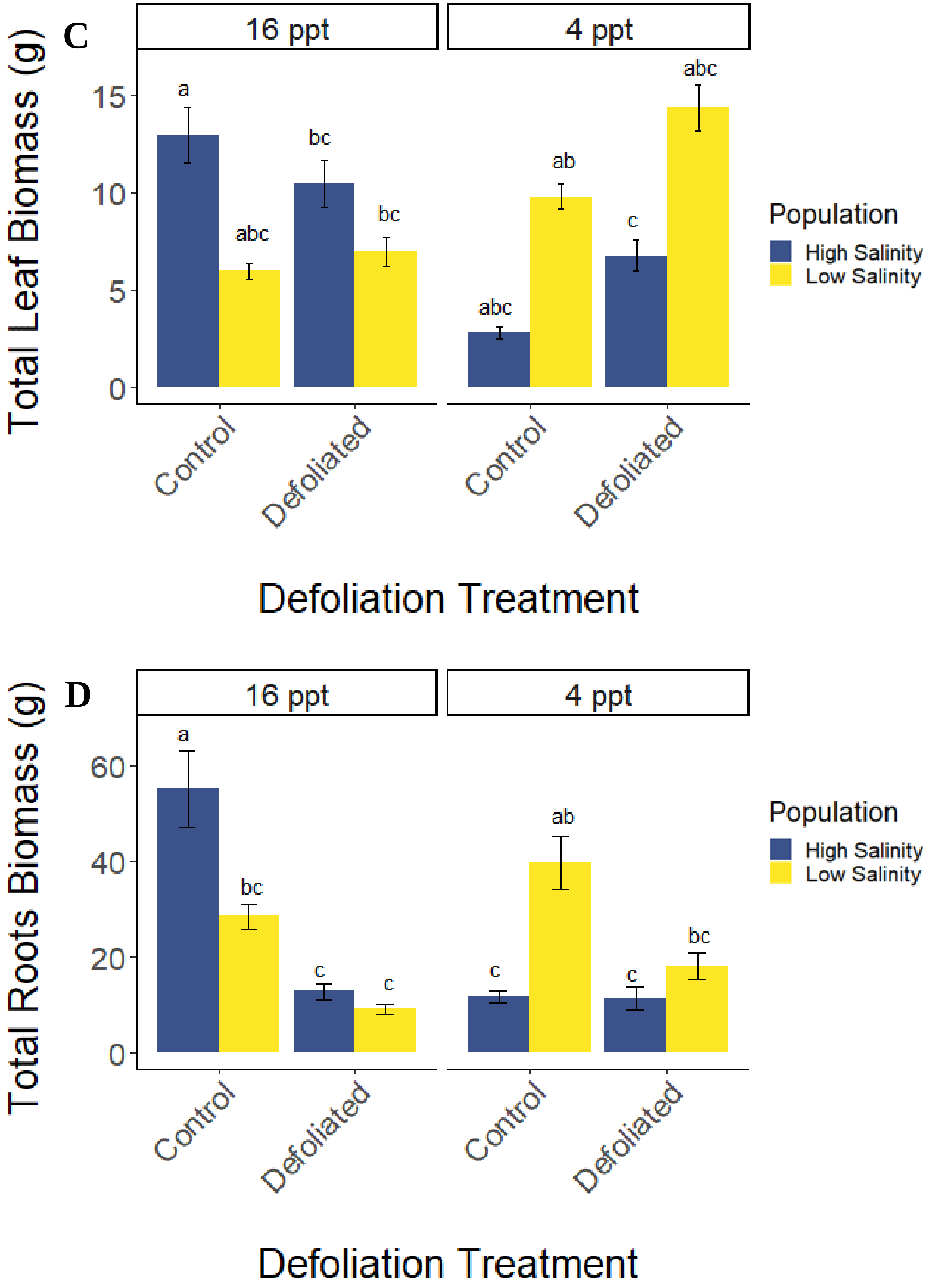

C
16 ppt
4 ppt
Total Leaf Biomass (g)
0
5
10
15
a
abc
bc
bc
abc
ab
c
abc
Control
Defoliated
Control
Defoliated
Defoliation Treatment
Population
High Salinity
Low Salinity
D
16 ppt
4 ppt
Total Roots Biomass (g)
0
20
40
60
a
bc
c
c
c
ab
c
bc
Control
Defoliated
Control
Defoliated
Defoliation Treatment
Population
High Salinity
Low Salinity

Fig 3.

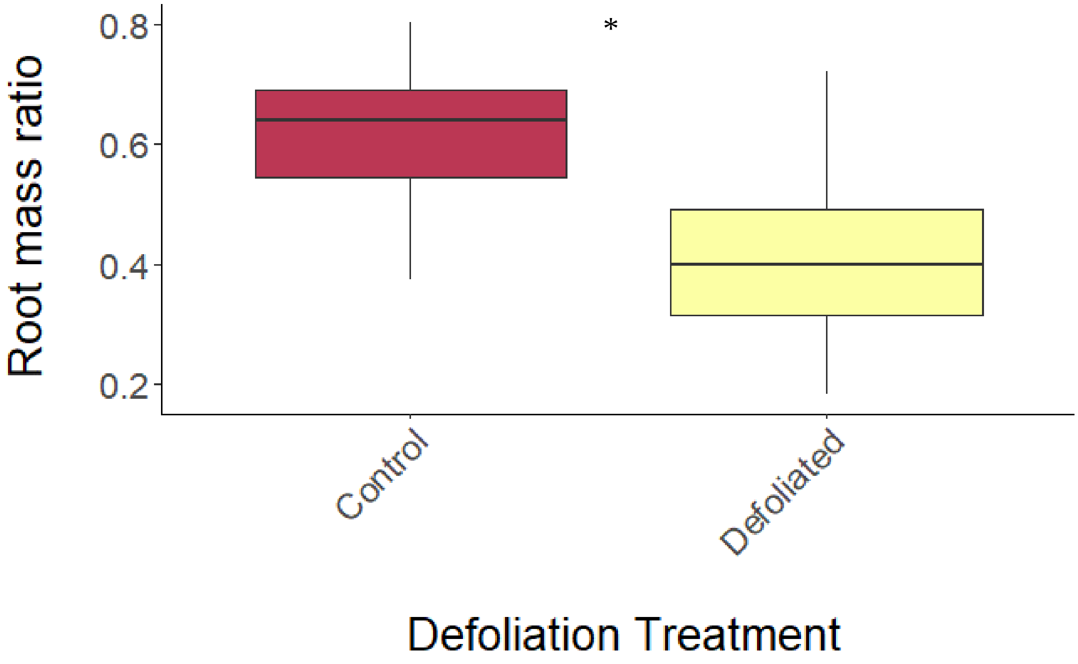
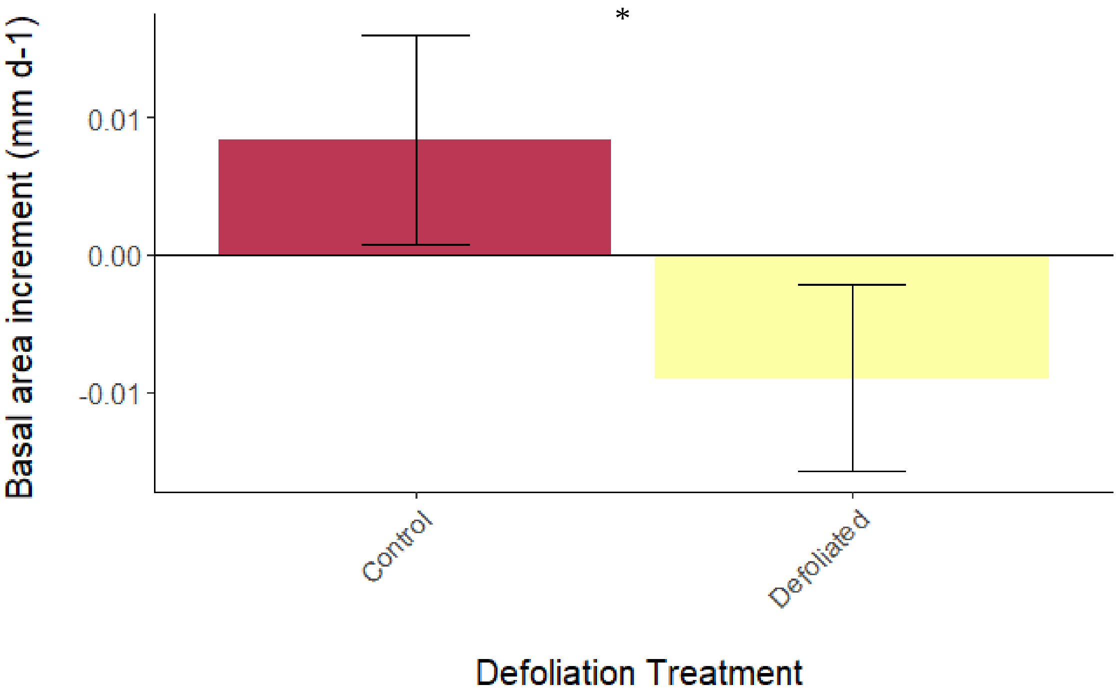

*
0.8
0.6
0.4
0.2
Root mass ratio
Control
Defoliated
Defoliation Treatment

Fig 4.


*
Basal area increment (mm d-1)
0.01
0.00
-0.01
Control
Defoliated
Defoliation Treatment