Shade intolerance and physiological responses of *Tamarix ramosissima*:

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Abstract

Invasive species, such as *Tamarix ramosissima*, pervade riparian habitats throughout the American Southwest. *Tamarix ramosissima* poses a threat to native plant community structure due to its fast growth rate, high water use, and stress tolerance. Therefore, it is of great importance to find long-term, viable strategies to mitigate its invasion. Here, we test the hypothesis that *Tamarix ramosissima* is a shade intolerant plant, as understanding the plant's ecology is necessary to employ an effective conservation strategy. We measured several functional traits, morphological traits, and flower number of *Tamarix ramosissima* within open and canopy habitat types to detect physiological responses to shade. The data show significant differences in photosystem efficiency, chlorophyll content, stomatal density, stomatal aperture, and flower number between open and canopy plants; there is also evidence of a reproductive tradeoff in shade plants between increased photosystem efficiency and chlorophyll content, and decreased flower number. These data indicate shade intolerance in *Tamarix ramosissima*, potentially suggesting that promoting native plant canopy cover could be an effective conservation strategy to restore native community structure.

Introduction

The invasion of exotic plants across fragile ecosystems is ubiquitous. While many nonnative plants do not interfere with the growth and reproduction of native plants, others readily outcompete their native counterparts, posing a serious threat to the community structure and the biodiversity of these ecosystems (Shaforth et al. 2005, Tomaso 1998, Craine et al. 2015). There are several traits that are characteristic of invasive plants, in part explaining their success in so many variable environments. Firstly, they are characteristically stress tolerant, allowing them to compete with and outperform native plants in areas of extreme temperature, high salt concentrations, water scarcity, or extreme light environments (Alpert et al. 2000). Due to their stress tolerance, they typically grow in areas of disturbance, suggesting that human development, among other types of disturbance, might promote the establishment of harmful, invasive species (Alpert et al. 2000). They also characteristically have a very fast establishment phase, allowing them to colonize areas of available habitat before native plants can establish (Van Kleunen et al. 2010). Additionally, many invasive plants have high rates of recruitment and establishment, through their production numerous, small seeds and high germination success (Van Kleunen et al. 2010). When an invasive plant begins to negatively alter native plant community structure, and in particular, when they begin to affect neighboring human populations, conservationists attempt to employ various strategies to mitigation their population growth.

Tamarix ramosissima (hereafter *Tamarix*) is one such invasive species that is of particular concern to conservationists. *Tamarix* was first brought to the United States as late as the 1950's to be used as a bank stabilizer in riparian habitats. While *Tamarix* is

exceptionally effective as a bank stabilizer, there are many ecological and anthropological repercussions. Firstly, *Tamarix* can outcompete or displace native riparian plant species through colonizing particular areas of riverbank (Bay & Sher 2008). This is possible for many reasons; characteristic of many invasive species, *Tamarix* is exceptionally stress tolerant. It is a drought tolerant species that is able to grow in arid habitats, making it an effective competitor in areas throughout the Southwest (Lovell et al. 2009). This is in part because mature *Tamarix* act as facultative phreatophytes, and have been shown to significantly depress the surrounding water table, consequently altering the riparian geomorphology (Dahm & Cleverly 2002). High salinity can also induce drought stress; *Tamarix,* however, often preferentially grow in alkaline and saline soil conditions, as they sequester and excrete salts in surrounding soils, inhibiting germination of their native competitors (Brotherson & Winkel 1986, Tomaso 1998). In addition, *Tamarix* is a heat tolerant plant. Due to its phreatophytic behavior, *Tamarix* has plastic water use and therefore can have high transpiration rates (Sala & Smith 1996); increased transpiration maintains a boundary layer around the leaves, one strategy that plants use to regulate heat load (Heschel & Hausmann 2001). The ecological consequences of *Tamarix's* stress tolerance are many: using disproportionate amount of available water, reducing necessary shade by outcompeting *Populus* species in the establishment phase, forming prolific colonies, and thus changing native flora and fauna population dynamics. (Busch & Smith 1995, Lesica & Miles 2001, Tomaso 1998). Anthropologically, *Tamarix* has gained a reputation throughout the American Southwest for its notably high water use. Water use studies began as an effort to identify water resources for human populations. Many of these studies indicate that

phreatophytes, of which *Tamarix* and *Populus* are some, use disproportionally high rates

of water and therefore threaten human populations' water accessibility, providing an

impetus for the removal of phreatophytes (Stromberg et al. 2009). The emphasis put upon

phreatophyte removal in these studies partially influenced a shift in conservation

ideology from ecosystem restoration to single-species eradication (Stromberg et al.

2009). *Tamarix* has been targeted as one of these species. However, physical eradication

of *Tamarix* individuals has proven ineffective due to their significant below ground

biomass, keeping them well established (Brotherson & Winkel 1986). Additionally,

Tamarix control through the release of the Northern tamarisk beetle (*Diorhobda*

carinulata) resulted in significant defoliation, but also a compensatory increase in the

plant's water use (Craine et al. 2016). Lastly, further studies suggest that habitat quality

and presence of native species is more important in limiting *Tamarix* than chemical

eradication (Bay & Sher 2008). These strategies target *Tamarix* individually and do not

take into account habitat restoration as a whole.

One such strategy that takes into account whole habitat restoration is the

reintroduction of natural flood regimes. Dams disrupt natural flooding regimes, which

have coevolved with many native species in riparian habitats. For example, flooding is

necessary in *Populus* establishment (Sher et al. 2002). Therefore, the termination of

periodic flooding could make native plants more vulnerable to invasion. *Tamarix* is well

able to grow in areas downstream of dams, as the lack of sediment deposition and

nutrient availability can induce salt and drought stress, favoring *Tamarix* establishment

over *Populus* establishment (Glenn & Nagler 2005, Pataki et al. 2005). In areas of

flooding or habitats upstream of dams, however, native plants, such as *Populus* and *Salix*,

outcompete *Tamarix* in the establishment phase, and thus do not experience stress of invasion (Lovell et al. 2009). Reestablishing natural flooding effectively takes into account the physiology and ecology of *Tamarix* in an effort to control its invasion.

Another potential strategy that utilizes a systems approach to conservation is inducing shade stress on *Tamarix* populations. *Tamarix*, like all other plants, will plastically adjust its physiology depending upon light environment. A typical response to shade stress is to increase maximum photosynthetic rate, as plants in sun environments have naturally higher carbon assimilation rates (Heschel et al. 2004). However, when a plant allocates energy towards maximizing photosynthetic potential, there are often reproductive tradeoffs. Higher maximum carbon assimilation is generally associated with higher respiration rates, suggesting that the synthesis of organs and compounds for cellular work is more costly in shade plants. Additionally, plants in the shade are more sensitive to increases in light level, typically having both a lower Light Compensation Point (LCP) and a Higher Apparent Quantum yield (Aqe) (Larcher 1995). Morphologically, shade plants will increase leaf area and decrease leaf thickness to maximize light absorption at a lower cost of production (Abrams & Kubiske 1989). Sun-shade studies typically investigate the morphological and physiological responses to light intensity (Heschel et al. 2004, Abrams & Kubiske 1989, Larcher 1995). There is some evidence of morphological changes in *Tamarix* due to shade stress, including changes in stem basal area, mortality, canopy height, and percent live foliage (Dewine & Cooper 2007). Here, we focus on *Tamarix* physiological and morphological responses to shade stress.

This study aims to investigate *Tamarix* ecology and physiological responses to light environments and suggest a conservation strategy that limits *Tamarix* reproduction and growth, while promoting native plant community structure. We do this through measuring several functional traits, morphological traits, as well a rough measure of fitness. We measured stomatal conductance to quantify *Tamarix* water use. Water use can be indicative of the heat and drought stress the plant is experiencing. It is likely that if a plant is drought stressed it will decrease its stomatal conductance to conserve water. Conversely, if a plant is heat stressed, it might increase its stomatal conductance to maintain a sufficient boundary layer and thus cool its leaves (Heschel & Hausmann 2001). Leaf temperature is another functional trait measured to quantify heat stress. We expect that leaf temperatures will be highly variable across plants, as leaf temperature is not reflective of ambient temperature and is greatly affected by the plant's microhabitat. Consistent leaf temperatures could indicate that the plant is not well able to regulate heat stress on leaves; similarly, if the plants experience a range of leaf temperatures, this could indicate that the plant is an effective adapter to quickly changing environmental conditions.

To quantify the plant's foliar photosynthetic potential, we measured photosystem efficiency, a functional trait that measures the percentage of red light absorbed by individual leaves. The more efficiently a plant can absorb available light, the greater its photosynthetic potential. Photosystem efficiency is not a direct measurement of photosynthetic rate, but rather is a factor that influences photosynthetic rate. Drought, heat, or other environmental stressors can impact the efficiency of a plant, making photosystem efficiency a useful trait to measure, as it is likely impacted by changes in

light intensity. Chlorophyll content is another useful functional trait that can be indicative of a plant's foliar photosynthetic potential. Chlorophyll content provides a relative measurement of the concentration of chlorophyll in an individual leaf. Therefore, an increase in chlorophyll content could result in increased photosynthetic potential. Synthesizing chlorophyll, of course, has an energetic cost, so we suspect a physiological tradeoff as a result of increased chlorophyll content. Taken together, photosystem efficiency and chlorophyll content provide a relative measure of foliar photosynthetic potential.

Additionally, we measured stomatal density and stomatal aperture, two morphological traits that also likely impact photosynthetic potential. Stomatal density measures the number of stomata in a given area of leaf. Stomatal aperture measures the relative area of the individual stomata, including the guard cells. An increase in stomatal density and stomatal aperture indicate increased potential for transpiration, contributing to increased photosynthetic potential.

Lastly, we quantified flower number of individual plants. Although flower number does not directly reflect the number of viable seeds, we used flower number as our proxy for fitness in this study. Measuring fitness provides a metric to predict the relative survivorship and reproductive success of plants. Previous studies show that *Tamarix* under significant environmental stressors experience decreased fitness, likely as they are allocating energies to reduce these stressors, rather than to produce reproductive units (Craine et al. 2016). Therefore, as fitness predicts the reproductive ability of the plant, it is the most direct indicator of its success. Fitness is also influenced by functional

traits, some of which are measured in this project, so taken together can be indicative of the plant's overall response to shade as a potential stressor.

We combine functional traits, morphological traits, and flower number to address the following question: is *Tamarix* shade intolerant? If *Tamarix* performs poorly in shady environments, we can potentially utilize knowledge of its ecology and environment to promote the establishment and maintenance of shady riparian habitats to mitigate its invasion. Furthermore, we sought to understand if some of *Tamarix's* native competitors are more important in promoting or deterring *Tamarix* establishment, and therefore if *Tamarix* significantly impacts native plant community structure. We focused on Plains Cottonwood (*Populus deltiodes*) (hereafter *Populus*), a native species to the American Southwest that most readily competes with *Tamarix* and shares a similar habitat. Based on a 2015 dataset included in our analysis, as well as observational data, we hypothesized that *Tamarix* would show typical shade stress responses, including reduced stomatal conductance in the shade, increased photosystem efficiency and chlorophyll content in the shade, and decreased stomatal density and stomatal aperture in the shade. Additionally, we expected that *Tamarix* in shady environments would have lower reproductive potential. We also hypothesized that *Populus* would be negatively correlated with *Tamarix* density, as it provides the majority of shade in riparian habitats and thus would stunt *Tamarix* growth more significantly.

Methods

Study System

The two datasets analyzed in this study were collected in a riparian forest along Fountain Creek in Fountain, CO, during the summer months of 2015 and 2017. This riparian habitat shares populations of native species, such as *Populus deltoides* and *Salix exigua*, as well as invasive species, such as *Tamarix ramosissima* and Russian Olive (*Elaeagnus angustifolia*). This species makeup is very common in the American Southwest (Reynolds & Cooper, 2010). In arid areas such as these, riparian forests provide specialized habitat structure for native fauna, maintaining a relatively high degree of biodiversity (Berger et al. 2001). The introduction of invasive plant species, such as *Tamarix*, has potential to disrupt the native community structure. It is therefore important to understand *Tamarix* life history strategies, as well as its impact on native plant species.

Study Sites

Three study sites were identified along Fountain Creek, named A, B, and C, and were used in both 2015 and 2017 research. Sites were chosen by similar habitat structure and relative proximity to one another. In 2015, 231 *Tamarix* plants were measured across all sites, and the sites represented a similar number of plants. In 2017, 174 *Tamarix* plants were measured. Site A had 46 *Tamarix* individuals that were measured, while Site B had 24 individuals, and Site C had 104 individuals. Sites A and C had substantial canopy cover by *Populus* in parts, while Site B had none.

Experimental Design

Site environment measurements

A Light Scout PAR (Photosynthetically Active Radiation) quantum meter (Spectrum Technologies, Aurora, IL) was used to measure ambient light levels, and a

TDR Moisture Reader (Campbell Scientific, Logan, UT) was used to measure volumetric water content (VWC) across all three sites to quantify environmental conditions. PAR light and VMC were measured at evenly spaced points along transects in sites A and C and at the base of each marked plant in site B. Environmental measurements were taken to ensure similar growing conditions across sites, allowing for viable physiological comparisons.

Plant selection and exposure categorization

  Tamarix individuals selected for measurement were haphazardly chosen throughout the three sites in both observationally shady and open areas. The number of plants selected in each site depended upon the relative sizes of the sites, as the plants selected were meant to be a representative sample of the population. Plants of similar size were chosen for measurement to control for relative age. In addition, stem diameter of the branch used for functional trait measurements was measured on each plant to control for developmental age using a caliper. Stem diameter was comparable across sites A, B and C. The mean stem diameter (+/- 1 SE) in site A was 2.01 +/- 0.10 cm, in site B was 2.32 +/- 0.15 cm, and in site C was 2.16 +/- 0.10 cm. Stem diameter was also comparable across exposure type. Mean stem diameter (+/- 1 SE) in open habitats was 2.29 +/-0.10 cm, and in canopy habitats was 1.99 +/-0.08 cm.

  In 2015, plants were categorized as "open" or "canopy" exposure types based on light quantity values, as well as a subjective assessment of canopy cover. 123 *Tamarix* individuals were classified as canopy, and 108 *Tamarix* individuals were classified as open. In 2017, plants were categorized as "open" or "canopy" exposure types based on

the mean Red:Far-red (R:FR) ratio taken on the canopy edge of each plant, compared to the median value of all R:FR readings across all sites. All values above the median were considered "open," while all values below the median were considered "canopy." 86 *Tamarix* individuals were classified as canopy and 88 *Tamarix* individuals were classified as open.

Functional traits

For functional traits measurements, one branch on each marked plant was selected for measurement. This branch was determined by finding the first red-green colored branch with viable leaves and no inflorescences closest to the base of the first branch off of the main axis of the plant. The leaves used for measurements were selected by finding several viable leaves near the center of the branch.

Stomatal conductance was measured using a Leaf Promoter (Decagon Devices SC1). All measurements were taken between 9:30hr and 14:00hr. Three adjacent leaves selected for measurement were clamped in the sensor head. If stomatal conductance values read above 300 mmol/m^{2}s^{-1} or below 40 mmol/m^{2}s^{-1}, we assumed human error in applying the sensor head to the leaves, and retook measurements. These readings represent the stomatal conductance of individual branches, controlled for age, rather than the whole plant.

Percent photosystem efficiency was measured using a fluorometer (EARS). As many leaves as needed were taken from the branch selected for measurement to completely cover the sensor. Leaves were held parallel against a black square piece of paper and then the UV light from the fluorometer was projected on the leaves. Leaf temperature was recorded with an infrared temperature probe (ExTech) at the same time

as recording photosystem efficiency. The infrared temperature probe was held just above the leaves and measurements were taken multiple times at slightly different angles. The highest reading was recorded.

Chlorophyll content index was measured using a SPAD meter (CCM 200 plus, Opti-Sciences Inc.). Two adjacent leaves selected for measurement were clamped in the sensor head. If the SPAD meter was unable to produce a measurement, the positions of the leaves were slightly adjusted and recorded again. If after few times the SPAD meter could not produce a measurement, new leaves on the same branch were selected.

Leaf morphology was evaluated by measuring stomatal density and stomatal aperture. An equal number of sample leaves were collected from selected plants at site C in 2015. Using a scanning electron microscope (SEM), stomatal density was measured by counting the total number of stomata on a single image twice and calculating a mean. Leaf area was estimated and used to calculate density. To quantify stomatal aperture, six sample images (12 total) were used from each exposure type. Two stomata on each image were selected and their areas estimated. To limit the margin of error in measurements, SEM images were enlarged and the two stomata were measured using calipers. The scale bar on each image was used to convert the caliper measurements into micrometers.

Relative Densities

We established line transects through sites A and C to quantify the relative densities of *Tamarix*, *Populus*, and *Salix*, in an attempt to describe how *Tamarix* impacts community structure. We established two transects through site C and six through site A. In site C, all transects spanned the length of the site and varied in length from 100m to 250m. In site A, transects were established in areas of clusters of *Tamarix* individuals,

and so varied in length from 15m to 50m. No transects were established in Site B, as the values of relative densities obtained from transects in Site A and C were assumed to generally describe the relative densities at Site B.

Using 50 meter transect tape, we established linear transects throughout the sites stopping every 10m to record the number of individual *Tamarix, Populus*, and *Salix* in a 10x10m square area. The 10x10m square area was a visual approximation from the ten-meter mark on which we were standing to the previous ten-meter mark. We counted individual *Tamrix, Populus* and *Salix* plants using clicker counters.

Fitness estimates

We used total flower number on the first branch off the main axis as a rough proxy for fitness (Heschel, unpublished data). We counted of the number of flowering stems, defined as a stem containing more than one inflorescence, on the first branch off the main axis twice, and obtained a mean. Flower number has been used as a proxy for fitness in previous studies quantifying *Tamarix* fitness (Craine et al. 2016).

Data Analyses

All data analyses were performed with JMP **(**version 5.1.2, SAS Institute). Two-way ANOVAS were used to test for differences between functional traits and flower number between sites and exposure type. Sites and exposure types were considered fixed, and site was used as a blocking factor to control for environmental differences across sites A, B, and C. For any significant interaction between site and exposure ($P < 0.05$), planned t-tests were used to compare trait values between individual exposure types within sites. SEM data were examined for exposure differences with a one-way ANOVA.

Stomatal conductance and leaf temperature values were adjusted for time of day by regressing trait values against time, and adding residuals to the grand mean of each trait (Craine et al. 2016). Residual distributions and histograms were evaluated for each trait, and adjusted using log10 transformations where necessary to meet assumptions of normality. In order to log10 transform densities and flower number, one number was added to all values within any trait containing at least one zero value.

Regression analyses were used to evaluate the effects of *Populus* and *Salix* densities on *Tamarix* densities within exposure types. Multivariate correlation analyses were used to evaluate the relatedness of functional traits to one another and to flower number (Heschel and Riginos 2005).

Site environment measurements, VWC and PAR, were evaluated by comparing site means to one another. These measurements were recorded to quantify any notable differences in environmental conditions.

Results

Site Environmental Conditions

Temperature and precipitation data were taken from a weather station in Butts Army Airfield in Fort Carson, CO, within 16 km of the site. The mean temperatures from June through August in 2015 ranged from 19.4°C to 21.67°C, and in 2017 from 19.4°C to 22.22°C (Fig. 1). The mean precipitation in this time in 2015 ranged from 0.20 cm to 0.84 cm and from 0.03 cm to 0.64 cm in 2017.

PAR light, R:FR light, and VWC measurements were taken across all sites in 2017. Mean PAR (+/- 1 SE) in Site A was 1205.09 +/- 137.43 mol m^{-2}s^{-1}, in Site B was 1099.90 +/-132.43 mol m^{-2}s^{-1}, and in Site C was 1248.18 +/- 98.07 mol m^{-2}s^{-1}. Mean R:FR (+/- 1 SE) in Site A was 0.91 +/- 0.03, in Site B was 0.89 +/- 0.04, and in Site C was 0.90 +/- 0.02. Mean R:FR (+/- 1 SE) in open habitats was 1.03 +/- 0.02 and in canopy habitats was 0.77 +/-0.01. Mean VWC (+/- 1 SE) in Site A was 8.65 +/- 0.76%, in Site B was 8.98 +/- 0.39%, and in Site C was 4.17 +/-0.25%.

Regression analyses were performed between *Populus* density and *Tamarix* density within exposure types in 2017. There was no significant effect of *Populus* density on *Tamarix* density for open or for canopy exposure types (canopy: β=0.20, r²=0.02, df=62, P=0.28; open: β: 0.40, r²= 0.06, df=33, P=0.18). While this relationship is not significant, the majority of the canopy cover at the study site was provided by *Populus* (personal observation).

Tamarix Functional Traits

Stomatal Conductance - Exposure type had a significant effect on stomatal conductance in 2015 (Table 1). Mean stomatal conductance was lowest in canopy habitats and highest in open habitats (Figure 2). In 2017, exposure type did not have a significant effect on stomatal conductance (Table 1). Mean stomatal conductance was higher in open habitats than in canopy habitats (Figure 3). Site also had a significant effect on stomatal conductance in both 2015 and 2017 (F=15.34, *P*<0.0001; F=3.16, *P*=0.044). An increase in stomatal conductance can occur to reduce foliar heat load. In

2015, stomatal conductance was significantly positively correlated with leaf temperature (r= 0.34, *P*<0.001).

Leaf morphology - Exposure type had a significant effect on stomatal density in 2015 (F=12.8, *P*=0.003). Mean stomatal density was higher in open habitats and lowest in canopy habitats (Figure 4). Exposure type also had a marginally significant effect on stomatal aperture in 2015 (F=3.97 *P*=0.077). Mean stomatal aperture was highest in open habitats and lowest in canopy habitats (Figure 5).

Leaf temperature - Exposure type had a significant effect on leaf temperature in both 2015 and 2017 (Table 1). In 2015, mean leaf temperature was highest in open habitats and lowest in canopy habitats (Figure 2). In 2017, mean leaf temperature was highest in canopy habitats and lowest in open habitats (Figure 3). In addition, site had a significant effect on leaf temperatures in both 2015 and 2017 (F=5.73, *P*=0.017; F=31.88, *P*<0.0001). Leaf temperature can be indicative of the plant's heat stress.

Photosystem Efficiency - Exposure type had a significant effect on photosystem efficiency in both 2015 and 2017 (Table 1). In both years, mean photosystem efficiency was highest in canopy habitats and lowest in open habitats (Figure 2, 3). In 2017, photosystem efficiency was significantly negatively correlated with leaf temperature (r=-0.0176, *P*= 0.02). Site also had a significant effect on photosystem efficiency in 2015. (F=14.88, *P*<0.0001)

Foliar Chlorophyll Content - Exposure type had a significant effect on foliar chlorophyll content in both 2015 and 2017 (Table 1). In both years, mean chlorophyll content was highest in canopy habitats and lowest in open habitats (Figure 2, 3). Site also

had a significant effect on chlorophyll content in 2017 (F=11.53, $P<0.0001$). Higher chlorophyll content should translate into higher photosynthetic potential.

Tamarix Flower Production

Exposure type had significant effect on flower number in both 2015 and 2017 (Table 1). In both years, mean flower number was highest in open habitats and lowest in canopy habitats (Figure 6). In 2015, flower number was significantly positively correlated with leaf temperature and stomatal conductance (r= 0.26, $P = 0.0001$; r= 0.125, $P= 0.057$). In 2017, flower number was significantly negatively correlated with leaf temperature (r= -0.158, $P= 0.038$).

Discussion

Tamarix's response to shaded environments is exemplary of many typical shade-stress strategies. Previous studies have suggested that the primary way a plant compensates in shade-stressed conditions is through increasing photosynthetic potential, as sun leaves are generally able to fix more carbon at high light levels (Heschel et al. 2004, Pearcy & Sims 1994). Our data show that *Tamarix* experience lower gas exchange in shaded conditions, reducing its overall photosynthetic potential, and therefore must significantly increase the efficiency with which it absorbs red light, along with its chlorophyll concentration. These two mechanisms of increasing photosynthetic potential appear to have a significant reproductive cost to the plant, as seen in significantly decreased flower number in canopy habitats.

Effect of environment on *Tamarix* physiology

In 2017, light availability and soil moisture remained similar across sites, suggesting that all three sites pose similar environmental conditions for plant growth. These data indicate universally dry conditions, and these are comparable to previous studies (Craine et al. 2016). Relatively consistent environmental conditions reduce variability in the data and support the hypothesis that physiological differences in *Tamarix* are due to shade stress.

Another environmental factor potentially influencing *Tamarix* physiology is differences in ambient temperatures across years. 2015 experienced slightly higher mean temperatures during the summer months than 2017, potentially prompting changes in physiology. For example, canopy *Tamarix* in 2015 had significantly lower rates of gas exchange, and while this trend is emerging in 2017, is not significant. This could be explained by higher temperatures imposing greater heat stress on leaves, and requiring the opening of stomata more frequency to relieve the heat load. The lesser heat stress in 2017 may not have required the opening of stomata to such a degree that the difference is significant.

Effect of habitat on *Tamarix* regulation of heat load

Tamarix is an exceptionally heat tolerant plant, and utilizes many morphological and physiological strategies to regulate its heat load. First, our data suggest significant variation in leaf temperature. Each data point provides a point-in-time snapshot of the plant's heat stress, influenced greatly by time of season, time of day, and ambient temperature. Therefore, the fluctuations of leaf temperature across years indicates that

Tamarix is well able to adjust to rapidly changing environmental conditions and regulate its heat load, making it a very successful competitor in these habitats. Second, increased stomatal conductance is another mechanism for managing heat stress. The more frequently the plant opens its stomata, the better able it is to maintain a boundary layer and thus reduce heat load (Heschel & Hausmann 2001). Third, *Tamarix* significantly changes its leaf morphology in open habitats, assisting in the reduction of heat load. Increased stomatal density allows for more efficient gas exchange, as more stomata are present in the same area. Increased stomatal density is a trait typically observed in sun leaves (Lichtenthaler et al. 1981). Similarly, increased stomatal aperture allows for more gas exchange to occur per stomata, also making gas exchange more efficient. In this study, open plants have higher stomatal conductance, as well as higher stomatal density and aperture, suggesting that these plants are better able to regulate heat load. These plants, therefore, participate in more gas exchange, increasing photosynthetic potential.

Reproductive cost of shade stress

Canopy plants absorb significantly more red light for photosynthesis than open plants. The overall light availability in canopy habitats is reduced, so the plant's efficient use of this limited light is crucial. In addition, canopy plants have a significantly increased chlorophyll concentration when compared to open plants. Interestingly, there is some evidence showing that while shade leaves experience decreased levels of water in their leaves overall, significantly more of the leaf water is allocated towards chloroplast production and function (McCain et al. 1988). This corroborates our findings as sun leaves tend to transpire more than shade leaves, resulting in an increase in total water in sun leaves; the increased allocation of total water towards chloroplasts in shade leaves

might suggest that chloroplast production in the shade is costly, especially in arid environments. Increasing photosystem efficiency and chlorophyll content are two strategies for maximizing photosynthetic potential where light is limited; these are exemplary of typical shade-stress responses.

These shade responses, however, appear to have a reproductive cost. Canopy plants produce significantly less flowers, potentially suggesting that leaf production under a canopy is very costly. This reproductive tradeoff could be partially explained by the early successional life history strategies of many invasive plants. Invasive plants typically grow quickly in establishment, often to avoid being outcompeted by shade from neighboring plants (Martin et al. 2010). In later developmental stages, growth slows and exposure to shade is more likely, potentially impacting fitness. Martin et al. identified a fitness tradeoff between exposure to shade in later developmental stages, and overall survivorship in invasive *Ailanthus altissima* (Martin et al. 2010). Given the significant reproductive cost of maximizing photosynthetic potential in the shade, these data suggest that *Tamarix* is a shade intolerant species, supporting our primary hypothesis.

Effect of heat stress and shade stress on fitness

Heat stress does not appear to have a significant effect on *Tamarix* fitness. *Tamarix* experiencing heat stress increase stomatal conductance, stomatal density, and stomatal aperture. These plants also have a higher mean flower number, likely related to increased fitness. Therefore, the physiological and morphological changes made to accommodate heat stress do not seem to have a negative impact on flower number. Our analysis of flower number is not precise in that it does not directly represent the number of viable

seeds an individual plant produces. For the scope of this study, however, flower number provides a useful fitness index, and is used to explore broad trends in reproductive potential.

Flower number data support the hypothesis that canopy plants experience shade-stress at a reproductive cost. The overall trends in the data suggest that open plants might be more fit than canopy plants. As detected here, open plants posses a different suite of physiological traits than canopy plants, and increased fitness might suggest that these suites of traits are selected for in different light environments. Previous studies suggest that there might be selection for shade avoidance traits, such as internode length and flowering date, in open *Impatiens capensis* plants (Donohue et al. 1999). Selection was not directly tested for in this study, but in the future could be useful in informing which traits most contribute to reproductive fitness. Overall, flower number data suggest that the energetic cost of leaf production and photosynthesis in open plants might be reduced, allowing for the increased allocation of energy towards flower production. This is a strong indicator that canopy plants are shade-stressed, and suffer from lower reproductive potential.

Conservation implications

Tamarix is likely a shade-stressed species that experiences a reproductive cost for maximizing photosynthetic potential in low light environments. This suggests that sufficient canopy cover, notably from *Populus*, in shared habitats could effectively mitigate *Tamarix* population growth over time.

Although we detected no significant relationship between *Populus* density and *Tamarix* density, the majority of the shade under which *Tamarix* grow is provided by *Populus* within our study site. Additionally, *Tamarix* growing under a *Populus* canopy generally appear smaller in size than those growing in open habitat (personal observation). So while there is evidence of canopy cover by *Populus* affecting *Tamarix* growth, our measurement of *Populus* density is not the most accurate metric for predicting *Tamarix* density. Other factors that might better predict *Tamarix* density are *Populus* stand age or *Populus* canopy size, as these variables could better explain differences in amount of available light.

Overall, the observed trend that presence of *Populus* influences *Tamarix* growth might indicate that *Populus* and *Tamarix* are effective competitors, in that both species grow in similar habitats, and depending upon environmental conditions, one often appears more successful than the other. There are many potential explanations for why one species might outperform the other; success of *Populus* in shared habitats might be driven by post-establishment competition, in that shading by *Populus* might primarily stunt the growth and reproduction of mature *Tamarix* plants. Secondly, persistence of one species over another might be driven by habitat quality during the establishment phase. Previous studies suggest that *Tamarix* most effectively invades habitats of human-induced disturbance, and does not act as an aggressive invader, and so in areas of predominant native plant community, *Tamarix* is less likely to colonize (Sher et al. 2002). This is likely influenced by the fact that *Tamarix* primarily competes with *Populus* in the establishment phase, as both species are early successional phraetophytes (Lesica & Miles, 2001). Therefore, a well-established canopy will make *Populus* establishment

more likely. This suggests that the primary factor limiting *Tamarix* establishment is minimal disturbance and presence of already established native species. For these reasons, employing conservation strategies that seek to promote native plant community structure, rather than to actively remove *Tamarix* from existing communities, might be more viable in mitigating *Tamarix* population growth.

In addition to the maintenance of *Populus* canopy, other conservation strategies could be employed that promote native plant community structure. For example, the reestablishment of natural flooding regimes likely favors native plants. In areas where natural flooding occurs, *Tamarix* grows more slowly and is naturally outshaded by *Populus* and *Salix* (Lovell et al. 2009). This is because *Populus* establishment relies on flooding, so under natural flooding conditions, *Populus* will likely perform better than *Tamarix* in the establishment phase, when invasion is most probable. This strategy acts to limit the recruitment of *Tamarix*, thus promoting native plant community structure, arguably the most viable, long-term conservation strategy.

Promoting native plant community structure in riparian habitats is important for a multitude of reasons. Firstly, riparian areas are exceptionally biodiverse. This is due to relatively more abundant water, allowing for the growth of larger and various plant species, adding vertical heterogeneity to the landscape. Many bird species rely on and choose nesting locations based on particular structural habitats (Berger et al. 2001). Additionally, riparian restoration projects are often designed to improve habitat structure for native avian community (Shanahan et al. 2011). Invasion by *Tamarix* greatly impacts this habitat structure, and therefore can significantly reduce regional avian biodiversity (Brand et al. 2008). As riparian habitats house 80% of avian diversity in the arid

Southwest, this is of significant concern (Berger et al. 2001). *Tamarix* also do not provide significant food sources for mammals and insects, with the exception of woodrats (*Neotoma* spp.), the desert cottontail (*Sylvilagus audubonii*), and the Northern tamarisk beetle (*Diorhabda carinulata*) (Tomaso 1998). Additionally, invasive plants characteristically form monocultures if environmental conditions permit (Tomaso 1998); for this to occur, the invader must outcompete native species, thus inherently reducing native plant biodiversity.

Secondly, water scarcity defines much of the community structure and species interactions in arid ecosystems. Phraetophytes, such as *Tamarix*, can have exceptionally high water use, and limit available water to neighboring plants (Lovell et al. 2009). Furthermore, plants with high water use pose a threat to adjacent human populations, who also rely on the surrounding water supply for subsistence. Therefore, the maintenance of native plant community structure could potentially reduce water usage from plants overall, benefitting the flora community, as well as neighboring human communities.

Table 1. ANOVA results for sites (A, B, and C) and exposure type (open and canopy) on functional traits and total flowers. g_{st} = stomatal conductance; %PE = percent photosystem efficiency; CCI = chlorophyll content index. *F* values are reported from effect tests.

2017	Log g_{st}	%PE	Log CCI	Log Leaf Temp.	Total Flowers
Site	3.1649*	0.278	1.5384***	31.8863***	0.7631
Exposure	2.2517	5.5339*	7.176**	1.3735	4.4785*
Site x Exposure	0.9389[+]	6.1807**	0.9097	7.192**	1.3065

2015	Log g_{st}	%PE	Log CCI	Log Leaf Temp.	Total Flowers
Site	15.4351***	14.8814**	1.6465	62.1073***	4.6724
Exposure	13.72***	102.4186*	2.7556[+]	5.7388*	15.9087***
Site x Exposure	2.0148	4.0837	1.2268	4.0899	0.2421

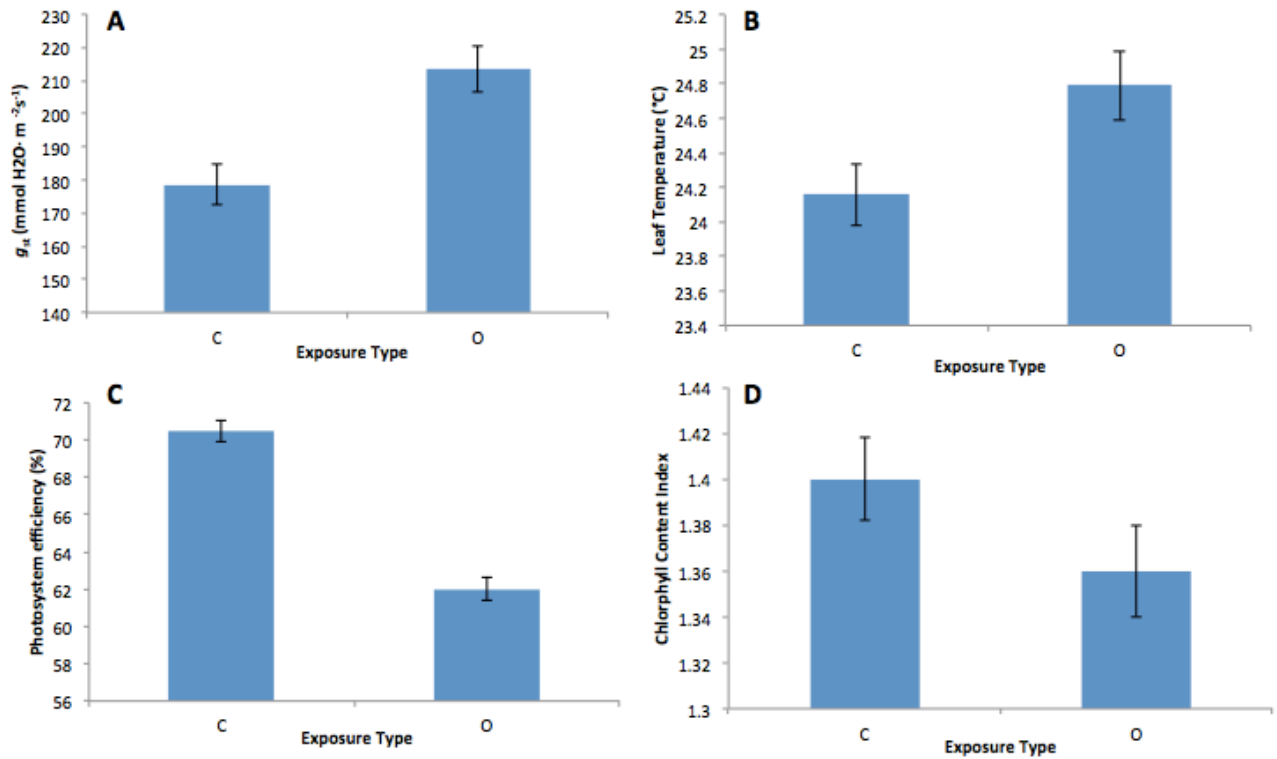[+] $P < 0.1$ ** $P < 0.01$
*$P < 0.05$ *** $P < 0.001$
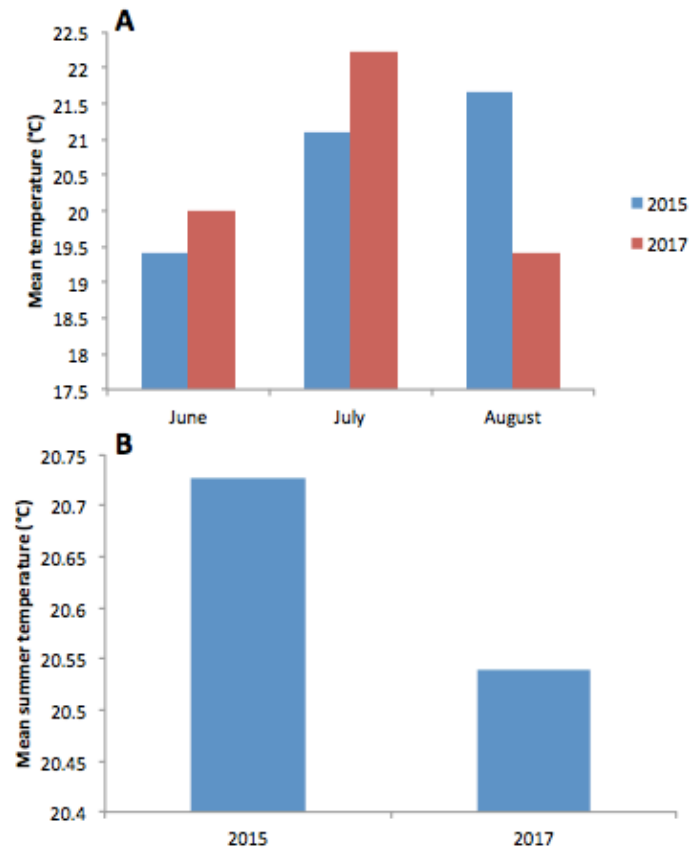
Fig. 1.
A. Mean ambient temperatures in Fountain, CO in June, July, and August of 2015 and 2017. **B.** Mean ambient temperatures in Fountain, CO for the summer months of 2015 and 2017.

Fig. 2.
Functional trait measurements (+/- 1 SE) for *Tamarix* individuals in canopy (C) and open (O) exposure types in 2015. **A.** Mean stomatal conductance (g_{st}). Conductance values were adjusted for time. **B.** Mean leaf temperature. Temperature values were adjusted for time. **C.** Mean photosystem efficiency values. **D.** Mean chlorophyll content index values.
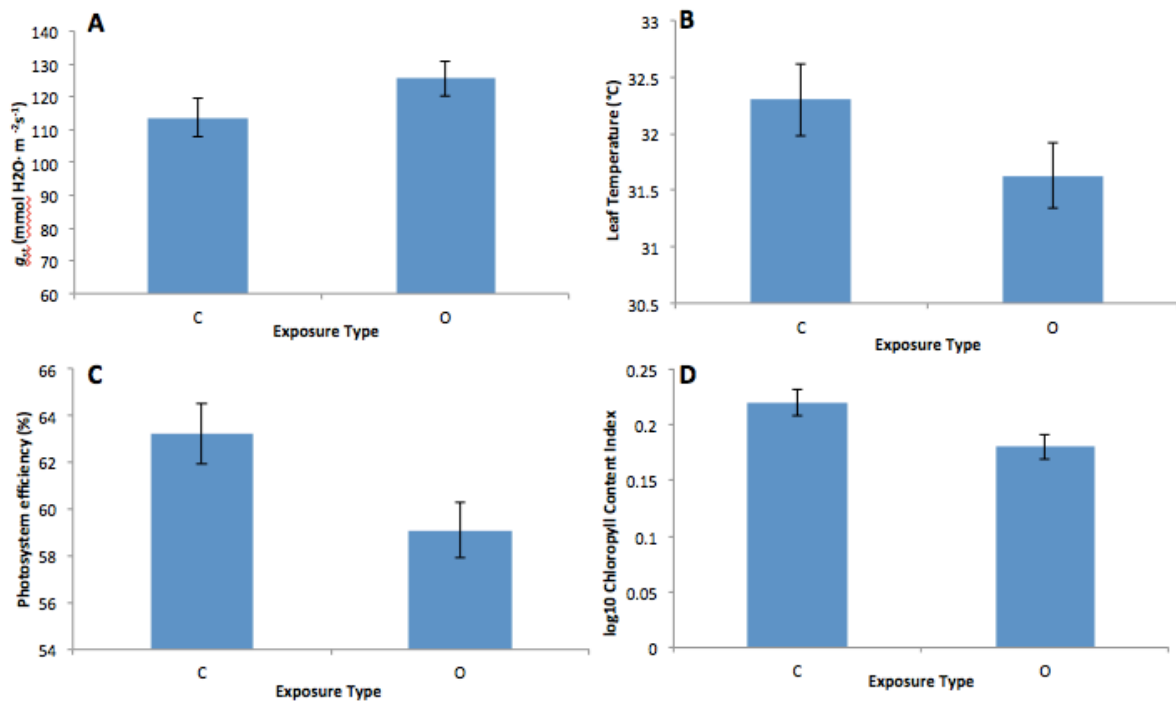
Fig. 3.
Functional trait measurements (+/- 1 SE) for *Tamarix* individuals in canopy (C) and open (O) exposure types in 2017. **A.** Mean stomatal conductance (g_{st}). Conductance values were adjusted for time. **B.** Mean leaf temperature. Temperature values were adjusted for time. **C.** Mean photosystem efficiency values. **D.** Mean chlorophyll content index values.
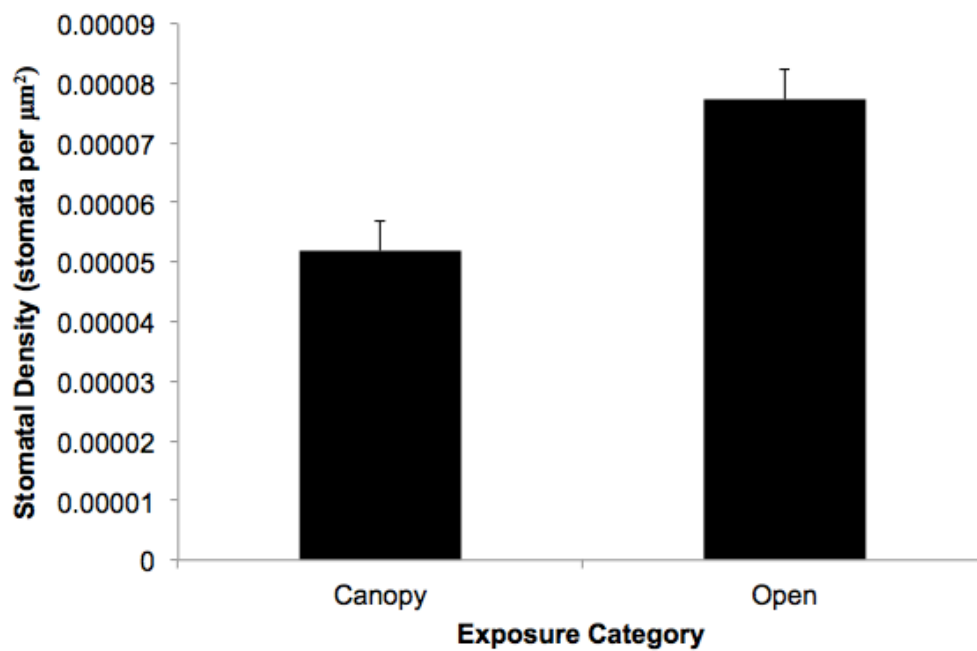
Figure 4. Mean stomatal density (+/- 1 SE) of *Tamarix* individuals in canopy (C) and open (O) exposure types in 2015
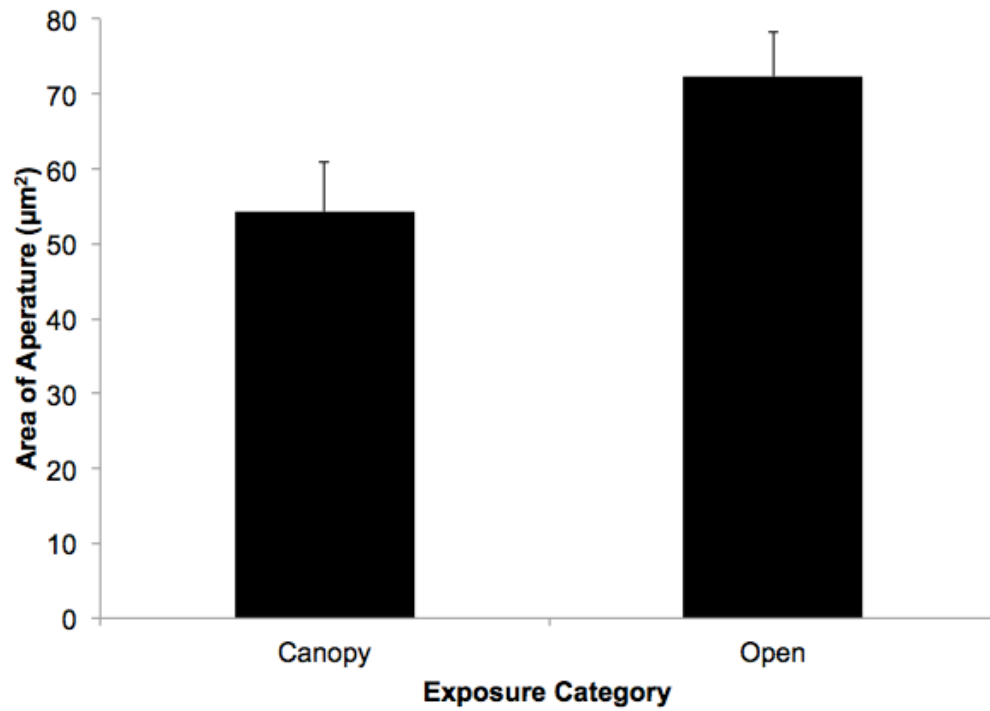
Figure 5. Mean stomatal aperture (+/- 1 SE) of *Tamarix* individuals in canopy (C) and open (O) exposure types in 2015.
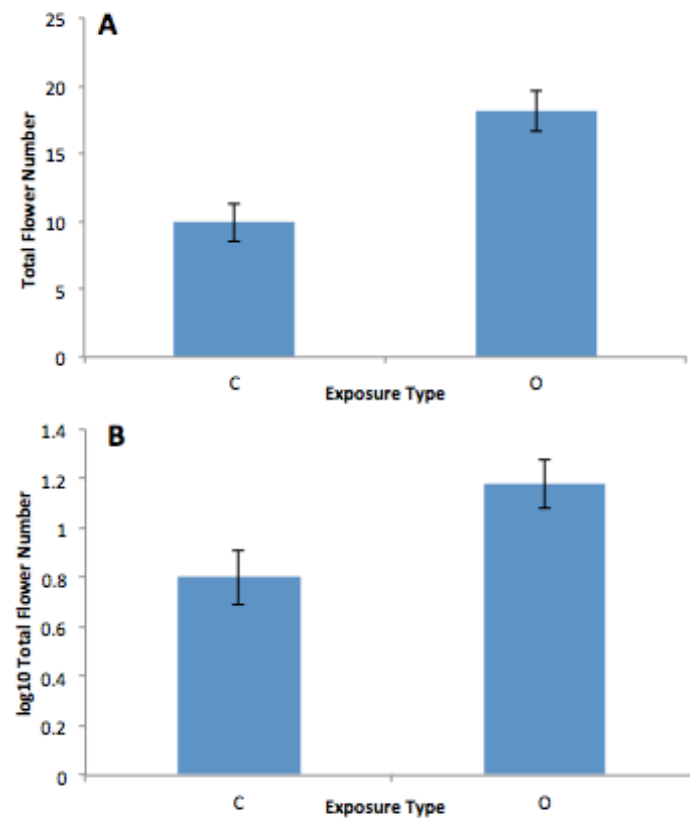
Fig. 6.
A. Mean total flower number (+/- 1 SE) of *Tamarix* individuals in canopy (C) and open (O) exposure types in 2015. **B.** Mean total flower number (+/- 1 SE) of *Tamarix* individuals in canopy (C) and open (O) exposure types in 2017.

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