TAMARIX RAMOSISSIMA WHOLE PLANT AND LEAF LEVEL PHYSIOLOGICAL RESPONSE TO INCREASING SALINITY

by

JACOB CARTER

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Major Professor
Jesse Nippert

Abstract

In 1902, President Theodore Roosevelt signed and enacted the Reclamation Act, which would fundamentally alter the lowland hydrology of the arid southwest over the next century. Flow regulations, groundwater pumping, damming, and river channel changes have led to decreases in water table heights and periodic overbank flooding, and subsequently, increased soil salinity in the arid Southwest. During this period, native riparian tree species have declined significantly and an invasive tree species, *Tamarix ramosissima*, has increased in abundance and distribution. Increases in soil salinity negatively impact the physiology of native riparian tree species, but the impacts of soil salinity on *Tamarix* physiology are incompletely known. I studied the impact of increasing soil salinities on the physiology of *Tamarix* in both field and controlled environments. I first studied the impacts of increasing soil salinities on *Tamarix* physiology at two semi-arid sites in western Kansas. I concluded that physiological functioning in *Tamarix* was maintained across a soil salinity gradient from 0 to 14,000 ppm illustrating robust physiological responses. Using cuttings from *Tamarix* trees at both sites, I subjected plants to higher NaCl concentrations (15,000 and 40,000 ppm). *Tamarix* physiology was decreased at 15,000 ppm and 40,000 ppm. *Tamarix* physiological functioning was affected at the induction of treatments, but acclimated over 30-40 days. These results reveal a threshold salinity concentration at which *Tamarix* physiological functioning decreases, but also illustrate the advantageous halophytic nature of *Tamarix* in these saline environments. Many arid and semi-arid environments are predicted to become more saline, however, results from both studies suggest that increasing salinity will not be a major barrier for *Tamarix* persistence and range expansion in these environments.

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Dedication

This thesis is dedicated to my grandfather, Bill Stracener. Pappaw never wasted a day with my visits in the summer teaching me how to love plants and always let me help in the garden. His love for plants has inspired me more than he will ever know and I hope to continue in his footsteps.

CHAPTER 1 - Introduction

Surpassing natural barriers by modern day travel, humans have become the principal global dispersers of vascular plants (Mack and Lonsdale, 2001). Human dispersal of vascular plants is both accidental (Muenscher, 1955) and intentional (Mack, 1999). Intentional introductions of foreign plants have helped humans survive for many years. Fossil records suggest that cultivation of plants far from their home ranges has occurred for thousands of years (Godwin, 1975). However, human dispersal of plants is not always beneficial. Foreign plants can become prolific in their new range and can cause economic losses, reduction of biodiversity, and other environmental problems (Pimentel et al., 2000; Vitousek et al., 1996). These plant species are referred to as naturalized species, invaders, or weeds (Richardson et al., 2000).

Economic and environmental costs of invading species have been reported extensively (Born et al., 2005; Brown and Sax, 2004; Gaudet and Keddy, 1988; Pimentel et al., 2000; Pimentel et al., 2005). In the year 2000, it was estimated that 50,000 non-native species had been introduced to the United States. Of the 50,000 species, 79, over a period of 85 years, had caused approximately $97 billion in damages (Pimentel et al., 2000). Invading species can also cause environmental damages. Invasive plant species have been shown to reduce native plant species richness (Martin, 1999) and diversity (Pysek and Psyek, 1995). These species can also alter belowground processes (Weidenhamer and Callaway, 2010) and fire cycles (Brooks et al., 2004; Kurdila, 1995). These changes ultimately alter community composition (Levine et al., 2003; Pimentel et al., 2000). Some invading plant species have even been termed transformer

species, as they change the character, condition, form, or nature of ecosystems over a substantial area relative to the extent of that ecosystem (Richardson et al., 2000).

By investigating the mechanisms of plant invasions, scientists can understand and predict future expansion and persistence of the invader. In this thesis, I have studied the physiological ecology of a transformer species, *Tamarix ramosissima* Ledeb. (hereafter referred to as *Tamarix*), in the context of changing riparian ecosystem salinities to elucidate the future persistence and range expansion of this tree species. This introduction will focus on the history of the *Tamarix* spp. invasion and discuss past and current research.

An Invader Promoted

An 1818 inventory from the Harvard Botanic Garden is the earliest mention of a *Tamarix* specimen growing in North America (Peck, 1818; Figure 1-1). However, the arrival and establishment of the invader into North America remains obscure. Commercial distribution of *Tamarix* began as early as 1823 (Robinson, 1965), although this documentation is incomplete. As early as 1868, six *Tamarix* specimens were growing at the United States Department of Agriculture (USDA) arboretum in Washington, D.C. (Robinson, 1965) and army engineers had already begun to propagate *Tamarix* for channel stabilization by 1886 (Mansfield, 1886). At this point in time, *Tamarix* spp. were regarded as desirable horticultural species and started to become popular as ornamental plants.

As enthusiasm grew for *Tamarix* trees, the USDA's section of Foreign Seed and Plant Introduction (SPI) was formed. This section of the USDA was responsible for retrieving foreign plants that would be economically valuable to North America. One of the section's main responsibilities was to find uses for introduced plants. *Tamarix* was already well known by the USDA and becoming increasingly popular, but the plant lacked a valuable use. One of the

section's staffers, Mark Carleton, grew *Tamarix* on his own farming land. In his special article for *Science*, Carleton described *Tamarix* plants as the most drought resistant and hardiest of all trees and shrubs (Carleton, 1914). He also noted that *Tamarix* was easily propagated from cuttings so many plants could be obtained from a small initial investment (Carleton, 1914). In 1916, J.J. Thornber, a botanist, revealed tests in the University of Arizona's *Timely Hints for Farmers* that also described *Tamarix* as a hardy plant (Thornber, 1916). Thornber noted that the uses of *Tamarix* would be constrained as ornamentals, hedges, windbreaks, and shade for small livestock. However, University of Texas Professor and Dean of Engineering Thomas Taylor would find another use: channel stabilization.

Army engineers had already used *Tamarix* for channel stabilization, but did not document their findings. Taylor wrote a paper entitled, "Tamarisk on Guard," in which he claimed *Tamarix* reduced sedimentation problems at McMillan Reservoir on the Pecos River, New Mexico (Chew, 2009). Taylor also noticed that *Tamarix* created such dense stands that the flow of the river was greatly diminished. In 1927, the Association of American Geographers would decide that the benefits of *Tamarix* outweighed the negative consequences based on Taylor's research. *Tamarix* trees would be propagated on rivers across the Southwestern U.S. (Bryan and Hosea, 1934).

An Ecosystem Altered

Very sparse and highly variable precipitation makes the American Southwest ill suited for dry farming. Water diversions and canals were already in place, but water storage and flood control were still lacking (Pisani, 2002). In 1902, President Theodore Roosevelt signed and enacted the Reclamation Act that would found the Bureau of Reclamation (Pisani, 2002). Hundreds of dams would be constructed along major riverways creating some of the largest reservoirs in the U.S. Rivers that once flooded every spring would now only flood when dam

infrastructure was overwhelmed. Native riparian trees that were phenologically adapted to spring floods were left without the annual renewal of bare sediments that their seeds require for germination. These native tree species were also subjected to prolonged, drowning flows (Chew, 2009). *Tamarix* trees were well adapted to handle droughted conditions and did not require the renewal of bare sediments to reproduce. Altering the regional hydrology created an ecological subsidy for *Tamarix*, but this went unnoticed for several years. (Chew, 2009).

Problems Arise

In the American southwest, farmers competed for water due to scarcity of the resource. Water law grew to become complicated due to this competition. In the southwest, water law is mainly based upon one principle, "first in time, first in right," or senior claims trump junior ones. New farmers, therefore, began to search for new water. Their solution was to curtail non-beneficial uses of surface water to free new water. Farmers turned to previous work by Oscar Meinzer, hydrologist and founder of the term, "Phreatophyte," which translates to "water loving plant." Meinzer suggested using phreatophytes to locate surface water in an earlier paper (Meinzer, 1926), but never suggested removing phreatophytes would create water savings.

The National Resources Committee formed in 1935 and its successor Planning Board formed in 1939 began investigations into water issues of the Upper Rio Grande and Pecos River. Investigation along the Pecos River included studies on Lake McMillan. It was at this time that *Tamarix* was labeled as a non-beneficial user of water, but how much water *Tamarix* consumed was unknown. Studies conducted at Lake McMillan estimated water use by phreatophytes with full-scale field data, but methods were different for each plant and were not described well (Chew, 2009). Results concluded that *Tamarix* was consuming more water than any other plant along the Pecos, but the results were doubted (Natural Resources Planning Board (NRPB),

1942). However, the NRPB decided *Tamarix* was consuming lots of water and that if it were eliminated, the water savings would be significant.

About the same time, water problems were on the rise in the Arizona Safford Valley along the Gila River. The Phelps Dodge Corporation (PDC), a mining company, had just made its home upstream of the Safford Valley. The mining corporation contained all rights to the land and all ore bodies. After the Pearl Harbor bombings, the government issued an order for PDC to increase its copper production by 80%, which would require much more water. PDC began to search for "new" water, as new farmers were doing, and began to investigate the possibility of water savings by removing phreatophytes. PDC gave rights to the United States Geologic Survey (USGS) to investigate these potential water savings. The Lower Safford Valley study used six methods to compute water use by phreatophytes and documented the highest transpiration rate for *Tamarix*, 7.2 acre-feet of water per acre (Gatewood et al., 1950). The 1950 report never mentioned how much water would be salvaged, but the solution was the same as it was at Lake McMillan, *Tamarix* elimination would produce water savings. At this time, hydrologist Thomas Robinson, also known as "Mr. Phreatophyte," began to build his career upon stereotyping *Tamarix* as a prodigious water-user (Johnson, 1972). *Tamarix* was no longer regarded as the exquisite ornamental, it was now labeled as an invader and a water-spending monster.

Current Research

Tamarix has remained a subject of interest for a long time and there is a vast array of literature describing the invasive species. Research has shown that *Tamarix* has many physiological adaptations that allow it to persist and expand its range in North America: (1) high seed production (Glenn and Nagler, 2005); (2) rapid germination and seedling establishment (Brotherson and Field, 1987); (3) high growth rates (Friederici, 1995); (4) drought tolerance

(Cleverly et al., 1997); and (5) extreme salt tolerance (Glenn et al., 1998). New methods have allowed scientists to accurately estimate transpiration of *Tamarix* compared to native riparian vegetation. Sap-flow measurements, the Bowen Ratio, and eddy covariance flux towers have been used to show evapotranspiration rates of *Tamarix* plants are similar to native vegetation (Cleverly et al., 2002; Devitt et al., 1998; Nagler et al., 2001, 2004; Sala et al., 1996). Studies investigating the effects of salinity on *Tamarix* were first published by Kleinkopf and Wallace (1974), but the effects of salinity on the physiological response of *Tamarix* trees have not been documented comprehensively. This thesis investigated how *Tamarix* leaf-level and whole-plant physiological responses vary as a function of increasing salinities. In chapter two I investigate these responses over a broad salinity gradient in the field. In chapter three I extend the NaCl concentration found in field measurements to *Tamarix* cuttings grown in a controlled environment. Finally, in chapter four I conclude these results and implicate future directions for *Tamarix* and salinity research.

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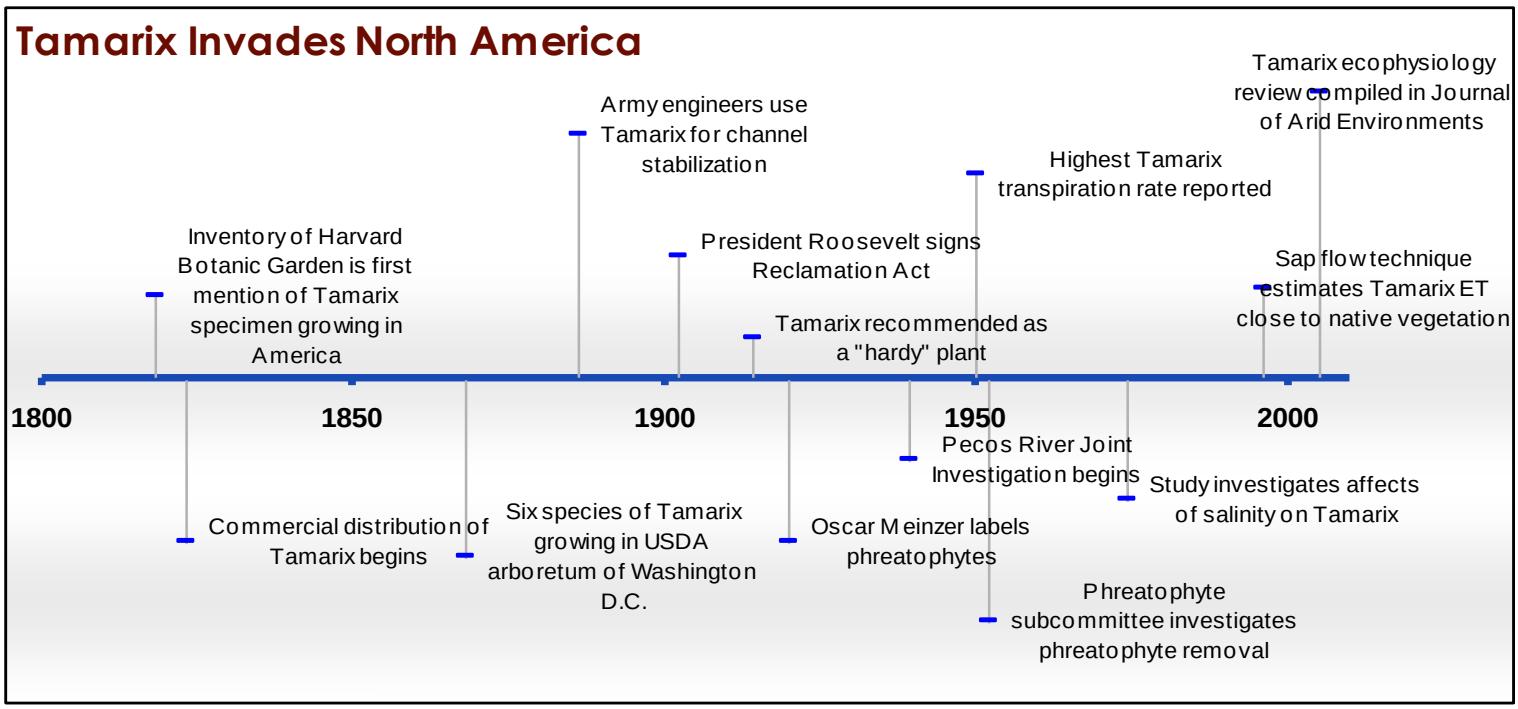
Figures and Tables

Figure 1-1 A timeline representing important biological and historical dates during the introduction of *Tamarix ramosissima* until present time (2010).



CHAPTER 2 - Leaf-Level Physiological Response of *Tamarix ramosissima* to Increasing Salinity[1]

Abstract

Over the past century, the invasive halophytic shrub *Tamarix ramosissima* Ledeb. has increased in abundance and distribution in riparian ecosystems of western North America. These increases coincide with anthropogenic modification of river systems which decrease the rate of periodic overbank flooding, leading to an increase in soil salinity. Increased soil salinity negatively impacts the physiology of native riparian tree species, but the impact of increased soil salinity on *Tamarix* physiology is incompletely known. To measure the impacts of soil salinity on *Tamarix*, I measured leaf-level responses across a broad range of salinity concentration at two sites in western Kansas. Photosynthesis at 2000 µmol m^{-2} s^{-1} ($A_{at\ 2000}$), stomatal conductance to water (g_s), intercellular CO_2 concentration (C_i), and leaf $\delta^{13}C$ showed little change over soil salinities from 0.5 to 17.65 mmhos/cm. The small variation in leaf physiological responses suggests robust functioning by *Tamarix* across a broad range of soil salinity. Leaf-level physiology and $\delta^{13}C$ responses were assessed by canopy position, but responses were not significantly different. These results are among the first to show broad acclimation and robust physiological functioning for many leaf-level processes measured on mature trees grown across a wide soil salinity gradient in the field.

[1]This chapter has been formatted for submission to *The Journal of Arid Environments*

Introduction

Over the past century, major river modifications including damming, flow alterations, and diversions for water use have led to decreased periodic overbank flooding in semi-arid and arid riparian ecosystems (DiTomaso, 1998; Everitt, 1980). These alterations have decreased soil moisture content and increased soil salinity, both of which influence community composition in riparian ecosystems (Glenn and Nagler, 2005; Pan, 2001; Ruan et al., 2009; Smith et al., 1998; Stromberg et al., 2007). Reduction of habitat quality in riparian ecosystems has contributed to the decline of native mesic tree species and opened a niche for invasion by *Tamarix ramosissima* Ledeb. (hereafter *Tamarix*) in the western United States (Busch and Smith, 1995; Ladenburger et al., 2006; Ruan et al., 2009; Stromberg et al., 2007).

Tamarix is a Eurasian shrub or tree that is common around ephemeral waters of semi-arid and arid climates (Baum, 1967; Chew, 2009). *Tamarix* is halophytic (salt-loving plant) and a facultative phreatophyte (water-loving plant) (Busch et al., 1992; Sala et al., 1996). The halophytic nature of mature *Tamarix* trees is one mechanism hypothesized to explain increased abundance in altered riparian ecosystems (Busch and Smith, 1995; Cui et al., 2010; Glenn and Nagler, 2005; Sala et al., 1996; Vandersande et al., 2001). *Tamarix* is reportedly tolerant of high salinities (Busch and Smith, 1995; Vandersande et al., 2001). However, increased saline conditions can impart metabolic stress even for halophytes (Khan et al., 2000; Moghaieb et al., 2004; Tal et al., 1979). Salt stress (e.g., NaCl) impacts plant physiology through a decline in leaf-level gas exchange, suppressed growth, osmotic effects, and the creation of reactive oxygen species (Parida and Das, 2005).

Plants have developed biochemical and molecular mechanisms to tolerate salt stress (Parida and Das, 2005). Examples of these mechanisms include exclusion of ions,

compartmentalization of ions, and synthesis of compatible solutes (Tester and Davenport, 2003). *Tamarix* shows non-selectivity in ion exclusion from salt glands, which is hypothesized as one mechanism by which *Tamarix* maintains an acceptable salt balance (Berry, 1970). The tolerance of *Tamarix* to saline soils might be a result of the synthesis of compatible solutes to protect enzymatic activity and cellular osmotic potential (Ding et al., 2009; Ruan et al., 2007; Ruan et al., 2009; Solomon et al., 1994). Solomon et al. (1994) showed that *Tamarix jordanis* synthesizes N-methyl-L-proline (MP) and N-methyl-*trans*-4-hydroxy-L-proline (MHP) in the presence of high NaCl content. The two solutes are effective for maintaining the carboxylating activity of Rubisco in *Tamarix jordanis* Boiss. Studies conducted along the Tarim River, China, showed *Tamarix* accumulated soluble sugars under salt stress which might contribute to the tolerance to high salinity in the species (Ruan et al., 2009). However, compatible solutes are energetically expensive to synthesize and may reduce plant growth or impact other physiological processes (Ding et al., 2009; Kleinkopf and Wallace, 1974; Tester and Davenport, 2003).

Few studies have reported how increasing salinity impacts physiological responses in *Tamarix*. Glenn et al. (1998) grew a mix of shrubs and trees, including *Tamarix ramosissima*, in a greenhouse over a salinity gradient from 0 to 32 g l^{-1} NaCl. *Tamarix* had a minor 2% reduction in relative growth rate, but transpiration decreased between 16 and 32 g l^{-1} NaCl (Glenn et al., 1998). Leaf-level processes such as transpiration, photosynthesis, and stomatal closure are sensitive to salinity stress (Parida and Das, 2005). Busch and Smith (1995) investigated how hydrologic variation and varying salinity in floodplain environments affects ecophysiological responses of dominant woody taxa including *Tamarix*. Physical site differences were subtle, and soil salinity did not vary significantly in areas sampled. Kleinkopf and Wallace (1974) found increasing salinity had a small effect on leaf-level gas exchange. Growth decreased in *Tamarix*

at higher salt levels, which the authors attributed to a greater energy demand to transport salt to leaf salt glands.

To elaborate on the responses of *Tamarix* to soil salinity, I measured several leaf-level physiological responses over a wide salinity gradient in western Kansas. Our specific questions were: (1) Does increasing salinity reduce leaf-level photosynthesis, stomatal conductance to water, intercellular CO_2 concentration, pre-dawn and mid-day water potentials, alter the natural abundance of ^{13}C and ^{15}N, or C:N? And (2) do leaf-level photosynthesis, stomatal conductance to water, intercellular CO_2 concentration, pre-dawn and mid-day water potentials, the natural abundance of ^{13}C, and C:N vary as a function of *Tamarix* canopy structure across a salinity gradient?

High salinity is known to disrupt water-uptake of plants as well as to cause ionic toxicity (Tester and Davenport, 2003). High soil salinity lowers soil water potential disrupting the soil-plant-atmosphere-continuum on which plants take up water through bulk flow (Mahjan and Tuteja, 2005). This water stress can be reflected as lower leaf-level water potential (Ψ_w) and therefore, I predicted that leaf-level water potential would decline as soil salinity increased. Plants also lose water during leaf-level gas exchange processes when stomates are open (Cruiziat et al., 2002). Photosynthesis, stomatal conductance to water, and intercellular CO_2 concentration, are all measurements that reflect gas exchange through the stomates of leaves. Many plants close stomates during periods of water stress to conserve water and leaf-level gas exchange stops or occurs at a reduced rate (Chen et al., 2010). As high salinity causes water stress, I predicted that *Tamarix* leaf-level photosynthesis, stomatal conductance to water, and intercellular CO_2 concentration would be reduced at higher soil salinities. Furthermore, Na^+ is highly toxic in the

cytoplasm of plant cells and can disrupt enzymatic functioning resulting in reduced photosynthetic rates (Parida and Das, 2005).

Stomatal regulation can be monitored by measuring the natural abundance of the stable isotope, ^{13}C. As stomates close, the carboxylating enzyme RuBisCO uses more ^{13}C, which results in a heavier $\delta^{13}C$ value (Dawson et al., 2002). As water stress is predicted to increase at higher soil salinities, and stomates close during water stress, I predicted that $\delta^{13}C$ would be heavier in *Tamarix* leaves at higher salinities. Salinity can also affect a plant's ability to acquire nitrogen. Therefore, I predicted that leaf C:N would be highest in plots with higher salinities.

Salinity stress is exacerbated in leaves that are shaded (Parida and Das, 2005). Trees arrange leaves in a mosaic like framework to intercept as much incoming light as possible. Leaves at the bottom of the canopy receive less sunlight and typically do not have gas exchange rates as high as leaves that are exposed to more direct sunlight at the top of the canopy (Yoshimura, 2010). During salinity stress, shaded leaves tend to show signs of stress first (Parida and Das, 2005) and therefore, I predicted that shaded leaves, or leaves at the bottom of *Tamarix* canopies, would have the lowest photosynthesis, stomatal conductance to water, intercellular CO_2 concentration, water potentials, higher C:N, and the heaviest $\delta^{13}C$ values.

Materials and Methods

Study Area

This research was performed at two sites in western Kansas. The Ashland research site is a Kansas Geological Survey and Kansas State University research site located adjacent to the Cimmarron River, Ashland, Kansas, USA (37°11'19"). *Tamarix* is the predominant species at this site, but other herbaceous species are intermixed among the *Tamarix* and include *Sporobolus*

airoides (Torr.), *Panicum virgatum* L., and *Schizachyrium scoparium* (Michx.) (Nippert et al., 2010). Soil textures at this site consist of coarse silts through medium sands. Cedar Bluff State Park is near Ellis, Kansas, USA (38°48'N and 99°43'W) and managed by the Kansas Department of Wildlife and Parks (KDWP). The size of Cedar Bluffs Reservoir varies year by year and receives intermittent flow from the Smoky Hill River in eastern Colorado. Riparian areas are dominated by juvenile and adult *Tamarix* as well as other vegetation including *Sporobolus compositus* (Michx.), *Schizachyrium scoparium*, and *Populus deltoides* (Bartr.).

Salinity Analysis

In May 2009, four 10m X 5m plots were established at each site. Four or five soil core samples were collected from each plot at 15 cm depth in May and September, 2009. All soil cores were homogenized into a single sample per plot. Analyses were conducted at the Kansas State University Soil Testing Center. Samples were sieved, dried, made into a soil paste, and the electrical conductivity (EC) of the soil paste was measured in mmhos/cm.

Plant Physiology

Five *Tamarix* individuals, each approximately 1.5 meters in height, were randomly selected in each plot and the same individuals were measured during June, July, August, and September, 2009. Physiological measurements were conducted at three canopy locations that were categorized as bottom of the canopy, middle of the canopy, and top of the canopy for each replicate. On each sampling date, gas exchange measurements were taken using a LiCor-6400 infra-red gas analyzer with a red/blue light source and a CO_2 injector (LiCor, Lincoln, Nebraska, USA). Irradiance inside the cuvette was 2,000 µmol m^{-2} s^{-1}, CO_2 concentration was 400 ppm and relative humidity was maintained at ambient. Gas exchange measurements were made on

new, mature leaves growing in full sunlight between 0800 and 1700 hours Central Daylight Time (CDT). Measurements included photosynthetic rate at 2000 µmol m^{-2} s^{-1} ($A_{at\ 2000}$), stomatal conductance to water (g_s), and intercellular CO_2 concentration (C_i). Measurements occurred on clear days and projected leaf area within the gas exchange cuvette was estimated using a LiCor 3100 leaf area meter (LiCor, Lincoln, Nebraska, USA). Water potential measurements were conducted at both predawn (0300-0600 hours CDT) and midday (1300-1500 hours CDT) using a Scholander pressure bomb (PMS instruments, Albany, Oregon, USA). One leaf sample per individual per canopy position per plot was measured from June-September. Data were analyzed using a mixed effects model ANOVA in SAS 9.1 (Cary, North Carolina, USA), where site, plot nested within site, and canopy location were fixed effects, whereas sampling date was a random effect to account for repeated measures in the design. Gas exchange measurements were not recorded during September at either site and water potential data were not collected for the Ashland research site in September due to inclement weather.

Stable Isotopic Analysis

Leaf samples were collected from each individual at each canopy position for each sampling period except for the Ashland research site in September, 2009. Samples were dried at 60ºC for 48 hours and ground to a fine powder. Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopic signature by using a Finnigan Delta-plus continuous flow isotope ratio mass spectrometer connected to an elemental analyzer. The within run precision was <0.15‰ for $\delta^{15}N$ and <0.05‰ for $\delta^{13}C$. Between run variation was <0.2‰ for $\delta^{15}N$ and <0.08‰ for $\delta^{13}C$. C:N values were obtained from an elemental analyzer.

Results

Electrical conductivity (EC) values varied between and within both study sites with a range between 0.5 to 17.65 mmhos/cm (Table 2-1). All statistical results are reported in Table 2-2. No trends were evident across sites or across plots nested within sites for leaf-level gas exchange responses (Fig. 2-1 a, b, c). $A_{at\ 2000}$ values significantly varied by plot nested within site and by canopy position ($p<0.05$), but not across sites ($p>0.05$). Photosynthetic rates ranged from 15 to 27 μmol CO_2 m^{-2} s^{-1} across all plots (Fig. 2-1 a). C_i values were not significantly different between sites ($p>0.05$), but did vary significantly among plots nested within sites ($p<0.05$) and by canopy position ($p<0.05$). C_i values ranged from 203 parts per million (ppm) to 264 ppm across all plots. Stomatal conductance to water (g_s) rates ranged from 0.19 to 0.4 mol H_20 m^{-2} s^{-1} and did not vary significantly between sites ($p>0.05$), but did vary significantly across plots nested within site ($p<0.05$) and by canopy position ($p<0.05$). Photosynthesis, intercellular CO_2 concentration, and stomatal conductance to water significantly varied by canopy position, but no trends were evident across canopy positions (Figure 2-3 a, b, c) and there was not a significant salinity*canopy interaction ($p>0.05$).

Pre-dawn water potentials ranged from -0.9 to -1.3 MPa and mid-day water potentials ranged from -1.5 to -2 MPa (Fig. 2-2a). Pre-dawn water potentials did not vary significantly by canopy position ($p>0.05$) or between sites ($p>0.05$), but did vary significantly across plots nested within site ($p<0.05$). Mid-day water potentials did not vary significantly between sites ($p>0.05$), but did vary significantly across plots nested within site ($p<0.05$) and by canopy position ($p<0.05$). C:N values varied significantly between sites ($p<0.05$). At Cedar Bluffs Reservoir, C:N varied significantly between plots ($p<0.05$), but did not vary significantly by canopy

position ($p>0.05$). At the Ashland research site, C:N values significantly varied across plots ($p<0.05$) and by canopy position ($p<0.05$). C:N values ranged from 16:1 to 31:1 across all plots.

Leaf samples had the heaviest $\delta^{13}C$ signatures at the Ashland research site as compared to Cedar Bluffs Reservoir (Fig. 2-2 b). Leaf $\delta^{13}C$ values varied significantly between sites ($p<0.05$). Leaf $\delta^{13}C$ varied significantly by canopy position at the Ashland research site, but not between plots. At Cedar Bluffs Reservoir, leaf $\delta^{13}C$ values significantly varied between plots ($p<0.05$) and by canopy position ($p<0.05$). Leaf $\delta^{15}N$ values significantly varied between sites ($p>0.05$) with heavier $\delta^{15}N$ signatures at Cedar Bluffs Reservoir (Fig. 2-2 d). Leaf $\delta^{15}N$ values significantly varied between plots at the Ashland research site and Cedar Bluffs Reservoir ($p>0.05$). Leaf $\delta^{15}N$ did not significantly vary by canopy position at either site ($p>0.05$).

Discussion

Increasing salinity causes salt stress in most plants and this stress is reflected in leaf-level physiological measurements (Khan et al., 2000; Leport et al., 2006; Tester and Davenport 2003). Salt stress inhibits photosynthesis, suppresses growth, affects protein synthesis, and alters energy and lipid metabolism (Parida and Das, 2005). In this study, soil EC varied broadly across both study sites. I expected leaf-level physiological measurements to decline as soil EC increased (Gulzar et al., 2003; Parida et al., 2004; Parida and Das, 2005). However, I found no support that leaf-level physiological responses of *Tamarix* varied as a function of soil EC over the salinity gradient measured.

Tamarix physiological functioning was maintained across all soil EC values, suggesting that *Tamarix* is able to accommodate a broad range of salinities, which is consistent with other studies (Brotherson and Winkel, 1986; Busch and Smith, 1995; Ruan et al. 2009). As salinity increased among all plots between sites, water potential did not significantly change. Soil

salinity disrupts the soil-plant-atmosphere-continuum on which plants obtain water (Mahajan and Tuteja, 2005). I predicted that leaf-level water potentials would decrease as soil salinity increased. As plants become more water stressed, leaf-level gas exchange is typically reduced and $\delta^{13}C$ values become heavier (Parida and Das, 2005; Tester and Davenport, 2003). Photosynthesis, stomatal conductance to water, and intercellular CO_2 concentration did not significantly change as salinity increased. It may be hypothesized, then, that the driver of physiological responses in *Tamarix* is soil moisture, not salinity. However, it is also possible that the threshold salinity to elicit a physiological decline from *Tamarix* was not reached. Previous results from a greenhouse study by Glenn et al. (1998) suggest that *Tamarix* leaf-level physiology exhibited marginal decreases until 29 mmhos/cm (20,000 ppm) EC. When tested under field conditions, our results are consistent with Kleinkopf and Wallace (1974), who showed there were only marginal effects on *Tamarix* leaf-level gas exchange over a salinity gradient from 0 to ~17.5mmhos/cm.

Kleinkopf and Wallace (1974) did observe a reduction in *Tamarix* growth as salinity increased. The authors attributed this growth decline to diversion of energy for use in salt pumping and energy production through respiration. Indeed, salt is exuded through salt glands of *Tamarix* species via an apoplastic xylem pathway (Campbell et al., 1974; Arndt et al., 2004). Since regulation of salinity is an energy-requiring process, I expected to see declines in leaf-level physiology for *Tamarix* trees by canopy position.

Sun and shade leaves have varying leaf morphologies and physiologies (McClendon, 1962; Oberbauer and Strain, 1986; Wylie, 1951). Shaded leaves tend to be less photosynthetically efficient than sun leaves and typically show signs of salt stress first (Oberbauer and Strain, 1986; Stephens et al., 2009). Therefore, I expected less energy to be

contributed to leaf maintenance in shaded leaves and thus, a larger decline in leaf-level photosynthesis, stomatal conductance, and intercellular CO_2 concentration in shaded leaves. Leaf-level gas exchange, $\delta^{13}C$, and mid-day water potential varied significantly ($p<0.05$) by canopy position, but a significant canopy*salinity interaction did not exist. Over the range of salinities measured, physiological responses to increasing salinity did not impact shaded leaves in the bottom of the canopy proportionally more than leaves in the top of the canopy. It is interesting to note that leaves at the bottom of the canopy exhibited reduced photosynthesis and increased intercellular CO_2 concentration. These results are likely a reflection of higher C:N at the bottom of the canopy suggesting these leaves have lower foliar nitrogen content. Low nitrogen content can cause lower photosynthetic rates regardless of irradiance (Cai et al., 2008). Furthermore, shaded leaves tend to have lower nitrogen concentrations than sun leaves (Evans, 1993; Evans and Poorter, 2001).

C:N varied significantly between the Ashland research site and Cedar Bluffs Reservoir. Cedar Bluffs Reservoir had much lower C:N values suggesting *Tamarix* leaves had a higher foliar nitrogen content at this site. Drivers of $\delta^{15}N$ likely varied between sites. $\delta^{15}N$ increased between soil conductivities of 8.55 and 17.65 mmhos/cm, which corresponded to an increase in soil pH from 7.3 to 8.5 (Figure 2-2d). Pataki et al. (2005) showed $\delta^{15}N$ increased significantly in saline *Tamarix* leaves compared to non-saline *Populus* leaves. The response of ^{15}N was attributed to increased soil pH associated with saline soils. High soil pH results in the volatilization and loss of NH_3 which enriches the remaining substrate in ^{15}N. At Cedar Bluffs Reservoir, $\delta^{15}N$ values were much higher than the Ashland research site, but showed no trends over the salinity gradient. High $\delta^{15}N$ and high C:N values at Cedar Bluffs Reservoir likely reflect higher nitrogen availability. Craine et al. (2009) showed a correlation between $\delta^{15}N$ and

nitrogen availability, suggesting that $\delta^{15}N$ increases as soil nitrogen availability increases. The results at Cedar Bluffs Reservoir do not suggest that high salinity resulted in higher ^{15}N responses because the salinity gradient at this site was much narrower than the Ashland research site. Alternate explanations for the carbon and nitrogen dynamics could be changes in soil textures between sites (McLauchlan, 2006; Mclnerney and Bolger, 2000) or differences in precipitation (Austin and Sala, 2002; Knapp and Smith, 2001). However, the Ashland research site and Cedar Bluffs Reservoir received similar precipitation amounts for 2009 (550mm) and soil textures were also similar, consisting of coarse silts through medium sands.

The overall objective of this study was to assess leaf-level physiological responses of *Tamarix* to increasing salinity. Our results illustrate robust physiological response for many leaf-level variables measured on mature *Tamarix* trees grown across a wide soil salinity gradient in the field. Our findings support other results that high salinities might contribute to the competitive advantage of *Tamarix* (Busch and Smith, 1995; Glenn et al., 1998; Ruan et al., 2009). Arid and semi-arid environments are predicted to become more saline (Jolly et al., 2008). However, results from this study suggest increasing salinity will not be a major barrier for *Tamarix* persistence and range expansion in these environments.

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Figures and Tables

Plot	Electrical Conductivity (mmhos/cm)	Soluble Na paste (meq/100g)	pH	Estimated CEC (meq/100g)
ARS (A)	1.65	1.3	7.3	21
ARS (B)	12.2	3.4	7.7	13
ARS (C)	17.65	1.67	8.5	7
ARS (D)	8.55	2.02	7.4	8
CBR (E)	2.35	0.09	7.3	21
CBR (F)	1.6	0.12	7.3	17
CBR (G)	0.9	0.05	4.6	17
CBR (H)	0.5	0.06	6.9	14

Table 2-1 The electrical conductivity, soluble Na paste, pH, and estimated CEC among plots between the Ashland Research Site (ARS) and Cedar Bluffs Reservoir (CBR).

Response Variables	Canopy Position	Plot (Site)	Site	ARS Plots	ARS Canopy Position	CBR Plots	CBR Canopy Positions
$A_{at\ 2000}$	*	*					
g_s	*	*					
C_i	*	*					
$\Psi_{w\ predawn}$		*					
$\Psi_{w\ midday}$	*	*					
$\delta^{13}C$			*		*	*	*
$\delta^{15}N$			*	*		*	
C:N			*	*	*	*	

Table 2-2 Results from all statistical tests; an asterisk (*) denotes significance ($p<0.05$) for a response variable in a MIXED model ANOVA. ARS represents plots at the Ashland Research Site and CBR represents plots located at Cedar Bluff's Reservoir.

Figure 2-1 *Tamarix ramosissima* mean (±1 SE) a) photosynthetic rate at 2000 µmol m^{-2} s^{-1} (A_{at2000}), b) intercellular CO_2 concentration (C_i), and c) stomatal conductance to water (g_s) sampled across a wide range of salinity concentrations as expressed by electrical conductivities (EC). Gas-exchange measurements were not recorded on September, 2009 at either site due to inclement weather.

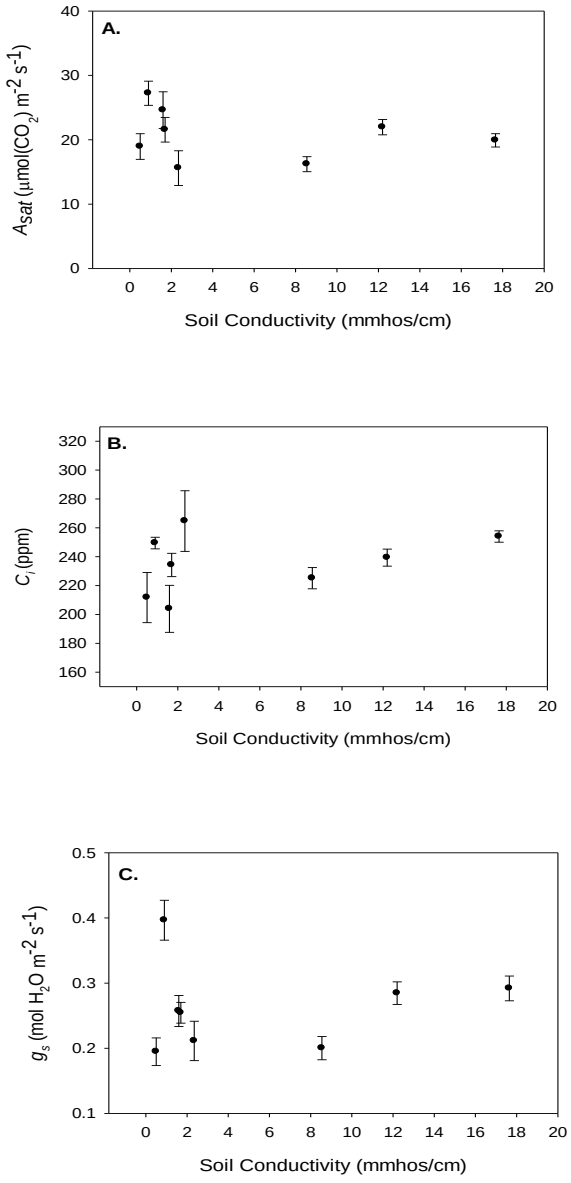


Figure 2-2 *Tamarix ramosissima* mean (±1 SE) a) pre-dawn (black circles) and mid-day (white circles) water potential, b) stable carbon isotopic signature ($\delta^{13}C$) for the Ashland Research Site (ARS) and Cedar Bluffs Reservoir (CBR), c) C:N for both ARS and CBR, and d) stable nitrogen isotopic signature ($\delta^{15}N$) for ARS and CBR. ARS data are denoted by black circles and CBR data are denoted by white circles. ARS was not sampled in September, 2009 because of inclement weather.

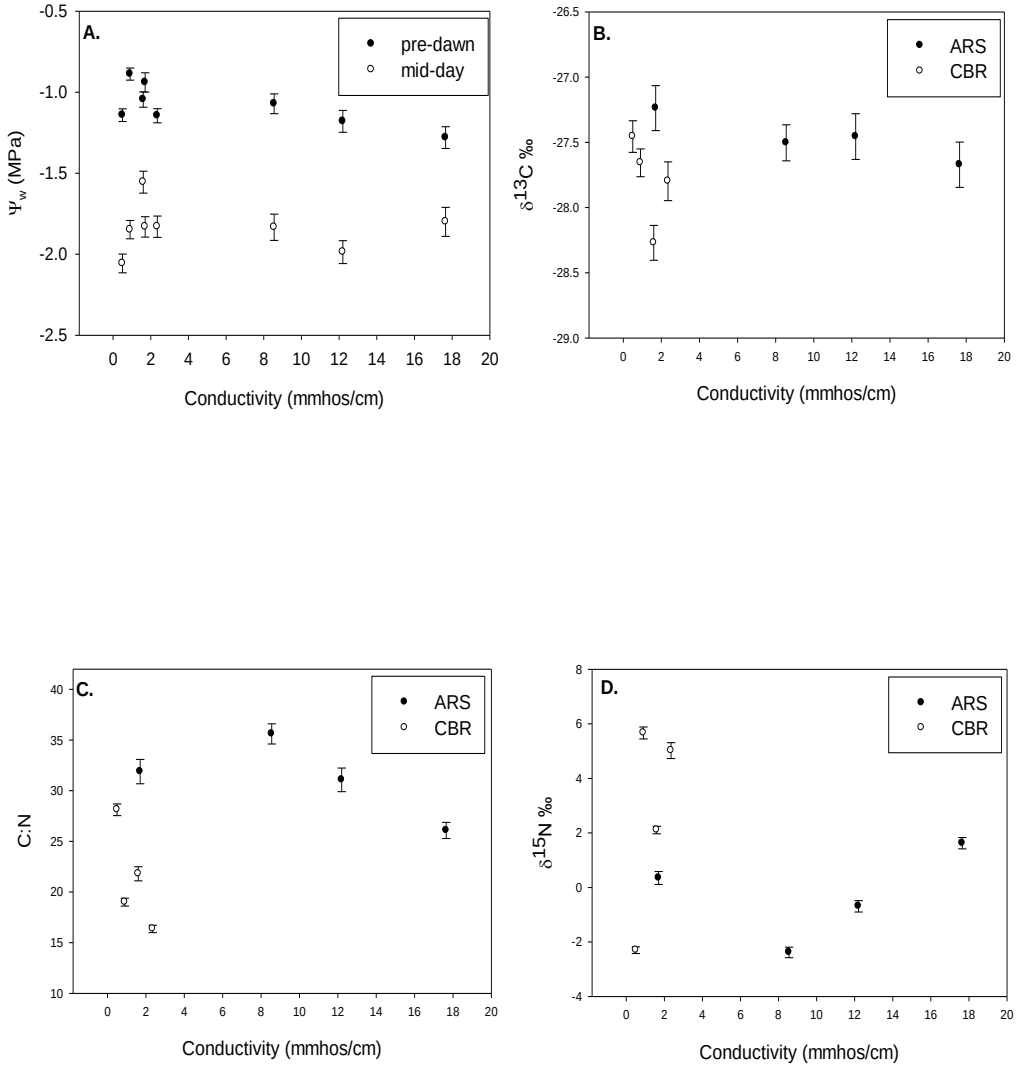
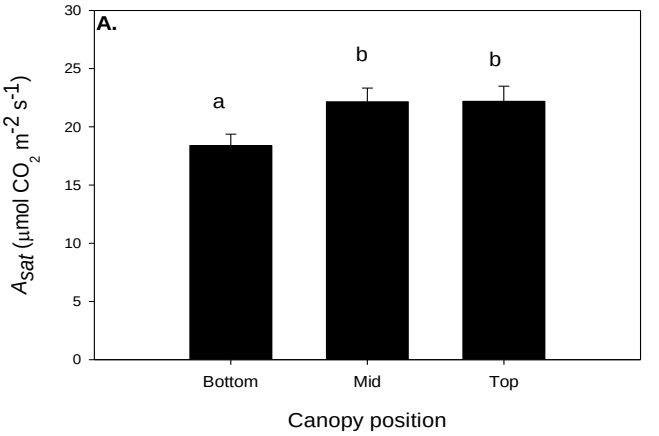
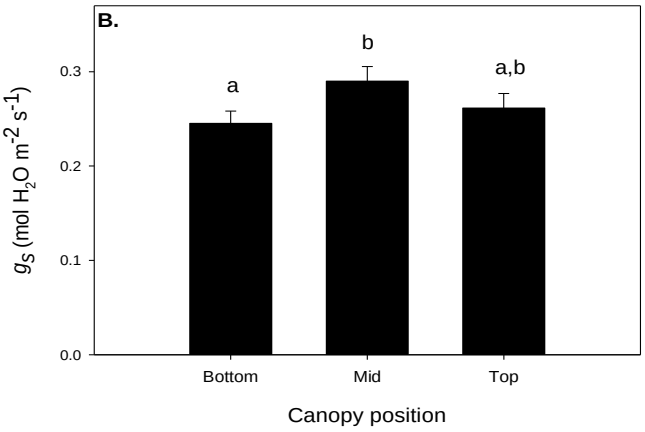


Figure 2-3 *Tamarix ramosissima* mean (±1 SE) a) photosynthetic rate at 2000 µmol m^{-2} s^{-1} (A_{at2000}), b) intercellular CO_2 concentration (C_i), and c) stomatal conductance to water (g_s) response by canopy position.





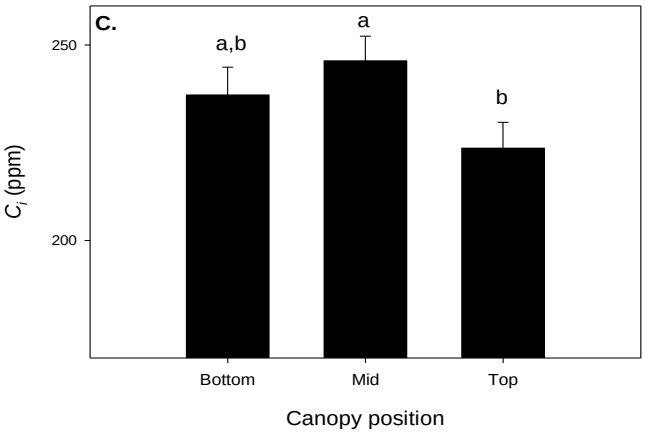
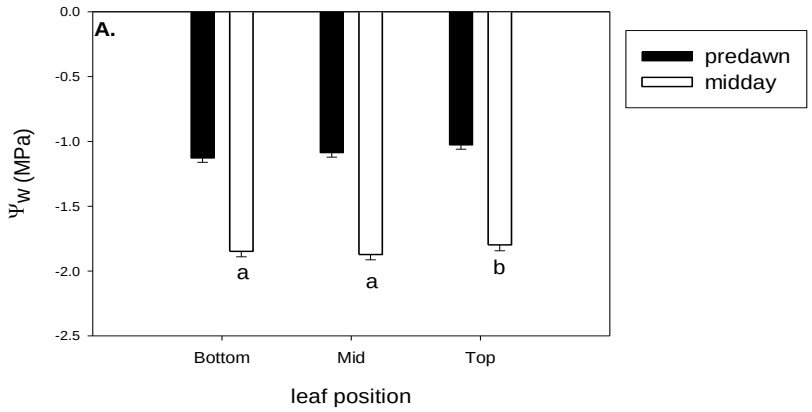
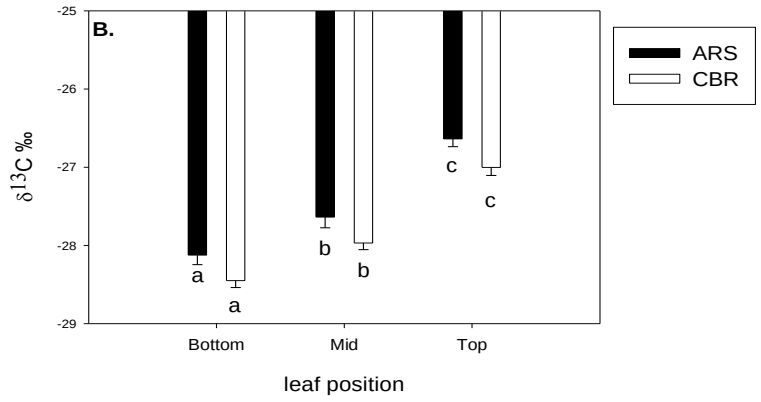
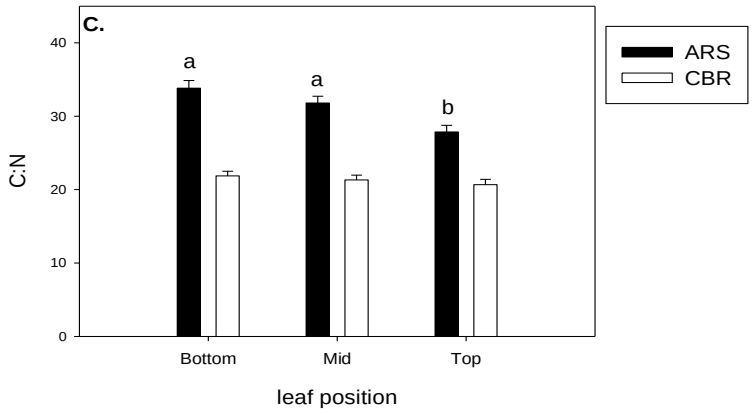


Figure 2-4 *Tamarix ramosissima* mean (±1 SE) a) pre-dawn (black bars) and mid-day (white bars) water potential, b) stable carbon isotopic signature ($\delta^{13}C$) for the Ashland research site (ARS) (black bars) and Cedar Bluffs Reservoir (CBR) (white bars), and c) C:N for both ARS (black bars) and CBR (white bars).







CHAPTER 3 - Physiological Responses of *Tamarix ramosissima* to a NaCl Concentration Gradient[1]

Abstract

Alterations of hydrologic regimes and geomorphology can shift disturbance regimes and the timing of resource availability, which might lead to a change in species with a different suite of life-history traits. In western North America, the lowland hydrology has been fundamentally altered leading to lower water tables and increased salinity. These hydrologic alterations over the past century have contributed to the establishment and spread of an introduced species, *Tamarix ramosissima* Ledeb., which now dominates riparian ecosystems of this region. *Tamarix* is a halophytic species and its salt tolerant strategies may contribute to its widespread occurrence in western North America. However, the physiological responses of *Tamarix* to salinity stress are incompletely known. I measured several whole plant and leaf-level physiological responses in a controlled environment over a gradient of NaCl concentrations. *Tamarix* photosynthesis (A_{at2000}), stomatal conductance to water (g_s), water potential (Ψ_w), and the maximum quantum yield of photosystem II (*Fv/Fm*) decreased at 15 and 40 g l^{-1} NaCl compared to control treatments. However, *Tamarix* was able to acclimate to high NaCl treatments after approximately 35 days as indicated by increasing photosynthetic rates, dark adapted chlorophyll fluorescence, and stomatal conductance to water, which might correspond to increases of proline. This acclimatization response suggests the salt tolerant strategies of *Tamarix* are effective, even at extremely high NaCl concentrations.

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Introduction

Plant growth and production can be adversely affected by various biotic and abiotic stressors. Plants are frequently exposed to a wide variety of stressful conditions including salinity, drought, flooding, low temperatures, heat, pathogens, fungi, and bacteria (Mahajan & Tuteja, 2005). Among abiotic stressors, salinity is considered one of the major causes of decreased primary production, particularly in crop yield loss (Albassam, 2001; Huang et al., 2009; Nublat et al., 2001; Zhang et al., 2010). Approximately 20% of the word's cultivated land is affected by salinity and nearly half of all irrigated lands are affected by salinity (Ashraf & Harris, 2004; Huang et al., 2009; Mahajan & Tuteja, 2005; Munns, 2002; Sairam & Tyagi, 2004; Tester & Davenport, 2003). Salinity impacts crop production and is also a determinant of the ecological distribution of plant species (Moghaieb et al., 2004).

Salinity Stress

Although salts exist in many natural forms, plant salinity stress is primarily caused by NaCl and its associated ions (Parida & Das, 2005). NaCl imposes two major stress effects, ionic and osmotic (Khan et al., 2000; Parida & Das, 2005; Rosental et al., 1979; Slama et al., 2008; Tester & Davenport, 2003). Both Na^+ and Cl^- can accumulate to high concentrations in the leaves of plants, and both are toxic in the cytoplasm of plant cells (Tester & Davenport, 2003). However, Na^+ is more toxic to plant cells than Cl^- (Parida & Das, 2005). High Na^+ concentration competes with K^+ in plant cells for binding sites that are essential for cellular function (Munns, 2002; Zhang et al., 2010). K^+ is required by plants for maintaining osmotic balance, opening and closing stomata, and is an essential co-factor for many enzymes such as pyruvate kinase (Mahajan & Tuteja, 2005). For example, Slama et al. (2008) grew *Sesuvium portulacastrum* cuttings and subjected these cuttings to drought, salinity, or a combination of drought and

salinity. When cuttings were subjected to droughted conditions, K^+ concentration decreased, but not as drastically as when cuttings were subjected to salinity.

NaCl can also induce osmotic effects in both plants and soils. Salinity alters soil physical properties, causing a decline in soil structure because of increased swelling, dispersion, and slaking that is due to soil wetting and then crusting or hardsetting upon drying. These alterations in soil physical properties cause declines in permeability, infiltration, hydraulic conductivity, and osmotic potential (Wong et al., 2010). Lower osmotic potential in soil disrupts the soil-plant-atmosphere continuum (SPAC) on which plants move water through xylem. Leaf-level water potential, stomatal conductance, and transpiration rates all decline under salinity stress (Parida & Das, 2005). For example, *Atriplex halimus* plants irrigated with 600 mM NaCl decreased Ψ_s as low as -7.0 MPa in leaves (Bajji et al., 1998).

Salinity Tolerance

Although salinity might adversely affect the production and growth of many plants, some plants have adapted to tolerate highly saline environments. These plants are termed halophytes, which means, "salt loving plants." One mechanism to tolerate high salinities is to regulate Na^+ transport to shoots and leaves. This is important to maintain a high K^+:Na^+ ratio, as one basis of Na^+ toxicity is competition with K^+ for K^+ binding sites (Amtmann & Sanders, 1999; Cuin et al., 2003). Salts can be excluded from leaves by the selectivity of uptake by root cells, although it is unclear which cell types control this selectivity (Munns, 2002). Some halophytic species have specialized salt glands or salt bladders that exude salt from the plant via apoplastic pathways (Agarie et al., 2007; Park et al., 2009).

Compartmentalization and the creation of compatible solutes are also important salt tolerating mechanisms. Many halophytes compartmentalize Na^+ in cell vacuoles to limit toxicity in the cytoplasm (Mahajan & Tuteja, 2005; Munns, 2002; Parida & Das, 2005; Tester & Davenport, 2003). Compartmentalization of Na^+ disrupts the osmotic balance in cells between the vacuole and cytoplasm. Plants may synthesize compatible solutes (e.g., proline, glycine betaine) in the cytoplasm to reestablish osmotic balance. These low-molecular-mass compounds do not interfere with normal biochemical reactions (Zhifang & Loescher, 2003). However, compatible solutes are energetically expensive, requiring as much as 52 ATP per mol for synthesis (Raven, 1985).

The Case for Tamarix ramosissima

In the western United States, soils in riparian areas have become saline (Beauchamp et al., 2009). Soils are saline due to low precipitation, which allows salts to accumulate in the soil. This salinization is often exacerbated by flow regulation, groundwater pumping, and river channel changes that decrease the frequency of overbank flooding which washes salts away (Beauchamp et al., 2009; Meritt & Poff, 2010; Stromberg et al., 2007). Timing of resource availability and disturbance regimes is often altered with shifts in geomorphic and hydrological regimes, which in turn alter competitive hierarchies and favors species with a different suite of life-history traits (Stromberg et al., 2007; Tickner et al., 2001). Many introduced species have become introduced to areas in which fluvial alterations are present (Hobbs & Huenneke, 1992; Holmes et al., 2005; Meeks et al., 2010). One such invading species is *Tamarix ramosissima* Ledeb. (hereafter referred to as *Tamarix*).

Tamarix is a small tree or shrub that is common around ephemeral waters and is native to Eurasia (Chew, 2009; DiTomaso, 1998; Everitt, 1980). *Tamarix* has many physiological

adaptations hypothesized to allow the species to persist along disturbed riparian corridors. These adaptations include high seed production, high growth rates, drought tolerance, ability to resprout after fire or grazing, facultative phreatophytic nature, and extreme salt tolerance (Busch & Smith, 1995; Cleverly et al., 1997; Glenn et al., 1998; Glenn & Nagler, 2005; Nippert et al., 2010).

Tamarix is a halophytic species and imparts various salinity tolerance mechanisms. Most notably, *Tamarix* develops salt glands that secrete excess salts that would be accumulated by non salt-tolerant species (Berry, 1970; Wilkinson, 1966). Salt is excreted in solution through specialized salt glands via an apoplastic pathway to alleviate metabolic stress caused by Na^+. *Tamarix* also accumulates compatible solutes when under salinity stress. Studies conducted along the Tarim River, China (Ruan et al., 2007, 2009) and the Yellow River, China (Cui et al., 2010) suggest *Tamarix* creates compatible solutes (proline and soluble sugars) during salinity stress to maintain internal osmotic balance. Solomon et al. (1994) also showed that *Tamarix jordanis* Boiss. synthesizes N-methyl-L-proline (MP) and N-methyl-*trans*-4-hydroxy-L-proline (MHP) in the presence of high NaCl content. Evidence suggests both solutes are effective at maintaining the carboxylating activity of Rubisco.

Although *Tamarix* has salt-tolerating mechanisms, physiological responses of *Tamarix* to salinity stress are incompletely known and few studies have reported how increasing salinity impacts these responses. Studies by Kleinkopf and Wallace (1974) found increasing salt levels had very little effect on the net exchange rates of carbon and water in *Tamarix*. Kleinkopf and Wallace (1974) also measured a decrease in *Tamarix* growth as salinity increased. They attributed this energy loss to salt gland pumping. Glenn et al. (1998) grew a mix of shrubs and trees, including *Tamarix*, in a greenhouse and subjected plants to a salinity gradient from 0 to 32

g l^{-1} NaCl. *Tamarix* transpiration decreased markedly between 16 and 32 g l^{-1} NaCl, but growth rate showed only a minor reduction (2%).

The halophytic nature of *Tamarix* has been hypothesized as a factor contributing to the spread and establishment of the species (Glenn et al., 1998; Glenn & Nagler, 2005; Hayes et al., 2009; Kleinkopf & Wallace, 1974). Because salinity varies in concentration spatially, it is important to understand how *Tamarix* physiology is impacted by this variation. To elaborate on the responses of *Tamarix* to soil salinity, I measured several whole plant and leaf-level physiological responses on cuttings grown over a salinity gradient in a controlled environment. I tested the following questions: (1) what is the concentration of NaCl at which *Tamarix* may no longer maintain photosynthesis, intercellular CO_2 concentration, stomatal conductance to water, water potential, the maximum quantum yield of photosystem II, and the natural abundance of ^{13}C and ^{15}N? And (2) how does photosynthesis, intercellular CO_2 concentration, stomatal conductance to water, water potential, the maximum quantum yield of photosystem II, and the natural abundance of ^{13}C and ^{15}N change over time as salinity stress is induced?

Materials and Methods

Experimental Design and Procedures

Branch tip cuttings of *Tamarix ramosissima* were collected from trees growing at two sites. The Ashland Research Site (ARS) is on the Arnold Ranch adjacent to the Cimmaron River near Ashland, Kansas, USA (37°11'19"). Cedar Bluff Reservoir (CBR) is near Ellis, Kansas, USA (38°48'N and 99°43'W). Cuttings were kept moist, cut at the stem base (approximately 0.6 cm in diameter) and auxin was applied to promote root development. Cuttings were propagated in a Conviron (Pembina, North Dakota, USA) growth chamber at Kansas State University

(Manhattan, Kansas, USA) in plastic nursery pots (19.3 cm diameter, 17.8 cm deep). Prior to transplanting cuttings to pots, soils were soaked in a nutrient solution made up of 20% nitrogen 20% phosphoric acid, 20% soluble potash, 0.02% boron, 0.05% chelated copper, 0.15% chelated iron, 0.05% chelated manganese, 0.0009% molybdenum, and 0.05% chelated zinc. Pots contained 550 g of a mixture of potting soil and native soil (1:1 v/v). Controlled environment conditions were set on a 12-hour photoperiod. Humidity was set at 65%, average temperature was maintained at 25°C, CO_2 concentration was maintained at 600 ppm, and PAR measured at 330 µmol m^{-2} s^{-1}.

Salinity treatments were implemented for each cutting. Distilled water was added to NaCl to make solutions of 0, 15, and 40 g l^{-1} NaCl. Salinity trials were initiated by irrigating pots with 400 ml of NaCl solution over a four day period (100 ml per day) to not shock cuttings. Plant physiological responses were measured biweekly on each cutting after the total 400 ml of solution was added. Measurements continued until all plants within the 40 g l^{-1} treatment were dead, which was between 65-75 days. A total of 48 cuttings were used in the experiment. The control treatment contained 12 cuttings, whereas the 15 and 40 g l^{-1} treatments contained 18 cuttings each. *Tamarix* cuttings from both sites were assigned to treatments at random.

Plant Physiology

Gas exchange measurements were taken using a Licor-6400 infra-red gas analyzer with a red/blue light source and a CO_2 injector (Licor, Lincoln, Nebraska, USA). Irradiance inside the cuvette was 2,000 µmol m^{-2} s^{-1}, CO_2 concentration was 400 ppm and the relative humidity was maintained at ambient. Measurements included photosynthetic rate at 2000 µmol m^{-2} s^{-1} (A_{at2000}), stomatal conductance to water (g_s), and intercellular CO_2 concentration (C_i). Projected leaf area within the gas exchange cuvette was estimated using a Licor 3100 leaf area meter

(Licor, Lincoln, Nebraska, USA). Water potentials were measured by using a Scholander pressure bomb (PMS instruments, Albany, Oregon, USA) and the maximum quantum yield of photosystem II (*Fv/Fm*) was measured by a chlorophyll fluorometer (Walz instruments, Germany). Measurements from the last date of survival for each cutting were analyzed by a mixed model ANOVA in SAS 9.1. (Cary, North Carolina, USA). NaCl concentration was treated as a fixed effect in the model whereas date of sampling was considered random to account for repeated measures in the experimental design.

Stable Isotope Analysis

Leaf samples were collected from each individual cutting on each date sampled. Samples were dried at 60ºC for 48 hours. Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using a Finnigan Delta-plus continuous flow isotope ratio mass spectrometer connected to an elemental analyzer. Within run precision was <0.04‰ for $\delta^{13}C$ and <0.05‰ for $\delta^{15}N$, while between run variation was <0.12‰ for $\delta^{13}C$ and <0.15‰ for $\delta^{15}N$.

Proline Determination

Free proline was determined spectrophotometrically following methods from Bates et al. (1972). A standard curve was generated using *L*-Proline. Approximately 0.5 g of plant material was homogenized in 10 ml of 3% sulfosalicylic acid. The homogenate was filtered through Whatman #2 filter paper and then reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid for 1 hour at 100°C in a test tube. The reaction was stopped by placing test tubes in an ice water bath and then mixing vigorously with toluene. The chromophore containing toluene was separated and absorbance read at 520 nm using toluene as a blank. To react at least 0.5 g of plant

material with 3% sulfosalicylic acid, leaf samples per salinity treatment were amalgamated by sampling date.

Results

All plants subjected to the 40 g l^{-1} NaCl concentration treatment died between 60-75 days after induction of the treatment. Leaf-level gas exchange measurements suggest *Tamarix* physiological functioning varied as a function of increasing salinity (Figure 3-1). Photosynthetic rates ranged from 0.2 to 37 µmol CO_2 m^{-2} s^{-1} among all treatments. Photosynthesis declined 50%, but did not vary significantly by salinity treatment ($p>0.5$; Figure 3-1a). Stomatal conductance to water values ranged from 0.01 to 0.48 µmol H_2O m^{-2} s^{-1} among treatments. Stomatal conductance to water values significantly declined nearly 75% from 0 g l^{-1} NaCl concentration to 40 g l^{-1} NaCl concentration ($p<0.05$; Figure 3-1c). Leaf-level stomatal conductance and photosynthetic rates were also reduced at 15 g l^{-1} NaCl concentration (Figure 3-1a, c). Intercellular CO_2 concentration ranged from 19 to 417 ppm among treatments. Intercellular CO_2 concentration did not vary significantly by NaCl concentration ($p>0.05$; Figure 3-1b).

Decreases in the maximum quantum yield of photosystem II (*Fv/Fm*) suggest *Tamarix* metabolic functioning significantly declined as salinity increased from 15 to 40 g l^{-1} NaCl concentration ($p<0.05$; Figure 3-2a). Mean *Fv/Fm* for the 40 g l^{-1} treatment was 0.76±0.015, whereas average *Fv/Fm* for control plants was 0.81±0.007. The maximum quantum yield of photosystem II ranged from 0.59 to 0.84. Water stress varied significantly as salinity increased as suggested by Ψ_w values ($p<0.001$; Figure 3-2b). Water potentials ranged from -0.3 to -4.0 among treatments. Mean Ψ_w values are nearly two times lower in 40 g l^{-1} NaCl concentrated

treatments compared to controls. Neither above-ground nor below-ground biomass were significantly affected by increasing salinity ($p>0.05$; Figure 3-2c,d).

Leaf $\delta^{13}C$ significantly varied as salinity increased ($p<0.05$; Figure 3-3a). Leaf $\delta^{13}C$ was heaviest in 40 g l^{-1} NaCl concentration and lightest in control treatments. $\delta^{13}C$ values ranged from -28.1 to -36.9 among treatments. $\delta^{15}N$ values did not vary significantly as a function of increasing salinity ($p>0.05$; Figure 3-3b). $\delta^{15}N$ values ranged from -7.9 to 5.0 among treatments. $\delta^{15}N$ values were heaviest when salinity was added as compared to lower mean values in control treatments (Figure 3-3b).

Tamarix physiological functioning acclimated to salinity over time (Figure 3-4). Photosynthetic rates declined immediately after initial NaCl additions, but began to increase after approximately 35 days (Figure 3-4a). Acclimation is also suggested by the maximum quantum yield of photosystem II, stomatal conductance, and proline concentrations because these parameters increased over time (Figure 3-4b, c, d). However, of the 3 treatments, *Tamarix* cuttings within 40 g l^{-1} NaCl concentrated treatment consistently showed lower photosynthesis, stomatal conductance to water, dark-adapted chlorophyll fluorescence, and the highest proline concentrations (Figure 3-4).

Discussion

It has been hypothesized that *Tamarix* persists and expands its range in western North America due to greater physiological tolerance compared to native riparian species, especially important is the halophytic nature of *Tamarix* (Arndt et al., 2004; Ladenburger et al., 2006; Shafroth et al., 1995; Vandersande et al., 2001). Increasing salinity is known to cause physiological stress in most species (Khan et al., 2000; Leport et al., 2006; Tester & Davenport, 2003). Salt stress inhibits photosynthesis, suppresses growth, affects protein synthesis, and

energy and lipid metabolism (Parida & Das, 2005). Few studies examining the physiological responses of *Tamarix* to salinity exist, and the results of those few studies are contradictory. Kleinkopf and Wallace (1974) showed *Tamarix* leaf-level gas exchange was only marginally affected as salinity increased. However, results from Glenn et al. (1998) show marked decreases in transpiration as salinity increased. Our results are consistent with Glenn et al. (1998), suggesting that *Tamarix* leaf-level physiological responses decrease at high NaCl concentration.

Salinity imparts both ionic and osmotic stress in plants (Parida & Das, 2005; Tester & Davenport, 2003). Data in this study suggest *Tamarix* is both ionically and osmotically affected. However, *Tamarix* appears to be more stressed osmotically because high NaCl concentration reduces stomatal conductance by nearly 75% and lowers Ψ_w values by over 50%. Plant water status is highly sensitive to saline soils and, therefore, can be a dominant factor determining a plant's response to stress (Huang et al., 2009; Yeo et al., 1985). Even low-level salt exposures can cause extensive modifications in plant-water relations (Touchette et al., 2009a, 2009b). It is difficult to partition changes in physiological functioning to water stress or salt-specific effects as these changes can be co-dependent over time. After minutes to hours, growth rates and physiological responses instantaneously decline as salinity concentrations increase. Typically there is a partial recovery after initial declines, but growth rates and physiological functioning still remain low when under salt stress (Munns, 2002; Parida & Das, 2005; Tester & Davenport, 2003). These quick declines also occur in plants where KCl, mannitol, or polyethylene glycol (PEG) have been added, suggesting these responses are not solely salt-specific (Slama et al., 2007; Yeo et al., 1991).

In the present study, *Tamarix* plants subjected to 40 g l^{-1} NaCl show marked physiological declines after 14 days (Figure 3-4a). Declines in dark-adapted chlorophyll

fluorescence, photosynthesis, and stomatal conductance were consistent after 28 days. However, these parameters began to increase after 40 days. It is interesting to note that free proline accumulation began to increase in all treatments after 28 days. Free proline accumulation is an indicator of water stress (Bates et al., 1972; Bhaskaran et al., 1985; Singh et al., 1972). Proline is an organic solute that decreases tissue osmotic potential to help maintain turgor pressure (Bai et al., 2008; Silveria et al., 2009). It is possible that *Tamarix* is able to maintain physiological functioning, including water status, by accumulating proline. Similar results have been shown by Solomon et al. (1994) for *Tamarix jordanis*.

Proline also accumulated in control treatments after 28 days. Because all *Tamarix* cuttings were placed in the same growth chamber and were therefore within close proximity, this response by control cuttings may have been triggered by adjacent salt-stressed cuttings. Recent advances in ethylene research have shown that ethylene is responsive to abiotic stresses and may signal nearby plants to begin responses to such stressors (Lin et al., 2009). Furthermore, recent research is beginning to show that ethylene increases in some species when subjected to high salt concentrations (Kukreja et al., 2004; Shibli et al., 2007; Zapata et al., 2007). After 14 days, proline concentration varied by treatment. After 28 days, proline concentration was relatively the same for all treatments. I hypothesized that *Tamarix* cuttings exposed to NaCl are producing ethylene, which may signal nearby cuttings to begin synthesizing proline. However, I am not aware of any studies that show ethylene accumulation signals proline synthesis or studies that show ethylene accumulation increases under salt stress in *Tamarix*. It is also possible that *Tamarix* may synthesize proline during development as the specie is frequently exposed to high salinity.

Proline accumulation is not the only tolerant strategy that halophytic species may impart to maintain osmotic balance. Guard cells may be triggered to close around stomatal pores to conserve water when under osmotic stress (Boyer, 1965; Kaufmann, 1982). This is typically the first response of all plants to acute water deficit and is referred to as hydropassive closure and it is regulated by abscisic acid (Mahajan & Tuteja, 2005). It is possible to interpret the integrated stomatal behavior of a plant by measuring the $\delta^{13}C$ stable isotopic signature as an estimate of water use efficiency (Dawson et al., 2002). Our results suggest high salinity triggers guard cell closure in *Tamarix*. Values of leaf $\delta^{13}C$ were, on average, heavier under 40 g l^{-1} treatments. Similarly, our gas exchange data show reduced stomatal conductance under 40 g l^{-1} NaCl. It is also interesting to note that $\delta^{15}N$ values were heavier when NaCl was added. High salinity causes increases in soil pH which results in the volatilization and loss of NH_3, which enriches the remaining substrate in ^{15}N (Pataki et al., 2005).

Reduced stomatal regulation and lighter $\delta^{13}C$ could be a reflection of anisohydric stomatal behavior. Plants that exhibit anisohydric stomatal behavior open and close guard cells around their stomatal pores depending on the surrounding environment and climate (Rogiers et al., 2009; Schultz, 2003; Tardieu & Simmioneau, 1998). Contrary to anisohydric stomatal behavior, plants that exhibit isohydric stomatal behavior maintain a relatively low and constant Ψ_w by keeping guard cells closed around stomatal pores during most of the day (Tardiue & Simmioneau, 1998). *Tamarix* maintained reduced photosynthesis and still accumulated biomass under high salinity and water stress. In controlled outdoor experiments it has been shown that *Tamarix* is able to maintain higher leaf conductances (low stomatal resistance) when under water or salt stress (Busch & Smith, 1995; Carter & Nippert, *in review*; Glenn & Nagler, 2005; Nagler et al., 2003).

The overall objective of this study was to assess whole plant and leaf-level physiological responses of *Tamarix* to a NaCl concentration gradient. Previous results suggested that *Tamarix* maintained physiological functioning in the field from 0 to 14 g l^{-1} NaCl (Carter & Nippert, *in review*). In this study, *Tamarix* begins to show decreases in gas exchange, dark-adapted chlorophyll fluorescence, and water relations at 15 g l^{-1} NaCl. Physiological functioning changed over time as salinity stress was induced. *Tamarix* physiological functioning decreased after NaCl was added, but over time *Tamarix* acclimated to this stress. Results from this study suggest that NaCl concentrations of 15 g l^{-1} or higher may decrease *Tamarix* physiological functioning, but the halophytic nature is well adapted to acclimate to these conditions, which may impart a greater competitive advantage to *Tamarix*.

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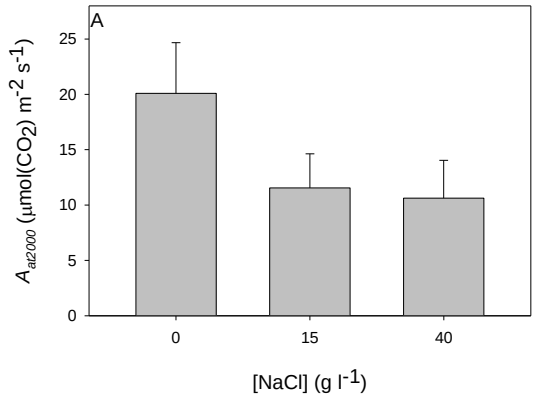
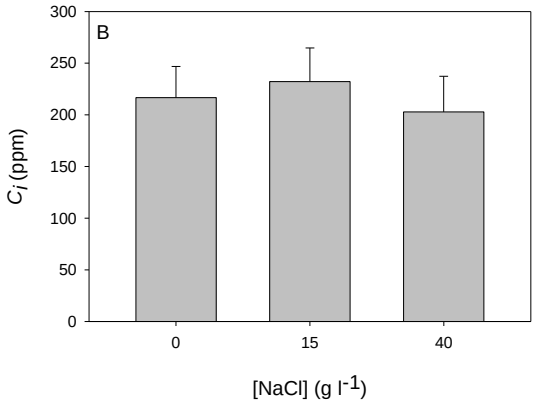
Figures and Tables

Figure 3-1 *Tamarix ramosissima* mean (±1 SE) a) photosynthetic rate at 2000 µmol m^{-2} s^{-1} ($A_{at\ 2000}$), b) intercellular CO_2 concentration (C_i), and c) stomatal conductance to water (gs) among three concentrations of NaCl.





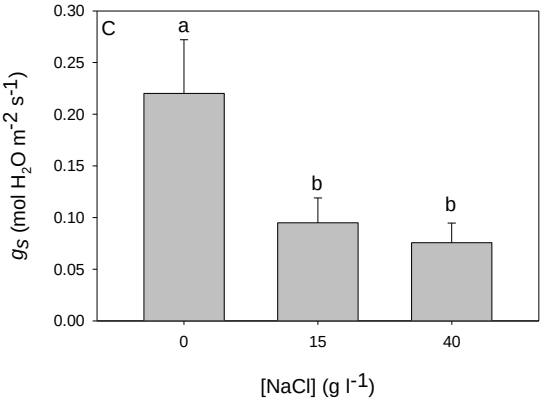


Figure 3-2 *Tamarix ramosissima* mean (±1 SE) a) maximum quantum yield of photosystem II (*Fv/Fm*), b) water potential (Ψw), c) above-ground biomass, and d) below-ground biomass among three concentrations of NaCl.

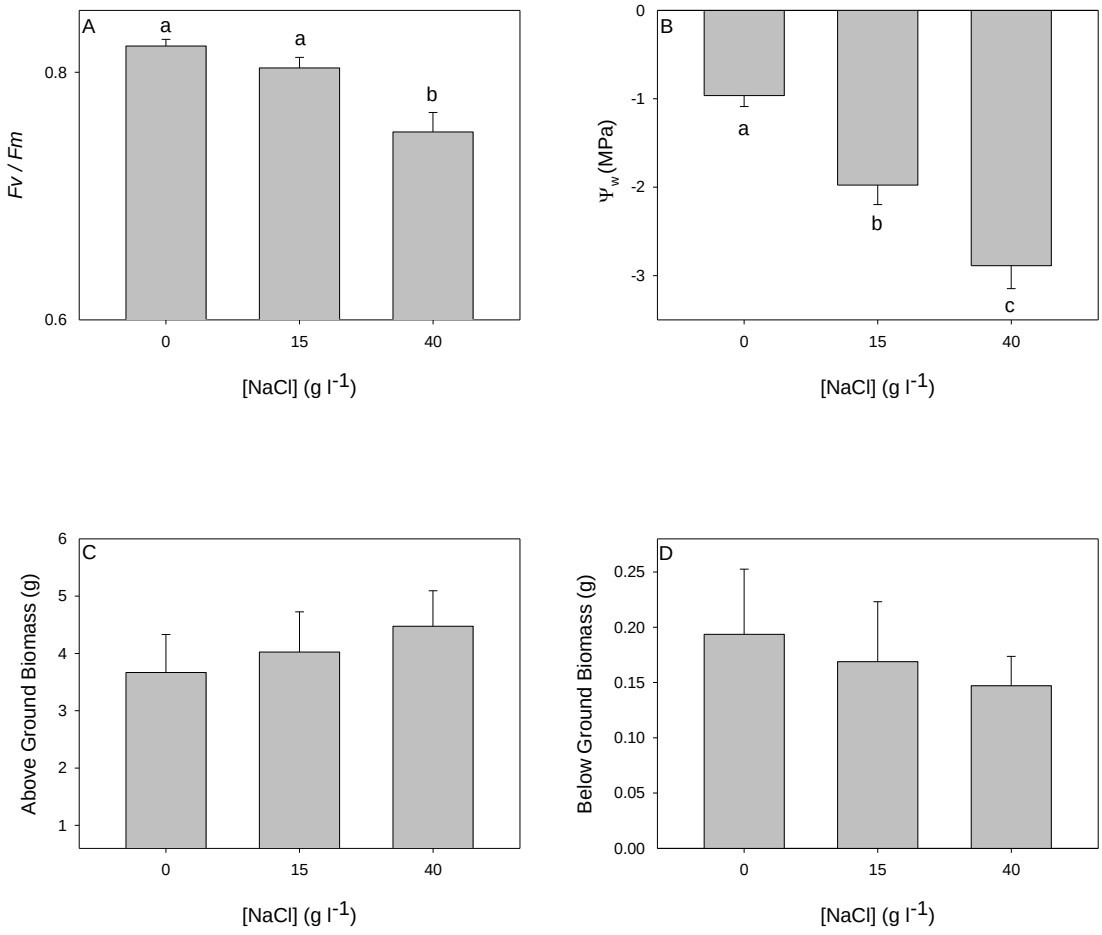


Figure 3-3 *Tamarix ramosissima* (±1 SE) a) leaf $\delta^{13}C$ stable isotopic signature and b) leaf $\delta^{15}N$ stable isotopic signature among three NaCl concentrations.

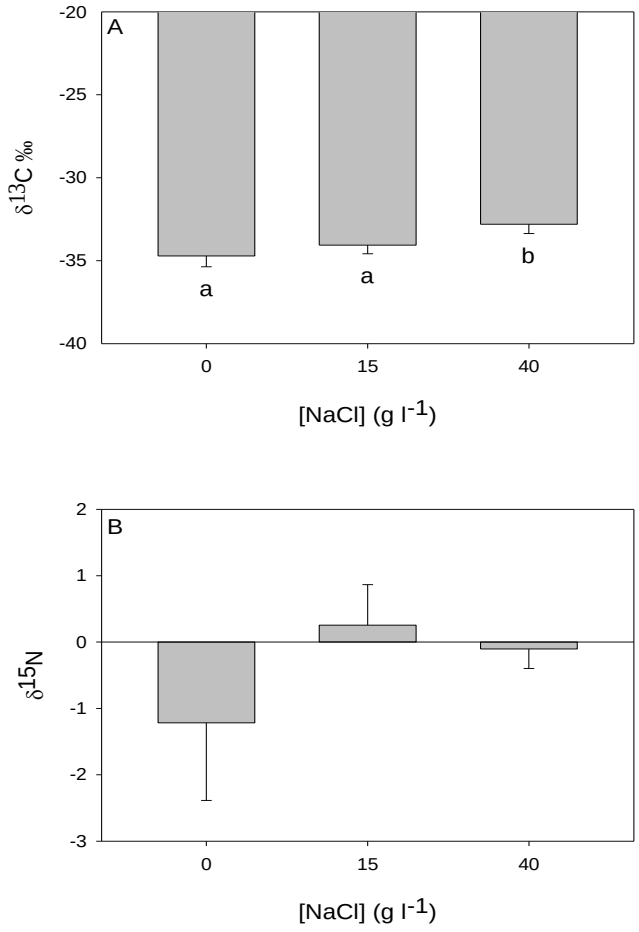
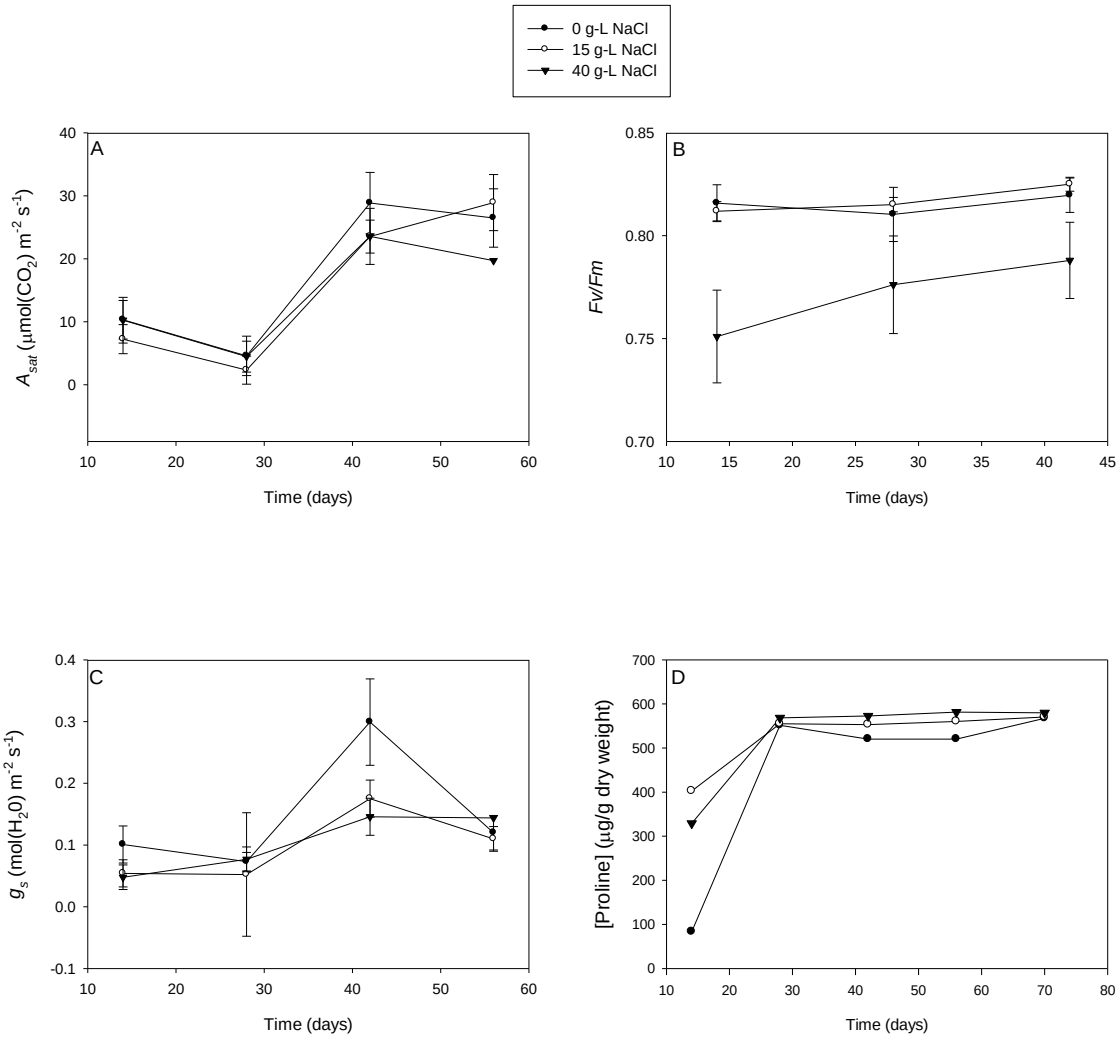


Figure 3-4 *Tamarix ramosissima* a) photosynthetic rate at 2000 µmol m^{-2} s^{-1} (A_{at2000}), b) maximum quantum yield of photosystem II (*Fv/Fm*), c) stomatal conductance to water (g_s), and d) proline concentration over time across three NaCl concentrations (closed circles=0 g l^{-1} [NaCl], opened circles=15 g l^{-1} [NaCl], closed triangles=40 g l^{-1} [NaCl]).



CHAPTER 4 - Conclusions

Riparian corridors in arid and semi-arid western North America are becoming more saline. This increase in salinity is a reflection of not only the low precipitation characteristic of this region, but also anthropogenic modifications of fluvial regimes over the past century. It is of no coincidence that native trees of this region are declining and the invasive shrub, *Tamarix*, is expanding its range (Stromberg et al., 2007). Due to the halophytic nature of *Tamarix*, and both the spatial and temporal variation in soil salinity, it is important to understand the underlying physiology of the invading species to this environmental stressor. Plant physiological understanding can improve predictions of future invasions.

Soil salinization is a growing concern for cultivation (Ashraf & Harris, 2004; Huang et al., 2009; Mahajan & Tuteja, 2005; Munns, 2002; Sairam & Tyagi, 2004; Tester & Davenport, 2003) and has been hypothesized as a contributor to the competitive advantage of *Tamarix*, but little is known about *Tamarix* physiological responses to increasing salinity. Previous studies investigating physiological responses of *Tamarix* to increases in salinity are few and contradictory (Glenn et al., 1998; Kleinkopf and Wallace, 1974). Therefore, the overall goal of this thesis was to assess whole plant and leaf-level physiological responses of *Tamarix* to increasing soil salinity. To address this gap in knowledge, I studied responses of *Tamarix* in both field and controlled environments. I first evaluated physiological responses of *Tamarix ramosissima* across a soil salinity gradient (0-14,000 ppm total dissolved solids) at two field sites, the Ashland research site (ARS) and Cedar Bluffs Reservoir (CBR).

Both sites have characteristics of semi-arid environments and *Tamarix* is highly abundant. Also, between site variations in salinity concentrations provided me with an ideal situation to study *Tamarix* physiological responses to increasing salinity. Through measuring

gas exchange, water-relations, and stable isotopic signatures of both ^{13}C and ^{15}N, I was able to assess *Tamarix* physiological functioning across a wide range of salinities.

In Chapter 2, I concluded that *Tamarix* has a robust physiological response across a wide salinity gradient. Gas exchange and water-relations of *Tamarix* species across this gradient did not change significantly. These results were notable as salt stress is known to inhibit gas exchange and water-relations of plants (Parida and Das, 2005; Tester and Davenport, 2003). I predicted leaf-level physiological measurements would decline as salinity increased. Our data suggested that *Tamarix* is able to accommodate a broad range of salinities, which reflect its advantageous tolerance to salts as a halophytic species. Additionally, I concluded that *Tamarix* would most likely continue to spread across these semi-arid saline regions even as salinity is predicted to increase (Jolly et al., 2008).

I also examined physiological responses to increasing salinity by canopy position (bottom, middle, or top of the canopy). Halophytic species are tolerant of high salt concentrations, but regulating salt toxicity is an energy-requiring process. Trees have both sun and shade leaves, which have varying leaf morphologies and physiologies (McClendon, 1962; Oberbauer and Strain, 1986; Wylie, 1951). Shaded leaves are less photosynthetically active (Oberbauer and Strain, 1986; Stephens et al., 2009) and, during salt stress, are typically the first leaves to be senesced (Parida and Das, 2005). I expected less energy to be contributed to leaf maintenance from *Tamarix*, but our results suggest that a salinity*canopy interaction was not present.

Chapter 2 results suggest the threshold salinity concentration, which would elucidate a decline in *Tamarix* leaf level physiology, was not reached. Therefore, I initiated a second experiment in which I could control NaCl concentration and hold other environmental variables

constant. I took *Tamarix* cuttings from both field sites and grew them in plastic nursery pots in a growth chamber. I subjected cuttings to three treatments: 0, 15, or 40 g l^{-1} NaCl. Treatments were applied to 48 *Tamarix* cuttings at random and whole plant and leaf level physiologies were recorded. In Chapter 3, I concluded that salinity concentrations of 15 and 40 g l^{-1} (15,000 and 40,000 ppm, respectively) would elucidate leaf-level physiological decline in *Tamarix*. Additionally, I illustrated physiological responses of *Tamarix* to increasing NaCl concentrations change over time. Photosynthetic rate, stomatal conductance to water, the maximum quantum yield of photosystem II, and leaf-level proline accumulation show acclimation over time. I report this acclimation is most likely a reflection of both proline synthesis and salt gland secretion. The halophytic nature of *Tamarix* is effective at acclimating physiological functioning over time as salt concentration increases. I also discovered that proline was synthesized in control cuttings. Although I am unsure of the mechanisms behind this result, I hypothesized that the increase in proline accumulation might be due to adjacent *Tamarix* cutting production of ethylene. Increases in proline concentration in control cuttings may also be a result of *Tamarix* proline synthesis during plant development. Research shows that ethylene may be produced as salinity stress is induced (Kukreja et al., 2004; Shibli et al., 2007; Zapata et al., 2007). Our data also suggest *Tamarix* continues to photosynthesize and accumulate biomass even when under salt stress; these results are consistent with other studies (Busch & Smith, 1992; Carter & Nippert, *in review*; Glenn & Nagler, 2005; Nagler et al., 2003).

Arid and semi-arid regions of western North America suffer to a continually human altered environment and, as a consequence, have lost historically native tree species and become dominated by the invader, *Tamarix ramosissima*. As key environmental factors that have contributed to this invasion become noted, it is important to understand how they may affect the

physiology of *Tamarix*. The underlying physiology of *Tamarix* to such environmental factors can help us to predict future invasions of the species and serve as an intrinsic value to the scientific community.

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