***Tamarix* dieback and vegetation change following release of the northern tamarisk beetle**

***(Diorhabda carinulata)* in Western Colorado**

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Abstract

The northern tamarisk beetle (*Diorhabda carinulata*) was released in 2001 as a biocontrol agent for *Tamarix* spp., an invasive tree that dominates riparian ecosystems throughout the southwestern United States. The factors that influence its effectiveness at controlling *Tamarix*, and the effects of control on plant communities, are not well known. Here we report patterns of *Tamarix* dieback, mortality, and vegetation composition at ten of the early *D. carinulata* release sites in western Colorado. Across the ten release sites, 265 permanently marked *Tamarix* trees were measured over a six year period (2008-2014). Vegetation composition and woody debris adjacent to each of these trees were measured annually for four years (2010-2014). We examined relationships between site factors (soil properties, hydrology, and land use history), *Tamarix* dieback, and vegetation composition. *Tamarix* mortality was observed at seven of ten sites, where it ranged from 15-56% after six years. Overall, *Tamarix* crown cover decreased by more than half (54%) while crown volume decreased by 63% in the first two years of the study. Neither total plant cover nor fallen woody debris increased under *Tamarix* trees over the last four years of the study. Combined cover of classified noxious weeds and other non-native species was greater than native plant cover at eight of ten sites. *D. carinulata* proved to be effective in controlling the *Tamarix* invasion locally. However, the high cover of noxious weeds will continue to be a management problem, with or without *Tamarix* control by the northern tamarisk beetle.

Keywords: *Diorhabda carinulata*, *Tamarix* spp., riparian ecosystems, southwestern United States

1. Introduction

Since their introduction to the western United States from Asia over a century ago, members of the genus *Tamarix* (tamarisk, salt cedar) have become the third most common woody species along rivers in the southwestern US and the second-most dominant in cover (Friedman et al. 2005). *Tamarix* gained this dominance in part by taking advantage of human alterations in riparian systems, such as altered flood regimes brought about by dams (Stromberg et al. 2007). Once established, *Tamarix* can be a powerful ecosystem engineer, leading some researchers to describe it as both a passenger and a driver of ecosystem change (e.g., Johnson 2013). The presence of *Tamarix* in western North America has been cited for reducing water availability (Brotherson and Field 1987), reducing biodiversity (Bailey et al. 2001), displacing native vegetation (particularly cottonwood and willow tree species) (Di Tomaso 1998) and reducing wildlife habitat quality (Hink and Ohmart 1984). However, recent studies have shown that although native riparian vegetation is preferable, *Tamarix* may contribute some ecosystem services, particularly as wildlife habitat, and it is not as profligate in water use as once was thought (Shafroth et al. 2005, Stromberg et al. 2009, Bateman et al. 2013, Nagler and Glen 2013, Cleverly 2013).

Tamarix control has been a major priority of private and public land management for decades (Douglass et al. 2013). Traditional control strategies such as mechanical removal, fire, and herbicidal treatments are very costly (Tamarisk Coalition 2008), particularly considering the enormous scale of the landscape requiring treatment. In 1996, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) approved the saltcedar leaf beetle (*Diorhabda elongata* Brulle) from Central Asia for use as a *Tamarix* biocontrol agent

(DeLoach et al. 2003; Bean et al. 2013). Although originally identified as *D. elongata*, the beetles used in this study have been reclassified as *D. carinulata* Desbrochers, the northern tamarisk beetle (Tracy and Robbins 2009). In 2001, *D. carinulata* was released in multiple locations across the Western U.S. where it has spread much faster than originally predicted, at least in some locations (Nagler et al. 2014). By 2015, it could be found along river corridors in Utah, Colorado, Nevada, Arizona, New Mexico, Texas, Oklahoma, Kansas, Mexico, Wyoming, Idaho and Oregon (Tamarisk Coalition 2015).

Diorhabda carinulata feeds exclusively on *Tamarix* foliage resulting in foliage desiccation and subsequent leaf drop (i.e. defoliation) that lasts several weeks (Dudley and Kazmer 2005). Repeated defoliation either within one season or over several consecutive growing seasons results in carbon starvation that reduces foliated production and growth, in some cases killing the tree (Hultine et al. 2015). Early studies of *D. carinulata* show that stand level mortality rates of up to 80% are possible after five years (Pattinson et al. 2011), but mortality varies considerably among stands (0-100%; Hultine et al. 2015). Why some *Tamarix* stands experience mortality faster than others is still an open question, but mortality is likely influenced by several factors including, the timing of defoliation, soil conditions, plant access to resources (Hultine et al. 2015), stand age, tree growth rate (Hultine et al. 2013), the number of defoliation events (but see Hultine et al. 2015) and population genetics (Williams et al. 2014). The widespread distribution of *D. carinulata* will likely reduce the competitive ability of *Tamarix* populations by lowering water resource use (Pattinson et al. 2011, Sueki et al. 2015) and shifting water, nutrient, and carbon cycling processes (reviewed in Hultine et al. 2009). If resource monopolization by *Tamarix* has inhibited the growth of other plants, dieback of

Tamarix canopies or outright mortality could release associated plants from competition. Where *Tamarix* dieback and mortality is extensive, the resulting effects on plant communities could be large-scale. Since defoliation is a relatively recent process, few studies are available on changes in vegetation communities as *Tamarix* canopies decline (Sher 2013, Hultine et al. 2009). While replacement vegetation is critical for bank stabilization and erosion control, wildlife habitat enhancement, and other ecosystem services, of equal concern is the colonization of non-native plants in areas released from competition due to *Tamarix* defoliation (Hultine et al. 2009). The flux of woody litter from dead *Tamarix* crowns to the ground is not well studied but is also important since it could alter microsite conditions for plants as well as microbial communities involved in decomposition and nutrient cycling.

From 2005-2007 *D. carinulata* beetles were released at ten riparian study sites in the upper Colorado River watershed in western Colorado. The ten sites span a range of hydrologic conditions from ephemeral canyon drainages to banks of the Colorado River. In this paper, we describe the patterns of beetle-induced defoliation, crown dieback, and mortality of *Tamarix* trees, and changes in vegetation composition and woody debris near affected *Tamarix* trees. We also explore how the observed patterns relate to site conditions, including soil properties, hydrology, and land use history.

2. Material and Methods

2.1 Study sites

Ten long-term monitoring sites were selected in Mesa County, Colorado, USA (Fig. 1) where the Colorado Department of Agriculture, Palisade Insectary had released northern tamarisk beetles

from 2005-2007 (Table 1). All sites were located in *Tamarix* stands, but the ten sites represented a variety of riparian and hydrologic conditions ranging from sites adjacent to the Colorado River to sites along ephemeral streams. The Colorado, Gunnison, and Dolores Rivers all have dams upriver from the field sites yet still experienced episodic flooding. The perennial creeks and ephemeral streams are unregulated and experienced flooding associated with heavy rains and snowmelt.

Eight of the sites are managed by the Bureau of Land Management and the remaining two sites are located on private property that is used primarily as range for grazing cattle. Two of the sites were burned by wildfires in 2007, one accidentally (Knowles) and the other intentionally (SYBurned). At one site (Salt Creek 2), *Tamarix* trees were mulched by hydroax in 2010 (Torrent EX40 mulching head mounted on a lightweight minimum impact Kubota excavator). *Tamarix* trees resprouted after both the fires and mulching treatment so these sites were kept in the present study, although no measurements were made in the mulched site in the year it was mulched.

Between 5,000-15,000 *D. carinulata* adults were released at each site, with most sites requiring more than one release over two to four years to establish adequate beetle populations (Table 1). At each site, beetles were released onto the same *Tamarix* tree, located at the center of the site, called the release tree.

Climate data from Grand Junction, CO reports a mean annual precipitation of 219 mm, mean monthly maximum temperature of 34.2°C in July and mean monthly minimum of -9.7°C in January (Western Regional Climate Center <http://www.wrcc.dri.edu/local-climate-data/>).

Other than *Tamarix*, vegetation at the study sites was a mix of both riparian and upland vegetation. Cottonwood trees (*Populus fremontii* S. Watson) were present at sites located along the larger rivers (Colorado, Gunnison, Dolores). Common native shrubs across all sites included rubber rabbitbrush (*Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom and Baird), yellow rabbitbrush (*Chrysothamnus viscidiflorus* (Hook.) Nutt. , greasewood (*Sarcobatus vermiculatus* (Hook.) Torr.), fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.), coyote willow (*Salix exigua* Nutt.), and big sagebrush (*Artemisia tridentata* Nutt.). Native grasses included inland saltgrass (*Distichlis spicata* (L.) Greene). A mix of non-native invasive grasses (cheatgrass (*Bromus tectorum* L.)) and forbs (Russian knapweed (*Rhaponticum repens* (L.) Hidalgo) and Russian thistle (*Salsola tragus* L.) were also common, as will be discussed later in this paper (nomenclature follows USDA PLANTS database, except for *Rhaponticum repens* which follows Hidalgo et al. 2006).

2.2. Estimating *D. carinulata* abundance and *Tamarix* defoliation, crown dieback, and mortality

At the time of the first beetle release at each site, 25 *Tamarix* trees were selected in a stratified random method and permanently marked: the release tree, 12 trees spaced within 100 m of the release tree, and another 12 trees spaced between 100-200 m of the release tree. The exception was at the first release site (Horsethief) where a total of 40 *Tamarix* trees were permanently marked. A range of tree sizes was selected, from small to large trees. A GPS unit was used to identify each tree's coordinates to within approximately 1 m.

Diorhabda carinulata abundance on trees, tree health, and tree size were measured from 2008-2014, although sampling frequency differed among sites due to time constraints and

accessibility. When possible, sites were monitored at least three times per season (late May to early June, early July, and August), although some sites were only measured once or twice a season (the total number of sampling dates for each site is shown in Table 1).

Abundance of *D. carinulata* adults and larvae was visually estimated for each study tree by a minimum of two trained observers. Observers carefully scanned all branches for presence of larvae and adults and reached consensus on one of the following abundance categories (representing the range of larvae or adults per tree): 0, 1-10, 11-50, 51-100, 101-500, 501-1000, and 1000+. Midpoints of these categories were used to calculate averages per site.

Defoliation and crown dieback of each study tree was visually estimated by a minimum of two trained observers using the color of foliage and percentage of dead branches, respectively.

Foliage damaged by *D. carinulata* turns brown before falling from branches, therefore foliar damage by beetles was estimated for each tree as the percentage of foliage remaining on the tree that had turned brown (with the sum of brown and green foliage equaling 100%). We refer to this measurement as leaf desiccation. Complete defoliation of a tree by beetles was indicated by the appearance of 100% brown foliage or no foliage throughout the tree crown.

Dead branches were estimated to the nearest 10% of the total branches for each tree, using branch flexibility, color (red versus gray), and foliage presence or absence, to help determine whether branches were living or dead. Entire trees were considered dead when 100% of their branches were observed to be dead. Percent dead for each site was then calculated as the number of trees considered dead of all monitored trees at that site.

The crown area and crown volume of each tree was estimated by measuring crown diameter in two directions (longest and shortest widths) and total tree height. For each of these measurements, only living green foliage was included, therefore a large tree that was mostly dead would result in a small crown diameter and possibly height, depending on the position of the living foliage. Crown widths were used to calculate crown area of each tree as an ellipse ($\text{Area} = \frac{1}{4} \pi \cdot w_1 \cdot w_2$). Crown widths and height were used to calculate an overall crown volume as an ellipsoid ($\text{Volume} = \frac{1}{6} \pi \cdot h \cdot w_1 \cdot w_2$).

2.3. Understory vegetation composition

Plant composition under or near *Tamarix* trees was estimated once during each growing season from 2011 to 2014. Adjacent to each marked *Tamarix* tree, two 1 m² non-permanent plots were established approximately 1 m from the east and west sides of the trunk. In each plot, each plant species was identified and its percent canopy cover was estimated using the following cover classes: 0-5, >5-25, >25-50, >50-75, >75-95, >95-100%. Cover was also estimated for fallen woody debris < 2.5 cm diameter, and woody debris > 2.5 cm diameter. Midpoints of cover classes were used for statistical analyses.

Plant species were grouped into the following life forms for analysis: annual grasses, annual forbs, perennial grasses, perennial forbs, shrubs and trees. Species were also grouped into the following weed classes for analysis: classified as a noxious weed ("classified"), non-native species not classified as noxious weeds (non-native), and native species. Cover and frequency of plants designated as wetland indicators (OBL or FACW) was calculated. Wetland indicator status and weed status follows the USDA Plants Database.

2.4. Soil pH, salinity, and texture

In 2013-2014, soil samples were collected near 10 randomly selected study trees at each site to depths of 0-15 cm and 15-30 cm. In the lab, slurries were made by mixing 25 g of air-dried soil with 50 mL distilled water and tested for pH and soil conductivity using Hach brand portable meters (sensION+ pH1 and sensION+ EC5, respectively). For each site, individual soil samples were bulked and a composite sample measured for soil texture using the hydrometer method of measuring particle size fractionation based on differential sedimentation rates.

2.5. Site hydrological characteristics

Several parameters related to site hydrology were characterized to examine potential patterns between site hydrology, *Tamarix* decline, and vegetation composition. We noted if the water flow was permanent or not (i.e., intermittent or ephemeral). Then, aerial pictures from Google Earth were used to calculate the river width (m) and the shortest distance from the center of the site to the margin of the main channel (m). The lowest depth of the groundwater was calculated as the difference in elevation from the ground at the center of the site to the summer river water level (or to the lowest point in the channel if the stream was ephemeral) using a Spectra Precision Laser HL450 Laserometer© with an auto-leveling rotating transmitter (vertical accuracy = 10 cm). If depth to groundwater could not be estimated it was replaced with the median of all sites. Although the water table level in floodplains may deviate from horizontality at gaining and losing rivers, relative elevation is accepted as a good proxy of depth to groundwater (e.g., Stromberg et al., 1996; Battaglia et al., 2002). Both distance to the main

channel and depth to groundwater / relative elevation were used as proxies of the local frequency and intensity of flooding (Shafroth et al., 2008).

2.5. Statistical analysis

Pearson correlations were calculated at the tree-level (n= 265 trees) between the increase in crown dieback (change over time in % dead branches) and the following: *D. carinulata* larvae abundance, *D. carinulata* adult abundance, and leaf desiccation (% brown leaves). Since most crown dieback occurred during 2008-2010 and remained relatively constant from 2011-2014, correlations were calculated separately for these two time periods. Pearson correlations were also calculated at the tree-level between the increase in crown dieback (change in % dead branches over 2008-2010 and 2011-2014) and soil salinity, pH, and change in total plant cover for 2011-2014. Site-level correlations were calculated between the increase in crown dieback (change in % dead branches over 2008-2010 and 2011-2014) and the following site characteristics: percent sand, elevation above river, river width, distance from river, and soil salinity. Only significant correlations (at the 5% significance level) with $R > 0.30$ are reported. To determine if *Tamarix* mortality increased significantly over the study period, we used a chi-squared test for two population proportions, pairing the proportion of dead trees in 2008 and 2014 within each site. To determine if total plant cover increased under tamarisk trees, total plant cover in 2011 and 2014 were compared using T-tests.

We used non-metric multidimensional scaling (NMDS) to explore patterns between vegetation and sites, running separate models for plant life forms and individual plant species. We overlaid site factors (soil properties, hydrology, and land use history) on the ordinations to explore

possible relationships between plant life forms or species and site factors. NMDS is a non-parametric ordination method for determining similarities (or more precisely, dissimilarities) between objects (e.g., plant communities) in a relatively low-dimensional space (Kruskal, 1964). The ordination plots are interpreted by noting that sites that are near each other are more similar with respect to cover by plant life form or species. A site that is near the origin has the most in common with the other sites. Life forms (or species) located near a site are most abundant in that site.

The program R (R Core Team, 2015) was used for all analyses except the NMDS analysis for which the R package *vegan* was used (Oksanen et al. 2015). In employing the *vegan* package, we used the standard distance metric and settings recommended by the package authors (e.g., the Bray-Curtis distance) (Bray and Curtis, 1957).

3. Results

3.1. *D. carinulata* abundance and *Tamarix* defoliation, crown dieback, and mortality

The average abundance of both *D. carinulata* larvae and adults on study trees was highly variable between years (Fig. 2a and b), with notable declines in 2011 and 2014. The abundance of *D. carinulata* larvae and adults also varied considerably between sites (Table 2). The percent desiccation (as indicated by brown leaves) tended to follow the general pattern of *D. carinulata* larvae abundance over time (lag zero cross correlation $R^2=0.642$ $p=0.12$; Fig. 2c) while *D. carinulata* adult patterns preceded leaf desiccation by a year (lag -1 cross correlation $R^2=0.735$ $p = 0.05$). The percentage of dead branches in *Tamarix* crowns increased from an overall

263 average of 23% the first year of monitoring to 55% after six years (Fig. 2d). There was
264 considerable variation between sites however, with the percent of dead branches in the final
265 season of measurement ranging from 17 to 81% (Table 2).

266 *Tamarix* mortality (defined as 100% of branches dead) was observed at seven of ten sites,
267 where it ranged from 15-56% after six years (Fig. 3). The increase in *Tamarix* mortality over the
268 study period was significant at four of the ten sites. No *Tamarix* mortality was recorded at
269 three study sites, despite two of these sites having moderate levels of crown dieback in the last
270 year of the study (55% at Gateway and 45% at Salt Creek 1). The other site with no *Tamarix*
271 mortality (Rattlesnake) had low levels of *D. carinulata* larvae, adults, defoliation and crown
272 dieback (Table 2).

273 Averaged across all sites, *Tamarix* crown cover decreased by more than half (54%, or from 11 to
274 5 m²) and crown volume decreased by 63% (from 32 to 12 m³) in the first two years of the study
275 (Fig 4). There was little change in crown cover and volume during the last four years of the
276 study period.

277 A positive relationship between the leaf desiccation (% brown leaves) and crown dieback (%
278 dead branches) at the tree-level was detected for the period from 2008-2010 ($R^2 = 0.40$, $p <$
279 0.0001). All other comparisons showed no correlation.

280 3.2. Patterns of mortality related to site factors

281 The average increase in crown dieback (% dead branches) over the study period was positively
282 correlated with sand% in soils ($R^2 = 0.39$, $p < 0.0001$) and negatively correlated with river width

283 ($R^2=-0.41$, $p<0.0001$) and elevation above river ($R^2=-0.32$, $p<0.0001$). Soil salinity, soil pH, and
284 distance from river showed no significant correlations.

285

286 3.3. Vegetation cover and composition

287 3.3.1. Cover by life form and dominant species

288 Total plant cover under *Tamarix* trees did not increase from 2011-2014 ($p = 0.71$; Fig 5). At the
289 tree-level, there was no correlation between change in crown dieback from 2011-2014 and the
290 change in total plant cover over the same time period ($R=-0.14$, $p=0.02$). As a group, annual
291 grasses had the highest cover averaged over the study period while trees had the lowest. Each
292 of the life forms tended to be dominated by a single species. Cheatgrass (*Bromus tectorum*)
293 comprised 94% of annual grass cover, the native inland salt grass (*Distichlis spicata*) comprised
294 59% of perennial grass cover, the non-native Russian knapweed (*Rhaponticum repens*)
295 comprised 79% of perennial forb cover, and the native rabbitbrush (*Ericameria nauseosa*)
296 comprised 52% of shrub cover. Although annual forb cover was less dominated by a single
297 species than other life forms, the two most abundant annual forbs, lambs quarters
298 (*Chenopodium album*) and kochia (*Kochia scoparia*) combined comprised 50% of annual forb
299 cover. Non-*Tamarix* tree cover averaged less than 1% throughout the study, and was comprised
300 of only cottonwood (*Populus fremontii*) and Russian olive (*Elaeagnus angustifolia*). All species
301 with frequencies greater than 20% are shown in Table 3.

302 Cover of woody debris, which averaged 11%, did not increase over the study period (data not
303 shown).

3.3.2. *Plant cover by non-native species*

Non-native plant cover exceeded native plant cover at eight of the ten study sites (Fig. 6). Averaged across study sites and years, non-native plant cover averaged 35% compared to 23% native plant cover. 73% of non-native cover was comprised of classified noxious weed species. Only two species, cheatgrass and Russian knapweed, comprised 91% of classified noxious weed cover. There were more native species than non-native species however, with 30 native species and 22 nonnative species found across all sites.

3.3.3. *Abundance of wetland species*

Only 10% of species found were OBL or FACW species. Average frequency of these eight species among plots was 23%, average cover was <1%, and average cover when present was 16%.

3.3.4. *Plant composition patterns*

The NMDS model using life forms was a good fit to the data for the typical two-dimensional ordination (computed stress value was 0.0911, where <0.25 is considered a good fit). This analysis revealed several associations of life forms and sites that persisted over the four year study period (Fig. 7). The close position of annual grasses and annual forbs in the ordination plot suggests that these two life forms are similarly distributed among sites. In some cases, the association of a life form and a site was due to the presence of only a few species. For example, sites that were associated with perennial forbs had high cover of Russian knapweed (Horsethief, Rattlesnake, and Bedrock). Sites associated with annual grasses had a high cover of

cheatgrass. Sites associated with perennial grasses were dominated by either inland salt grass (Gateway), alkali sacaton (Gateway, Flume), or crested wheatgrass (*Agropyron cristatum*; SYBurned). No environmental factors were found to be significant at the 5% level, therefore environmental gradients are not included in the ordination plot. The NMDS model using plant species was a weak fit and had contradictory results for the significant environmental factors, therefore these model results are not shown.

4. Discussion

4.1. High variation in *Tamarix* defoliation, crown dieback, and mortality

The effectiveness of *D. carinulata* at causing *Tamarix* defoliation, crown dieback, and mortality was highly variable among sites. While this variability may not be surprising given the wide range of site conditions, only a few patterns emerged relating site factors to dieback and mortality. *Tamarix* crown dieback tended to be higher at sites with smaller streams and sandier soils. This pattern may suggest that water stress caused by lower average soil moisture could contribute to branch death. Our results differ from those of Hultine et al. (2015) who found *Tamarix* crown dieback increased with soil salinity. We did not find a significant correlation with crown dieback and soil salinity in our study, and in fact, the site with the highest soil salinity (Gateway) experienced no *Tamarix* mortality.

4.2. Overall reduction in *Tamarix* crown cover and volume

Although *D. carinulata* did not cause mortality at all sites, crown dieback resulted in substantial reductions in crown cover and volume at all sites except one. Excluding this site, crown cover declined by 66% from the beginning to the end of the study period (9.8 to 3.2 m²). The amount

of crown dieback and reduction in crown cover found in this study are generally consistent with other studies from Nevada that report leaf area reductions of 50% (Nagler et al. 2014) to 90% (Pattinson et al. 2011) following beetle damage.

This reduction in *Tamarix* crown cover will have effects on understory plants by increasing direct sunlight. The 72% reduction in crown volume (from 27.3 to 7.7 m³ among sites with dieback) would correspond to a reduction in transpiring canopy volume, presumably increasing available soil moisture. Studies that measured changes in transpiration due to defoliation have found decreases as large as 75% (Pattinson et al. 2011). This reduction in crown cover and volume may be more indicative of how *D. carinulata* is changing community structure, function, and composition than overall mortality. One site (Bedrock), for example, had *Tamarix* mortality of less than 50% but near complete reduction of *Tamarix* crown cover and volume (96% and 95%, respectively).

4.3. Lack of increase in plant cover and woody debris

The lack of increase in total plant cover under *Tamarix* trees is unexpected, particularly given the reduction in *Tamarix* crown cover. This observed lack of change in total plant cover may be due to the timing of the plant census which began in 2011, after most reduction in crown cover and volume had taken place (2008-2010). Therefore, it is possible that the plant census missed significant changes in plant cover that may have occurred. However, examining changes at the tree-level do not support this explanation, since crown dieback of *Tamarix* after 2011 was not correlated with change in understory plant cover. A lack of change in plant cover following *Tamarix* control has been found in another study by Ostojia et al. (2014) who reported that

herbaceous plant density did not differ between areas following mechanical *Tamarix* removal and controls.

The lack of increase in woody debris was also unexpected, but in contrast to plant responses, the flux of woody material from dead crowns to the soil surface may occur over longer time periods. As the increasing proportion of dead wood in *Tamarix* crowns suggests, most dead branches remained in canopies at the time of the study. Changes in downed woody debris will be an important parameter to continue monitoring given how important it could be in altering microsite conditions for plants in addition to microbial communities involved in decomposition and nutrient cycling. Studies by Uselman et al. (2011) showed that beetle-induced increases in leaf litter quality (increased nitrogen and phosphorus concentrations and decreased C:N, C:P, N:P, and lignin:N ratios) can lead to a short-term increase in nutrient cycling. However, addition of woody debris may counteract that effect by increasing C:N ratios, C:P, and lignin:N ratios.

4.4. Dominance of non-native species and noxious weeds

The general dominance of non-native plant species, particularly classified noxious weeds, across sites is a discouraging pattern but not unexpected. In a meta-analysis, González et al. (in review) found noxious weeds were almost ubiquitous after *Tamarix* control. However, some studies of more active *Tamarix* removal have shown that non-native invasive species did not always increase after mechanical and chemical *Tamarix* removal. For example, Sher et al. (2008) found a decrease of cheatgrass cover following both chemical and mechanical *Tamarix* removal. It is possible that more active methods of *Tamarix* control (mechanical and chemical) may be

387 better at controlling secondary weed invasions than biocontrol. However, the relationship
388 between control-related disturbance and weeds is likely to be species-specific with different
389 species having different responses to different treatments. For example, Ostoja et al. (2014)
390 found lower non-native annual grass cover but higher non-native annual forb cover in plots
391 treated with mechanical *Tamarix* removal. In addition to disturbance intensity, the temporal
392 scale over which biocontrol operates differs from mechanical and chemical controls; invasive
393 species may respond to the effects of biocontrol over much longer time periods than
394 mechanical or chemical controls.

395 This high cover of weedy species may explain the why vegetation patterns were not
396 significantly related to environmental factors such as site hydrology and soil. The establishment
397 of opportunistic weedy species (e.g. cheatgrass, Russian knapweed,) is not dependent on a
398 narrow range of environmental conditions. Likewise, many of the dominant native species
399 found at the sites (e.g. rabbitbrush, greasewood, and sagebrush) can also establish in a wide
400 range of site conditions. Wetland indicator species which require specific hydrologic conditions
401 were very low in abundance (average cover < 1%).

402 Without knowing nonnative cover prior to the release of beetles, it is difficult to determine if
403 nonnative cover increased at sites or was already high before beetle release. While non-native
404 plant cover increased over the study period (2011-2014) at half of the sites, it decreased in half
405 of sites. Nevertheless, the high dominance of non-native cover stresses that active restoration
406 may still be required following *Tamarix* control using *D. carinulata*. Harms and Hiebert (2006)
407 concluded that removal of *Tamarix* without active revegetation does not usually result in
408 replacement by native species. At our study sites, the conditions that allowed *Tamarix*

infestation still persist and may continue to favor weed establishment unless more active restoration treatments are applied. Continued monitoring of these sites will reveal if the secondary weeds decline spontaneously with time or persist and require secondary weed control. The fact that weed cover was dominated by two species (cheatgrass and Russian knapweed) may be advantageous in that fewer weed control methods may be needed to control invasives. Also, the higher species richness among native species indicates that seed sources may be available on site to help reestablish native plants after the control of non-native species.

5. Conclusions

This study shows that the northern tamarisk beetle can be a highly effective biocontrol agent, but levels of beetle-induced mortality are highly variable and not always predictable based on site characteristics. However, even where overall *Tamarix* mortality is low, *D. carinulata* can lower the dominance of *Tamarix* in plant communities by reducing crown cover and volume. Beetle damage at our study sites did not appear to strongly influence successional change of the plant community already in place. However the high frequency and cover of non-native plant species such as Russian knapweed and cheatgrass found at these study sites underscores the importance of site evaluation prior to biocontrol implementation, and post-implementation monitoring, both of which may indicate the need for additional restoration actions such as active revegetation at sites where other non-native species are already well established and where native plant recovery is the management goal.

Acknowledgements

430 USDA APHIS CAPS agreements 06-8534-0013 to 10-8564-0013 providing partial funding for field
431 site monitoring from 2006 through 2010. EG participation in this project was supported by a
432 Marie Curie International Outgoing Fellowship within the 7th European Community Framework
433 Programme (ESFFORES project grant number 299044). We would like to thank Andrew Norton
434 and Janet Hardin of Colorado State University for assisting with monitoring the Horsethief site.

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Figure captions

Fig. 1. Location of the ten study sites in western Colorado.

Fig. 2. Annual observed maximums of A) *D. carinulata* larvae abundance/tree on *Tamarix* trees, B) *D. carinulata* adult abundance/tree on *Tamarix* trees, C) percentage of *Tamarix* crowns with brown foliage (representing desiccation and subsequent defoliation), and D) percentage of dead branches on *Tamarix* trees (crown dieback) over the six year study period. For each graph, each point represents the average (across study sites) of the maximum level observed at each site (± 1 SE). Samples sizes (# study sites) for each year are: 2008=8, 2009=5, 2010=8, 2011=10, 2012=8, 2013=9, and 2014=10.

Fig. 3. Percentage of *Tamarix* trees observed to be dead at each study site each over the six year study period. Trees were considered dead when 100% of branches were observed as dead. Site abbreviations and significance of increase in mortality over time are: FL=Flume ($p<0.001$), K=Knowles ($p=1$), SY=Stan Young ($p=0.003$), BR=Bedrock ($p<0.001$), SC2=Salt Creek 2 ($p=0.03$), SYB=SYBurned ($p=0.10$), HT=Horsethief ($p=0.26$), GW=Gateway (NA), RS=Rattlesnake (NA), SC1=Salt Creek 1 (NA).

Fig. 4. *Tamarix* crown area and crown volume averaged across study sites for each year of the study period (± 1 SE). Samples sizes (# study sites) for each year are: 2008=8, 2009=5, 2010=8, 2011=10, 2012=8, 2013=9, and 2014=10.

Fig 5. Percent cover by life form averaged across the ten study sites for 2011-2014 (± 1 SE).

Samples sizes (# study sites) for each year are: 2011=10, 2012=9, 2013=9, and 2014=10.

576 Fig 6. Total cover of native and non-native species at each study site averaged over 2011-2014
577 (or 2011 and 2014 for site K). Sites are arranged from highest (left) to lowest (right) native
578 species cover as a percent of total cover. Site abbreviations are: GW=Gateway, RS=Rattlesnake,
579 SC1=Salt Creek 1, FL=Flume, SY=Stan Young, BR=Bedrock, K=Knowles, SYB=SYBurned, SC2=Salt
580 Creek 2, HT=Horsethief.

581 Fig. 7. Non-metric dimensional scaling analysis plots examining patterns between plant life
582 forms and study sites from 2011-2014. Life form groups are: AG=annual grasses, PG=perennial
583 grasses, AF=annual forbs, PF=perennial forbs, S=shrubs, T=trees.

Graphical Abstract

Table 1. Characteristics of the ten study sites monitored in this study.

Site name	Adjacent river or stream	River/stream type ^a	Property ownership ^b	Grazing level ^c	Soil texture (sand%)	Soil pH	Soil conduct. (uS/cm)	Ht above water level (cm)	Dist. to chan. (m)	River width (m)	Earliest beetle release year	No. of releases	Samp. freq. ^d	Additional info.
Stan Young	E. Salt Creek	ephe	private	1	51	8.7	1096.9	253	15	2.1	2007	4	7 (19)	
SY burned	E. Salt Creek	perm	private	2	50	8.5	1010.7	219	4	1.2	2007	4	7 (21)	burned 2007
Salt Creek 2	W. Salt Creek	ephe	BLM	2	40	8.3	1279.4	163	10	1.6	2006	1	6 (12)	mulched 2010
Salt Creek 1	W. Salt Creek	perm	BLM	1	34	8.5	1292.7	188	32	8.4	2006	3	7 (20)	
Knowles	Colorado R.	perm	BLM	1	55	8.1	398.4	350	100	84.7	2006	2	3 (3)	burned 2007
Horsethief	Colorado R.	perm	BLM	1	41	8.6	765.2	322	24	106.5	2005	1	4 (4)	
Flume	unnamed	ephe	BLM	0	74	8.9	499.4	251	1	0.5	2007	2	6 (13)	
Rattlesnake	Gunnison R.	perm	BLM	0	33	8.2	599.0	320	30	61.0	2007	2	6 (11)	
Gateway	Dolores R.	perm	BLM	2	65	8.8	3951.9	171	117	33.6	2006	1	7 (21)	
Bedrock	Dolores R.	perm	BLM	0	51	8.8	500.8	275	56	15.7	2007	2	6 (18)	

^a ephe = ephemeral stream; perm = permanent (perennial) stream or river

^b BLM = Bureau of Land Management

^c 0 = none currently; 1 = moderate; 2 = high

^d For sampling frequency, the first number represents the number of years that a site was sampled (out of a maximum of seven); number in parentheses represents the total number of sampling periods for the entire study (out of a maximum of 21).

Table 2. Average number of *Diorhabda* larvae, adults, percentage leaf desiccation of *Tamarix* trees, and percentage of dead branches on *Tamarix* trees at ten monitoring sites. For larvae abundance, adult abundance, and percent desiccation, the average maximums were calculated using the sampling dates with the highest response for each year across the study period (2008-2014). The percentage of dead branches is the highest value reported during the last year of the study (2014).

	<i>Diorhabda</i> larvae (#)	<i>Diorhabda</i> adult (#)	leaf desiccation (%)	dead branches 2014 (%)
Bedrock	142 (64)	132 (79)	53 (18)	79 (4)
Flume	54 (34)	1 (1)	21 (11)	81 (6)
Gateway	229 (85)	90 (23)	82 (5)	55 (4)
Horse Thief	5 (3)	6 (4)	45 (22)	44 (5)
Knowles	21 (12)	24 (20)	51 (25)	70 (7)
Rattlesnake	7 (4)	32 (21)	9 (5)	17 (4)
Salt Creek 1	133 (75)	57 (27)	23 (10)	45 (5)
Salt Creek 2	49 (46)	10 (6)	16 (7)	45 (8)
Stan Young	119 (73)	13 (8)	35 (12)	71 (6)
SY burned	125 (66)	56 (35)	33 (11)	41 (8)

Table 3. Plant species found in vegetation sampling plots at the ten study sites. Species are ordered from most frequent to least frequent. Weed status indicates C=classified noxious weed, N=native, NN=non-native. For classified noxious weeds, "Weed classification" refers to designation that the species has in either Colorado (CO) or California (CA). Wetland status refers to species' status as wetland species, where FAC=facultative, FACW=facultative wetland, OBL=obligate (wetland). Weed classification and wetland status follows USDA Plants Database.

<i>Species</i>	Common name	Weed status	Weed classification	Wetland status	Average cover	Average cover when present	Frequency
<i>Bromus tectorum</i>	cheatgrass	C	C (CO)		16.7	22.6	93%
<i>Ericameria nauseosa</i>	rabbitbrush	N			6.9	21.0	85%
<i>Distichlis spicata</i>	saltgrass	N		FAC	5.3	16.9	83%
<i>Sarcobatus vermiculatus</i>	greasewood	N			1.5	15.9	75%
<i>Chenopodium album</i>	lambs quarters	NN			2.1	12.4	70%
<i>Descurainia sophia</i>	flixweed	NN			0.8	4.9	68%
<i>Artemisia tridentata</i>	big sagebrush	N			1.8	17.0	55%
<i>Acroptilon repens</i>	Russian knapweed	C	B (CO)		8.1	23.8	53%
<i>Kochia scoparia</i>	kochia	NN			2.1	7.4	50%
<i>Eremopyrum triticeum</i>	annual wheatgrass	NN			1.1	5.9	50%
<i>Salsola tragus</i>	Russian thistle	C	C (CA)		0.9	6.2	50%
<i>Iva axillaris</i>	poverty sumpweed	N		FACW	0.8	14.1	50%
<i>Chorispora tenella</i>	blue or musk mustard	C	B (CA)		0.5	6.9	40%
<i>Cardaria draba</i>	hoarycress or white top	C	B (CO)		0.5	7.4	38%
<i>Agropyron cristatum</i>	crested wheatgrass	NN			1.4	21.9	35%
<i>Lepidium perfoliatum</i>	clasping pepperweed	NN			0.3	4.2	35%
<i>Phalaris arundinacea</i>	reed canarygrass	N		FACW	1.3	18.3	33%
<i>Sisymbrium altissimum</i>	tumble mustard	NN			0.5	6.9	33%
<i>Salix exigua</i>	coyote willow	N		FACW	0.7	15.8	30%
<i>Lepidium latifolium</i>	tall whitetop	C	B (CO)	FAC	0.4	9.8	30%
<i>Lactuca serriola</i>	prickly lettuce	NN			0.6	8.5	23%
<i>Suaeda spp.</i>	seepweed	N		OBL	0.2	10.9	23%
<i>Thlaspi arvense</i>	field pennycress	NN			0.2	7.0	23%
<i>Atriplex canescens</i>	four-wing saltbush	N			0.2	14.6	23%
<i>Conyza canadensis</i>	horseweed	N			0.1	4.8	23%

<i>Glycyrrhiza lepidota</i>	wild licorice	N		FAC	0.1	5.2	23%
<i>Rhus trilobata</i>	skunk bush	N			1.4	25.3	20%
<i>Convolvulus arvensis</i>	field bindweed	C	C (CO)		0.1	6.9	20%

Figure 1

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