

DISSERTATION

IMPACTS AND MANAGEMENT OF THE INVASIVE RUSSIAN OLIVE (*ELAEAGNUS
ANGUSTIFOLIA* L.) IN A HETEROGENEOUS RIPARIAN ECOSYSTEM

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ABSTRACT

IMPACTS AND MANAGEMENT OF THE INVASIVE RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA* L.) IN A HETEROGENEOUS RIPARIAN ECOSYSTEM

Russian olive is an exotic actinorhizal tree intentionally introduced to the U.S. in the early 1900's. It has become a dominant component of riparian ecosystems throughout the western U.S. Unlike most other riparian trees in the semi-arid west, Russian olive germinates and grows both in the open and in the understory of mature cottonwood stands. As an actinorhizal species, it forms an endosymbiosis with soil actinobacteria in the genus *Frankia* that allows for atmospheric N₂-fixation. This leads to higher soil N concentrations and mineralization rates underneath the tree's canopy than outside. Russian olive's high abundance and impact on soil N suggest it may alter plant communities, but these impacts have not been previously demonstrated. I investigated the impacts of Russian olive on shading, soil N availability, and plant communities and documented how those impacts varied across a semi-arid riparian ecosystem along the South Fork of the Republican River in eastern Colorado. Of the suite of environmental variables I measured, presence or absence of cottonwood canopy had the largest effect on Russian olive impacts. Russian olive increased shading, soil N availability, and proportion exotic plant and forb cover more in the open than underneath a cottonwood overstory.

Actinorhizal endosymbioses provides an important N source in terrestrial ecosystems, but N₂-fixation rates decrease due to high exogenous N and low photosynthetically active radiation (PAR). The amount that these environmental variables reduce N₂-fixation in host-*Frankia* symbiosis types dictates the strength and duration of those symbioses' impacts on ecosystems. To understand how the two main types of endosymbioses (*Alnus*- and *Elaeagnus*-*Frankia*) differ

in their response to environmental variability, I conducted a greenhouse experiment comparing growth and nodulation between two genera of actinorhizal species, *Elaeagnus* and *Alnus*, across exogenous N and PAR levels. Overall, *Elaeagnus* species had higher nodulation rates and tissue % N than *Alnus* species. Nodulation rate and growth response to nodulation were both lower at low PAR than high PAR for both genera. The reduction in the growth response to nodulation at high exogenous N was lower in *Elaeagnus-Frankia* symbiosis than *Alnus-Frankia* symbiosis. These results suggest that *Elaeagnus* species are more likely to cause a greater and longer-lasting increase in soil N than *Alnus* species.

A main objective of exotic species management is to increase native plant cover. However, few studies monitor plant community response to exotic species management, and the few that have suggest secondary invasion is likely, particularly when effects of the target invasive persists and management efforts cause disturbance. To measure the role of these two factors in plant community response to Russian olive removal, I monitored soil N availability and plant communities along the South Fork of the Republican River two years before and three years after the tree's removal. Russian olive's impact on soil N availability persisted, with levels staying high around removed Russian olive stems three years after removal. The plant community around removed Russian olive also had no increase in native plant cover but a dramatic increase in kochia (*Bassia scoparia*) cover following removal. My research demonstrates that Russian olive increases exotic plant cover in areas it invades and simply removing the tree does not promote native species recovery.

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DEDICATION

To my family, who continually provide unwavering support for me and my goals. Thank you for being a foundation throughout my life.

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CHAPTER 1: LOCAL ENVIRONMENTAL CONTEXT CONDITIONS THE IMPACT OF RUSSIAN OLIVE IN A HETEROGENENOUS RIPARIAN ECOSYSTEM¹

SUMMARY

Local abiotic and biotic conditions can alter the strength of exotic species impacts. To better understand exotic species impacts on invaded ecosystems and to prioritize management efforts, it is important that exotic species impacts are put in local environmental context. We studied how differences in plant community composition, PAR, and available soil N associated with Russian olive (*Elaeagnus angustifolia* L.) presence are conditioned by local environmental variation within a western U.S.A. riparian ecosystem. In four sites along the South Fork of the Republican River, Colorado, we established 200 pairs of plots (underneath and apart from Russian olive) to measure invasion impacts across the ecosystem. We used a series of *a priori* mixed models to identify environmental variables that altered the impacts of Russian olive. For all response variables, models that included the interaction of environmental characteristics, such as presence/absence of an existing cottonwood canopy, with the presence/absence of Russian olive canopy were stronger candidate models than those that just included Russian olive canopy presence as a factor. Compared to reference plots outside of Russian olive canopy, plots underneath Russian olive had higher relative exotic cover (exotic/total cover), lower perennial C4 grass cover, and higher perennial forb cover. These effects were reduced, however, in the presence of a cottonwood canopy. As expected Russian olive was associated with reduced PAR and increased N, but these effects were reduced under cottonwood canopy. Our results demonstrate that local abiotic and biotic environmental factors condition the impacts of Russian

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olive within a heterogeneous riparian ecosystem, and suggest that management efforts should be focused in open areas where Russian olive impacts are strongest.

MANAGEMENT IMPLICATIONS

Understanding how invader impacts vary across the landscape is crucial to developing more efficient management strategies. Current strategies primarily treat landscapes as homogeneous; therefore, management efforts are often inefficient because they do not preferentially target the areas of most concern (Albers et al. 2010). Using a more targeted management approach, where early intervention is applied only to areas of the landscape likely to experience strong invader impacts, would be much more cost effective (Albers et al. 2010). Based on our results, we suggest that ideally Russian olive should be removed from all habitat types because it is associated with an increase in soil N and proportional exotic plant cover. However, when management funding is limited, we suggest prioritizing control efforts on locations where it is growing in the absence of a cottonwood canopy. In these areas, Russian olive has the largest impact on soil N and proportional exotic cover. Since Russian olive appears to cause an ‘invasional meltdown’ by facilitating the invasion of other exotic species (Simberloff and Von Holle 1999), particularly in open canopies, research on effects of removal is warranted, to see if it reverses Russian olive impacts. If the increased soil N associated with Russian olive presence persists after the tree’s removal, secondary invasion after the disturbance from the removal process is likely (Pearson et al. 2016). Additionally, favoring cottonwood establishment over Russian olive can be accomplished by promoting flood disturbance by avoiding channel stabilization (i.e. by riprap) and construction (Katz and Shafroth 2003, Katz et al. 2005).

INTRODUCTION

Exotic plants have been shown to impact invaded ecosystems by competitively displacing native species, degrading habitat for wildlife, altering nutrient and water cycles, and changing disturbance regimes (e.g., Liao et al. 2008, Vilà et al. 2011). However, these effects vary greatly across ecosystems (Crooks 2002). Parker et al. (1999) conceptualized the impact of invasive species as a function of range, abundance, and per capita effects. The range and abundance of a species result from its ability to disperse widely and to become established and persist in a range of environments (invasiveness), while the per capita effects are a result of its influence on the recipient community (impacts). Much of invasion ecology research has focused on identifying the species traits and environmental characteristics influencing invasiveness of exotic species (e.g. Levine 2000, Kolar and Lodge 2001, Stohlgren et al. 2003), with a recent growing body of research also focused on how traits of invaders influence their impacts (e.g., Vilà et al. 2011, Barney et al. 2013, Gaertner et al. 2014). Less work has focused on identifying how local-scale environmental factors affect the per capita impacts of specific exotic species across environmental gradients or within heterogeneous landscapes (Melbourne et al. 2007, Hulme et al. 2013). This information is crucial for understanding overall invader impacts and developing effective management strategies (Albers et al. 2010).

Once exotic plants invade an area, their impacts on the recipient ecosystems have been explained in terms of attributes of the invader, characteristics of the recipient community, and local site conditions. One key impact mechanism is the introduction of novel functional traits (e.g., N-fixation) via invasion, which produce impacts by transforming ecosystem dynamics (Ehrenfeld 2010, Strayer 2012). Characteristics of the recipient community (e.g., species and/or functional group composition) and the invaded site (e.g., climate, soils) also influence the

strength of invader impacts (Maron and Marler 2008, Pyšek et al. 2012, Hulme et al. 2013, Castro-Díez et al. 2014). For example, a meta-analysis of factors influencing exotic species impacts Gaertner et al. (2014) found that the likelihood of invaders causing reinforcing feedbacks (e.g., impacts to soil nutrient cycling) varied based on specific combinations of invader life form and recipient ecosystem. For instance, trees were more likely to affect nutrient dynamics in dunelands than forests. Similarly, Castro-Díez et al. (2014) found that impacts of exotic plants on N cycling were greatest in warm moist climates and where there were large functional differences between the invader and native species. These recent investigations produced generalizations from meta-analyses of a variety of specific invader-ecosystem combinations (where each invader is present in a single habitat type), but did not examine how impacts of single species vary with environmental context. Focusing on one invader across a heterogeneous environment allows for a clearer understanding of the influence of site conditions on specific invader impacts (Hulme et al. 2013).

Across its entire invaded range and even within a single invaded ecosystem, each invader is likely to encounter heterogeneous biotic communities and abiotic conditions that will influence the type and magnitude of its impact. In a few cases, impacts of single exotic plant species have been shown to vary with environmental conditions, such as precipitation and temperature gradients, and with variations in disturbance regimes or recipient plant community composition (reviewed in Ehrenfeld 2003, and Hulme et al. 2013). For example, invasion by the African grass *Melinis minutiflora* across an elevation gradient in Hawaii Volcanoes National Park produced similar changes to fire regimes at all elevations, but the response of native plant communities varied across the elevation gradient due to differences in species and functional group composition (D'Antonio et al. 2000). In New Zealand tussock grasslands, the impact of the

exotic forb *Hieracium pilosella* on N mineralization rates was influenced by aspect and recipient community composition (Scott et al. 2001). Understanding the overall impact of an exotic plant species requires assessment of its impact across the range of environmental conditions characteristic of the invaded ecosystem.

We investigated how the impacts of the exotic Russian olive (*Elaeagnus angustifolia* L.) vary across a heterogeneous western U.S. riparian ecosystem in eastern Colorado. Russian olive is a small deciduous tree in the family Elaeagnaceae. It is actinorhizal, forming a N-fixing symbiosis with actinobacteria in the genus *Frankia*. Russian olive is currently the fourth most frequently occurring and the fifth most dominant riparian tree species in the western U.S. (Friedman et al. 2005). First introduced to the U.S. from western Asia and southern Europe in the early 1900's (Katz and Shafroth 2003), it is now found in all western U.S. states (Friedman et al. 2005) and the southern Canadian provinces (Nagler et al. 2011). Based on habitat suitability models, Russian olive is predicted to continue its expansion further north and west in North America (Jarnevich & Reynolds 2011). The fact that occurrence of naturalized Russian olive is well-predicted by nearby occurrence of planted individuals suggests the population is still spreading at the subcontinental scale (McShane et al. 2015). Due to concerns about its potential impacts, Russian olive is classified as a noxious weed in Colorado, New Mexico, Washington, Wyoming, and Connecticut and is a regulated plant in Montana (USDA, NRCS 2013). Currently, federal, state, and local land managers have initiated multiple removal projects using mechanical and herbicide control techniques (O'Meara et al. 2010).

Russian olive possesses several traits that could cause large impacts on recipient ecosystems. First, as a N-fixing tree invading into ecosystems where this trait is uncommon, the impact of Russian olive on N cycling is likely to be high (Liao et al. 2008). Rates of N-fixation

by Russian olive are higher than rates of all native riparian taxa in the western U.S. except red alder (*Alnus rubra*), and Russian olive leaves contain higher % N and lower C:N molar ratios than native cottonwoods (Follstad Shah et al. 2010). Inputs of Russian olive leaf litter causes elevated soil N (Simons and Seastadt 1999, Follstad Shah et al. 2010). Indeed, mineralized soil N levels underneath Russian olive were significantly higher than levels outside its canopy on the Rio Grande in New Mexico, USA (DeCant 2008, Follstad Shah et al. 2010). Second, since seedling recruitment can occur on litter, Russian olive establishes in previously unforested meadows and wetlands not usually suitable for native cottonwood or willow recruitment, which need bare soil to germinate (Katz et al. 2001). Third, Russian olive is shade tolerant, allowing it to grow as an understory tree in cottonwood-willow gallery forests of the western Great Plains, where there are few native understory trees (Katz & Shafroth 2003). Because of its abundance in western US riparian ecosystems and concern over its ecological impacts, a growing body of literature assesses the impacts of Russian olive on terrestrial wildlife and aquatic ecosystems (reviewed in Katz and Shafroth 2003, Collette and Pither 2015). However, no published peer-reviewed studies have documented the impact of Russian olive on riparian plant communities. One study, Reynolds and Cooper 2011, examined the effects of Russian olive removal on understory plant communities, but did not compare invaded areas to un-invaded reference locations.

Western U.S. riparian systems are ideal for exploring how site characteristics condition invasive species impacts because they have high heterogeneity (Patten 1998), are often heavily invaded (Rood et al. 2010), and are ecologically valuable (Naiman et al. 1993). Riparian systems in the semi-arid western U.S. show high spatial and temporal variation in soil nutrient dynamics (Boggs & Weaver 1994, Vought 1994), soil texture (Lyon & Gross 2005), hydrology (Toner &

Keddy 1997), and vegetation patterns (Boggs & Weaver 1994; Lyon & Gross 2005, Wintle & Kirkpatrick 2007) driven by fluvial-geomorphic processes (Friedman et al. 1996, Patten 1998). Vertical and horizontal distance from the river channel (thalweg) is responsible for much of the variation in local abiotic and biotic conditions (Merigliano 2005). We tested the influence of physical conditions (e.g., distance to and height above the thalweg, soil texture) and forest canopy (i.e., presence or absence of cottonwood gallery forest overstory) on Russian olive impacts on plant community composition, available soil N, and photosynthetically active radiation (PAR). These environmental variables (physical condition and forest canopy) were chosen to describe the heterogeneity of the riparian landscape because they are important in modifying ecosystem functioning in other semiarid riparian ecosystems (Nakamura et al. 1997, Merigliano 2005). We predicted that Russian olive would be associated with higher proportional exotic plant cover and soil N and reduce PAR, and that these impacts would be conditioned by soil texture and vertical and horizontal distance from the river.

METHODS

Study Area

Our study was located in eastern Colorado, USA, at the boundary of the Western Short Grasslands and the Central and Southern Mixed Grasslands ecoregions (Ricketts et al. 1999) in the western Great Plains (Osterkamp et al. 1987). The climate of the Great Plains is characterized by large variation in daily, monthly, and yearly temperature and precipitation, high potential evaporation, and frequent and severe storms (Rosenberg 1986). In the year preceding 2010 sampling (August 2009 through July 2010) total precipitation was 68.6cm (158% of average). Precipitation in the second year of the study was 56.4cm (130% of average) (Colorado Climate Center 2010).

We selected four sites along an unregulated section of the South Fork of the Republican River in Kit Carson and Yuma Counties, Colorado, USA. The flood of record occurred on this river after heavy thunderstorms on May 30-31, 1935. This flood greatly widened the channel and initiated a multi-decade period of channel narrowing and establishment of a broad gallery forest of plains cottonwood (*Populus deltoides* W. Bartram ex Marshall ssp. *monilifera* (Aiton) Eckenwalder) and peachleaf willow (*Salix amygdaloides* Anderss.) on the flood-widened channel bed (Katz et al. 2005). These are patchily distributed across the floodplain, with patches ranging from single trees to several hectares in size interspersed with areas of open meadow. Almost all cottonwood recruitment in the study area occurred in the two to three decades following the 1935 flood and no saplings occurred within our transects. Russian olive first became established in the study area in the 1970's, and it now occurs both in open meadow habitats and in the understory of the cottonwood gallery forest. There are few other tree or shrub species present at these sites, and these are only rarely found. For example, Katz et al. found that the next most common tree/shrub at our sites, *Juniperus virginiana* represented only 0.6% of the individual trees or shrubs present in the study area. At each site, Russian olive stands extended at least 100 m away from the river and at least 400 m along the river's length. See Katz et al. 2005 for a detailed description of the riparian forest at our sites. All riparian areas used in the study were seasonally grazed.

Study Design and Data Collection

Our four study sites were distributed along an 8-km stretch of the South Fork of the Republican River. We set up a total of ten transects, 2-3 per site. Transects originated at the edge of the river and were perpendicular to the active river channel. These extended at least 100 m from the river bank up to the distal edge of Russian olive stands. Transects were located at least

100 m apart. Along each transect, we established 40 paired, 1x1 m plots (386 in 2010 and 383 in 2011), 20 underneath the canopy of a Russian olive tree (Russian olive plots) and 20 located at least three meters from the nearest Russian olive and its canopy drip line (reference plots), at the same height above and distance from the river. The median and 95% quantile for distance between invaded plots along each transect was 23 (7.2 – 123.01) m. The median distance between an invaded plot and a reference plot was 8.2 (3.5 – 20.86) m. Locating the reference plots at least three meters from the Russian olive dripline avoided Russian olive influence on soil N (Decant 2008) and light. Each plot was further classified as occurring under the cottonwood forest canopy (cottonwood) or in an open grassland area (open) (Fig. 1.1).

Biotic response variables (plant community characteristics)

To account for the effect of variation in temperature and precipitation on plant growth, we collected plant community data for two years. In late July 2010 and early August 2011, we visually estimated percent cover of each vascular plant species originating in each plot. Plants were identified to species using Weber and Wittman 2001 and Shaw 2008. We used the U.S.D.A. PLANTS database (USDA, NRCS 2013) for taxonomic nomenclature, for classification of each species as exotic or native, and for assignment of each species to one of 6 functional groups: annual grass, annual forb, perennial C3 grass, perennial C4 grass (Shaw 2008), perennial forb, and woody. The low incidence of sedges (*Carex* spp.) and rushes (*Juncus* spp.) at our sites prevented the mixed models from converging when these were included as a separate functional group. Consequentially these taxa were combined with perennial C3 grasses for analysis.

Abiotic response variables (PAR and available N)

Light intensity (photosynthetically active radiation, PAR) was measured in July 2010 using a LICOR LI-185 PAR sensor. To determine percent light attenuation, we compared average light intensity values one meter above the ground surface in each of the four plot corners and center (five total readings) to the light intensity in full sun immediately before sampling in each plot between 8am and 5pm. We used ion exchange resin bags to measure available soil N (Binkley 1984). Nylon mesh bags containing 15 mL of mixed bed ion-exchange resin were buried at a 5-10 cm depth in the corner closest to the nearest Russian olive tree in 12 plot pairs (Russian olive / reference) on two transects at each site (192 plots total). One bag was buried in each of the selected plots. The 12 plots represented every other pair of plots along each transect plus the middle and last pairs. Resin bags were buried for 4-month intervals (August-November, December-March, and April-July). After bags were retrieved from the field, we extracted ions with 75 mL of 2M KCL and measured ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) concentrations using an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station TX) at the Colorado State University Natural Resource Ecology Laboratory.

Environmental variables

We recorded the location of each study plot in order to determine its position relative to the present river channel. Plot locations were recorded using a 2012 Trimble Geo 6000 XT GPS connected to an external Trimble Zephyr II antenna at 2 m height. We also recorded the position of the river thalweg (the point of lowest elevation within the active river channel) every ten meters along the river at each site. We used these coordinates to measure height above (m) and distance from (m) the thalweg for each plot. Russian olive and reference plots were a similar

distance from (185.1m(mean) \pm 10.1(SEM) vs. 182.4m \pm 10.2, $t_{306}=-0.19$, $p=0.85$) and height above the thalweg (1.91m \pm 0.08 vs. 1.95m \pm 0.08, $t_{306}=0.37$, $p=0.71$). Of the 386 plots used in our analysis, 248 occurred outside of the cottonwood canopy (open) and 138 occurred underneath the cottonwood canopy (cottonwood). Compared to cottonwood plots, open plots tended to occur further from the channel (213.2m \pm 8.8 vs. 136.9m \pm 11.1, $t_{306}=5.41$, $p<0.01$) and at a lower height above the channel (1.76m \pm 0.07 vs. 2.19m \pm 0.09, $t_{306}=-3.51$, $p<0.01$).

We measured soil texture in the 192 plots where we deployed resin bags. At each plot, we collected soil cores from each of the three corners without a resin bag using a 2 x 30 cm soil probe. The three soil cores were then aggregated for each plot. We then used the hydrometer method (Bouyoucos 1936) to measure soil texture (% sand, silt, clay) on a 40 g dried and sieved sub-sample. Since all three soil textures were highly correlated (data not shown), we only included percent sand in our statistical analyses. Percent sand in the plots ranged from 17.5% to 96.3%. Russian olive plots had similar % sand to reference plots (62.9% \pm 2.2 vs. 62.6% \pm 2.2, $t_{146}=-0.11$, $p=0.91$). Open plots tended to have higher % sand than cottonwood plots (67.2% \pm 1.9 vs. 56.2% \pm 2.3, $t_{146}=3.63$, $p<0.01$).

Statistical Analysis

We used the SAS 9.3[®] MIXED procedure (2012, SAS Institute, Cary, NC) to run several *a priori* general linear mixed models to identify environmental variables that conditioned the impact of Russian olive on plant community structure, PAR, and soil N. Our set of candidate models all contained Russian olive canopy (categorical: presence/absence) as a fixed factor, plus other variables previous research has shown were important modifiers of semi-arid riparian ecosystems (e.g. Merigliano 2005; Bagstad et al. 2006; Bechtold and Naiman 2006). Thus, the candidate models were designed to determine which environmental factor(s) were associated

with greater or lesser effects of Russian olive (Table 1.1). Fixed factors examined in the models were presence of cottonwood overstory (categorical: cottonwood/open), soil percent sand (continuous), and distance to and elevation above thalweg (both continuous) and each of these variables interaction with Russian olive. Because an AIC model selection approach requires that only a limited set of a priori models be examined, we did not examine all possible combinations of environmental variables with and without Russian olive, and instead focused our models on those that could test whether or not Russian olive's effect on the response variables was modified by other environmental factors. Within any one model, evidence for effect modification is a significant interaction between Russian olive and an environmental variable. The response terms used in the models were relative PAR (measured in July 2010), total available soil N concentration (for August 2010 through July 2011), total plant cover in 2010 and 2011, relative exotic plant cover (exotic/total cover) in 2010 and 2011, plant species richness in 2010 and 2011, and plant functional group cover in 2010 and 2011. Separate models were run for each year of data collection.

Plant functional group cover was modeled using a repeated measures structure (Holland 2006). To do this, we created a class variable "functional group", and added it along with an interaction with every other fixed effect into the model structure used above. This variable was the repeated factor, and a heterogeneous compound-symmetric covariance structure was used to account for a lack of independence among functional groups. This covariance structure produced the lowest AIC values of the structures tested (variance components, compound symmetric, heterogeneous compound symmetric, and unstructured).

Using a single model for functional cover each year allowed us to compare overall differences in functional group composition and differences between specific functional groups.

SAS's subject option was used to ensure that plot remained the unit of replication. Available soil N concentrations and plant functional cover data were $\ln +0.01$ transformed and total plant cover was sqrt transformed to better meet the model assumptions of normality and homoscedasticity.

To assess model strength, we compared weighted Akaike's Information Criterion scores ($w_i(\text{AIC})$) between models that added additional environmental factors and their interaction with Russian olive presence as fixed factors. A weighted AIC score is calculated by dividing the relative likelihood of each model by the sum of the relative likelihoods for all candidate models. A weighted AIC score can be interpreted as the probability that a selected model is the best model (minimizes Kullback-Leibler discrepancy; Burnham and Anderson 2002). To keep comparisons of weighted AIC scores consistent between models, we used a dataset that eliminated any plots with missing values for any of the explanatory variables. Once we selected models for each of the eight response variables, we used F-tests on the full datasets to identify significant factors within the selected model and performed t-tests to compare least squares means between groups. If the selected model had a weighted AIC value below 90% (Johnson and Omland 2004), we also examined any other models with $w_i(\text{AIC})$ values greater than 10%. We did not adjust our p-values for multiple tests, because such adjustments make p-values a function of the number of tests conducted. Determining how many tests to include in an adjustment is arbitrary, making adjusted p-values difficult to interpret (Gotelli and Ellison 2004).

RESULTS

Environmental Influence on Russian Olive Impacts

Presence of Russian olive was associated with higher proportion exotic plant cover, perennial forb cover, and soil N concentration and lower perennial C4 grass cover and PAR. However, the impact of Russian olive on biotic and abiotic components of the riparian ecosystem

was conditioned by environmental context. For all response variables, models containing additional explanatory factors were more informative (had higher weighted AIC scores) than models containing Russian olive alone. For all response variables, the most informative models all contained Russian olive presence, cottonwood presence, and their interaction. For relative exotic species cover, the most informative models also included distance to and height above the thalweg and those terms' interactions with Russian olive. The selected models for total cover, functional group composition, soil N concentration, and PAR were strongly supported as being the best of the candidate models (weighted AIC scores above 90%; Table 1.1).

The selected models for richness and relative exotic cover in both years were not as strongly supported, with weighted AIC scores between 52 and 83%. For each of these response variables, one other candidate model was included as an alternative (weighted AIC score above 10%). The second most informative model for plant richness in 2010 contained only Russian olive presence as an explanatory variable. For plant richness in 2011, the second most informative model contained Russian olive presence, height above the thalweg, and those terms' interaction. For proportion exotic cover in 2010 and 2011, the second most informative models included soil texture (percent sand) in addition to the terms in the most informative model (Table 1.1).

Biotic Response Variables: Plant Community Composition

Over both years, we found a total of 138 species over all plots. Of these, 99 were native, 31 were exotic and 8 were only identified to genus and could not be unambiguously categorized as native or exotic. Reference plots contained a total of 122 species (91 native, 24 exotic, and 7 that were only identified to genus), while Russian olive plots contained a total of 111 species (72 native, 31 exotic and 8 that were only identified to genus). Based on percent cover, the ten most

abundant species were *Panicum virgatum* L., *Bromus inermis* Leyss. spp. *inermis*, *Ambrosia psilostachya* DC., *Elymus canadensis* L., *Nepeta cataria* L., *Bassia scoparia* (L.) A.J. Scott, *Spartina pectinata* Bosc ex Link, *Cirsium arvense* (L.) Scop., *Sporobolus cryptandrus* (Torr.) A. Gray, and *Bromus tectorum* L (full species list available as Appendix A).

Based on mixed-model ANOVA of the factors included in the AIC-selected most informative models, Russian olive presence had a significant effect on all biotic response variables except richness in 2010. The interaction between Russian olive and cottonwood canopy had a significant effect on total cover in 2010 and 2011, relative exotic cover in 2011, functional group composition in 2011, and plant richness in 2011. For relative exotic cover in 2010 and 2011, the interaction of Russian olive with distance to thalweg and height above thalweg were significant (Table 1.2).

Russian olive had a strong influence on plant community composition, but the strength of that influence was generally reduced under a cottonwood canopy. Compared to reference plots, Russian olive plots had higher relative exotic cover (exotic/total cover), lower perennial C4 grass cover and higher perennial forb cover (Table 1.3, Figs. 1.2 & 1.3). Presence of a cottonwood canopy, however, significantly altered the impact of Russian olive for five of the eight vegetation variables measured over two years (Table 1.2). For example, in both 2010 and 2011, the presence of a cottonwood canopy reduced the increase in relative exotic cover attributable to Russian olive (Table 1.3).

The second best models for exotic cover (weighted AIC = 33.3% for 2010, 23.2% for 2011) included percent sand in addition to the variables included in the best model (Table 1.1). When sand was included, none of the variables and interactions in the model had a significant effect on relative exotic cover. This is likely the result of reduced sample size, as we did not

measure soil texture on all plots. The second best model for species richness in 2010 (weighted AIC = 23.5%) contained only Russian olive presence as an independent variable (Table 1.1). However, there was no significant effect of Russian olive in this model ($F_{1,192}=0.00$, $p=0.95$). In the alternative model for richness in 2011 (weighted AIC = 15.8%), distance to thalweg had a significant effect ($F_{1,257}=4.51$, $p=0.03$), but its interaction with Russian olive ($F_{1,217}=0.00$, $p=0.99$) and Russian olive alone did not ($F_{1,201}=2.09$, $p=0.15$). In this model, the number of species per plot decreased as height above the thalweg increased (-0.32 ± 0.14 #species/plot/m, $t_{302}=-2.34$, $p=0.02$).

Abiotic Response Variables: PAR and Available N

In the AIC-selected models for the abiotic response variables, Russian olive presence and the interaction of Russian olive and cottonwood canopy was significant for PAR, but only Russian olive presence was significant for soil N concentration (Table 1.2). Russian olive decreased PAR in the open and underneath cottonwood, but the effect was greater in the open (Table 1.3, Fig. 1.4). In the open, Russian olive plots had less than a third the PAR level of reference plots. While underneath cottonwood canopy, Russian olive plots had about half the PAR level of reference plots. However, Russian olive plots had similar PAR regardless of whether they were under cottonwood canopy or in the open ($t_{381}=1.29$, $P=0.20$), meaning the significant effect cottonwood canopies on Russian olive impact on PAR was due to differences in reference plot PAR values. Russian olive increased available soil N most in open areas (Table 1.3, Fig. 1.4). Under cottonwood, Russian olive plots had 1.7 times higher available soil N than reference plots, while they had 3.1 times higher available N in the open. Compared to Russian olive plots under cottonwood, Russian olive plots in the open had 1.7 times higher available soil N ($t_{1,99.7}=-2.04$, $P=0.04$).

DISCUSSION

This study demonstrates that Russian olive substantially impacts riparian plant communities, and that those impacts are conditioned by environmental factors within a heterogeneous riparian ecosystem. Specifically, the per capita impacts of Russian olive on the understory plant community (i.e., increased relative exotic cover, decreased perennial C4 grass cover, and increased perennial forb cover), PAR, and soil N, were influenced primarily by presence or absence of cottonwood overstory canopy, with generally greater impacts in open areas outside of the cottonwood gallery forest. Understanding variations in impact of single plant invaders is important for determining the characteristics of ecosystems that make them most vulnerable to invasion-induced changes, and for identifying the specific combinations of invader traits and ecosystem characteristics that yield the greatest impact (Ehrenfeld 2003, Hulme et al. 2013).

Our results highlight the importance of native broadleaf pioneer tree species (i.e., cottonwoods and willows) in dampening invader impacts in western US riparian ecosystems. Under cottonwood canopies, Russian olive had less effect on plant community composition, PAR, and available soil N than in open areas. A reduction in total vegetation cover was the only effect of Russian olive that occurred under cottonwood and not in the open. Previous studies have documented the critical role of riparian cottonwoods in providing habitat for wildlife (Sedgwick & Knopf 1986) and reducing bank erosion (Henderson 1986), and have raised concerns about the conservation status of cottonwood-willow dominated ecosystems in arid regions (Braatne et al. 1996). We add to the understanding of cottonwood's importance by demonstrating that riparian gallery forests also diminish the impacts of a widespread and abundant non-native tree species.

The reduction in per capita impacts of Russian olive under the cottonwood canopy was likely caused by the smaller size and lower growth rates of Russian olive individuals growing in this environment. Russian olive trees had greater basal diameters in open areas than under cottonwood canopies at our study sites (40.1 ± 20.8 cm vs. 29.2 ± 12.4 cm, G. Tuttle, *unpublished data*). The larger size of Russian olive individuals in the open could have been due to either earlier establishment in these areas, or faster growth there. However, Katz et al. (2005) found no evidence that Russian olive had established earlier in open habitats than in the cottonwood understory in our study area. Thus, the larger size of Russian olive individuals in the open was likely due to faster growth rates there, where light levels were 1.5 times higher than under the cottonwood canopy. This idea is supported by Shafroth et al. (1995), who found greater biomass of Russian olive seedlings grown in the sun compared to the shade in an experimental study. Faster growth would lead to denser and larger Russian olive canopies and more strongly reduced PAR levels in the open, as we observed. Better conditions for photosynthesis in the open would also lead to increased N fixation (Vitousek et al. 2002) and higher N concentrations in Russian olive foliage, which is consistent with our observation of higher soil N under Russian olive in the open than under a cottonwood canopy. Thus, enhanced growth and performance of Russian olive in open habitats was likely responsible for its greater impacts on riparian abiotic and biotic conditions.

An increase in available soil N likely drove the increase in relative exotic cover underneath Russian olive, as the highest proportion of exotic cover occurred under Russian olive in open areas where available soil N was also the highest. Increased N availability may benefit fast growing exotics at the expense of slow growing natives adapted to low resource availability (Chapin 1980). In experimental studies, elevated soil N has been shown to increase the

abundance of exotic species (e.g., Paschke et al. 2000, Brooks 2003), likely because the flux of unused resources can be exploited by invaders (Davis et al. 2000). However, DeCant (2008) argued that N enhancement by Russian olive was unlikely to facilitate exotic plant invasion on the Rio Grande floodplain because soil microbial growth experiments showed limitation by C not N. He suggested instead that the effects of shading by Russian olive on PAR and soil moisture may be more responsible for changes in community composition. DeCant's hypothesis seems implausible for our study sites because shading by cottonwood did not produce the increase in exotic species that we observed under Russian olive. Our results suggest that an 'invasional meltdown' is occurring (Simberloff and Von Holle 1999), since Russian olive appears to facilitate the invasion of other exotic species, particularly outside of the cottonwood canopy.

As in other riparian systems, variations in soil texture and horizontal and vertical distance from the river influenced biotic and abiotic conditions at our study sites, though these effects were less important than the effects of Russian olive and cottonwood. For example, differences in soil texture, soil moisture, flooding frequency, and soil N concentrations, driven largely by flooding disturbance history, had large impacts on plant community composition along the Snake River in Idaho (Merigliano 2005). In our study, the effect of N on plant community composition may have been complicated by differences in soil texture (Bechtold and Naiman 2006), denitrification rates, and plant N uptake among riparian geomorphic surfaces (Pinay et al. 1995). However, soil texture (percent sand) was not included as an explanatory factor in any of our best models, and was only included in two second-best models (exotic cover in 2010 and 2011). This result supports Decant (2008), who found that in riparian areas invaded by Russian olive, variation in available soil N was best explained by Russian olive presence and not soil texture.

Ultimately, the presence or absence of Russian olive and cottonwood were the strongest determinants of riparian abiotic and biotic conditions in our study plots, with riparian soil and geomorphic factors playing relatively minor roles.

Table 1.1: Weighted AIC ($w_i(\text{AIC})$); relative likelihood of model / sum of relative likelihoods for all models; shown as %) for *a priori* selected mixed models describing Russian olive impacts on plant richness, exotic cover, functional group composition, ionic soil N concentration, and light availability, as conditioned by environmental variables. In all models, Russian olive and Russian olive interaction with each additional term were included. For functional group cover, models all include a functional group interaction for each term included. The terms used in the models were “Ro” indicating underneath or outside Russian olive canopy, “Cott” indicating underneath or outside cottonwood canopy, “Dst” indicating distance to thalweg, “Hgt” indicating height above thalweg, and “Sd” indicating percent sand. For each response variable we considered models with weighted AIC scores greater than 10%. Bold text indicate models with the highest $w_i(\text{AIC})$ scores.

Model Terms	2010 Total Cover	2011 Total Cover	2010 Rel. Exotic Cover	2011 Rel. Exotic Cover	2010 Func. Group	2011 Func. Group	2010 Rich.	2011 Rich.	PAR	Soil N
Ro	0.14	0.01	<0.01	<0.01	<0.01	<0.01	23.54	0.34	<0.01	3.88
Ro Cott	99.56	99.73	7.44	8.55	99.99	99.99	67.27	82.53	99.99	95.61
Ro Cott + Ro Dst	<0.01	0.07	0.26	0.35	<0.01	<0.01	0.03	0.03	<0.01	<0.01
Ro Cott + Ro Dst + Ro Hgt	<0.01	0.03	52.27	63.15	<0.01	<0.01	0.01	0.87	<0.01	<0.01
Ro Cott + Ro Sd	0.09	0.15	3.51	3.31	<0.01	<0.01	0.34	0.37	<0.01	0.09
Ro Cott + Ro Dst + Ro Hgt + Ro Sd	<0.01	<0.01	33.33	23.23	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ro Dst	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ro Dst + Ro Hgt	<0.01	<0.01	0.10	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ro Dst + Ro Hgt + Ro Sd	<0.01	<0.01	0.06	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ro Hgt	0.20	<0.01	3.02	1.34	<0.01	<0.01	8.66	15.85	<0.01	0.87
Ro Sd	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.14	<0.01	<0.01	<0.01

Table 1.2: Mixed-model ANOVA of AIC-selected models for plant species richness, total cover, exotic plant cover, plant functional group composition in 2010 and 2011, PAR, and soil N described by the interaction of Russian olive, cottonwood canopy, distance to thalweg, and height above thalweg. For functional cover, all terms are interactions with functional group. Missing values (e.g. distance to thalweg for 2010 or 2011 cover) occur when an independent variable is not present in the AIC selected model.

Model Term	Total Cover 2010	Total Cover 2011	Rel. Exotic Cover 2010	Rel. Exotic Cover 2011	Functional Group Comp. 2010	Functional Group Comp. 2011	Richness 2010	Richness 2011	PAR	Soil N
Russian olive	F_{1,191}=7.94 p<0.01	F _{1,187} =1.85 p=0.17	F_{1,175}=15.72 p<0.01	F_{1,172}=12.94 p<0.01	F_{5,2100}=9.14 p<0.01	F_{5,2082}=14.31 p<0.01	F _{1,192} =0.02 p=0.90	F_{1,191}=10.32 p<0.01	F_{1,192}=251.29 p<0.01	F_{1,58.8}=29.32 p<0.01
Cottonwood	F_{1,213}=27.78 p<0.01	F_{1,254}=34.66 p<0.01	F _{1,228} =0.34 p=0.56	F _{1,219} =0.82 p=0.37	F_{5,2100}=36.35 p<0.01	F_{5,2082}=35.88 p<0.01	F_{1,240}=4.29 p=0.04	F_{1,227}=4.92 p=0.03	F_{1,229}=10.26 p<0.01	F _{1,70.7} =0.98 p=0.32
Russian olive* Cottonwood	F_{1,203}=6.66 p=0.01	F_{1,195}=12.31 p<0.01	F _{1,164} =1.72 p=0.19	F_{1,160}=5.26 p=0.02	F _{5,2100} =2.03 p=0.07	F_{5,2082}=2.62 p=0.02	F _{1,204} =0.42 p=0.52	F_{1,209}=10.85 p<0.01	F_{1,374}=41.68 p<0.01	F _{1,60.7} =3.26 p=0.08
Distance to Thalweg	.	.	F _{1,111} =2.18 p=0.14	F_{1,150}=7.85 p<0.01
Russian olive* Distance to Thalweg	.	.	F _{1,154} =1.16 p=0.28	F _{1,153} =0.69 p=0.41
Height above Thalweg	.	.	F _{1,221} =2.55 p=0.11	F _{1,271} =0.90 p=0.34
Russian olive* Height above Thalweg	.	.	F _{1,192} =0.19 p=0.67	F _{1,188} =0.28 p=0.60

Table 1.3: T-tests of differences between Russian olive and reference plot least square means underneath cottonwood canopy and in the open. Values for total cover and soil N have been back-transformed. Significant differences at $\alpha=0.05$ between Russian olive and reference plots are bolded.

Response Variable	Cottonwood					Open				
	Russian olive – Reference plots	Std. Error	DF	T-value	P-value	Russian olive – Reference plots	Std. Error	DF	T-value	P-value
Total Cover 2010	-11.70	0.51	198	-3.36	<0.01	-0.52	0.02	195	-0.17	0.86
Total Cover 2011	-9.55	0.50	194	-3.04	<0.01	5.07	0.21	191	1.83	0.06
Rel. Exotic Cover 2010	19.99	4.70	159	4.25	<0.01	28.11	3.65	156	7.70	<0.01
Rel. Exotic Cover 2011	20.03	4.28	155	4.67	<0.01	32.99	3.34	153	9.88	<0.01
Richness 2010	-0.15	0.30	199	-0.48	0.63	0.10	0.22	195	0.43	0.66
Richness 2011	-0.02	0.37	202	-0.06	0.95	1.50	0.27	197	5.45	<0.01
PAR	-25.91	4.46	202	-5.81	<0.01	-61.93	3.32	197	-18.65	<0.01
Soil N	15.35	1.24	56.7	2.47	0.02	39.61	1.23	63.3	5.27	<0.01

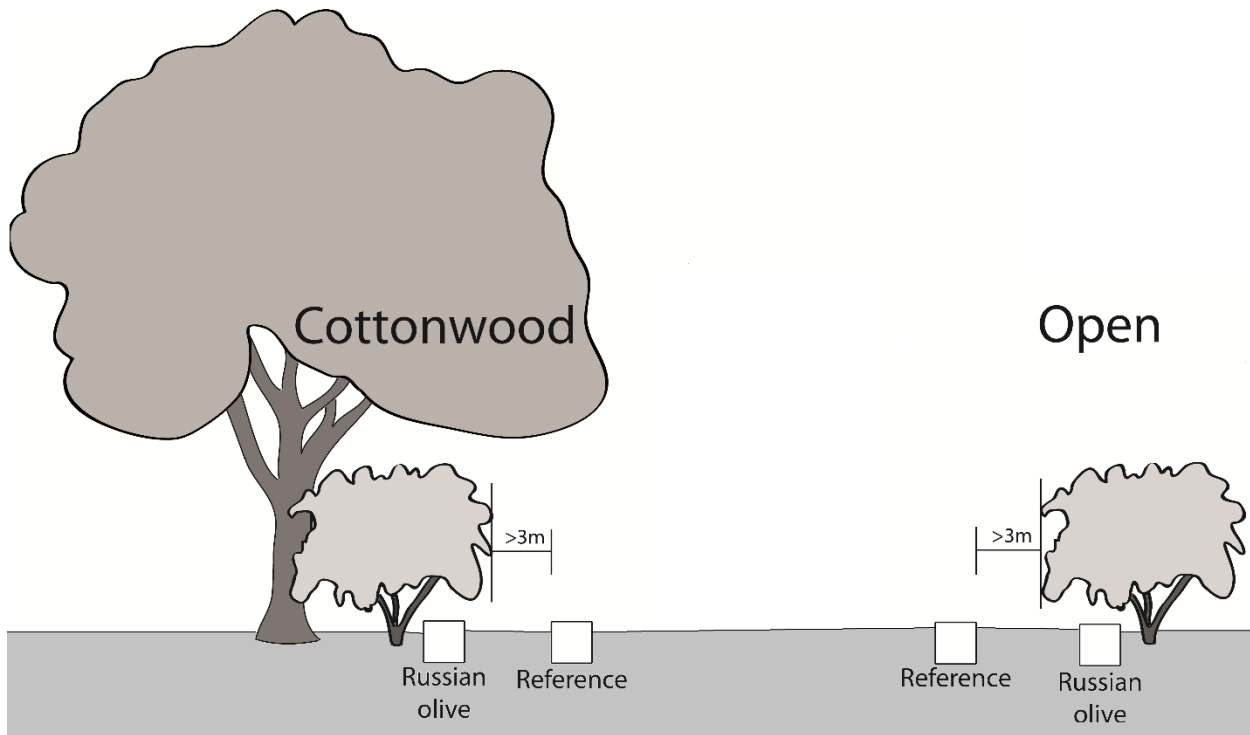


Figure 1.1: Diagram of two Russian olive and reference plot pairs, showing locations underneath cottonwood canopy (cottonwood) or outside the canopy (open). Note that the reference plot for each pair is located either in the open or under cottonwood canopy, depending on where the corresponding Russian olive plot for each pair is located.

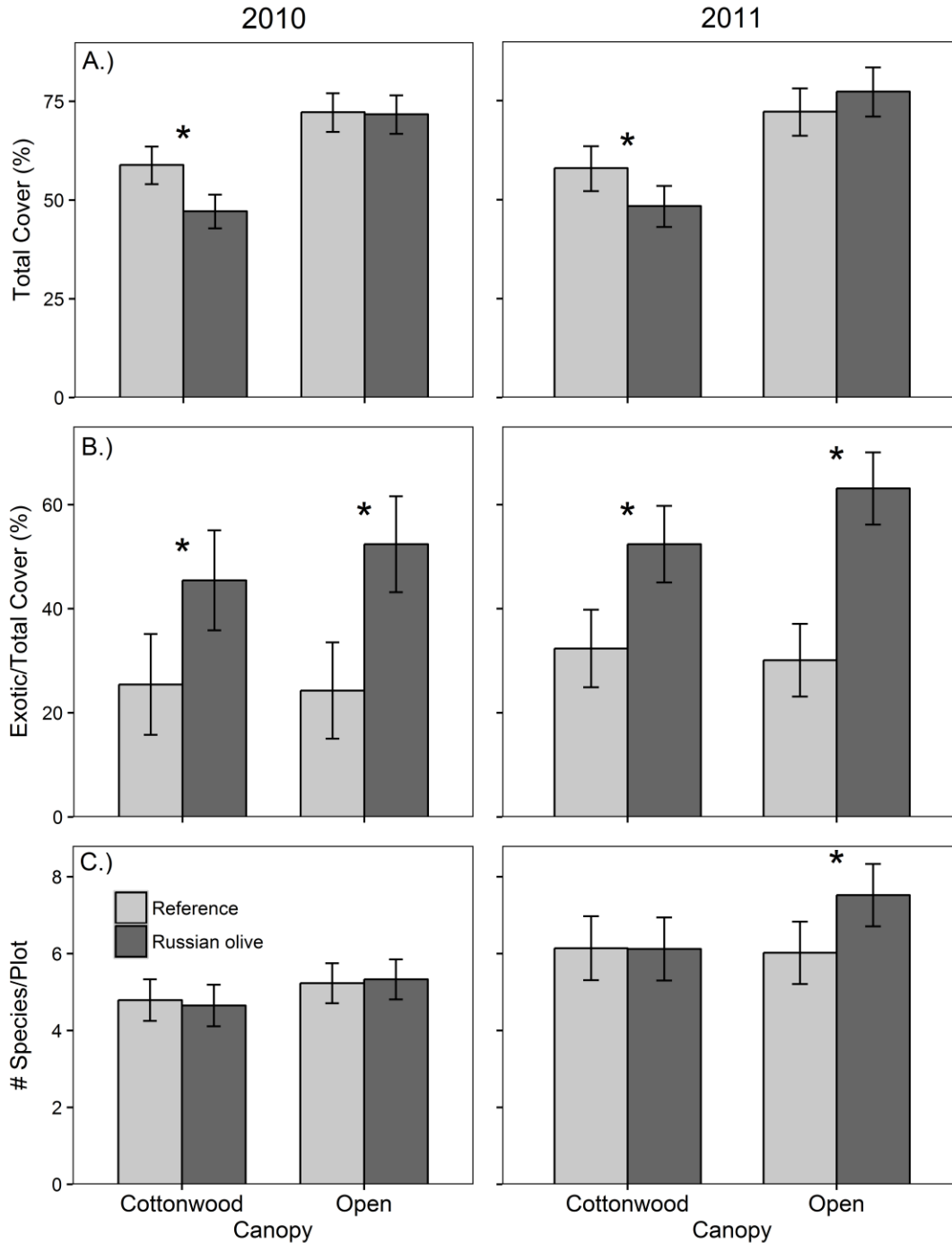


Figure 1.2: Least squares means \pm SE for biotic response variables measured in August 2010 and 2011, as conditioned by presence/absence of Russian olive and cottonwood forest overstory: A.) total plant cover (A), proportion exotic cover (B), plant species richness(C). Russian olive plots were located underneath a Russian olive canopy, and reference plots were located at least three meters away from a Russian olive canopy dripline. Cottonwood plots occurred underneath the cottonwood gallery forest overstory, and open plots did not. Values in plots A and C have been back-transformed. * indicates significant difference ($\alpha = 0.05$) between Russian olive and reference plots.

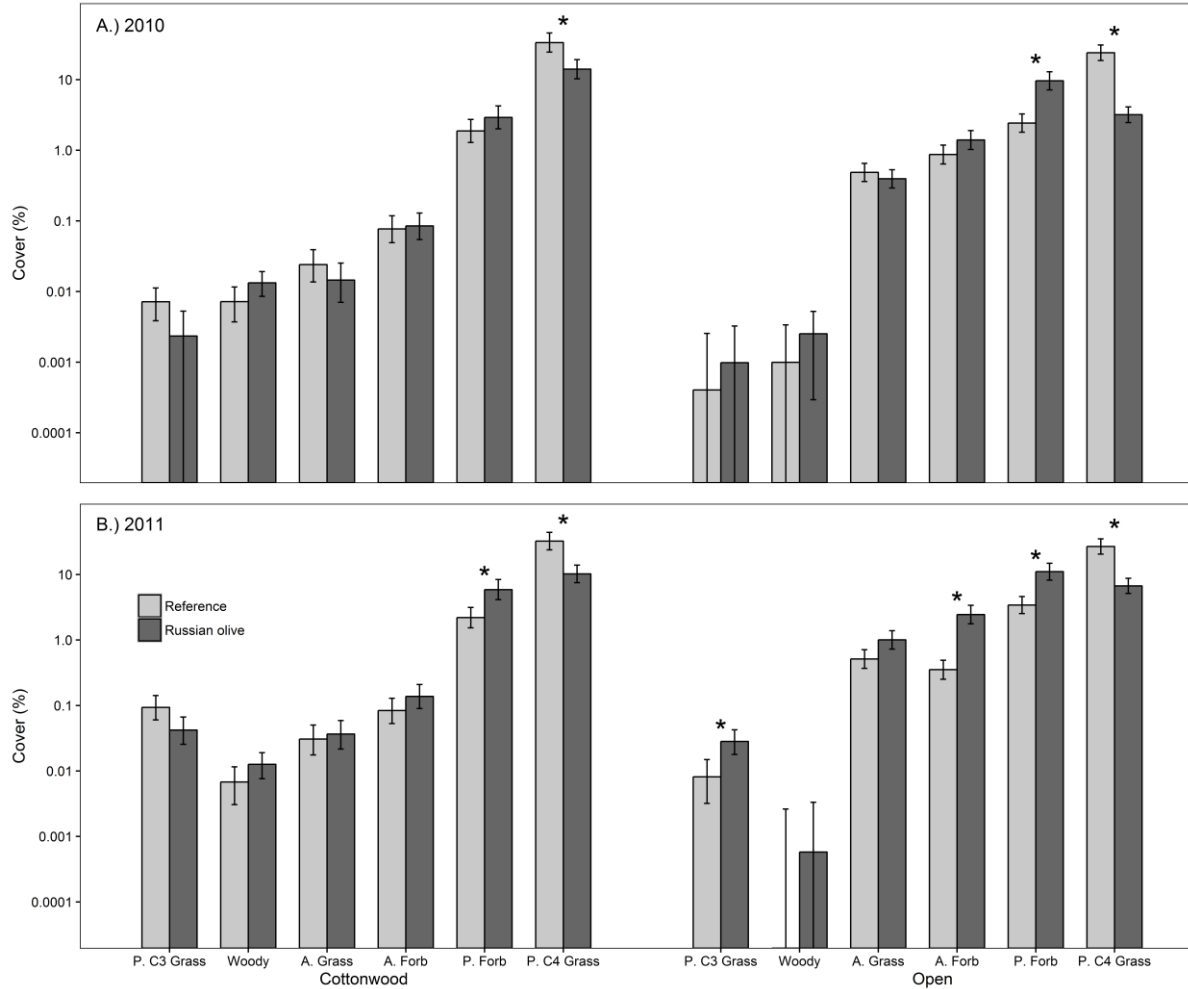


Figure 1.3: Least squares means \pm SE for plant functional group cover measured in August 2010 (A) and 2011 (B), as conditioned by presence/absence of Russian olive and cottonwood forest overstory. Values have been back-transformed. * indicates significant difference ($\alpha = 0.05$) between Russian olive and reference plots. Note: y-axis is in log₁₀-scale to show functional groups with lower cover.

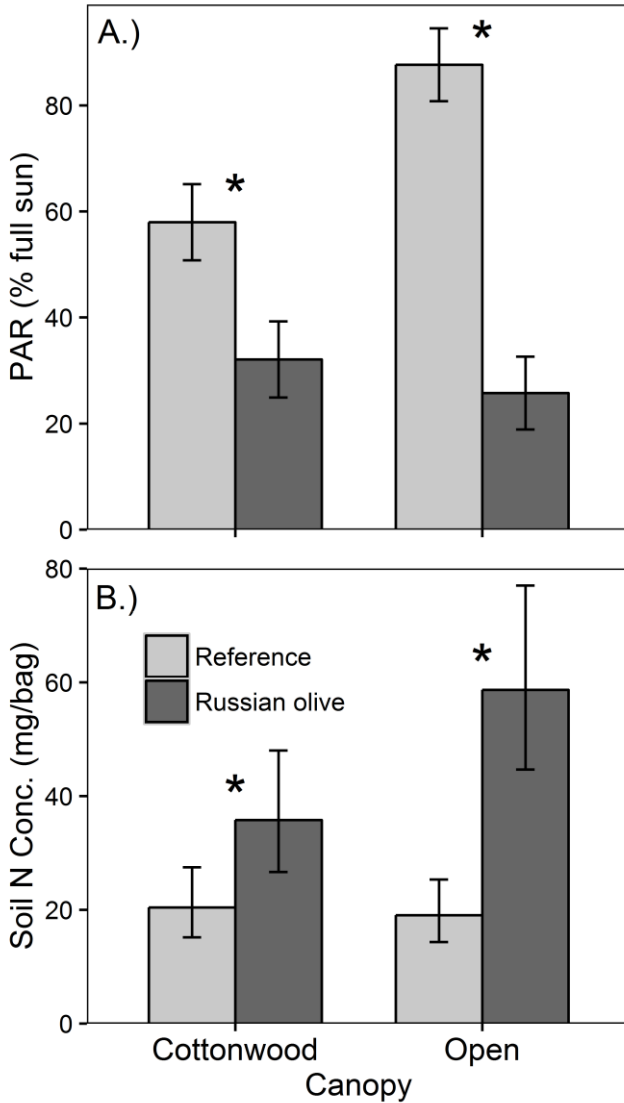


Figure 1.4: Least squares means \pm SE for abiotic response variables, as conditioned by presence/absence of Russian olive and cottonwood forest overstory: A.) Photosynthetically active radiation (PAR) in August 2010, B.) Inorganic soil N concentration (August 2010 through August 2011). Plot locations described in Fig. 1.3. Values in B have been back-transformed. * indicates significant difference ($\alpha=0.05$) between Russian olive and reference plots.

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CHAPTER 2: SOIL N AND PLANT COMMUNITY RESPONSE TO RUSSIAN OLIVE REMOVAL FROM A SEMI-ARID RIPARIAN ECOSYSTEM

SUMMARY

Removing exotic invasive plant species (EIPS) does not necessarily lead to an increase in native species cover and may instead facilitate secondary invasion due to a combination of EIPS legacy effects and disturbance from removal. Despite uncertainty about EIPS removal achieving management objectives, few studies have monitored community response to EIPS removal. We measured the impacts of Russian olive (*Elaeagnus angustifolia*), an invasive N-fixing tree, on soil N availability and plant community composition in a semi-arid riparian ecosystem from 2010-2014. Half of the trees at two of our three sites were removed in winter and spring 2012, which caused soil disturbance at those sites. The third site acted as an undisturbed control. In the two years prior to removal, areas underneath Russian olive had higher soil N availability and proportion exotic plant cover than areas away from the tree. Three years after removal, soil N availability was not significantly lower next to removed trees than trees left intact. NMDS and MRPP analyses showed that three years after removal plant communities were significantly different around removed Russian olive, Russian olive left intact, and reference areas away from the tree's canopy. There was not a large difference in proportion exotic cover between removed and intact Russian olive, but the exotic species found in each treatment were different. Areas next to removed Russian olive had higher cover of the exotic annual forb kochia, which may be due to a combination of high N and soil disturbance during removal. Kochia has the potential to maintain high soil N and high densities, delaying native species from returning to those areas.

These results suggest that land managers should consider the legacy effect of Russian olive on soil N availability when developing management strategies for this tree.

IMPLICATIONS FOR PRACTICE

In the three years following mechanical removal and cut-stump treatment of invasive N₂-fixing Russian olive trees, areas around removed Russian olive maintained high soil N availability and became dominated by the exotic, annual forb kochia. The increase in cover of kochia and other exotic forb species was likely due to a combination of the legacy effect of Russian olive (i.e., high plant available soil N) and physical soil disturbance from the removal process. The high kochia cover has the potential to persist for decades due to a reinforcing feedback between kochia's competitive dominance in high N soils and microbial decomposition of kochia leaf litter that causes high C and N mineralization rates (Vinton and Burke 1995). To reduce kochia invasion following Russian olive removal, we suggest restoration strategies that can ameliorate high available N, such as amending the soil or mixing topsoil with lower soil layers (Malcolm et al. 2008), which would prevent this feedback from originating (Wali 1999). Removing Russian olive in the late summer and fall when kochia seeds are rare in the seedbank and less likely to germinate the following spring (Schwinghamer & Van Acker 2008), and seeding with desirable species after removal could further reduce kochia's competitive ability.

INTRODUCTION

The main objective of exotic invasive plant species (EIPS) management is to reduce EIPS impacts on the invaded ecosystem and promote community recovery, such as an increase in native and decrease in exotic cover (Hulme 2006; Barney 2016). To measure if management actions achieve their objectives, studies need to monitor the ecosystem properties before, during, and after management (Blossey 1999). Most studies that monitor after EIPS management focus

only on efficacy of target EIPS removal and not on response of the community (Reid et al. 2009; Downey 2011; Pearson et al. 2016). Of the few studies that measure community response following EIPS management, many found high rates of secondary invasion by other EIPS (Reid et al. 2009; Pearson et al. 2016), likely due to a combination of target EIPS legacy effects and management side effects (Pearson et al. 2016). This has led some researchers to question whether current EIPS management strategies that focus solely on removing species should be continued (e.g. Ogden et al. 2005; Reid et al. 2009; Larson & Larson 2010; Downey 2011; Pearson et al. 2016). Understanding the factors that influence plant community response to EIPS management is crucial for developing effective restoration strategies (Hobbs 2007; Grman et al. 2013).

In some cases, the effects of EIPS on invaded ecosystems may persist after their removal, particularly when EIPS impact soil conditions. If these EIPS legacy effects occur, then return to pre-invasion community structure and function can be delayed (Corbin & D'Antonio 2012). EIPS can alter ecosystem soil N pools and cycling rates (Ehrenfeld 2003), which can facilitate secondary exotic invasion (Simberloff & Von Holle 1999). The continuance of EIPS effects on soil N after it has been removed seems to vary by species and ecosystem. For example, soil N availability remained high for at least 22 months after removal of the N-fixing Scotch broom (*Cytisus scoparius*), which facilitated secondary invasion and delayed native plant recovery in formally forested sites in the Pacific northwestern U.S. (Grove et al. 2015). Alternatively, plant community recovery 2 years after Russian olive (*Elaeagnus angustifolia*) removal in an arid riparian ecosystem in Canyon de Chelly National Monument, AZ was not influenced by elevated soil N availability around removed trees (Reynolds & Cooper 2011). Currently, too few studies have been conducted on EIPS legacy effects to gain a clear understanding their likelihood of

occurring and their role in ecosystem recovery following EIPS management (Corbin & D'Antonio 2012; Wurst & Ohgushi 2015; Tekiela & Barney 2017).

The combination of disturbance from management activities and EIPS legacy effects could cause the ecosystem more harm than not managing the EIPS (Barney 2016). Alterations to the natural disturbance regime often favor exotic species over natives, particularly when nutrient addition coincides with the disturbance (Hobbs & Huenneke 1992). Disturbance caused by EIPS management can have negative or positive direct or indirect effects on other EIPS depending on population sizes and environmental conditions (Buckley 2007). If legacy effects on soil N occur, then the disturbance from management and follow-up restoration can shift communities toward faster-growing exotic species (Maron and Jefferies 1999). Alternatively, restoration that involved mixing upper soil that had high soil N concentrations with lower soil layers that had lower soil N reduced the persistence of black locust (*Robinia pseudoacacia*) effects on soil N. This likely helped quicken plant community recovery in the Albany Pine Bush, NY after black locust removal (Malcolm et al. 2008).

Russian olive (*Elaeagnus angustifolia* L.) is an exotic invasive actinorhizal (N₂-fixing) tree that has the potential to have effects that persist after removal. Presence of Russian olive is associated with higher soil N availability and exotic plant cover, as well as reduced photosynthetically active radiation (PAR) compared to areas outside the tree's canopy (Tuttle et al. 2016). The removal of Russian olive often utilizes heavy machinery and causes significant soil disturbance. A previous study by Reynolds and Cooper (2011) in the Canyon de Chelly National Monument, Arizona found soil N availability was higher around removed Russian olive stumps than away from the tree, but exotic plant cover decreased and native plant cover increased in the two years following the tree's removal. From these results, it is unclear how soil

N availability changed due to Russian olive removal and how that change influenced plant community composition. Our study monitored how soil N and the plant community in areas around Russian olive and away from the tree's canopy responded to Russian olive being removed and the disturbance from removal in a semi-arid, riparian grassland in eastern Colorado. This allowed us to see if there are Russian olive legacy effects, and, if so, how those influence plant community changes following disturbance. We predicted that in our study area, (1) the effects of Russian olive on soil N availability would persist into the third year following removal, and (2), unlike in Reynolds and Cooper's (2011) study, cover by exotic species would increase and native species cover would decrease due to the persistence of high soil N availability and soil disturbance from removal facilitating secondary invasion.

METHODS

Study Site

Our study area consisted of three sites along a 3.5-km, unregulated section of the South Fork of the Republican River in Kit Carson and Yuma Counties, Colorado, USA. The river is located at the edge of the Western Short Grasslands and the Central and Southern Mixed Grasslands ecoregions (Ricketts et al. 1999) in the western United States Great Plains (Osterkamp et al. 1987). Drier upland areas at our sites have similar plant communities to the Western Short Grassland, while wetter areas have higher densities of tall-grass species (Albertson 1937). A full list of all herbaceous species found at our study area is available in Tuttle et al. 2016.

The climate of the Great Plains is characterized by large variation in daily, monthly, and yearly temperature and precipitation, high potential evaporation, and frequent and severe storms (Rosenberg 1986). This variability was realized during the five years of our study (2010-2014),

particularly for precipitation. Growing season (April-August) mean temperature was within 0.5°C of the 23-year average (20.7°C) for all years of our study with the exception 2012 and 2014, which were 1.9°C higher and 1.6°C lower than the average, respectively. Precipitation during the growing season was above average (5.9 cm) in 2010, 2011, and 2014. It was 89.9% of the average in 2012 and 86.4% in 2013 (Station IDL01, Colorado Agricultural Meteorological Network 2016).

Russian olive first became established in the study area in the 1970's (Katz et al. 2005), and it now occurs both in open meadow habitats and in the understory of the cottonwood gallery forest. At each site, Russian olive stands extended at least 100 m away from the river and at least 400 m along the river's length. All riparian areas used in the study were seasonally grazed by cattle every two years.

Study Design and Data Collection

We established the three study sites in Russian olive stands. Data were collected in 1x1 m plots (total plots = 240 in 2010, 240 in 2011, 235 in 2012, 233 in 2013, and 235 in 2014), spread along transects at each site. Transects originated at the edge of the active river channel, and extended at least 100 m perpendicular from the river bank up to the distal edge of Russian olive stands. Along each transect, we established 40 paired plots, 20 underneath the canopy of a Russian olive tree (Russian olive plots) and 20 located at least three meters from the nearest Russian olive and its canopy drip line (reference plots), at the same height above and distance from the river (site 1 = 3 transects (120 plots), site 2= 2 transects (80 plots), and site 3 = 1 transect (40 plots). We located the reference plots at least three meters from the Russian olive dripline to avoid Russian olive influence on soil N (Decant 2008) and light. Plot acted as our unit of replication for statistical analyses.

Russian olive removal treatment

In winter through spring 2012, Russian olive was removed from 51 of the 100 pairs of plots at two of the three sites using a Hydro-Ax™ feller buncher to cut and pile trees followed by application of triclopyr (Garlon 4 ©) to stumps to prevent re-sprouting. The third site acted as an undisturbed benchmark -- none of the Russian olive trees were removed, and therefore the site was undisturbed by the removal process. This design allowed us to track how plant communities respond to removing Russian olive and the disturbance from removal. At the first site, 30 of the 60 sample trees were removed, and 21 of 40 sample trees were removed at the second site (Fig. 2.1). We refer to these plots as Russian olive-removed = trees removed in 2012, Russian olive-disturbed = trees left intact at the two sites where removal occurred, and Russian olive-undisturbed = trees at the site with no removal. Reference-disturbed = reference plots at sites where removal occurred, and Reference-undisturbed = reference plots at the site with no removal. This design allowed us to measure the effects of disturbance from the removal process and the effects of removing the tree.

Soil N availability

We measured ionic soil N availability (ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-)) using ion exchange resin bags (Binkley 1984). One nylon mesh bag, containing 15 mL of mixed bed ion-exchange resin, was buried at a 5-10 cm depth in 12 plot pairs (Russian olive / reference) on two transects at each site (48 plots/site). The 12 plots represented every other pair of plots along each transect plus the middle and last pairs. Each bag was buried for a 4-month interval (August-November, December-March, and April-July) in the corner of each plot closest to the nearest Russian olive tree. We extracted N ions from bags retrieved from the field using 75 mL of 2M KCL and measured NO_3^- , NO_2^- and NH_4^+ availability using an AlpKem Flow Solution

IV Automated wet chemistry system (O.I. Analytical, College Station TX) at the Colorado State University Natural Resource Ecology Laboratory.

Herbaceous plant cover

In late July to early August, we visually estimated percent cover of each vascular plant species originating in each 1 m² plot for the two years prior to removal (2010 and 2011) and three years after removal (2012-2014). We identified plants to species according to Weber and Wittman (2001) and Shaw (2008) and used the U.S.D.A. PLANTS database (USDA, NRCS 2013) for taxonomic nomenclature, classification of each species as exotic or native, and assignment of each species to one of six functional groups: annual grass, annual forb, perennial C₃ grass, perennial C₄ grass (Shaw 2008), perennial forb, and woody. Due to the low incidence of sedges (*Carex* spp.) and rushes (*Juncus* spp.) at our sites, we combined these taxa with perennial C₃ grasses for analysis.

Statistical Analysis

We used the SAS 9.3® MIXED procedure (2012, SAS Institute, Cary, NC) to run a Mixed-model repeated measures ANOVA for soil N availability. The model included the factorial interaction of three categorical terms: plot type (Russian olive, reference), year (2010-2014), and removal (removed, disturbed, and undisturbed) as fixed effects, site as a random effect, plot pair within site as subject, and year as repeated measure. We used compound symmetry covariance matrix and the Kenward and Roger (1997) covariance matrix fixed effect degree of freedom adjustment. Soil N availability was ln transformed to better meet the model assumptions.

We examined similarities in plant community composition using non-metric multi-dimensional scaling (NMDS) ordination in PC-ORD 6.08® (McCune & Mefford, 2011, MjM

Software, Gleneden Beach, OR). In NMDS, we used the Bray-Curtis distance measure, 90 runs with real data, stability criterion of 0.00001, and 15 iterations to evaluate stability. We used all plots across the 5 years of our study for this analysis. To allow the analysis to run, we removed 65 of 1190 total year-plot combination due to zero plant cover or distance measurements greater than 2.5 S.D. We also removed 113 of 169 plant species because they were found in fewer than 5 plots. For the NMDS analyses, we chose a three-dimensional solution, the simplest solution with a stress below 0.20. Groups were defined by the interaction of plot type (Russian olive, reference), removal treatment (removed, disturbed, undisturbed), and year (2010-2014). We used centroids for each group to graph their change in plant community composition over time and selected vectors of environmental variables and plant metrics with Pearson correlations above 0.2. Environmental variables examined were Russian olive basal diameter, soil texture, distance to and height above thalweg, soil N availability, and winter and summer leaf area indexes. Metrics examined to describe plant community composition were functional group cover (woody, perennial forb, perennial C₃ grass, perennial C₄ grass, annual forb, and annual grass), proportion exotic cover, native cover, richness, Shannon-Weiner diversity, and total cover.

We used multi-response permutation procedure (MRPP) in PC-ORD 6.08® to test for significant differences in plant community composition among groups. MRPP compares within-group mean pairwise Bray-Curtis distance measurements (observed delta) to a null distribution based on 4,999 permutations of pairwise Bray-Curtis distances between all samples (expected delta) (Zimmerman et al. 1985).

To further examine the effect of Russian olive removal on species composition three years after removal, we used Dufrene and Legendre's (1997) method to calculate species indicator values for the 82 species found at the disturbance sites in 2014 in PC-ORD 6.08®. This

method scores species based on their relative abundance and relative frequency, with a score of 100 meaning it is a perfect indicator of that group. Finally, we compared change in cover between 2010 and 2014 for the eight most common species with mean covers above 2% throughout the study using the same mixed-model repeated measure ANOVA used for soil N availability. Species cover data were $\ln +0.01$ transformed to better meet the model assumptions. The eight species combined comprised over 65% of average total plot cover.

RESULTS

Soil N Availability

Prior to removal, soil N availability was higher in Russian olive plots than reference plots at undisturbed and disturbed sites (Fig. 2.2). Removing Russian olive did not have a significant influence on soil N availability, which remained higher in Russian olive-removed plots than in reference-disturbed plots three years after the trees were removed. Neither the removal treatment main effect nor any of its interactions with the other independent variables were significant in this model (Table 2.1). Soil N availability in Russian olive-removed plots was not significantly different than Russian olive-disturbed plots prior to or after removal. However, soil N availability was significantly higher in Russian olive-removed than in reference-disturbed plots in all four years. At the undisturbed site, Russian olive plots had significantly higher soil N availability than reference plots in all years, except 2011 (Fig. 2.2).

Plant Community Composition

Over the 5 years of this study, we found 169 plant species rooted within our study plots. The most common species averaged across all years and treatments were; Cuman ragweed (*Ambrosia psilostachya* DC.), kochia (*Bassia scoparia* (L.) A.J. Scott), cheatgrass (*Bromus*

tectorum L.), Canada wildrye (*Elymus canadensis* L.), catnip (*Nepeta cataria* L.), switchgrass (*Panicum virgatum* L.), Kentucky bluegrass (*Poa pratensis* L.), and sand dropseed (*Sporobolus cryptandrus* (Torr.) A. Gray) (Fig. 2.3). Annual forb, native, proportion exotic, and total cover were the only vectors above $r^2=0.2$ in the NMDS. None of the other plant cover metrics or environmental variables were significantly correlated (Fig. 2.4).

Pre-removal

NMDS indicated that the plant communities in Russian olive plots had higher annual forb and proportion exotic cover than reference plots at both sites in the two years prior to removal (2010 and 2011), though the plant communities differed between the undisturbed and disturbed sites (Fig. 2.4). MRPP analysis showed, prior to removal, the plant communities in Russian olive plots were significantly distinct from reference plots at both the undisturbed and disturbed sites (Table 2.3). There were no significant differences in plant communities between Russian olive-removed plots and Russian olive-disturbed plots (2010: $A < 0.001$, $t = -0.16$, $p = 0.36$; 2011: $A < 0.001$, $t = 0.13$, $p = 0.50$).

Post-removal

NMDS analysis showed that, after the disturbance from Russian olive removal, there was an initial decrease in total plant cover in 2012 followed by an increase in annual forb and proportion exotic cover across all plot types at the disturbed sites. At all sites, plots showed an increase in annual forb and proportion exotic cover over the study period. However, the change was less pronounced for plots at the undisturbed site because they decreased proportion exotic cover in 2014. Plots at the disturbed sites did not show a decrease in annual forb and proportion exotic cover in 2014. Russian olive-removed plots diverged from Russian olive-disturbed plots, after removal (Fig. 2.4).

MRPP analyses showed that after removal the plant community in Russian olive-removed plots was different than Russian olive-disturbed plots. In the second and third year after removal (2013-2014), the plant communities in Russian olive-removed and intact plots significantly diverged (2013: $A=0.012$, $t=-4.76$, $p<0.01$; 2014: $A=0.016$, $t=-6.68$, $p<0.01$). At both the undisturbed and disturbed sites, all Russian olive plots had significantly different plant communities than reference plots for all years of the study (Table 2.4).

The overall proportion exotic cover was not different between Russian olive-removed and intact plots (Fig. 2.3), but the composition of exotic species diverged between the two plot types after removal. Species indicator values showed that in 2014 cheatgrass and catnip were more common in Russian olive-disturbed than Russian olive-removed plots, while kochia was more common in Russian olive-removed plots (Table 2.2). Over the study, cover of the exotic annual C_4 forb kochia increased more in Russian olive-removed than Russian olive-disturbed plots, though not significantly ($t_{984.5}=-1.33$, $p=0.18$). Kochia cover had a ten-fold increase from 2.5% in 2010 to 22.4% in 2014 in Russian olive-removed plots compared to an increase from 2.9% to 12.9% in Russian olive-disturbed plots. The exotic perennial C_3 forb catnip decreased more over the study in Russian olive-removed than intact plots, though not significantly (12.4% to 0.1% in removed vs. 14.4% to 3.3% in intact, $t_{985.4}=1.50$, $p=0.13$; Table 2.4; Fig. 2.3). The exotic annual C_3 grass cheatgrass (*Bromus tectorum*) increased less, but not significantly differently, in removed than intact Russian olive plots (0.9% to 3.5% in removed vs. 1.7% to 11.6% in intact, $t_{982.5}=0.92$, $p=0.36$; Table 2.4; Fig. 2.3).

A higher cover of the native perennial C_4 grass sand dropseed in Russian olive-removed plots than Russian olive-disturbed plots accounted for some of the difference in plant community between the two plots after removal (Table 2.2; Fig. 2.3). Sand dropseed cover increased more in

Russian olive-removed plots than Russian olive-disturbed plots from 2010 to 2014 (0.3 to 4.0% in removed vs <0.01 to 0.25% in intact, $t_{984.2}=-4.19$, $p<0.01$). In 2014, mean sand dropseed cover in reference-disturbed plots was 6.23% (Fig. 2.3).

DISCUSSION

Recent literature reviews found that few studies have monitored recovery of invaded communities after EIPS removal, which makes it difficult to assess how well EIPS management achieves restoration objectives (Reid et al. 2009; Pearson et al. 2016). Our study showed that, three years after Russian olive was removed, some native species increased in the plant community around Russian olive stumps, but the community became distinct from areas where the tree was left intact or areas devoid of Russian olive due to a change in exotic species composition. These results showed that without additional restoration, there was a delay in plant community recovery after Russian olive removal. This delay in recovery was likely due to a combination of Russian olive legacy effects on soil N and disturbance from the removal process.

Legacy effects of EIPS on soil chemistry can alter the recovery of plant communities following EIPS management (Corbin and D'Antonio 2012). Previous studies have shown that soil N availability is higher underneath Russian olive than outside the tree's canopy (DeCant 2008; Follstad Shah et al. 2010; Tuttle et al. 2016). Our study showed that elevated soil N availability persisted at least three years after Russian olive removal in this semi-arid riparian ecosystem, and that this legacy effect is likely impacting the plant community around removed Russian olive trees. The persistence of elevated soil N availability after Russian olive removal in our study may have been caused by Russian olive increasing N pools instead of just changing N fluxes (Corbin and D'Antonio 2004; Corbin and D'Antonio 2012). Elevated N fluxes, due to EIP tissue chemistries differing from that of native species, often revert to pre-invasion levels

relatively quickly following removal (reviewed in Corbin and D'Antonio 2004). In contrast, elevated soil N availability that results from changes in N pools often persists for years or decades after removal (e.g., Maron and Jefferies 2001; Marchante et al. 2008; Von Holle et al. 2013). Our results agree with Reynolds and Cooper (2011), who also found that soil N availability remained high in the second year after removal.

Continued high proportion of exotic species and annual forb cover around removed Russian olive trees may have been facilitated by the persistence of high soil N availability, but was also likely promoted by disturbance during the removal process. This appeared to be the case in our study because annual forb and proportion exotic cover increased more at the disturbed sites than the undisturbed sites over the course of the study, particularly where Russian olive was previously present (Fig. 2.4). Studies in tallgrass ecosystems found that elevated soil N was often associated with increased exotic plant and forb cover following disturbance (Seastedt et al. 1991; Averett et al. 2004). The continued elevation of soil N levels due to legacy effects of EIPS has also been shown to favor invasion of fast-growing EIPS in the Pacific northwestern U.S. (Grove et al. 2015).

The high proportion of exotic cover around removed Russian olive, after removal, was largely due to a dramatic increase in kochia cover. This increase more than compensated for the decrease in catnip and cheatgrass cover that occurred during that time (Table 2.4; Fig. 2.3). The increase in kochia cover was likely due to its ability to colonize faster after soil disturbance than natives and other exotics in the Great Plains (Wali 1999; Schwinghamer and Van Acker 2008). Further, kochia grows particularly well after a disturbance when N levels are high (McLendon and Redente 1991).

The increased cover of kochia in removed Russian olive plots has the potential to influence the plant community for decades, thus altering its successional trajectory. In an experimental study in the shortgrass steppe north of our study area, kochia became dominant in plots where moisture and N were added (Laurenroth et al. 1978). Twenty years after the original experiment was conducted, kochia persisted as the dominant species in those plots (Vinton and Burke 1995). The authors suggested this was due to a positive feedback, where kochia maintains its competitive advantage over other species because its tissue chemistry is favorable to microbial decomposition and, thus, produces high C decomposition and N mineralization rates. If this feedback occurs at our study sites, areas heavily invaded by kochia may persist for years or decades, potentially creating an alternative stable state (Suding et al. 2004). Over the next few years, it will become clearer whether kochia cover is persisting or if the plant community is returning to pre-invasion species compositions.

There were indications that the community around removed Russian olive trees became more like pre-invasion reference conditions. In the plant community around removed Russian olive, there was a larger decrease in the exotic perennial forb catnip and the exotic annual grass cheatgrass, as well as an increase in the native perennial C₄ grass sand dropseed than areas around intact trees. Both catnip and cheatgrass grow best under stable and relatively high soil moisture levels and stable temperatures (Thill et al. 1979; Augé et al. 2003). Sand dropseed increased in cover following drought years in other Great Plains pasturelands (Weaver and Hansen 1939). The responses to water availability for these species match the results at our study; there was a shift toward higher sand dropseed and lower catnip and cheatgrass cover following droughts in 2012 and 2013 (Fig. 2.3). The plant communities around intact and undisturbed Russian olive had smaller changes for these species than communities around

removed Russian olive. This difference was likely due to shading from Russian olive trees reducing the drought effect and limiting photosynthesis rates.

In addition to decreasing soil moisture availability, removing Russian olive's impact on shading likely influenced plant communities by increasing photon flux density. In a previous study along this stretch of river, we found that PAR levels underneath Russian olive canopy were around 30% of full sun (Tuttle et al. 2016). The mean July photon flux density for visible light during peak growing season in the area from 1998 to 2005 ranged between $906 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1708 \mu\text{mol m}^{-2} \text{s}^{-1}$ (National Renewable Energy Laboratory 2008). Grass and forb species of the Western Short Grasslands and the Central and Southern Mixed Grasslands ecoregions become light saturated between $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Polley et al. 1992; Turner and Knapp 1996). Since light response curves are asymptotic, and decrease exponentially below the saturation point, many species likely became light limited underneath Russian olive. C_4 species, such as sand dropseed, are particularly intolerant of low sunlight and are outcompeted by C_3 species, such as catnip and cheatgrass, in shaded areas (Ehleringer 1978). The difference in shade-tolerance between these species could further explain why the exotics, catnip and cheatgrass, decreased after removal and sand dropseed increased. It may also partially explain why the exotic C_4 annual forb kochia became dominant in the plant community around removed Russian olive trees only after the tree was removed (Table 2.3; Fig. 2.3).

As we predicted, there was a legacy effect of Russian olive on soil N availability. However, unlike our prediction, removing Russian olive did not result in increased exotic cover. Instead, it resulted in a shift in exotic cover from catnip and cheatgrass to kochia, which may delay native species establishment (Vinton and Burke 1995). Our work showed that the persistence of EIPS impacts can alter how the invaded ecosystem responds to EIPS removal,

delaying plant community recovery. Management of N-fixing EIPS needs to consider the impact of a persistent elevated N pool on management objectives. Return of the plant community to its pre-invasion state may require additional restoration efforts. Steps to mitigate high available N, for example by mixing soil (Malcolm et al. 2008) or the seeding of desirable species that are competitive in a high N environment, may need to be included in management practices to prevent the establishment of secondary weeds.

Table 2.1: Mixed-model ANOVA for soil N availability, modeled using the factorial interaction of year (2010-2014), plot type (Russian olive, reference), and removal treatment (removed, intact, undisturbed).

Type	Effect	Num DF	Den DF	F Value	Pr > F
Fixed	Year	3	208	9.71	<0.01
	Plot Type	1	219	49.47	<0.01
	Year*Plot Type	3	204	0.96	0.41
	Removal	2	2.66	1.12	0.44
	Year*Removal	6	208	1.92	0.08
	Plot Type*Removal	1	219	0.58	0.45
	Year*Plot Type*Removal	3	204	0.46	0.71
Type	Effect	Covariance Estimate	Std. Error	Z value	Pr > Z
Random	Site	0.15	0.26	0.58	0.28
	Site (Pair)	0.57	0.15	3.88	<0.01
	Residual	0.74	0.07	9.90	<0.01

Table 2.2: Species indicator values for the five species with the highest average values in Russian olive-removed, Russian olive-disturbed, and reference disturbed plots in 2014.

Treatment	Species	Common Name	Score
Russian olive-disturbed	<i>Bromus tectorum</i> (E, AG)	Cheatgrass	43
	<i>Nepeta cataria</i> (E, PF)	Catnip	35
	<i>Elymus canadensis</i> (N, P4)	Canada wildrye	29
	<i>Bassia scoparia</i> (E, AF)	Kochia	24
	<i>Elymus repens</i> (E, P4)	Quackgrass	21
Russian olive-removed	<i>Bassia scoparia</i> (E, AF)	Kochia	49
	<i>Setaria viridis</i> (E, AG)	Green bristlegrass	20
	<i>Sporobolus cryptandrus</i> (N, P4)	Sand dropseed	19
	<i>Salsola tragus</i> (E, AF)	Russian thistle	13
	<i>Ambrosia psilostachya</i> (N, PF)	Cuman ragweed	11
	<i>Hordeum jubatum</i> (N, P4)	Foxtail barley	11
Reference disturbed	<i>Panicum virgatum</i> (N, P4)	Switchgrass	39
	<i>Sporobolus cryptandrus</i> (N, P4)	Sand dropseed	29
	<i>Poa pratensis</i> (E, P3)	Kentucky bluegrass	23
	<i>Conyza canadensis</i> (N, AF)	Canadian horseweed	18
	<i>Bromus tectorum</i> (E, AG)	Cheatgrass	14

Table 2.3: Multi-response permutation procedure (MRPP) comparisons of plant communities between groups separated by year (2010-2014), plot type (Russian olive, reference), and removal treatment (removed, intact, undisturbed). Significant differences at $\alpha=0.05$ are bolded.

Site	Year	Plot Type 1	Plot Type 2	Observed Delta	Expected Delta	Delta Variance	A Value	t Value	Pr > t
Undisturbed	2010	Russian olive	Reference	0.8765	0.8942	0.2396	0.0198	-3.61	<0.01
	2011	Russian olive	Reference	0.8119	0.8434	0.2168	0.0373	-6.75	<0.01
	2012	Russian olive	Reference	0.8315	0.886	0.4262	0.0615	-8.34	<0.01
	2013	Russian olive	Reference	0.8489	0.8789	0.2184	0.0342	-6.43	<0.01
	2014	Russian olive	Reference	0.8586	0.8851	0.2286	0.0299	-5.54	<0.01
Disturbed	2010	Russian olive-disturbed	Reference	0.8209	0.8472	0.0267	0.0311	-16.13	<0.01
		Russian olive-removed	Reference	0.8295	0.8514	0.0234	0.0257	-14.31	<0.01
		Russian olive-removed	Russian olive-disturbed	0.8281	0.8284	0.0415	0.0004	-0.16	0.36
	2011	Russian olive-disturbed	Reference	0.8320	0.8582	0.0201	0.0306	-18.52	<0.01
		Russian olive-removed	Reference	0.8340	0.8621	0.0187	0.0325	-20.50	<0.01
		Russian olive-removed	Russian olive-disturbed	0.8033	0.8031	0.0305	0.0003	0.13	0.5
	2012	Russian olive-disturbed	Reference	0.8827	0.9023	0.0267	0.0217	-11.99	<0.01
		Russian olive-removed	Reference	0.8971	0.9069	0.0367	0.0108	-5.10	<0.01
		Russian olive-removed	Russian olive-disturbed	0.8847	0.8899	0.0767	0.0058	-1.87	0.05
	2013	Russian olive-disturbed	Reference	0.8680	0.8860	0.0225	0.0203	-12.01	<0.01
		Russian olive-removed	Reference	0.8895	0.8994	0.0209	0.0110	-6.83	<0.01
		Russian olive-removed	Russian olive-disturbed	0.8682	0.8787	0.0481	0.0119	-4.76	<0.01
	2014	Russian olive-disturbed	Reference	0.8563	0.8715	0.0184	0.0175	-11.23	<0.01
		Russian olive-removed	Reference	0.8401	0.8601	0.0208	0.0232	-13.85	<0.01
		Russian olive-removed	Russian olive-disturbed	0.8106	0.8238	0.0393	0.0161	-6.68	<0.01

Table 2.4: T-tests of differences between least square means between 2010 and 2014 for species cover for the eight species with the highest mean cover across the study, based on groups defined by the interaction of plot type (Russian olive, reference) and removal treatment (removed, intact, undisturbed). Values have been ln back-transformed. Estimated differences and 95% confidence intervals of those differences are proportional, meaning a value of 1 indicates that species cover was the same in 2014 and 2010 for that treatment group, 2 indicates the cover was twice as high in 2014 as 2010, and 0.5 indicates it was half as high in 2014 as 2010. Significant differences at $\alpha=0.05$ are bolded.

Type	Row Description	<i>Ambrosia psilostachya</i>	<i>Bassia scoparia</i>	<i>Bromus tectorum</i>	<i>Elymus canadensis</i>	<i>Nepeta cataria</i>	<i>Panicum virgatum</i>	<i>Poa pratensis</i>	<i>Sporobolus cryptandrus</i>
Russian olive-disturbed	Prop. Diff. CI Diff. T-value P-value	0.23 (0.07, 0.7) t₉₈₀=-2.60 p<0.01	18.25 (7.36, 45.26) t₉₈₃=6.28 p<0.01	5.83 (2.35, 14.4) t₉₈₁=3.83 p<0.01	0.22 (0.08, 0.59) t₉₈₂=-3.04 p<0.01	0.07 (0.02, 0.17) t₉₈₃=-6.04 p<0.01	0.68 (0.25, 1.83) t ₉₈₀ =-0.75 p=0.45	9.68 (3.85, 24.31) t₉₈₄=4.84 p<0.01	1.69 (0.76, 3.74) t ₉₈₃ =1.31 p=0.19
Russian olive-removed	Prop. Diff. CI Diff. T-value P-value	0.04 (0.01, 0.14) t₉₈₄=-5.41 p<0.01	43.64 (17.63, 107.97) t₉₈₆=8.18 p<0.01	3.21 (1.3, 7.91) t₉₈₄=2.54 p=0.01	0.14 (0.05, 0.37) t₉₈₆=-3.99 p<0.01	0.02 (<0.01, 0.06) t₉₈₈=-8.18 p<0.01	0.81 (0.3, 2.19) t ₉₈₃ =-0.39 p=0.69	4.23 (1.68, 10.61) t₉₈₈=3.09 p<0.01	18.63 (8.43, 41.16) t₉₈₅=7.25 p<0.01
Russian olive Undisturbed	Prop. Diff. CI Diff. T-value P-value	1.19 (0.2, 6.86) t ₉₈₆ =0.21 p=0.84	12.77 (2.89, 56.36) t₉₈₇=3.37 p<0.01	1.01 (0.21, 4.71) t ₉₈₅ =0.33 p=0.74	0.98 (0.2, 4.57) t ₉₈₈ =0.02 p=0.98	2.08 (0.53, 8.1) t ₉₉₀ =1.07 p=0.28	1.08 (0.21, 5.48) t ₉₈₄ =0.11 p=0.92	5.98 (1.32, 26.97) t₉₈₉=2.34 p=0.02	3.03 (0.82, 11.11) t ₉₈₆ =1.68 p=0.09
Reference Disturbed	Prop. Diff. CI Diff. T-value P-value	0.08 (0.03, 0.19) t₉₈₃=-6.08 p<0.01	8.34 (4.35, 15.98) t₉₈₅=6.41 p<0.01	0.29 (0.14, 0.58) t₉₈₄=3.94 p<0.01	3.29 (1.68, 6.47) t₉₈₅=-3.49 p<0.01	0.74 (0.40, 1.35) t ₉₈₇ =0.95 p=0.34	0.61 (0.3, 1.25) t ₉₈₂ =-1.32 p=0.19	18.53 (9.58, 35.82) t₉₈₇=8.70 p<0.01	5.57 (3.15, 9.85) t₉₈₅=5.94 p<0.01
Reference Undisturbed	Prop. Diff. CI Diff. T-value P-value	1.86 (0.33, 10.15) t ₉₈₁ =0.72 p=0.47	2.61 (0.61, 11.05) t ₉₈₄ =1.31 p=0.19	0.69 (0.16, 2.92) t ₉₈₂ =0.49 p=0.62	0.20 (0.04, 0.94) t₉₈₃=-2.03 p=0.04	1.32 (0.35, 4.96) t ₉₈₅ =0.43 p=0.67	0.89 (0.18, 4.32) t ₉₈₁ =-0.13 p=0.90	0.97 (0.22, 4.22) t ₉₈₆ =-0.03 p=0.98	1.22 (0.34, 4.31) t ₉₈₄ =-0.32 p=0.75

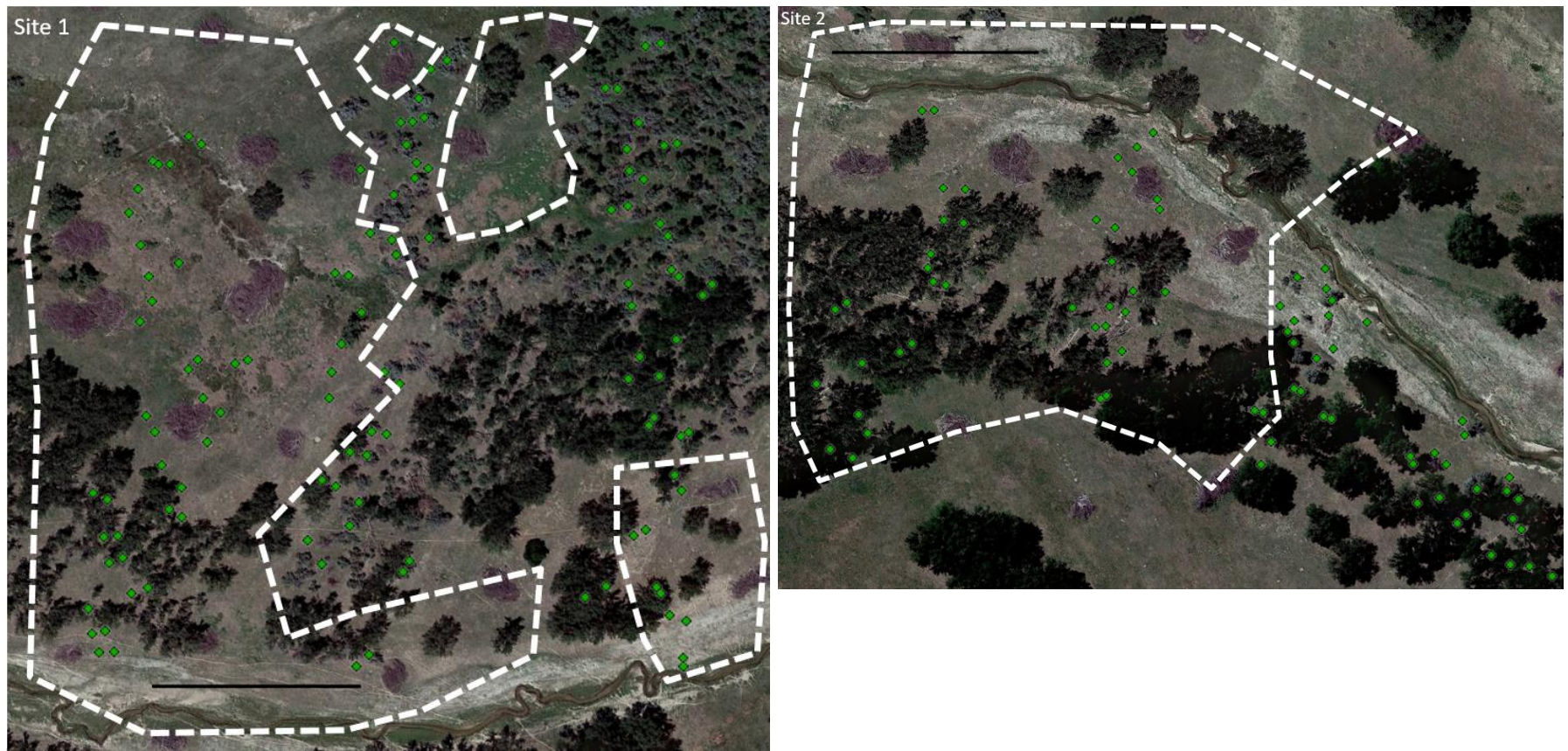


Figure 2.1: Plots (circles) at the two removal sites. Areas where Russian olive was removed in 2012 are outlined with dashes. Black bars show 100 m.

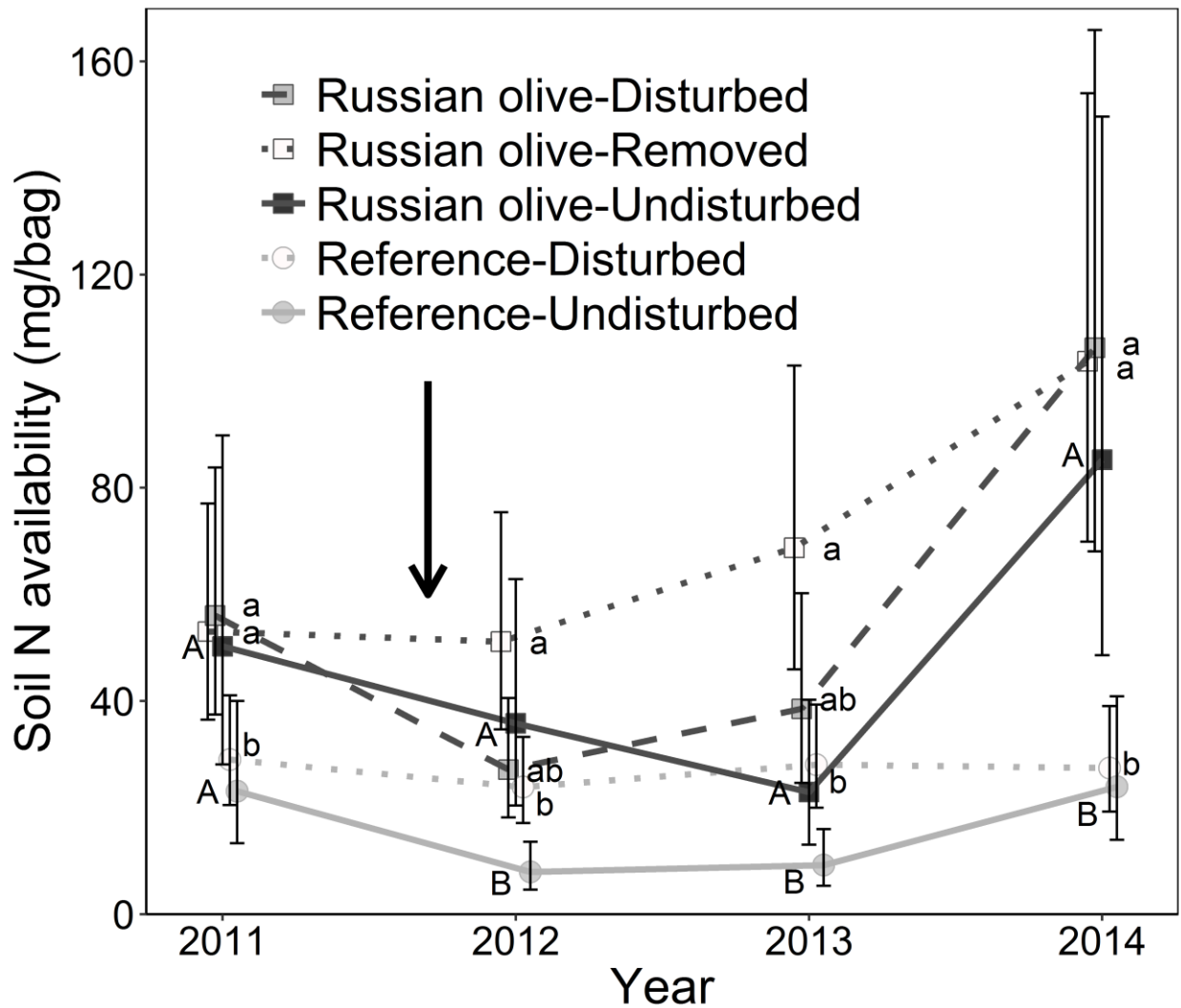


Figure 2.2: Back-transformed least square means for soil N availability collected with ion exchange resin bags from August 2010 – August 2014 (each year represents total ionic soil N extracted from September of the previous year to August of the year indicated) by plot type (Russian olive= underneath Russian olive canopy, Reference=at least 3 meters outside of Russian olive canopy) and removal treatment (Intact=Russian olive tree left intact and Removal=Russian olive tree removed) at the two sites where Russian olive was removed in winter through spring 2012 (Disturbed) and the site where no Russian olive occurred (Undisturbed). The arrow indicates when Russian olive was removed at the disturbed sites. Letters show significant differences between treatments for each year at $\alpha=0.05$. Capital letters are for the undisturbed site and lowercase are for the disturbed sites.

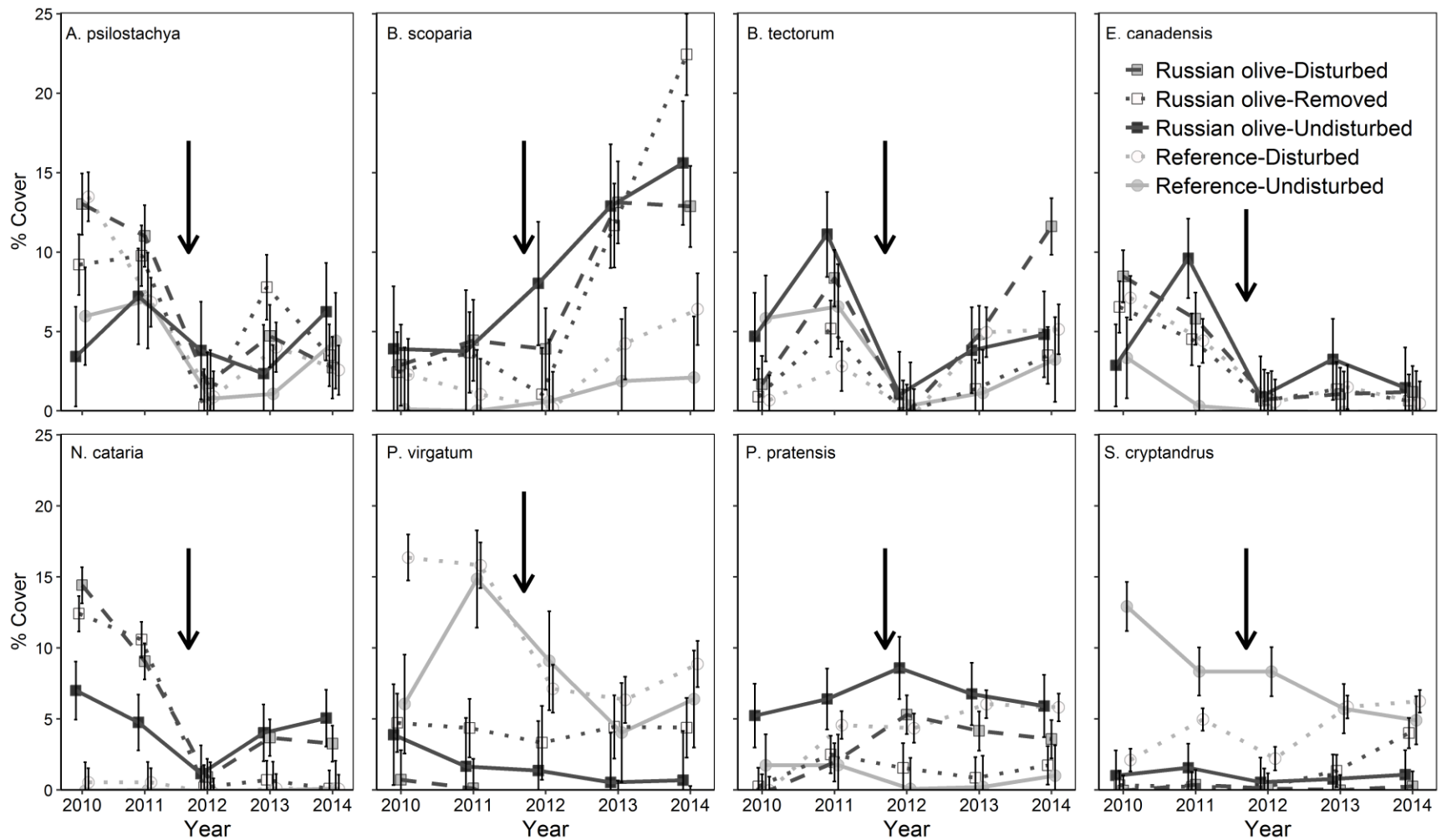


Figure 2.3: Least square means between 2010 and 2014 for species cover of the eight species with the highest mean cover across the study, based on groups defined by the interaction of plot type (Russian olive, reference) and removal treatment (removed, intact, undisturbed). The arrow indicates when Russian olive was removed at the disturbed sites. Total cover of the eight species accounts for over 65% of average total plot cover.

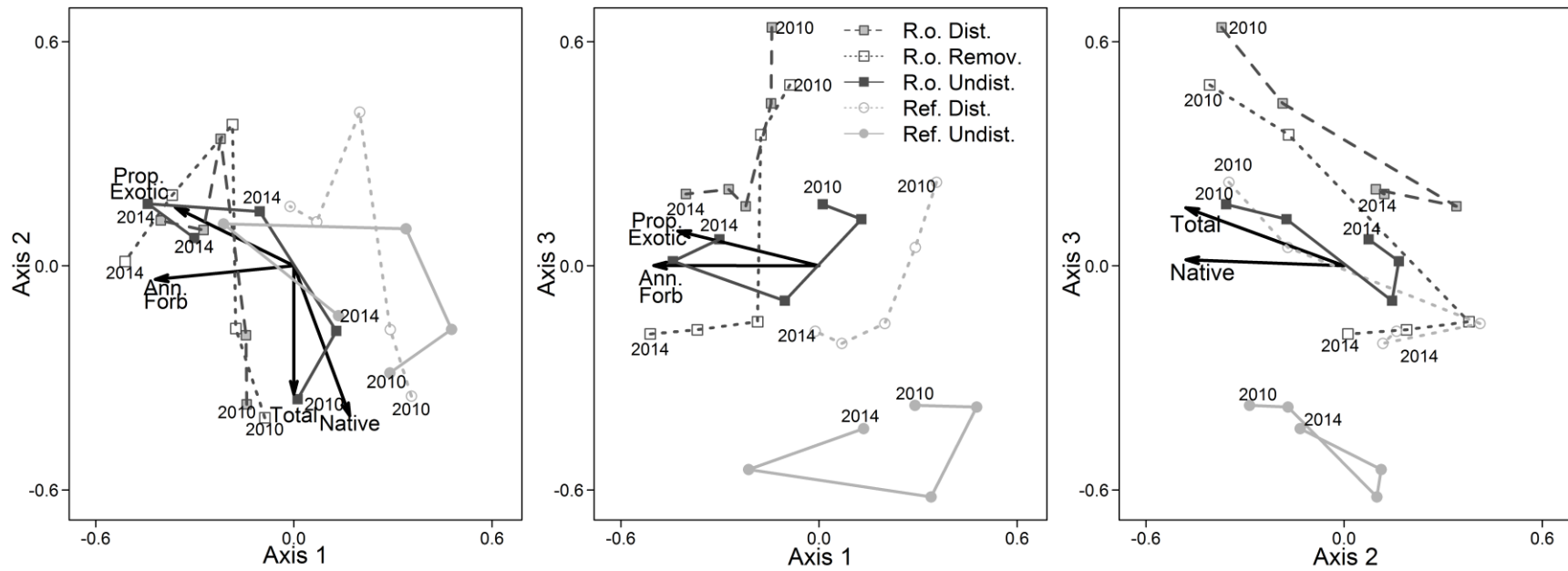


Figure 2.4: Centroids and vectors from NMDS analysis for plant community. Centroids were calculated from Bray-Curtis distance measures between plots and based on groups described in Fig. 2. Vectors all had Pearson correlations to an axis >0.2 .

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CHAPTER 3: *ALNUS*- AND *ELAEAGNUS*-*FRANKIA* ENDOSYMBIOSIS RESPONSE TO EXOGENOUS N AND PAR

SUMMARY

Actinorhizal endosymbioses between host trees and actinobacteria *Frankia* allow for atmospheric N₂-fixing, which provides an important N source in terrestrial ecosystems. Often N₂-fixation becomes limited in ecosystems due to high exogenous N and low photosynthetically active radiation (PAR), but the effect of these factors varies across actinorhizal species. Two groups of actinorhizal symbioses exist (*Alnus*- and *Elaeagnus*-*Frankia*), which differ in the type of root infection, the host species, and the *Frankia* strain involved. We conducted a greenhouse experiment to compare nodulation and growth response to nodulation between *Alnus*- and *Elaeagnus*-*Frankia* symbioses across a factorial combination of three exogenous N and two PAR levels. For 12 weeks, we grew three *Alnus* species (*A. incana*, *A. rubra*, and *A. viridis* subsp. *sinuata*) and three *Elaeagnus* species (*E. angustifolia*, *E. commutata*, *E. umbellata*). Across all treatments, *Elaeagnus*-*Frankia* symbiosis had higher nodule allocation, $\delta^{15}\text{N}$, and tissue %N but nodulation caused a smaller increase in total mass than in *Alnus*-*Frankia* symbiosis. From low to high exogenous N, *Elaeagnus*-*Frankia* symbiosis had a smaller increase in total biomass due to nodulation than *Alnus*-*Frankia* symbiosis. *Elaeagnus*-*Frankia* symbiosis also had a smaller decrease in nodule allocation and below:aboveground mass from low to high N than *Alnus*-*Frankia* symbiosis, but the differences between the two symbioses were not significant. We found no significant difference in response to the PAR treatment between *Alnus*- and *Elaeagnus*-*Frankia* symbioses for any of our six response variables. The combination of *Elaeagnus*-*Frankia* symbiosis being less influenced by exogenous N and resulting in higher tissue %N than *Alnus*-

Frankia symbiosis, suggests that *Elaeagnus* trees may have larger and longer-lasting N input to ecosystems than *Alnus* trees.

INTRODUCTION

Actinorhizal N₂-fixation is an important source of N in terrestrial ecosystems, particularly early in succession (Rhoades et al. 2008). Actinorhizal plants, through their symbiosis with soil actinobacteria in the genus *Frankia*, fix atmospheric N₂ in root nodules. This allows the plants to colonize recently disturbed soils with low N levels that many other species cannot, and it may help reduce N limitation expected under elevated atmospheric CO₂ (Norby et al. 2010). Over time, the fixed-N accumulates in the soil through leaf litter and dead root tissue, which builds up soil organic matter and creates more favorable conditions for other plants, thus, influencing later succession (Crocker and Major 1955, Lawrence et al. 1967, Chapin 1994, Paschke 1997). For example, in the early stage of succession in an Alaskan floodplain terrace, extractable soil N was more than four times higher under the actinorhizal shrub, buffalo berry (*Shepherdia canadensis*), than under willow (*Salix* spp.) canopy or between canopies (Rhoades et al. 2008).

N₂-fixation is more energetically costly than N uptake, which likely causes the general decrease in N₂-fixation rates and decline in N-fixing species abundance later in succession (Boring et al. 1988). These decreases occur due to reduced photosynthesis or an increase in soil N concentration (Vitousek and Howarth 1991, Rastetter et al. 2001). Fixation of atmospheric N₂ into ammonium, by the enzyme nitrogenase in *Frankia*, is an energetically costly process because it requires the breaking of N₂ triple bonds. Therefore, the host tree must invest a portion of the C fixed in photosynthesis to fuel this reaction (Benson and Silvester 1993, Berry et al. 2011). When photosynthetically active radiation (PAR) is reduced as canopies close later in succession, nitrogenase activity diminishes due to a reduction in photosynthates in roots

(Wheeler 1971). As actinorhizal tree stands age, soil total N and nitrate concentrations increase, which also causes a reduction in nitrogenase activity due to it being less costly for trees to acquire rather than fix N (Martin et al. 2003).

Experimental evidence has shown that low PAR and high exogenous N limit nodulation, nitrogenase activity, and growth in actinorhizal species. For example, Gordon and Wheeler (1978) found that European alder (*Alnus glutinosa* (L.) Gaertn.) grown at low PAR had lower nodule mass, nitrogenase activity, and growth than plants grown at high PAR. Similarly, Lundquist and Huss-Danell (1991) found nitrogenase activity in gray alder (*A. incana* (L.) Moench) decreased by 25% and amounts of nitrogenase proteins decreased by 60% after plants were exposed to 40 hours of darkness. Several studies have shown that high exogenous N level reduce nodulation in actinorhizal species (e.g. Markham and Zekveld 2007, Bélanger et al. 2011) and nitrogenase activity (e.g. Norby 1987, Rojas et al. 2002, Yamanaka et al. 2016). One study has shown that the effect of exogenous N can suppress the effect of PAR on nitrogenase activity in European alder (Benamar et al. 1995).

However, the nodulation and growth response of actinorhizal species to exogenous N and PAR appears to vary between symbiosis types (Mackay et al. 1987). *Frankia* strains are divided into four main phylogenetic clusters, which differ in their host specificity (Baker 1987), genetic and chemical signaling (Ribeiro et al. 2011), and method of root infection (Racette and Torrey 1989). The host tree likely controls the way *Frankia* infect roots because only one of the two methods of infection has been observed for each host genera (Miller and Baker 1986). *Alnus-Frankia* infection occurs through deformed root hairs (Berry et al. 1986) and *Elaeagnus-Frankia* infection occurs through intracellular penetration (Miller and Baker 1985). Tree hosts likely alter their chemical signal (auxin concentration) to *Frankia* based on abiotic environmental

conditions, such as soil N concentration or PAR, to regulate root infection and nodulation (reviewed in Froussart 2016). Research on single species in each symbiosis type suggest that these symbioses may respond to abiotic conditions differently. For example, Kohls and Baker (1989) found that high nitrate concentrations did not decrease or prevent nodulation in Russian olive (*Elaeagnus angustifolia*) as it did for the other three actinorhizal species in the experiment.

Differences in the way symbioses are effected by environmental characteristics, likely influence their impact on ecosystems. If a symbiosis is less influenced by high soil N and low PAR, than species in that genera are more likely to have larger and longer-lasting impacts on the ecosystem because there will not be a direct negative feedback of N accumulation or indirect negative feedback from N accumulation causing increased tree growth and shading (Vitousek and Field 1999). This experiment was designed to test whether the effects of exogenous N and PAR on nodulation and growth response to nodulation are different between *Alnus*- and *Elaeagnus-Frankia* symbioses. We hypothesized that *Elaeagnus-Frankia* symbioses would be less responsive to differences in PAR and exogenous N than *Alnus-Frankia* symbiosis. Further, we predicted that there would be an interaction of PAR and exogenous N, with the effect of high exogenous N masking the expected increase in nodulation at high PAR. To test these hypothesis, we grew three *Alnus* species and three *Elaeagnus* species (Table 3.1) in a full-factorial design with two PAR levels and three exogenous N concentrations. This design allowed us to observe the intrinsic (no nodulation) growth response of the trees in response to the abiotic changes, as well as, the interaction between PAR and exogenous N on nodulated seedlings.

METHODS

Experimental Design

Alnus and *Elaeagnus* genera represent the two main types of *Frankia* symbioses in actinorhizal species. We grew three *Alnus* species and three *Elaeagnus* species (Table 3.1) for 12 weeks at Colorado State University greenhouses to measure growth and nodulation of the two genera in response to PAR and soil N concentration. The experiment was a full-factorial design of three species in each of the two genera (*Alnus*, *Elaeagnus*), two inoculation treatments (nodulated, non-nodulated), two PAR levels (low, high), three soil N concentrations (low, medium, high), and four replications (total of 288 plants). Plants were grown in 37.5cm deep by 7.5cm top diameter cone pots filled with a sterilized mixture of 3:1 calcined montmorillonite (Turface®) to sand. Plants were started January 31 and harvested April 21. We separated racks of pots into two rows based on PAR level. Every two weeks, we randomly rearranged the pots within each rack and location of each rack along the row within each PAR level.

Plant Germination and Growth

All seeds were purchased from Sheffield Seed Company, cold stratified at -20°C for 90 days and scarified by soaking in water for 1 hour to 24 hours, depending on species. We germinated seeds in germination trays filled with a sterilized mixture of 3:1 Turface® to sand, which were subjected to the PAR and N treatments used in the experiment. *Elaeagnus* spp. seeds were started two weeks before *Alnus* spp. seeds to ensure synchrony of germination. After germination, we transferred two seedlings into each pot to compensate for seedling mortality. If both seedlings survived past week two, we selected one at random and removed the aboveground portion of the plant.

Inoculation Treatments

We randomly assigned plants to either a *Frankia* inoculation treatment (nodulated) or sterile control (non-nodulated). To prevent *Frankia* contamination, prior to planting we sterilized all Turface® and sand used in the experiment by autoclaving it at 121°C and 33.9kPa for 30 minutes. For the nodulated treatment, we inoculated seedlings with ground *Frankia* spp. nodules and soils surrounding roots of four of the six species used in the study. Nodules and soil from *A. rubra* and *E. umbellata* were collected from Forest Park, Oregon and along the Columbia River, Oregon, respectively. *A. incana* and *E. angustifolia* nodules and soil were collected from the Poudre Canyon, Colorado and Fort Collins, Colorado, respectively. Samples were stored for 30 days at 4°C until plants were ready for transplanting. We ground and homogenized equal portions of the nodules and soils from the four species and then mixed 12mL of that mixture to the top 5cm of each pot. For the sterilized treatment, the soil and nodule mixture was autoclaved at 121°C and 33.9 kPa pressure for 1 hour, stirred, and autoclaved at the same conditions for a second hour, prior to being ground, homogenized, and added to the pots. We performed a second inoculation 6 weeks later using the same materials as above that had been stored at 4°C. For this inoculation, 45 grams of homogenate were mixed in 1 liter of distilled water. We re-inoculated plants by applying 10ml of the slurry to the base of stems of the plants in the nodulated treatment and 10mL of water to the non-nodulated treatment. At that time, we also added a 30mL of 1.7g L⁻¹ concentration mixture of ecto- and endo-mycorrhizae (from Sheffield Seed Company) to each pot to increase plant phosphate uptake to improve nodulation (Gardner 1986).

Soil N Treatments

Plants were fertilized using a modified quarter strength no-N Hoagland's solution at a rate of 100ml twice a week for the first five weeks and 150ml twice a week from weeks six

through twelve. We added ammonium nitrate (NH_4NO_3) to the Hoagland's solution at three N concentrations (0.05, 1.03, 10.29 mg N L^{-1}). These concentrations were chosen to range from the minimum that would allow survival of sterilized plants to high levels observed in groundwater in western U.S. riparian areas (Spalding and Exner 1993). After six weeks, 9.5cm deep cups were added to the bottom of each pot to maintain higher water and fertilizer concentrations in the soil. We replaced cups bi-weekly during pot randomization.

PAR Treatments

The two PAR treatments (low= 142 ± 79 , high= 433 ± 213 $\mu\text{mol m}^{-2}\text{s}^{-1}$) were each divided on two benches so that the high PAR treatment could be placed underneath growth lights. Growth lights extended the photoperiod to 16h light: 8h dark from a 11h light: 13h dark natural photoperiod. We measured PAR levels with an Apogee MQ-100 quantum sensor 5-10cm over the tallest plant in the center of each rack. Measurements were taken bi-weekly throughout the experiment and were divided into three categories, morning (0800-1000), afternoon (1200-1600), and evening (1900-2000; when growth lights provided only lighting). During week 8 of the experiment, we measured soil temperature with a Ryobi infrared thermometer (model #IR001) at the four corners and one center pot in each tray. During weeks 6 and 10, we estimated soil evaporation by fully-hydrating two pots without plants and measuring mass change after 24h and 5d at both PAR levels. Compared to the low PAR treatment, high PAR was about eight times higher in the morning and evening and three times higher in the afternoon. Temperatures were over 5°C higher and evaporation was 10mL more over 24h and 5d in the full sun compared to the shade (Table 3.2).

Response Measurements

We measured six response variables, three describing nodulation (specific nodule number (SNN; number of nodules/root mass), nodule allocation (nodule biomass/total biomass), and $\delta^{15}\text{N}$) and three describing growth response to nodulation (total mass, below:aboveground mass, tissue %N). At the end of the experiment, we harvested above- and belowground portions of each plant, and then oven dried samples at 60°C for 30h, to obtain aboveground, below ground, and nodule biomass. A portion of nodules was removed during harvesting for later molecular work. Total nodule biomass was then estimated by calculating biomass per nodule for the remaining nodules and applying it to the original nodule number. SNN was calculated as number of nodules/ root biomass (mg^{-1}), and nodule allocation was calculated as nodule biomass/total biomass (%). Tissue samples were then ground and analyzed for $\delta^{15}\text{N}$ and %N using a Carlo Erba NA 1500 (Milano, IT) elemental analyzer coupled to a VG Isochrom continuous flow IRMS (Isoprime Inc., Manchester, UK) with an integrated thermal conductivity detector at Colorado State University EcoCore laboratory. We used plant $\delta^{15}\text{N}$ scores to infer non-directional variation in N_2 -fixation. We intended to use $\delta^{15}\text{N}$ results to calculate nitrogenase activity (%Ndfa) using the ' ^{15}N natural abundance' technique (reviewed in Boddey et al. 2000); however, the $\delta^{15}\text{N}$ values in our mineralized control plants overlapped with our test and fixation control plants.

Statistical Analysis

We used Proc Mixed in SAS 9.4 (SAS Institute Inc., Cary, NC) to run four-way ANOVA with genus (*Alnus*, *Elaeagnus*), inoculation (nodulated, non-nodulated), PAR (low, high), soil N concentration (low, medium, high) and their interactions as independent variables and SNN, nodule allocation, $\delta^{15}\text{N}$, total biomass, below:aboveground biomass, and tissue %N as

dependent variables. Replicate and species nested in genus were included as random factors in the models. Except for $\delta^{15}\text{N}$, all response variables were ln transformed to better meet model assumptions. Two plants in the inoculation treatment were removed from analyses because there was no apparent nodulation at the time of harvest.

RESULTS

Response to Inoculation

Allocation to nodules and $\delta^{15}\text{N}$ was higher in *Elaeagnus-Frankia* symbiosis than *Alnus-Frankia* symbiosis (Table 3.3; Fig. 3.1). For both genera, nodulated plants had higher plant mass and tissue % N and lower below:aboveground mass than non-nodulated plants. Nodulation had a greater effect on total mass and below:aboveground mass in *Alnus-Frankia* symbiosis than *Elaeagnus-Frankia* symbiosis, but the opposite was true for tissue %N (Table 3.4; Fig. 3.2).

Response to Exogenous N and PAR

For exogenous N, both genera had significantly higher SNN and effect of nodulation on tissue %N, as well as significantly lower $\delta^{15}\text{N}$ in low versus high exogenous N. SNN and effect of nodulation on tissue %N were nearly twice as high in the low exogenous N level as the high N level.

For PAR, both genera had higher SNN and lower nodule allocation and effect of nodulation on total mass in low versus high PAR. Plants at low PAR had about twice the SNN but about half the nodule allocation as plants at high PAR. Total plant biomass was about four times higher in the high PAR than low. PAR did not significantly influence $\delta^{15}\text{N}$ or the effect of nodulation on below:aboveground mass or tissue %N for either symbiosis (Table 3.5; Figs. 3.1 and 3.2).

Differences Between *Alnus*- and *Elaeagnus-Frankia* Symbioses

The effect of exogenous N on how much nodulation increased total mass (difference between nodulated and non-nodulated plants) varied between the two symbioses (Table 3.3). Nodulation caused a ten-fold greater increase in total mass at low exogenous N compared to high N for *Alnus* but only 1.5 times greater for *Elaeagnus* (Table 3.5; Fig. 3.2). Exogenous N also had a smaller effect on *Elaeagnus-Frankia* than *Alnus-Frankia* symbiosis for nodule allocation and below:aboveground mass, but the difference between the two symbioses was not significant (Table 3.5). The interaction of genus with exogenous N, PAR, and N*PAR on nodulation or the growth response to nodulation was not significant for any of the other response variables (Tables 3.3 and 3.4).

DISCUSSION

We designed this experiment to test for differences in the effect of exogenous N and PAR on nodulation and growth response to nodulation between *Alnus*- and *Elaeagnus-Frankia* symbioses. Our results show that *Alnus*- and *Elaeagnus-Frankia* symbioses do not differ in their response to PAR, but *Elaeagnus-Frankia* symbiosis is less influenced by exogenous N than *Alnus-Frankia* symbiosis. From low to high exogenous N concentrations, *Elaeagnus-Frankia* symbiosis had a smaller decrease in nodule allocation but a smaller effect of nodulation on growth than *Alnus-Frankia* symbiosis. Kohls and Baker (1989) had similar results to our study and conclude that maintaining higher nodulation inhibits *Elaeagnus* from growing as well as *Alnus* at high exogenous N concentrations. These findings suggest that *Elaeagnus* is not optimizing its utilization of N₂-fixation and is fixing when it would be more energetically beneficial for it to acquire N (Martin et al. 2003). At a wider range of exogenous N

concentrations than included in our experiment, there would likely have been a greater decrease in *Elaeagnus* growth due to continued nodulation at high exogenous N (Kohls and Baker 1989).

Overall, both symbiosis types responded to exogenous N in similar ways to species in other studies. At high N, both symbioses in our study had lower SNN and nodule allocation, while maintaining a consistent below:aboveground mass ratio and increasing in total mass. When similar results were observed in *A. viridis* subsp. *crispa*, the authors suggested the increased growth was due to more efficient nitrogen acquisition because $\delta^{15}\text{N}$ results showed proportion of nitrogen fixed by the plants had decreased (Markham and Zekveld 2007). High exogenous N levels reduce nodulation (e.g. Markham and Zekveld 2007, Bélanger et al. 2011) and nitrogenase activity (e.g. Norby 1987, Rojas et al. 2002, Yamanaka et al. 2016) because it becomes more viable for a plant to acquire N from the soil than through the energetically costly process of N_2 -fixation. Unfortunately, we cannot use our $\delta^{15}\text{N}$ values to show proportion of nitrogen fixed by the plants because our non-nodulated values varied too greatly. However, $\delta^{15}\text{N}$ was higher for both symbioses at high N than low N, which suggests a higher proportion of N was acquired from the fertilizer.

Based on the ability of *E. angustifolia* and *E. umbellata* to grow in the understory (Katz and Shafroth 2003; Yates et al. 2004), we expected *Elaeagnus-Frankia* symbiosis to be less influenced by PAR than *Alnus-Frankia* symbiosis, but the results indicated that the two symbioses did not differ in their responses. Plants in both symbioses grew larger and allocated more toward nodule growth in high PAR than low. These results match other studies that have shown a positive relationship between PAR, growth, and nitrogenase activity (e.g. McNeil and Carpenter 1978; Gordon and Wheeler 1978; Bormann and Gordon 1984; Joshi and Maikhuri 1996). In actinorhizal plants, a positive feedback occurs between photosynthesis and nitrogen

fixation; more photosynthesis leads to increased fixation, which leads to increased leaf growth and, thus, more photosynthesis (Arnone and Gordon 1990). In the shade, there are temporary changes to *Frankia* and host cell structure and function (Vikman et al. 1990), and plants decrease allocation of photosynthates to nodules, which reduces N₂-fixation (Bormann and Gordon 1984). These changes result in decreased plant growth (Benamar et al. 1995).

We expected SNN to be higher in the high PAR because photosynthesis is positively correlated with nodule mass and nitrogenase activity (Gordon and Wheeler 1978). Alternatively, decreased SNN may be due to sunlight stimulating flavonoids in the roots and root exudates that inhibit nodulation (Hughes et al. 1999). It is possible this mechanism is to increase investment in current nodules that are already fixing N₂ instead of investing in new nodules that will initially have lower N₂-fixation rates. This explanation matches our results because there was an increase in nodule allocation in high PAR, despite the decrease in SNN.

In summary, our results show that *Elaeagnus* species shade tolerance does not lead to increased nodulation or nitrogenase activity at low PAR compared to *Alnus* species, meaning *Elaeagnus* is not likely to continue to input N later in succession. However, the combination of *Elaeagnus-Frankia* symbiosis resulting in higher tissue %N and it being less influenced by exogenous N than *Alnus-Frankia* symbiosis, suggests that *Elaeagnus* trees may have larger N input to ecosystems than *Alnus* trees. In walnut (*Juglans nigra* L.) stands in Illinois, U.S., plantings that were interplanted with autumn olive had higher N mineralization and mineralized N availability than plantings interplanted with black alder (*A. glutinosa* L.) (Paschke et al. 1989). Actinorhizal fixation can account for about 70% of total N accumulation in an ecosystem during the growing season (Tripp et al. 1979). Even in the first year of growth, an estimated 0.23 mg N plant⁻¹ from fixation can accumulate (Huss-Danell et al. 1992). Though the role of N-fixing

species in an ecosystem diminishes over time (Vitousek and Howarth 1991), they can have a lasting impact on subsequent plant communities (Rhoades et al. 2008; Pink and Dawson 2014). Based on our results, *Elaeagnus-Frankia* symbiosis are likely to have large impacts on N cycling dynamics and plant communities in ecosystems where those species grow.

Table 3.1: Species characteristics for the three *Alnus* and three *Elaeagnus* species used in the experiment. Species names, common names, exotic status, wetland status, and U.S. range were obtained from U.S.D.A Plants database. Shade tolerances are between 0=intolerant and 5=tolerant and obtained from (Niinemets and Valladares 2006).

Species	Common Name	Exotic Status	Wetland Status	Shade Tolerance	U.S. Range
<i>Alnus</i> species					
<i>A. incana</i> (L.) Moench	gray alder	Native	FACW	2.5	West US
<i>A. rubra</i> Bong.	red alder	Native	FACW	1.8	N West US
<i>A. viridis</i> (Chaix) DC. subsp. <i>sinuata</i> (Regel) Á. Löve & D. Löve	Sitka alder	Native	FACW	1.5	North US
<i>Elaeagnus</i> species					
<i>E. angustifolia</i> L.	Russian olive	Exotic	FAC	1.4	All US
<i>E. commutata</i> Bernh. Ex Rydb.	silverberry	Native	FAC	-	All US
<i>E. umbellata</i> Thunb.	autumn olive	Exotic	FACU	-	N Central US

Table 3.2: Abiotic growth conditions at the two PAR (photosynthetically active radiation) levels. Values are means(medians) \pm SEM.

	Full Sun	Shade
Morning PAR ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	244.3(195) \pm 23.1	30.2(23) \pm 1.92
Afternoon PAR ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	433.1(417.5) \pm 30.4	142.4(130) \pm 11.4
Evening PAR ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	55.5(44) \pm 5.6	6.75(2.5) \pm 1.4
Soil Temperature ($^{\circ}\text{C}$)	29.5 \pm 0.6	24.1 \pm 0.5
24h Soil Evaporation (mL)	30	20
5d Soil Evaporation (mL)	60	50

Table 3.3: Mixed-model ANOVAs for the three nodulation response variables.

Fixed Effects	SNN				Nodule Allocation				Delta ¹⁵ N			
	Num	Den	F Value	Pr > F	Num	Den	F Value	Pr > F	Num	Den	F Value	Pr > F
PAR	1	126	41.45	<0.01	1	125	32.50	<0.01	1	122	0.05	0.83
Genus	1	4	0.51	0.52	1	125	4.23	0.04	1	3.98	10.37	0.03
Genus*PAR	1	126	1.35	0.25	1	125	0.04	0.85	1	122	2.48	0.12
N	2	126	18.27	<0.01	2	125	3.07	0.05	2	122	12.25	<0.01
N*PAR	2	126	0.48	0.62	2	125	1.40	0.25	2	122	5.73	<0.01
Genus*N	2	126	0.79	0.46	2	125	0.36	0.70	2	122	0.92	0.40
Genus*N*PAR	2	126	0.37	0.69	2	125	0.30	0.74	2	122	0.25	0.78
Random Effects	Est.	Std. Error	Z Value	Pr > Z	Est.	Std. Error	Z Value	Pr > Z	Est.	Std. Error	Z Value	Pr > Z
Replicate	0.00	0.00	.	.	0.00	0.00	.	.	<0.01	<0.01	0.32	0.37
Genus(species)	0.15	0.11	1.30	0.10	0.00	0.00	.	.	0.04	0.03	1.23	0.11
Residual	0.31	0.04	7.94	<0.01	0.47	0.06	7.91	<0.01	0.14	0.02	7.81	<0.01

Table 3.4: Mixed-model ANOVAs for the three growth response to nodulation variables.

Fixed Effects	Total Mass				Below:Above				Percent N			
	Num	Den	F Value	Pr > F	Num	Den	F Value	Pr > F	Num	Den	F Value	Pr > F
PAR	1	237	64.06	<0.01	1	236	116.43	<0.01	1	225	32.21	<0.01
Genus	1	4.01	2.47	0.19	1	4.02	0.01	0.95	1	4.1	2.90	0.16
Genus*PAR	1	236	1.81	0.18	1	235	13.15	<0.01	1	225	0.15	0.70
Inoc	1	236	426.39	<0.01	1	235	286.87	<0.01	1	223	830.26	<0.01
Inoc*PAR	1	237	47.60	<0.01	1	235	2.49	0.12	1	225	3.58	0.06
Genus*Inoc	1	235	59.99	<0.01	1	234	15.47	<0.01	1	223	14.96	<0.01
Genus*Inoc*PAR	1	236	0.99	0.32	1	235	0.33	0.57	1	224	0.50	0.48
N	2	235	106.87	<0.01	2	234	3.87	0.02	2	223	24.66	<0.01
N*PAR	2	235	0.39	0.68	2	234	6.20	<0.01	2	223	6.01	<0.01
Genus*N	2	235	18.53	<0.01	2	234	1.77	0.17	2	223	2.49	0.09
Genus*N*PAR	2	235	0.61	0.54	2	234	1.47	0.23	2	223	0.07	0.93
Inoc*N	2	235	25.99	<0.01	2	234	9.95	<0.01	2	223	33.06	<0.01
Inoc*N*PAR	2	235	1.64	0.20	2	234	0.89	0.41	2	223	1.02	0.36
Genus*Inoc*N	2	235	13.35	<0.01	2	234	0.98	0.38	2	223	0.39	0.68
Genus*Inoc*N*PAR	2	235	0.91	0.40	2	234	1.38	0.25	2	223	0.25	0.78
Random Effects	Est.	Std. Error	Z Value	Pr > Z	Est.	Std. Error	Z Value	Pr > Z	Est.	Std. Error	Z Value	Pr > Z
Replicate	<0.01	<0.01	0.23	0.41	<0.01	<0.01	0.58	0.28	<0.01	<0.01	0.33	0.37
Genus(species)	0.29	0.21	1.37	0.09	0.05	0.04	1.35	0.09	0.01	0.00	1.29	0.10
Residual	0.43	0.04	10.84	<0.01	0.16	0.01	10.82	<0.01	0.04	<0.01	10.54	<0.01

Table 3.5: Proportional differences between low and high values for the six response variables across exogenous N and PAR treatments. T-tests show significance of comparisons within each genus and between genera. A value of 1 indicates no difference between low and high treatment, while a value of 0.5 indicates high treatment values were twice as high as low treatment values.

	Response Variable	Alnus	Elaeagnus	Alnus vs. Elaeagnus
Exogenous N	SNN	1.89 (1.38, 2.6) $t_{126}=3.99, p<0.01$	1.99 (1.44, 2.75) $t_{126}=4.20, p<0.01$	0.95 (0.61, 1.5) $t_{126}=-0.22, p=0.82$
	Nodule Allocation	1.51 (1.02, 2.25) $t_{125}=2.06, p=0.04$	1.29 (0.85, 1.95) $t_{125}=1.22, p=0.22$	1.17 (0.66, 2.08) $t_{125}=0.55, p=0.58$
	$\delta^{15}\text{N}$	0.72 (0.58, 0.9) $t_{122.1}=-2.99, p<0.01$	0.64 (0.51, 0.8) $t_{122.4}=-3.99, p<0.01$	1.13 (0.83, 1.54) $t_{122.2}=0.77, p=0.44$
	Total Mass	10.14 (5.65, 18.17) $t_{235.4}=7.82, p<0.01$	1.51 (0.88, 2.58) $t_{235.2}=1.51, p=0.13$	6.72 (3.04, 14.84) $t_{235.4}=4.73, p<0.01$
	Below:Aboveground	0.61 (0.45, 0.83) $t_{234.4}=-3.20, p<0.01$	0.79 (0.60, 1.04) $t_{234.2}=-1.69, p=0.09$	0.78 (0.51, 1.17) $t_{234.4}=-1.21, p=0.23$
	Tissue %N	1.57 (1.28, 1.92) $t_{223.7}=4.36, p<0.01$	1.68 (1.43, 1.99) $t_{222.2}=6.23, p<0.01$	0.93 (0.72, 1.21) $t_{223.1}=-0.54, p=0.59$
PAR	SNN	2.03 (1.57, 2.63) $t_{126}=5.42, p<0.01$	1.63 (1.26, 2.12) $t_{126}=3.70, p<0.01$	1.24 (0.86, 1.79) $t_{126}=1.16, p=0.25$
	Nodule Allocation	0.50 (0.36, 0.69) $t_{125}=-4.24, p<0.01$	0.52 (0.37, 0.73) $t_{125}=-3.83, p<0.01$	0.96 (0.60, 1.53) $t_{125}=-0.19, p=0.85$
	$\delta^{15}\text{N}$	0.92 (0.77, 1.1) $t_{122.2}=-0.96, p=0.34$	1.12 (0.94, 1.34) $t_{122.3}=1.26, p=0.21$	0.82 (0.63, 1.05) $t_{122.3}=-1.58, p=0.12$
	Total Mass	0.27 (0.17, 0.44) $t_{237.4}=-5.37, p<0.01$	0.38 (0.25, 0.59) $t_{235.2}=-4.37, p<0.01$	0.72 (0.38, 1.38) $t_{236}=-1.00, p=0.32$
	Below:Aboveground	1.20 (0.94, 1.54) $t_{235.7}=1.46, p=0.14$	1.09 (0.87, 1.37) $t_{234.2}=0.74, p=0.46$	1.10 (0.79, 1.54) $t_{234.8}=0.57, p=0.57$
	Tissue %N	0.87 (0.74, 1.02) $t_{225.2}=-1.69, p=0.09$	0.94 (0.82, 1.07) $t_{222.5}=-0.94, p=0.35$	0.93 (0.75, 1.14) $t_{224.4}=-0.71, p=0.48$

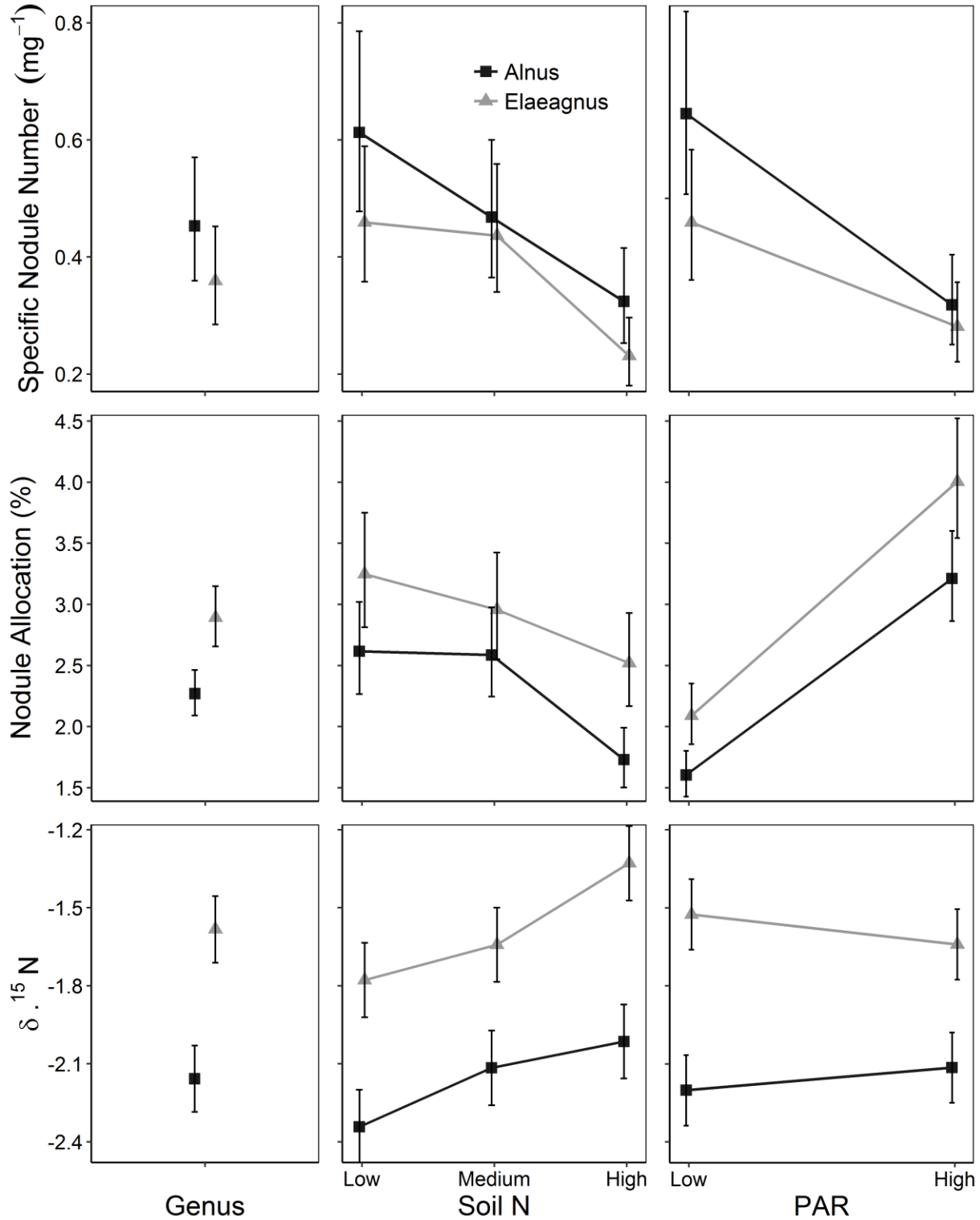


Figure 3.1: LSmeans for nodule allocation (nodule biomass/total plant biomass), specific nodule number (SNN; nodule number/root mass mg), and whole plant tissue $\delta^{15}\text{N}$ for both genera and across exogenous N and PAR. Values for nodule allocation and SNN have been ln back-transformed. Bars are SEM.

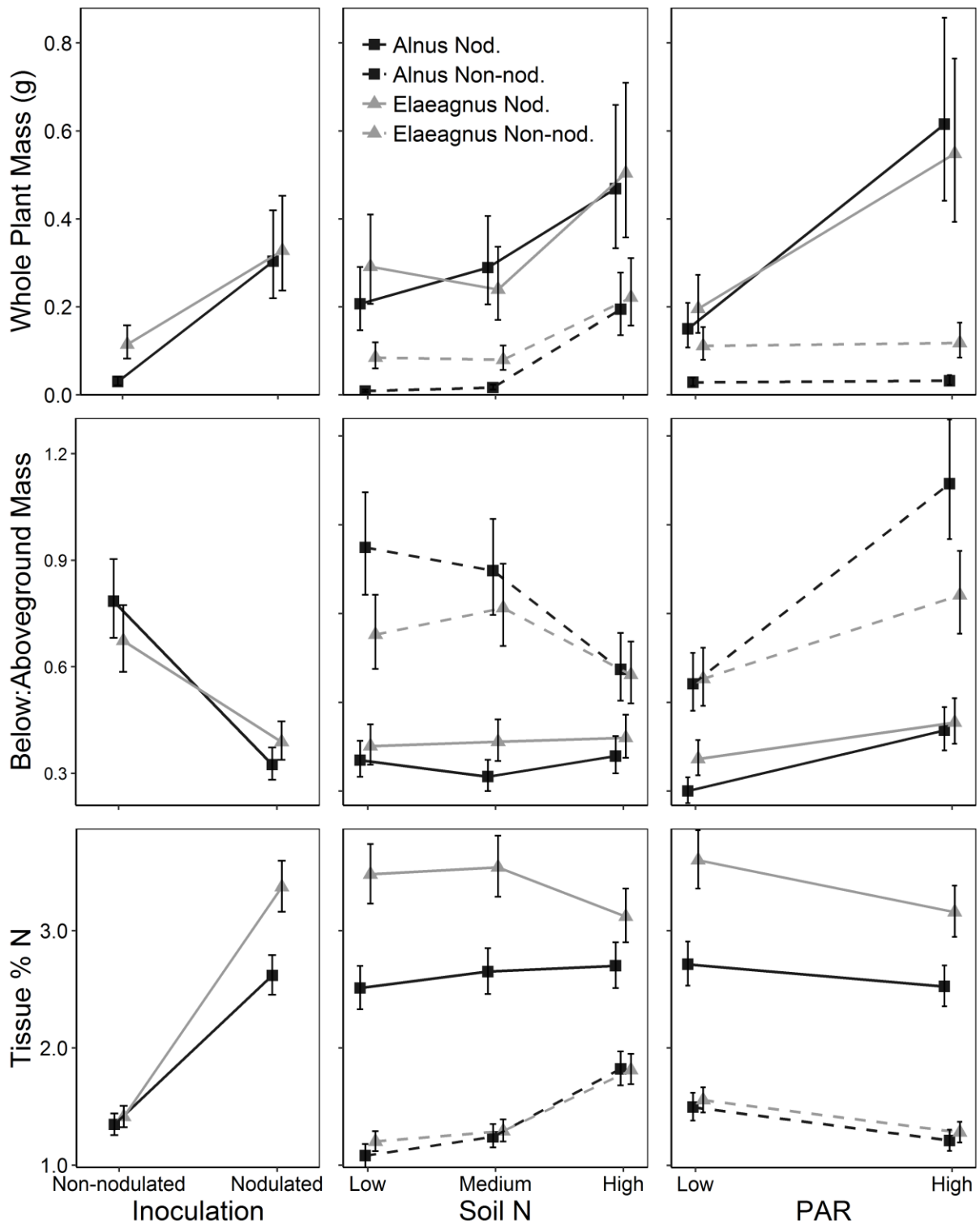


Figure 3.2: LSmeans for total plant biomass, belowground biomass: aboveground biomass, and whole plant tissue %N for response to nodulation in both genera and across exogenous N and PAR. Values have been ln back-transformed. Bars are SEM.

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APPENDIX 1: LIST OF PLANT SPECIES AT SOUTH FORK OF THE REPUBLICAN

Appendix 1.A: Mean % cover/plot for all species found in 2010 and 2011 at the South Fork of the Republican River, separated by Russian olive and cottonwood canopies. Species are sorted by 2010 mean cover.

Species	Status	Func. Group	2010					2011				
			Total	Cottonwood		Open		Total	Cottonwood		Open	
				Russian olive	Ref.	Russian olive	Ref.		Russian olive	Ref.	Russian olive	Ref.
			386	69	69	124	124	383	69	68	123	123
<i>Panicum virgatum</i>	Native	Per. C4 Grass	8.74	2.93	13.38	5.23	12.91	8.40	0.67	8.90	4.52	16.34
<i>Ambrosia psilostachya</i>	Native	Per. Forb	7.78	4.13	3.86	10.31	9.47	6.15	4.49	3.06	9.16	5.79
<i>Elymus canadensis</i>	Native	Per. C4 Grass	7.01	3.93	8.86	8.17	6.53	3.94	2.71	1.72	6.00	3.79
<i>Bromopsis inermis</i>	Exotic	Per. C3 Grass	6.91	17.04	15.07	2.14	1.49	6.33	15.70	13.84	2.60	0.67
<i>Nepeta cataria</i>	Exotic	Per. Forb	5.12	5.12	0.36	12.56	0.32	3.53	3.45	0.10	8.58	0.42
<i>Kochia scoparia</i>	Exotic	Ann. Forb	3.16	2.04	1.41	4.80	3.10	2.72	2.38	1.21	4.95	1.51
<i>Spartina pectinata</i>	Native	Per. C4 Grass	2.43	1.58	4.90	1.27	2.69	3.27	3.45	7.34	1.19	2.99
<i>Sporobolus cryptandrus</i>	Native	Per. C4 Grass	2.28	0	0.26	0.35	6.60	2.51	0.22	0.90	0.44	6.77
<i>Bromus japonicus</i>	Exotic	Ann. grass	1.96	0.11	0.20	3.25	2.68	4.49	0.51	0.66	8.86	4.48
<i>Cirsium arvense</i>	Exotic	Per. Forb	1.94	2.41	2.04	1.80	1.77	2.73	3.88	2.78	2.59	2.21
<i>Setaria viridis</i>	Exotic	Ann. grass	1.51	0.22	0.99	2.27	1.77	0.75	0.08	0.23	0.82	1.34
<i>Conyza canadensis</i>	Native	Ann. Forb	1.33	0.09	0.25	0.78	3.17	0.29	0	0.33	0.37	0.34
<i>Poa pratensis</i>	Exotic	Per. C3 Grass	1.03	0.43	1.41	1.05	1.13	3.62	2.16	4.22	4.47	3.26
<i>Elymus repens</i>	Exotic	Per. C4 Grass	1.00	2.03	0.38	0.68	1.10	1.36	1.55	1.25	1.59	1.08
<i>Chloris verticillata</i>	Native	Per. C4 Grass	0.93	0.12	0	0.03	2.81	1.21	0	0.16	0.28	3.40
<i>Agrostis gigantea</i>	Native	Per. C4 Grass	0.93	0	0.29	2.32	0.40	0.41	0.07	0.59	0.34	0.57
<i>Carduus nutans</i>	Exotic	Per. Forb	0.92	1.12	0.07	1.73	0.48	1.47	1.39	0.21	2.85	0.84
<i>Dactylis glomerata</i>	Exotic	Per. C4 Grass	0.92	0.29	0.19	2.34	0.27	1.44	0.49	0.29	3.61	0.43
<i>Bromopsis pubescens</i>	Native	Per. C3 Grass	0.90	0.10	1.46	0.42	1.52	0.04	0	0	0.12	0
<i>Hordeum jubatum</i>	Native	Per. C4 Grass	0.88	0.07	0.45	1.73	0.73	0.68	0.62	0.37	0.44	1.11
<i>Carex</i> spp.	Native	Per. C3 Grass	0.88	0.64	1.49	0.22	1.34	2.18	0.97	3.81	1.27	2.86
<i>Ambrosia trifida</i>	Native	Ann. Forb	0.65	0.45	0.42	1.10	0.45	0.97	0.41	0.34	2.16	0.44
<i>Chenopodium album</i>	Exotic	Ann. Forb	0.65	0.22	0.06	1.67	0.19	0.84	0.18	0.10	2.37	0.10
<i>Poa</i> sp.		Per. C3 Grass	0.62	1.23	0	0.58	0.65	0.15	0	0.15	0.37	0.02
<i>Physalis virginiana</i>	Native	Per. Forb	0.58	0.14	0.42	0.79	0.69	0.37	0.12	0.19	0.67	0.30
<i>Bidens frondosa</i>	Native	Ann. Forb	0.52	0	0.07	1.35	0.23	<0.01	0	0	0.02	0
<i>Echinochloa crus-galli</i>	Exotic	Ann. grass	0.44	0	0	0.24	1.13	0.14	0	0	0.05	0.39

<i>Helianthus annuus</i>	Native	Ann. Forb	0.42	0.04	0	0.61	0.67	0.35	0.12	0.04	0.15	0.85
<i>Calamovilfa longifolia</i>	Native	Per. C4 Grass	0.38	0.03	0	0.60	0.55	0	0	0	0	0
<i>Cucurbita foetidissima</i>	Native	Per. Forb	0.32	0	0.03	0.67	0.32	0.42	0.02	0	1.11	0.19
<i>Taraxacum officinale</i>	Exotic	Per. Forb	0.32	0.28	0.48	0.39	0.18	0.44	0.26	0.50	0.70	0.25
<i>Juncus sp.</i>	Native	Per. C4 Grass	0.25	0.06	0.33	0	0.56	0.14	0.01	0.38	0.16	0.04
<i>Euphorbia dentata</i>	Native	Ann. Forb	0.24	0.20	0.25	0.26	0.25	0.31	0.35	0.27	0.31	0.30
<i>Scirpus pallidus</i>	Native	Per. C4 Grass	0.23	0	0	0	0.73	0.52	0.33	1.09	0	0.85
<i>Andropogon gerardii</i>	Native	Per. C4 Grass	0.23	0	0.12	0.12	0.53	0.56	0	0.01	0	1.74
<i>Verbena stricta</i>	Native	Per. Forb	0.22	0	0.12	0.07	0.56	0.10	0	0.04	0	0.29
<i>Panicum capillare</i>	Native	Ann. grass	0.21	0	0.07	0.18	0.44	0.02	0	0.06	0	0.03
<i>Toxicodendron rydbergii</i>	Native	Per. Forb	0.21	0.28	0.33	0.22	0.10	0.34	0.62	0.44	0.22	0.25
<i>Fraxinus pennsylvanica</i>	Native	Tree	0.19	0.29	0.25	0.25	0.05	0.10	0.23	0.24	0.03	<0.01
<i>Bouteloua curtipendula</i>	Native	Per. C4 Grass	0.18	0	0	0.12	0.45	0.16	0	0	0.04	0.46
<i>Cyclachaena xanthifolia</i>	Native	Ann. Forb	0.17	0	0.06	0.10	0.40	0.23	0	0	0.33	0.38
<i>Solanum americanum</i>	Native	Ann. Forb	0.16	0.39	0	0.24	0.05	<0.01	0.01	0	<0.01	0.02
<i>Artemisia ludoviciana</i>	Native	Per. Forb	0.16	0.06	0.22	<0.01	0.33	0.06	0.04	0.03	0.04	0.11
<i>Rumex crispus</i>	Exotic	Per. Forb	0.16	0.22	0.30	0.20	0	0.10	0.16	0.03	0.12	0.07
<i>Thinopyrum ponticum</i>	Exotic	Per. C4 Grass	0.16	0.43	0.43	0	0	0.14	0.65	0.15	0	0
<i>Salvia reflexa</i>	Native	Ann. Forb	0.15	0.36	0.04	0.21	0.03	0.13	0.17	0	0.31	0
<i>Mirabilis nyctaginea</i>	Native	Per. Forb	0.14	0.13	0	0.23	0.13	0.14	0.06	0	0.28	0.13
<i>Bouteloua gracilis</i>	Native	Per. C4 Grass	0.14	0	0.07	0.18	0.21	0.22	0	0.34	0.28	0.23
<i>Cirsium vulgare</i>	Exotic	Per. Forb	0.13	0	0	0.27	0.15	0.20	0.09	0	0.48	0.08
<i>Verbena hastata</i>	Native	Per. Forb	0.12	0	0	<0.01	0.37	0.13	0	0	0	0.40
<i>Lactuca serriola</i>	Exotic	Ann. Forb	0.11	0.01	0.16	0.17	0.07	0.20	0.15	0.19	0.20	0.24
<i>Pascopyrum smithii</i>	Native	Per. C4 Grass	0.11	0.32	0.01	0.04	0.11	0.14	0.01	<0.01	0.13	0.30
<i>Salsola tragus</i>	Exotic	Ann. Forb	0.09	0.07	0.01	0.23	<0.01	0.36	0.18	0.08	0.66	0.31
<i>Chamaesyce glyptosperma</i>	Native	Ann. Forb	0.08	0	0.01	0	0.25	0.04	0	0.03	<0.01	0.09
<i>Callirhoe involucreta</i>	Native	Per. Forb	0.08	0	0.13	0.08	0.10	0.09	0	0.21	0.06	0.12
<i>Lycopus americanus</i>	Native	Per. Forb	0.08	0.36	0.10	0	0	0.07	0.25	0	0	0.07
<i>Schoenoplectus pungens</i>	Native	Per. C4 Grass	0.08	0.03	0.02	<0.01	0.23	0.43	0.07	0.21	0.36	0.84
<i>Viola nephrophylla</i>	Native	Per. Forb	0.08	0.20	0.07	0.09	0	0.05	0.09	0.18	0.02	0
<i>Distichlis spicata</i>	Native	Per. C4 Grass	0.06	0	0.22	0	0.08	0.12	0.04	0.59	<0.01	0.02
<i>Euphorbia marginata</i>	Native	Ann. Forb	0.06	0	0	0	0.19	<0.01	0.01	0	<0.01	0.02
<i>Artemisia filifolia</i>	Native	Per. Forb	0.05	0	0	0	0.17	0.05	0	0	0	0.16
<i>Solidago sp.</i>	Native	Per. Forb	0.05	0.22	0.06	0	0	0.12	0.12	0.43	0.03	0.02

Unknown Composite			0.05	0.04	0.01	0	0.12	0.09	0	0.07	0.04	0.20
<i>Glycyrrhiza lepidota</i>	Native	Per. Forb	0.04	0	0	0	0.14	0.11	0	<0.01	0	0.33
<i>Asclepias speciosa</i>	Native	Per. Forb	0.04	0	0	0.06	0.06	0.03	0	0	0.02	0.07
<i>Asclepias incarnata</i>	Native	Per. Forb	0.04	0.01	0.12	0.03	<0.01	0.12	0.07	0.29	0.02	0.16
<i>Plantago major</i>	Exotic	Per. Forb	0.04	0	0	0.09	0.02	0.05	0	0	0.13	0.02
<i>Juniperus virginiana</i>	Native	Tree	0.03	0.17	0	0	0	0.01	0.06	0	0	0
<i>Erigeron pumilus</i>	Native	Per. Forb	0.03	0.03	0	0	0.07	0	0	0	0	0
<i>Croton texensis</i>	Native	Ann. Forb	0.03	0	0	<0.01	0.07	0.07	<0.01	0.02	0.02	0.17
<i>Chamaesyce missurica</i>	Native	Ann. Forb	0.02	0.03	0	<0.01	0.05	0.03	<0.01	0	0	0.08
<i>Strophostyles leiosperma</i>	Native	Ann. Forb	0.02	0	0	0	0.07	0	0	0	0	0
<i>Populus deltoides</i>	Native	Tree	0.02	0.12	0	0	0	<0.01	0	0	0	0.02
<i>Solidago canadensis</i>	Native	Per. Forb	0.02	0	0.09	0.02	0	0	0	0	0	0
<i>Cenchrus longispinus</i>	Native	Ann. grass	0.02	0	0	0	0.06	<0.01	0	0	<0.01	0
<i>Artemisia dracunculus</i>	Native	Per. Forb	0.02	0	0	0	0.06	0.04	0.22	0	0	0
<i>Aster sp.</i>			0.02	0	0	0.02	0.04	0.15	0.01	0.26	0.11	0.19
<i>Euthamia occidentalis</i>	Native	Per. Forb	0.02	0	0	0	0.05	<0.01	0.04	0	0	0
<i>Rosa sp.</i>		Tree	0.02	0	0.01	0.04	0	0.05	0	0.04	0.14	0
<i>Cirsium undulatum</i>	Native	Per. Forb	0.01	0	0	0	0.04	0.02	0	0	0	0.07
<i>Elaeagnus angustifolia</i>	Exotic	Tree	0.01	0.06	0	<0.01	0	0.04	0.22	0	<0.01	0
<i>Ratibida columnifera</i>	Native	Per. Forb	0.01	0	0.06	0	<0.01	0	0	0	0	0
<i>Typha latifolia</i>	Native	Per. Forb	0.01	0	0.07	0	0	0.03	0	0.18	0	0
<i>Chamaesyce geyeri</i>	Native	Ann. Forb	0.01	0	0	0	0.03	0.06	0.03	0.12	0.03	0.07
<i>Leersia oryzoides</i>	Native	Per. C4 Grass	0.01	0	0	0	0.03	0.67	0.22	0.32	1.03	0.76
<i>Lemna sp.</i>			0.01	0	0	0.02	<0.01	0.07	0	0	0.20	<0.01
<i>Lithospermum incisum</i>	Native	Per. Forb	<0.01	0	0.04	0	0	0.04	0	0.06	0	0.10
<i>Polygonum convolvulus</i>	Exotic	Ann. Forb	<0.01	0	0	0.02	0	0	0	0	0	0
<i>Equisetum laevigatum</i>	Native	Per. Forb	<0.01	0.02	0.02	<0.01	<0.01	0.05	0.03	0.05	0.01	0.11
<i>Juncus arcticus</i>	Native	Per. C3 Grass	<0.01	0.03	<0.01	0	0	0.07	0.14	0.26	0	0
<i>Medicago lupulina</i>	Exotic	Ann. Forb	<0.01	0.01	0	<0.01	<0.01	0.04	0.25	0	0	0
<i>Melilotus officinalis</i>	Exotic	Per. Forb	<0.01	0.02	0	0	<0.01	0.01	<0.01	0	0.02	0.02
<i>Lythrum alatum</i>	Native	Per. Forb	<0.01	0	0	0	0.02	0.01	0	0	0	0.04
<i>Paspalum setaceum</i>	Native	Per. C4 Grass	<0.01	0	0	0	0.02	0.01	0	0	0	0.04
<i>Polygonum lapathifolium</i>	Native	Ann. Forb	<0.01	0	0.03	0	0	0	0	0	0	0
<i>Veronica sp.</i>	Native	Per. Forb	<0.01	0	0	0	0.02	0	0	0	0	0
<i>Polygonum ramosissimum</i>	Native	Ann. Forb	<0.01	0	0	<0.01	<0.01	<0.01	0	0	<0.01	0

<i>Cyperus erythrorhizos</i>	Native	Per. C4 Grass	<0.01	0	0	<0.01	<0.01	0	0	0	0	0
<i>Lepidium densiflorum</i>	Native	Ann. Forb	<0.01	0	0	0	<0.01	0	0	0	0	0
<i>Cyperus odoratus</i>	Native	Per. C4 Grass	<0.01	0	0	0	<0.01	0	0	0	0	0
<i>Eragrostis cilianensis</i>	Exotic	Ann. grass	<0.01	0	0	<0.01	0	0	0	0	0	0
<i>Mirabilis hirsuta</i>	Native	Per. Forb	<0.01	0	0	0	<0.01	0.02	0.04	0	0.02	0.02
<i>Mirabilis linearis</i>	Native	Per. Forb	<0.01	0	0	0	<0.01	<0.01	0	0	0	<0.01
<i>Bromus tectorum</i>	Exotic	Ann. grass	<0.01	<0.01	0	0	0	0.32	<0.01	0	0.45	0.55
<i>Chenopodium leptophyllum</i>	Native	Ann. Forb	<0.01	0	0	0	<0.01	<0.01	0	0	0	<0.01
<i>Juncus dudleyi</i>	Native	Per. C3 Grass	<0.01	<0.01	0	0	0	0	0	0	0	0
<i>Juncus torreyi</i>	Native	Per. C3 Grass	<0.01	<0.01	0	0	0	0.03	0	0	0	0.08
<i>Vulpia octoflora</i>	Native	Ann. grass	<0.01	0	0	0	<0.01	<0.01	0	0	0	<0.01
<i>Asclepias sp.</i>	Native	Per. Forb	0	0	0	0	0	<0.01	<0.01	0	0	0
<i>Bidens cernua</i>	Native	Ann. Forb	0	0	0	0	0	<0.01	0	0	0	<0.01
<i>Bromopsis sp.</i>		Per. C3 Grass	0	0	0	0	0	0.03	0.10	0.04	<0.01	<0.01
<i>Carex brevior</i>	Native	Per. C3 Grass	0	0	0	0	0	<0.01	0	0	0	<0.01
<i>Carex duriuscula</i>	Native	Per. C3 Grass	0	0	0	0	0	<0.01	0	0	0	<0.01
<i>Carex lasiocarpa</i>	Native	Per. C3 Grass	0	0	0	0	0	0.03	0.07	0.09	0	0
<i>Chenopodium fremontii</i>	Native	Ann. Forb	0	0	0	0	0	<0.01	0	0	<0.01	<0.01
<i>Chenopodium sp.</i>		Ann. Forb	0	0	0	0	0	0.14	0.04	0	0.41	0
<i>Conium maculatum</i>	Exotic	Per. Forb	0	0	0	0	0	0.04	0	0	0.11	0
<i>Convolvulus arvensis</i>	Exotic	Per. Forb	0	0	0	0	0	0.01	0	0.07	0	0
<i>Eleocharis sp.</i>	Native	Per. C3 Grass	0	0	0	0	0	0.10	<0.01	0.03	0.04	0.24
<i>Eriogonum annuum</i>	Native	Ann. Forb	0	0	0	0	0	<0.01	0	0	0	<0.01
<i>Hordeum pusillum</i>	Native	Ann. grass	0	0	0	0	0	<0.01	0	0	<0.01	0
<i>Lobelia siphilitica</i>	Native	Per. Forb	0	0	0	0	0	0.02	0	0	0	0.06
<i>Mentha arvensis</i>	Native	Per. Forb	0	0	0	0	0	<0.01	0.01	0	0	0
<i>Muhlenbergia pungens</i>	Native	Per. C4 Grass	0	0	0	0	0	<0.01	0	0.01	0	<0.01
<i>Oenothera curtiflora</i>	Native	Ann. Forb	0	0	0	0	0	<0.01	0	0	0	0.02
<i>Opuntia macrorhiza</i>	Native	Per. Forb	0	0	0	0	0	<0.01	0	0	0.02	0
<i>Parietaria pensylvanica</i>	Native	Per. Forb	0	0	0	0	0	0.21	0.26	0	0.47	0.02
<i>Polygonum aviculare</i>	Exotic	Ann. Forb	0	0	0	0	0	<0.01	0	0	<0.01	0
<i>Polygonum persicaria</i>	Exotic	Per. Forb	0	0	0	0	0	0.02	0.04	0	0	0.02
<i>Psoralidium linearifolium</i>	Native	Per. Forb	0	0	0	0	0	<0.01	0	0	0	<0.01
<i>Schizachyrium scoparium</i>	Native	Per. C4 Grass	0	0	0	0	0	<0.01	0	0	0	<0.01
<i>Solidago gigantea</i>	Native	Per. Forb	0	0	0	0	0	0.03	0	0	0.11	0

<i>Sonchus arvensis</i>	Exotic	Per. Forb	0	0	0	0	0	0.04	0	0	0.11	0
<i>Sorghastrum nutans</i>	Native	Per. C4 Grass	0	0	0	0	0	0.55	0.25	0.51	0.13	1.15
<i>Sphenopholis obtusata</i>	Native	Per. C3 Grass	0	0	0	0	0	<0.01	0	0	0	0.02
<i>Tragopogon dubius</i>	Exotic	Per. Forb	0	0	0	0	0	<0.01	0.01	0	<0.01	0
<i>Ulmus americana</i>	Native	Tree	0	0	0	0	0	0.02	0	0	0.05	0
Unknown grass			0	0	0	0	0	0.12	0	0	0.20	0.16