

THESIS

AN INTRODUCED PLANT AFFECTS THE STRUCTURE AND FUNCTION OF RIPARIAN FOOD WEBS

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ABSTRACT

AN INTRODUCED PLANT AFFECTS THE STRUCTURE AND FUNCTION OF RIPARIAN FOOD WEBS

Aquatic and terrestrial ecosystems are closely linked by the reciprocal flux of resource subsidies, but these dynamics can be uncoupled by anthropogenic change. Introduced species are easily spread by riverine systems, but the effects of non-native riparian vegetation on reciprocal arthropod subsidies are essentially unknown. I¹ studied the aquatic and terrestrial arthropod communities, and their flux into and out of the stream channel, in stream reaches in northwestern Colorado invaded and uninvaded by New Mexico locust (*Robinia neomexicana* A. Gray), a woody plant north of its native range. I also evaluated whether the aquatic- and terrestrial-derived diets of riparian songbirds were altered in stream reaches with New Mexico locust.

I found that reaches with New Mexico locust had fewer terrestrial arthropods collected from vegetation, particularly in spring. Consistent with these results, some songbird species using invaded sites displayed diet shifts towards aquatic-derived resources. Aquatic resources comprised approximately 34% of the songbird assemblage's diet, which highlights the importance of aquatic subsidies to riparian consumers. In contrast, there were no impacts of New Mexico locust on the aquatic insect community associated with the invasion. Overall, seasonal and annual variation best described patterns of arthropod subsidy production and consumption.

¹ Chapters 1 and 2, co-authored versions of manuscripts, use pluralized "we."

Since locust invasion did not drive strong patterns in insect communities, and because seasonal and annual factors provide little information about environmental drivers of resource subsidies, we also assessed the importance of vegetation and stream characteristics on aquatic and terrestrial arthropod communities. I found terrestrial insect biomass and richness increased with percent ground cover, forb cover, and vertical vegetation structure, and decreased with percent cover of New Mexico locust. Interestingly, vegetation characteristics best described emergence patterns of adult aquatic insects, but stream characteristics best described larval aquatic insect patterns.

Our results support the use of a multi-functional approach to assessing ecosystem alteration. By evaluating the multiple pathways through which a non-native species can affect riparian systems, I found a close connection between stream and riparian ecosystems. I found the impacts of invasion were most apparent for arthropod metrics directly related to vegetation (*i.e.*, arthropods collected from foliage), with weaker responses for indirect metrics (*i.e.*, aquatic insect biomass, songbird diet components).

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CHAPTER ONE

AN INTRODUCED PLANT AFFECTS RIPARIAN ARTHROPODS, BUT NOT AQUATIC OR TERRESTRIAL SUBSIDIES

INTRODUCTION

Increased interest in the permeable boundary between aquatic and terrestrial ecosystems has led to greater appreciation for linkages between them (Polis et al. 1997, Baxter et al. 2005, Tank et al. 2010). Food resources, known as resource subsidies, flow in multiple directions, and this reciprocal exchange of materials and energy stabilizes asynchronous ecosystems spatially and temporally (Polis et al. 1997, Nakano and Murakami 2001, Takimoto et al. 2002, Bartels et al. 2012). In particular, the bidirectional flux of aquatic and terrestrial arthropods into recipient ecosystems forms a crucial link between herbaceous biomass and food availability for riparian and instream consumers. For instance, terrestrial consumers in riparian communities can derive 40-50% of their dietary needs from aquatic primary production (Kautza and Sullivan 2016). Emergent aquatic insect biomass can constitute 26% of an entire bird assemblage's diet, or up to 67-98% for individual species, and terrestrial arthropods can constitute more than 45% of the annual energy budget for fish (Nakano and Murakami 2001, Baxter et al. 2005). These reciprocal exchanges likely explain why nearly 70% of all vertebrate species are riparian obligates (Raedeke et al. 1988).

Riparian plant invasions can restructure aquatic and terrestrial arthropod communities and influence ecosystem stoichiometry, primary productivity, and decomposition (Tibbets and Molles 2005, Hladyz et al. 2009, 2011, Mineau et al. 2011, 2012, Kominoski et al. 2012). In a

recent meta-analysis, Litt et al. (2014) found that non-native plants reduced terrestrial arthropod abundances in 62% of studies. Plant invasions along riparian zones can drive functional and compositional changes in aquatic insect communities by altering leaf litter quantity and quality, canopy openness, and algal growth rates (Moline and Poff 2008, Hladyz et al. 2011, Mineau et al. 2012). Yet, to our knowledge no studies have evaluated how non-native riparian plants influence cross-ecosystem subsidies provided by aquatic and terrestrial insect communities. Riparian zones are particularly prone to non-native species invasions because of downstream transport and flashy flow regimes (Richardson et al. 2007), and invasions have led to the loss of species and altered ecosystem function in riparian ecosystems globally (Vitousek et al. 1997, Tockner et al. 2010). Human activities centered on floodplains directly increase the prevalence of non-native species and indirectly alter species' ranges through climate change (Parmesan 1996, Walther 2010). New assemblages of taxa may alter the timing, duration, and magnitude of resource subsidies (Hobbs et al. 2014). These factors can interact to decouple cross-ecosystem subsidies (Richardson et al. 2007, Larsen et al. 2016), but it is unclear if plant invasion impacts will propagate similarly through terrestrial and aquatic ecosystems.

The objective of this study was to evaluate the effect of an introduced riparian plant species on arthropod communities and arthropod subsidies. Our focal species was New Mexico locust (*Robinia neomexicana* A. Gray; hereafter "locust"), which takes the form of a shrub or tree and is native to the southwestern United States, including southern Colorado (Little 1976, Pavsek 1993). Local understanding suggests homesteaders introduced locust to the Clear Creek drainage of northwestern Colorado, outside its native range, over 100 years ago to grow wood for fence posts (C. Tysse, Chevron, personal communication; Fig. 1.1). Due to its pervasive

growth in riparian areas, current landowners have attempted, with limited success, to remove locust using goat grazing and mechanical clearing. As a nitrogen fixer, locust is a successful pioneer species that can quickly become dominant because of its rhizomatous growth (Pavek 1993). The proximity of the locust introduction to its native range provides a unique opportunity to assess the potential consequences of poleward migrations predicted by climate change scenarios (Woodall et al. 2009, Walther 2010).

We posed the following hypotheses and predictions about the effect of the introduced locust on aquatic and terrestrial arthropod communities. First, we hypothesized terrestrial and aquatic arthropod communities and their respective flux into and out of the stream channel would be reduced in reaches invaded by locust (Litt et al. 2014). Second, we quantified the relative influence of native riparian shrub and tree species, locust, and stream characteristics on arthropod communities and subsidies. We predicted that the combination of locust and certain plant species or environmental characteristics could explain patterns of arthropod communities better than simply locust presence or absence.

METHODS

Study Area

The study was conducted along 1st and 2nd order streams in the Clear Creek drainage of the Piceance Basin in northwestern Colorado (Donnell 1961, Bartis et al. 2005; Fig. 1). The Piceance Basin is characterized by high mesas and steep canyons ranging from 1500 to 2700 m elevation. The surrounding oil shale geology produces predominantly fine sand and silt in stream channels, and larger substrate has a median intermediate diameter of 8 mm (Table 1.1). Big sagebrush (*Artemisia tridentate* Nutt.), pinyon pine (*Pinus edulis* Engelm.) and juniper

(*Juniperus* L.) dominate upland vegetation, and boxelder (*Acer negundo* L.), narrowleaf cottonwood (*Populus angustifolia* James), and Gambel oak (*Quercus gambelii* Nutt.) are common trees in and around riparian reaches.

Study Design

After initial reconnaissance and mapping of locust invasion in 2014, four pairs of sampling reaches matched by elevation (all sites within 1830 – 1959 m elevation) were selected in riparian forests with and without locust (hereafter “locust” and “reference” sites; Fig. 1.1). Sampling reaches included 180 m of stream channel and were separated by at least 300 m. Sampling transects that extended 5 m perpendicularly from both sides of the stream channel were placed at the beginning, middle, and end of each sample reach. At each transect, we collected terrestrial and aquatic arthropods and measured vegetation and channel characteristics. Data collection began at the downstream transect. Arthropod sampling and channel characteristic measurements were repeated 4 times at all sites, in spring and summer (May and July) of 2015 and 2016. Vegetation surveys were conducted in May and July of 2015, and vegetation communities were assumed to be similar for respective seasons in 2016.

Vegetation and Channel Characteristics

We conducted vegetation surveys and measured channel characteristics to evaluate other factors potentially associated with arthropod subsidy patterns and to assess potential environmental differences between locust and reference stream reaches. We measured vegetation composition and structure along each of the 3 sampling transects using 1 m² plots and 5 m vertical point line intercepts. The plots were placed on both banks 0, 2, and 4 m away from the stream’s edge. Ground cover of each growth form and species within the plot were

visually estimated. Plants taller than 0.6 m were included in this measurement, but only the portion of vegetation below this height. To measure vertical vegetation structure, a 5 m stadia rod was extended from 2-4 corners of the plot, depending on bank stability. The species identity and pole height was recorded for each vegetation contact point beginning at 0.6 m. Prior to statistical analysis, each vegetation metric was averaged across all 6 transect plots. At the first transect, we also measured stream water chemistry parameters: pH, specific conductivity, and temperature (YSI 30 probe, Yellow Springs, Ohio), and dissolved oxygen (DO) (YSI 550a probe, Yellow Springs, Ohio). At all 3 transects, we measured wetted and bankfull width and thalweg depth, and we used a clinometer and stadia rod to measure bank angle on both stream banks. We also used a densitometer to measure canopy cover from the center of each transect. We obtained current velocity by timing a neutrally buoyant object as it traveled $\geq$ 8 m downstream across the middle transect. At the upper and lower transect, we determined median substrate size using Wolman (1954) pebble counts of substrate pieces that were gravel sized and larger.

Arthropod Subsidies

Four methods of arthropod sampling were conducted at each sampling transect to evaluate aquatic and terrestrial communities and their contribution to resource subsidies. From these sampling methods, we quantified terrestrial and aquatic arthropod biomass as a measure of subsidy productivity, and richness and multivariate community composition as measures of potential ecosystem function. We collected benthic insects using a 0.10 m² Surber sampler placed once in a riffle nearest each sampling transect (Merritt et al. 1996). Samples were rinsed through a 350 μ m sieve and preserved in 100% ethanol in the field. Terrestrial invertebrate

communities were sampled semi-quantitatively by beating 3 shrubs or trees nearest the sampling transect for 20 seconds over a 0.3 m² stretched canvas beat sheet (BioQuip, Rancho Dominguez, California). Each sample was a composite of the three 20-second beats. We collected benthic and beat sheet samples before surveying channel and vegetation characteristics as to not disturb channel substrate or riparian vegetation. Fluxes of arthropod subsidies into and out of the stream channel were collected using 0.4 m² standing pan traps and 0.3 m² floating emergence traps (0.2 mm mesh), respectively (Wipfli 1997, Cadmus et al. 2016). Pan traps were filled to cover the base with about 5 cm of stream water filtered through a 350 µm sieve, and approximately 5 mL of biodegradable unscented surfactant was added to reduce surface tension. One pan trap was placed over each sampling transect, and one emergence net was placed over slow-moving water nearest each sampling transect. Both traps types were deployed simultaneously at all sites for 48 hrs, within a week of collecting benthic and beat sheet samples. Pan trap and emergence net contents were subsampled every 24 hrs. Arthropods collected from beat sheets, pan traps, and emergence nets were preserved in 80% ethanol.

In the laboratory, arthropod samples were sorted from sample debris, enumerated, and identified to the lowest taxonomic resolution possible (typically genus for benthic samples and family for all other samples) using standard taxonomic keys (Gray et al. 1983, Merritt et al. 1996, Ward et al. 2002, Triplehorn and Johnson 2005). After identification, we separated shredding insects from other functional groups in benthic samples to compare with the composition of insects colonizing leaf packs in the decomposition experiment (supplemental chapter; Wallace and Webster 1996). We sorted arthropods in pan trap samples by adult

aquatic insects or arthropods of terrestrial origin to determine proportion of subsidy available to consumers and to compare aquatic insect biomass with emergence net samples. Arthropods in emergence net and beat sheet samples were left unsorted. All arthropod samples, except beat sheet samples, were dried at 60°C for 48 hr to obtain biomass. We measured only abundance and not biomass for beat sheet samples because this is a semi-quantitative method. We obtained ash free dry mass (AFDM) of organic matter inputs and storage at each site by weighing the organic matter removed from pan trap and benthic samples, respectively. Organic matter samples were dried at 60°C for 48 hr and combusted at 500°C for 1 hr (Kominoski et al. 2011).

Data Analysis

Invasion Impacts on Arthropod Subsidies. - We tested for differences between reference and locust sites using repeated measures permutational analysis of variance (PERMANOVA). Biomass or abundance, richness, and multivariate community composition were tested separately for each sampling method. We used PERMANOVA because this method is adaptable for univariate and multivariate responses and is robust to violations of the assumption of a normal distribution compared to traditional ANOVA (Anderson 2001, 2005). The test statistic (*pseudo-F*, analogous to an F-ratio) is constructed from the distribution formed by exchangeable permutations of a symmetric distance matrix. Transformations of count (square root) and biomass data ($\ln(x+1)$) and were used to dampen effects of heteroscedasticity among treatments. We used Euclidean distance for univariate analyses and Bray-Curtis distance for multivariate analyses, and we considered Monte Carlo p-values ($MC(p)$) < 0.05 to be significant. Monte Carlo p-values are constructed when permutable units within observations are low

relative to total permutable units. The procedure creates a random sample distribution with equal mean and variance to the observed data, from which it approximates conservative within- and between-group difference for the test statistic. P-values in our analyses were generated using 9999 permutations (Anderson 2005).

Factors in the PERMANOVA model include riparian forest type (locust versus reference stream reaches), season and year (fixed effects, two levels each), and 2-way interactions between forest type and season or year. Site nested in forest type (random effect, eight levels) was also included to retain the repeated measures structure. Pairwise post-hoc tests were done on significant interactions. Using the PERMDISP function, we ensured homogeneity of variance for models with a significant forest type effect because PERMANOVA is not robust to assumptions of equal variance. We then used similarity percentage analysis (SIMPER) to identify taxa that contributed most to community composition differences. All comparison analyses were conducted using functions in the PERMANOVA+ add-on package for PRIMER v7 (Plymouth, United Kingdom, Anderson et al. 2008).

Environmental Impacts on Arthropod Subsidies. – We evaluated the influence of riparian vegetation and stream channel characteristics relative to locust invasion using a corrected Akaike Information Criteria (AICc) model selection approach (Burnham and Anderson 2002). AICc is preferred for small sample sizes, and it assigns each model a value indicative of its tradeoff between fit and complexity. The model with the smallest AICc value is considered the best fit, although models within $\Delta 2$ AICc can be equally informative (Burnham and Anderson 2002). For each sampling method and metric (*i.e.*, biomass and richness), model probability was calculated using Akaike weights and individual variable weights were calculated as the

cumulative weight of all top models containing that variable. For this analysis, we removed all *ad hoc* study design structure (*i.e.*, forest type, season, and year) because many environmental parameters collectively describe seasonal trends (*i.e.*, vegetation abundance increases and bare ground cover decreases in summer). This allowed us to explicitly test which underlying vegetation or aquatic parameters best explained patterns of resource subsidies and community richness. For ease of interpreting model coefficients, we did not evaluate models for multivariate community composition.

The model selection process began with 40 variables of interest sorted into three groups: 14 aquatic, 18 ground vegetation cover, and 9 vertical vegetation structure metrics (Table 1.1). We used a 2-step variable reduction processes, fit all possible model combinations, then used AICc values to select top models and weight variable importance. The 40 variables included the percent ground cover or vertical vegetation structure of native shrub and tree species that occurred along more than 5 transects to compare their influence on arthropod communities relative to locust. Other candidate vegetation parameters included vegetative richness and abundance, trees and shrubs (*i.e.*, ground cover and vertical structure), forbs, and grasses (*i.e.*, ground cover) (Appendix 1.1). Stream channel characteristics are known controlling factors of aquatic insect communities (Ward et al. 2002) and are often used as physical and chemical descriptors in stream assessments (*e.g.*, Ode et al. 2016). Channel characteristics were included as candidate parameters in models explaining emergence or benthic biomass and richness, and vegetation characteristics were considered for all models because our primary research question focuses on the role of riparian vegetation in determining resource subsidies.

To reduce the potential for spurious results, we eliminated variables with 2 steps. Prior to these steps, environmental variables were $\ln(x+1)$ transformed to dampen effects of heteroscedasticity, then normalized by subtracting the parameter's mean and dividing by its standard deviation. First, we examined Pearson correlation coefficients to check for redundant metrics within each group. Vertical vegetation structure was removed because it had $|R| > 0.75$ with vertical structure of boxelder. Next, we used principle component analysis (PCA) to eliminate environmental parameters that least contributed to differences across sites (after Ferreira et al. 2014). We eliminated metrics within each group that contributed less than average to the summation of the absolute value of the variable coefficients for the 1st PCA axis. This axis represents the best fit univariate gradient formed by all environmental variables in each group. For instance, the 1st PCA for stream characteristics poorly represents patterns in pH and DO, but describes a negative relationship between velocity and litter storage, depth, width, and bankfull width (Table 1.1). Using this approach, we eliminated 8 aquatic, 11 ground vegetation, and 4 vertical vegetation structure metrics (Table 1.1). Correlation and PCA analyses were carried out in PRIMER.

We used PROC REG adjusted- R^2 model selection in SAS v9.3 (SAS Institute, Cary, North Carolina) to run all possible combinations of remaining parameters. To meet linear regression assumptions, biomass data were $\ln(x+1)$ transformed and count data were square root-transformed. We considered all models within $\Delta 2$ AICc of the top model, but removed models if the addition of a variable with confidence intervals overlapping 0 resulted in a model within $\Delta 2$ AICc (*i.e.*, "pretending variables;" Burnham and Anderson 2002). Selected variables with

normalized coefficients <0.10 were considered weak and biologically insignificant, and models with adjusted- R^2 values $< 5\%$ were not considered because of their lack of explanatory power.

RESULTS

Locust comprised about 1.6% of ground cover and vertical vegetation structure in invaded sites (Table 1.1), which was somewhat less than boxelder, the dominant native tree (4% ground cover and vertical vegetation structure averaged across reference and locust sites). Vegetative ground cover and species richness was similar between reference and locust sites, except reference sites had approximately twice the shrub/tree ground cover and half the vertical vegetation structure and species richness above 0.6 m. Reference sites also had nearly a third of the percent ground cover of non-native plants (excluding locust; Table 1.1, Appendix 1.2).

All stream reaches had similar bank angles, substrate, water chemistry, litter inputs and litter storage in reference and locust sites (Table 1.1). Reference sites were slightly narrower, shallower, more steeply sloped, and had about 11% less canopy cover. As expected, there were strong seasonal differences in stream characteristics, and climatic variation also drove strong yearly differences. The upper Colorado River drainage encompassing our study sites received 1.65 cm more precipitation in water year 2016 compared to 2015 (NOAA 2016), and streams in our study reaches had approximately 2 times faster water velocities and depths in 2016.

Throughout the study, beat sheet, pan trap, benthic, and emergence samples were composed of 80, 139, 35, and 23 families or genera, respectively, and totaled 32,906 individuals. Beat sheet samples were dominated by dictynid spiders, aphids (Aphididae), centipedes (Chilopoda), and mites (Acari). Pan trap samples were also dominated by aphids, in

addition to thrips (Thripidae), leafhoppers (Cicadellidae), dark-winged fungus gnats (Sciaridae), and midges (Chironomidae). Benthic samples were dominated by baetid mayflies and black flies (*Simulium* sp.), and emergence samples were dominated by midges. Across all sites, arthropod biomass falling into streams exceeded emerging biomass by 25% in spring and 50% in summer. Overall, approximately 13% of aquatic-derived insect biomass returned to the stream channel.

Invasion Effects on Arthropod Subsidies

Over the 2-year study, there were differences between locust and reference sites in certain seasons and years, but differences were often not consistent across sampling periods (Fig. 1.2). Beat sheet samples from reference sites contained more than twice the abundance of terrestrial arthropods in the spring ($pseudo-F = 5.55$, $p(MC) = 0.0013$; Fig. 1.2), but not during summer, as indicated by a significant interaction with forest type and season (Table 1.2). Taxa richness in beat sheet samples did not differ between reference and locust sites ($p(MC) = 0.38$). The test for differences in community composition of beat sheet samples revealed a significant 2-way interaction between forest type and year ($pseudo-F = 1.8$, $p(MC) = 0.047$), although no pairwise comparisons were significant ($p(MC) > 0.070$ for 2015 and 2016, Fig. 1.2). No difference in biomass, richness, and community composition of pan trap, emergence, or benthic samples was detected between reference and locust sites. All other significant interactions of forest type with season or year were driven by significant pairwise comparisons of seasons or years—not between reference and locust sites.

Environmental Effects on Resource Subsidies

AICc models identified vegetation and channel characteristics that explained arthropod subsidy patterns better than ground cover or vertical structure of locust. Biomass of arthropods

in pan trap samples increased with vegetative ground cover and decreased with vertical structure of boxelder, but these relationships were relatively weak (Tables 1.3, Appendix 1.3). Richness of arthropods in pan traps was explained by a positive relationship with vegetative ground cover, which occurred in all models (*i.e.*, carried 100% model weight). Arthropod abundance in beat sheet samples was best explained by positive relationships with vertical structure of boxelder and forb cover, and negative relationships with rabbitbrush (*Ericameria nauseosa* (Pall. Ex Pursh)) and vertical structure of locust. Abundance of arthropods in beat sheets was also negatively correlated with richness of vertical vegetation, although richness of vertical vegetation averaged less than 1 at these sites (Tables 1.1, 1.3, Appendix 1.3). Models for richness of arthropods in beat sheet samples were not evaluated because they explained very little variation (adjusted- $R^2 < 1\%$). Emerging insect richness was best explained by terrestrial parameters: negative relationships with grass and forb cover and a positive relationship with ground cover (Tables 1.3, Appendix 1.3). Models for emerging insect biomass were not evaluated because they explained very little variation (adjusted- $R^2 < 5\%$). Benthic insect biomass and richness were the only arthropod metrics best explained by aquatic parameters, such as litter storage, water temperature, and velocity.

DISCUSSION

This study demonstrates the differential effects of an introduced plant on aquatic and terrestrial arthropod communities and their flux between ecosystems. In 3 of the 4 sampling periods we found reduced abundances of terrestrial arthropods collected directly from vegetation in reaches with locust. We believe these differences resulted from seasonal phenology of vegetation during spring sampling periods. In contrast to verdant native

vegetation, locust had not leafed out during our spring sampling. Arthropod communities less directly associated with terrestrial vegetation were less impacted by locust invasion. For instance, arthropods in pan traps at locust sites had lower richness in half of all sampling periods. This weaker effect of locust on terrestrial arthropods collected in pan traps could be explained by the sampling method, which presumably captures insects associated with vegetation, but also those deposited by wind or active movement. Additionally, locust had no effect on larval or emerging aquatic insects. Overall, seasonal and annual variation described arthropod communities and their fluxes better than locust, other vegetation, or channel characteristics. This is consistent with the vegetation and channel characteristics we tested that collectively describe seasonal trends, such as increased vegetative cover and richness in summer compared to spring, and the complex life cycles of arthropods which vary across years.

Although vegetation and channel characteristics identified by our models did not describe arthropod communities as well as seasonal and annual variation, they do provide useful information that can improve riparian management to optimize resource subsidies. Of the predictor variables that carried >50% model weight and had biologically significant coefficients (≥ 0.10), we observed positive relationships with vegetative ground cover and vertical vegetation structure for nearly all response metrics (Tables 1.3, Appendix 1.3). The abundance of terrestrial arthropods was lower in response to locust and rabbitbrush and higher with vertical vegetation structure, ground cover, and forb cover in most models. These results suggest that terrestrial arthropod communities and the subsidies they support will be most productive in riparian forests managed for abundant riparian vegetation and against the encroachment of locust or upland species like rabbitbrush. Furthermore, reaching the adult

phase of the aquatic insect life cycle may depend on the presence of an intact riparian vegetation community. Of the predictor variables that carried >50% model weight and had biologically significant coefficients, ground vegetation, grass cover, and forb cover best explained emergence richness. Grasses and forbs had negative coefficients, suggesting that shrub/tree cover (the remaining growth form) could be an important factor determining insect emergence. The only metrics explained by stream characteristics were benthic insect biomass and richness, indicating larval aquatic insects were most influenced by the local aquatic habitat features.

Averaged across all seasons, we found nearly 2 times higher abundances of arthropods in beat sheets at reference sites. Similarly, Ballard et al. (2013) also found nearly 2 times higher abundances of arthropods in experimental plots of native compared with non-native vegetation. A study of arthropod abundance and richness on native and exotic *Robinia* sp. in Arizona showed higher abundances of most feeding guilds on native *Robinia* sp. (Degomez and Wagner 2001). In contrast to our results, both of these studies reported that native plants supported higher species richness of terrestrial arthropods. We may not have detected a difference in richness because our beat sheet samples were a composite from shrubs or trees rather than a representative sample from a specific native or nonnative plant. Although locust sites had fewer terrestrial arthropods in beat sheets, we did not detect similar differences in pan trap samples. Pan traps and beat sheets appear to sample different guilds of arthropods because only 1 out of 4-5 dominant taxa were shared between these 2 methods. Researchers studying terrestrial arthropod subsidies in riparian reaches invaded by European bird cherry (*Prunus padus* L.) reported that sampling arthropods directly from vegetation and in pan traps

produced similar results: native riparian vegetation supported more than twice the abundance of arthropods (Roon et al. 2016). However, other researchers using pan traps alone have failed to detect arthropod biomass differences between forest types of differing successional phases (Wipfli 1997).

CONCLUSIONS

Our study found that an introduced species reduces abundance of terrestrial arthropods but has no consistent effect on aquatic insects or arthropod subsidies. Therefore, this invasion is unlikely to have strong long-term effects on riparian consumers. However, we observed seasonal and yearly differences between reference and locust sites for some arthropod community metrics. These differences may have short-term influences on riparian consumers or other ecosystem processes. For example, other research has shown that similar short-term pulses of aquatic subsidies influence habitat used by migrant birds (Nakano and Murakami 2001, Murakami and Nakano 2002, Uesugi and Murakami 2007).

Few studies have simultaneously measured aquatic insect emergence and terrestrial arthropod input, and no studies have evaluated these dynamics as a result of riparian species invasion. While this multi-pronged approach is labor intensive, we believe it is an important strategy to identify the potential interacting pathways through which non-native species can influence aquatic and riparian ecosystems. Understanding differential responses of aquatic and terrestrial arthropod communities to ecosystem alteration provides critical insight into the complex and potentially wide reaching ramifications of species invasions.

TABLES

Table 1.1 Mean ($\pm$ SE) values of environmental parameters evaluated in sampling reaches in the Clear Creek drainage of northwestern Colorado, and each parameter's score for the 1st axis of PCA. Bold text indicates factors retained for model selection because they contributed more than average to PC1. Shrub and tree species were tested in model selection if they occurred in >4 plots. Vertical vegetation structure of Douglas fir (*Psuedotsuga menziesii* (Mirb.) Franco), Gambel oak, currant (*Ribes* sp.), and rose (*Rosa* sp.); and ground cover of Saskatoon service berry (*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem), narrowleaf cottonwood, chokecherry (*Prunus virginiana* L.), currant, rose, Gambel oak, and snowberry (*Symphoricarpos albus* (L.) S.F. Blake) are not shown here because they contributed little to PC1.

Environmental parameters	Reference	Locust	PCA 1 st axis scores (variation explained)
<i>Vertical vegetation structure</i>			(28.5%)
Cover (%)	5.3 $\pm$ 0.76	9.4 $\pm$ 1.1	-
Richness	0.63 $\pm$.061	1.0 $\pm$ 0.053	0.51
Boxelder (%)	3.1 $\pm$.70	5.0 $\pm$ 0.83	0.41
Locust (%)	0 $\pm$ 0	1.6 $\pm$ 0.36	0.42
Narrowleaf cottonwood (%)	0.56 $\pm$ 0.18	0.31 $\pm$ 0.18	-0.37
Chokecherry (%)	0.076 $\pm$ 0.030	1.75 $\pm$ 0.46	0.32
<i>Ground cover</i>			(21.2%)
Cover (%)	46.4 $\pm$ 5.1	44.7 $\pm$ 6.4	0.46
Richness	5.6 $\pm$ 0.28	5.9 $\pm$ 0.39	0.38
Non-native (%)^a	4.6 $\pm$ 1.2	12.4 $\pm$ 3.0	0.36
Bare ground (%)	11.0 $\pm$ 2.2	17.5 $\pm$ 3.0	-0.24
Grasses (%)	12.5 $\pm$ 2.6	13.3 $\pm$ 2.9	0.32
Forbs (%)	12.9 $\pm$ 2.1	20.5 $\pm$ 4.0	0.40
Shrubs/Trees (%)	23.6 $\pm$ 3.5	11.2 $\pm$ 1.5	0.18
Boxelder (%)	3.1 $\pm$ 1.4	5.2 $\pm$ 1.2	0.011
Locust (%)	0 $\pm$ 0	1.5 $\pm$ 0.5	0.18
Rabbitbrush (%)	0.18 $\pm$ 0.071	0.45 $\pm$ 0.32	0.20

(Table 1.1 continued from previous page.)

<i>Stream characteristics</i>			(21.7%)
Thalweg Depth (cm)	21.1 ± 1.2	26.2 ± 1.3	-0.40
Wetted width (m)	1.9 ± 0.10	3.1 ± 0.11	-0.46
Bankfull width (m)	4.0 ± 0.26	4.7 ± 0.17	-0.36
Velocity (m/s)	0.59 ± 0.07	0.80 ± 0.11	0.25
pH	8.5 ± 0.14	8.4 ± 0.12	0.11
D.O. (mg/L)	8.6 ± 0.29	8.4 ± 0.25	0.17
Temperature (°C)	12.2 ± 1.2	12.4 ± 1.1	0.23
Specific Conductivity (µm)	615 ± 17	548 ± 33	0.17
Canopy Cover (%)	71.1 ± 2.3	82.7 ± 2.1	-0.049
Substrate (D ₅₀ mm)	7.4 ± 0.56	8.8 ± 1.10	0.058
Sum Bank Angle (°)	66.3 ± 2.1	66.4 ± 2.0	-0.14
Slope (%)	5.4 ± 1.1	2.6 ± 0.72	-0.22
Litter inputs (g m ⁻² d ⁻¹)	0.83 ± 0.16	0.75 ± 0.11	0.20
Litter storage (g m⁻²)	6.2 ± 0.92	6.6 ± 1.0	-0.26

^a See Appendix 1.2 for listing information at the time of research.

Table 1.2 The results of repeated measures PERMANOVA analyses comparing aquatic and terrestrial arthropod biomass, richness, and multivariate community composition sampled from stream reaches in the Clear Creek drainage of northwestern Colorado. Results are stratified by sampling method: pan trap and beat sheet samples capture primarily terrestrial arthropod taxa, and emergence and benthic samples capture aquatic insect taxa. Abundance was analyzed instead of biomass for beat sheet sampling because this is a semi-quantitative method. Factors in the models include forest type (reference or locust), season (spring or summer), year (2015 and 2016), interactions with forest type, and a random effect of site nested in forest type. Bold text highlights significant results with Monte Carlo p-values <0.05.

		Forest type			Season		Year		Forest type × season		Forest type × year		Site(forest type)	
Pan trap	Adj R ²	Pseudo-F _{1,6}	p(MC)	Pseudo-F _{1,177}	p(MC)	Pseudo-F _{1,177}	p(MC)	Pseudo-F _{1,177}	p(MC)	Pseudo-F _{1,177}	p(MC)	Pseudo-F _{6,177}	p(MC)	
Community	0.73	1.45	0.11	34.55	0.0001	8.67	0.0001	1.61	0.039	1.21	0.23	2.08	0.0001	
Richness	0.62	0.28	0.63	78.32	0.0001	0.82	0.37	0.091	0.76	6.19	0.014	2.75	0.015	
Biomass	0.84	1.16	0.33	16.62	0.0001	2.49	0.12	2.07	0.16	2.36	0.13	0.71	0.64	
Beat sheet	Adj R ²	Pseudo-F _{1,6}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{6,84}	p(MC)	
Community	0.68	1.64	0.11	12.09	0.0001	5.74	0.0001	1.69	0.064	1.8	0.047	1.97	0.0002	
Richness	0.36	0.90	0.38	97.09	0.0001	5.33	0.023	0.65	0.43	1.12	0.30	2.56	0.022	
Abundance	0.51	4.84	0.07	39.30	0.0001	0.053	0.81	4.85	0.033	2.66	0.11	2.1	0.066	
Emergence	Adj R ²	Pseudo-F _{1,6}	p(MC)	Pseudo-F _{1,176}	p(MC)	Pseudo-F _{1,176}	p(MC)	Pseudo-F _{1,176}	p(MC)	Pseudo-F _{1,176}	p(MC)	Pseudo-F _{6,176}	p(MC)	
Community	0.79	1.44	0.24	6.79	0.0001	12.59	0.0001	1.95	0.098	0.78	0.54	2.8	0.0005	
Richness	0.89	0.43	0.54	5.09	0.022	1.36	0.25	0.000055	0.99	0.099	0.75	2.45	0.028	
Biomass	0.97	1.78	0.23	0.054	0.82	0.26	0.60	0.21	0.64	0.43	0.51	0.68	0.66	
Benthic	Adj R ²	Pseudo-F _{1,6}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{1,84}	p(MC)	Pseudo-F _{6,84}	p(MC)	
Community	0.60	1.33	0.23	15.55	0.0001	6.62	0.0001	0.64	0.75	1.53	0.13	3.28	0.0001	
Richness	0.55	1.14	0.33	35.40	0.0001	16.16	0.0001	0.21	0.65	1.63	0.20	1.1	0.37	
Biomass	0.83	0.065	0.81	0.18	0.67	4.17	0.046	1.66	0.20	4.21	0.042	0.92	0.48	

Table 1.3 Variables selected using AICc model selection to explain biomass and richness of aquatic and terrestrial arthropod sampled in the Clear Creek drainage of northwest Colorado. Abundance was analyzed in place of biomass for beat sheet sampling because this is a semi-quantitative method. Cumulative weight describes each variable's probability of being selected in a model (*e.g.*, "1" is the highest possible weight and indicates the variable was selected in all models within 2 Δ AICc of the top model). Variables are sorted by their cumulative weight, asterisks denote significant variables, and bold text highlights significant variables with absolute coefficients ≥ 0.1 . See Appendix 1.3 for model details and adjusted-R² and AICc values.

Individual variables	Cumulative Weight	Top model β	β (SE)	95% Confidence limits	
<i>Pan trap biomass</i>					
Vegetation cover*	0.92	0.011	0.005	0.001	0.022
Boxelder structure*	0.86	-0.010	0.004	-0.019	-0.001
Vegetation richness	0.76	0.007	0.006	-0.005	0.018
Grass cover*	0.61	0.007	0.005	0.048	0.064
Cottonwood structure	0.43	0.005	0.004	-0.003	0.014
Locust structure*	0.27	0.013	0.006	0.001	0.025
Chokecherry structure	0.21	0.004	0.004	-0.004	0.013
Bare ground cover	0.16	0.005	0.004	-0.004	0.013
Forb cover*	0.15	0.013	0.004	0.005	0.022
Structural richness*	0.14	-0.012	0.006	-0.023	-0.001
Non-native cover	0.077	-0.009	0.006	-0.021	0.002
<i>Pan trap richness</i>					
Forb cover	1	-0.091	0.061	-0.21	0.030
Non-native cover	1	-0.035	0.051	-0.13	0.065
Vegetation cover*	1	0.22	0.055	0.11	0.33
Boxelder structure	0.37	-0.021	0.042	-0.10	0.061
Cottonwood structure	0.37	-0.009	0.044	-0.10	0.077
Structural richness	0.34	0.045	0.043	-0.040	0.13
<i>Beat sheet abundance</i>					
Boxelder structure*	1	0.32	0.16	0.01	0.64
Rabbitbrush cover*	1	-0.40	0.17	-0.73	-0.06
Forb cover*	1	0.80	0.25	0.31	1.30
Locust structure*	0.61	-0.60	0.19	-0.97	-0.23
Vegetation cover	0.55	0.40	0.21	-0.03	0.82
Structural richness*	0.49	-0.60	0.20	-1.00	-0.20
Cottonwood structure	0.38	0.25	0.16	-0.08	0.58
Non-native cover	0.31	-0.36	0.20	-0.75	0.036
Grass cover	0.28	0.26	0.16	-0.059	0.58

(Table 1.3 continued from previous page.)

<i>Emergence richness</i>					
Thalweg depth	1	-0.13	0.074	-0.28	0.013
Forb cover*	1	-0.18	0.058	-0.30	-0.068
Grass cover*	1	-0.14	0.059	-0.26	-0.022
Vegetation cover*	1	0.33	0.069	0.19	0.46
Velocity	1	0.084	0.054	-0.023	0.19
Wetted width	1	0.11	0.058	0.00	0.23
Vegetation richness	0.7	0.089	0.054	-0.018	0.20
Bare ground cover	0.33	0.060	0.044	-0.027	0.15
Non-native cover	0.25	-0.090	0.057	-0.202	0.022
Chokecherry structure	0.2	0.067	0.041	-0.014	0.15
Rabbitbrush structure	0.15	0.052	0.042	-0.032	0.14
<i>Benthic biomass</i>					
Bankfull Width*	1	-0.056	0.021	-0.10	-0.013
Litter storage*	1	0.075	0.022	0.03	0.12
<i>Benthic richness</i>					
Bankfull Width*	1	-1.37	0.45	-2.26	-0.49
Litter storage*	1	1.97	0.44	1.10	2.84
Water temperature*	1	1.44	0.41	0.62	2.27
Velocity*	1	-1.47	0.44	-2.36	-0.59
Bare ground cover	0.69	0.68	0.44	-0.19	1.55
Grass cover	0.31	-0.79	0.48	-1.74	0.17
Vegetation richness	0.31	0.69	0.49	-0.28	1.66

FIGURES

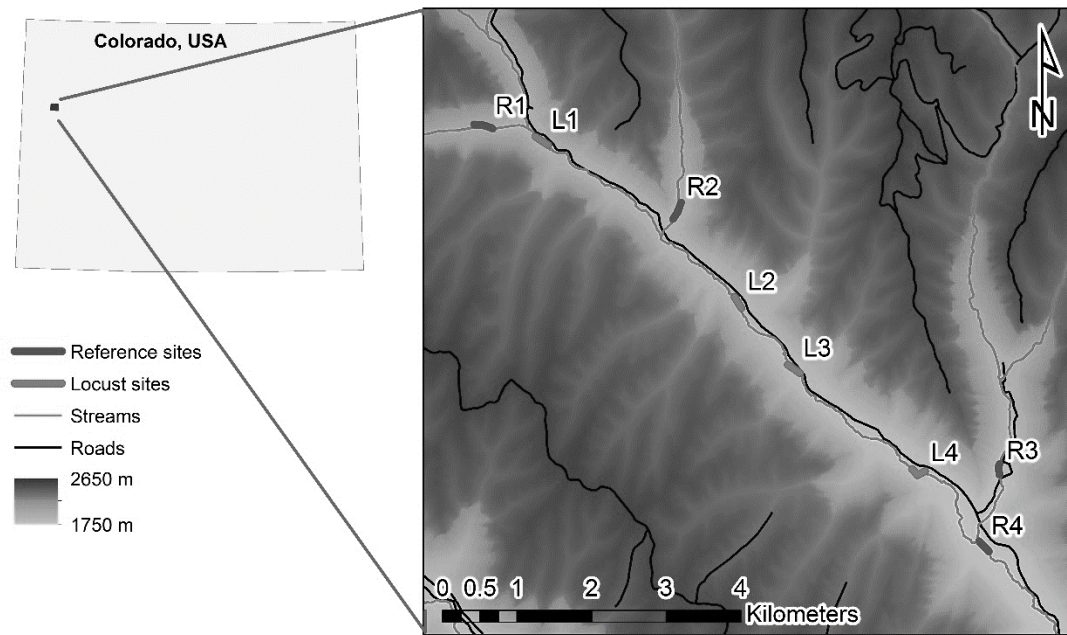


Figure 1.1 A map of the study area showing the 4 sampling reaches uninhabited by locust (reference, R) and 4 reaches invaded by locust (L) in the Clear Creek drainage, and the location of the study in Colorado.

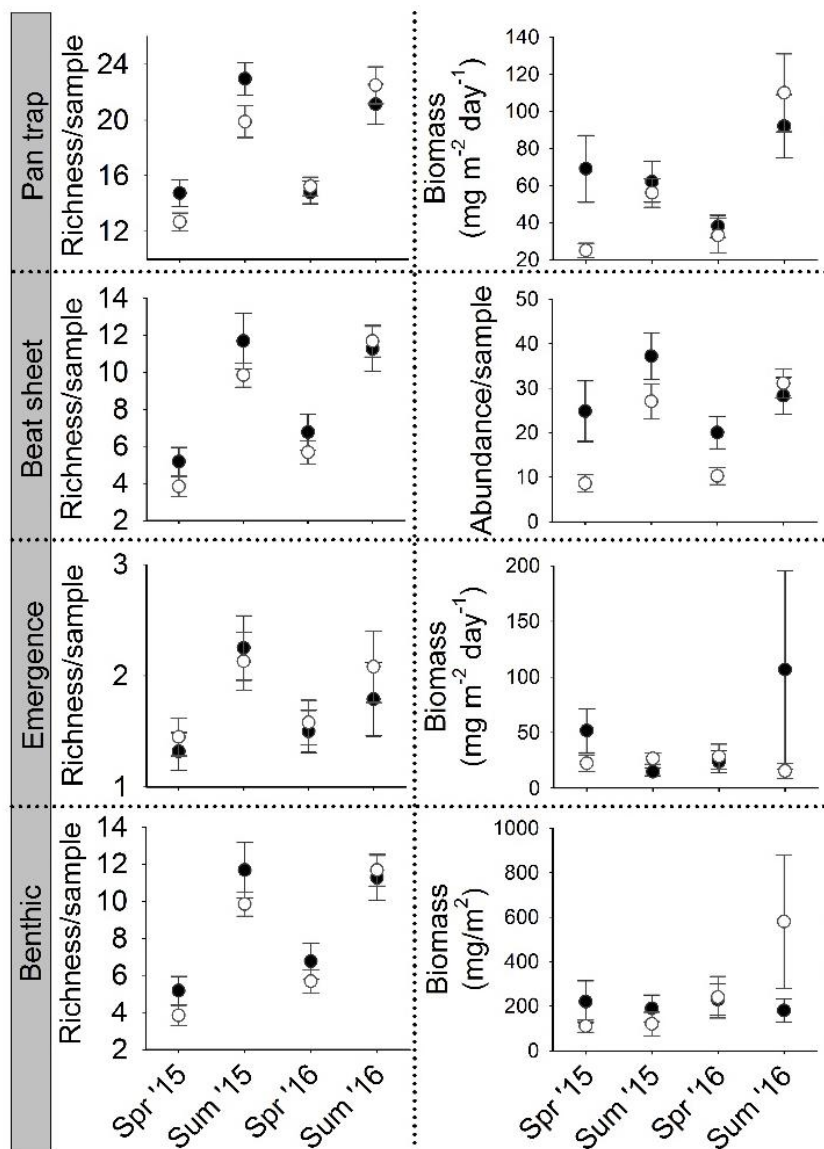


Figure 1.2 Mean ($\pm$ SE) values of richness (left column) and biomass or abundance (right column) of arthropod samples collected from sites invaded (open circles) and uninvaded (filled circles) by locust in northwest Colorado, during spring and summer sampling periods in 2015-2016. Pan traps and beat sheets captured primarily terrestrial arthropod taxa (top 2 rows), while emergence and benthic samples captured aquatic insect taxa (bottom 2 rows).

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CHAPTER TWO

AN INTRODUCED PLANT AFFECTS AQUATIC-DERIVED CARBON IN THE DIETS OF RIPARIAN BIRDS²

INTRODUCTION

Invasive species impact ecosystem structure and function (Ehrenfeld 2010) and lead to biotic homogenization of communities (Olden et al. 2004). Characteristics related to the life history, physiology and chemistry of invasive plants can drive fundamental shifts in primary production, nutrient cycling, water usage, and decomposition (Strong et al. 1984, Tallamy 2004, Ehrenfeld 2010, Hladysz et al. 2011). Recent syntheses have attempted to identify patterns in the mechanisms and consequences of invasion across diverse ecosystems, taxa and levels of ecological complexity (Vilà et al. 2011, Pyšek et al. 2012, Schirmel et al. 2016). However, few universal trends emerged from these assessments; rather, the effects of introduced plants appear to be highly context-specific, varying in direction and magnitude across ecosystems, taxa, and functional traits (Vilà et al. 2011, Pyšek et al. 2012, Schirmel et al. 2016). Equally apparent, studies tend to focus on a single ecosystem function and fail to address the interacting and potentially reinforcing mechanisms underlying invasion-driven ecosystem change (Levine et al. 2003). Resource subsidies, or fluxes of resources between ecosystems, can provide insight into the multiple ways invasive species alter biological communities (*e.g.*, Benjamin et al. 2011, Mineau et al. 2012).

² Co-authored with Lani Stinson, MS Candidate, Department of Fish, Wildlife and Conservation Biology, Colorado State University

Riparian plant and animal communities are particularly susceptible to invasion, which can lead to impacts on ecosystem services and human well-being (Dudgeon et al. 2006, Tockner et al. 2010). New assemblages of taxa, driven by species invasions and climate change (Hellmann et al. 2008, Walther et al. 2009, Cardinale et al. 2012) can alter the timing, duration, and magnitude of aquatic and terrestrial arthropod subsidies, and these factors can interact to decouple cross-ecosystem subsidies (Larsen et al. 2016). Changes in the riparian forest canopy, for example, can affect aquatic insect communities by altering litter input quality and quantity, canopy openness, and algal communities (Hladyz et al. 2011, Kominoski et al. 2013). Furthermore, compared to native vegetation, non-native plants often support decreased terrestrial arthropod abundance, biomass and richness (Simao et al. 2010, Litt et al. 2014). Changes in the relative availability of aquatic and terrestrial insects have potential to cascade through food webs, with bottom-up impacts on riparian insectivorous birds (Marczak et al. 2007, Hladyz et al. 2011).

Riparian bird assemblages are likely to be sensitive to invasion-mediated changes in resource subsidies because many insectivorous species consume aquatic prey. Aquatic insects can contribute substantially to avian insectivore diets, and some species are entirely dependent on aquatic food resources during certain times of year (Nakano and Murakami 2001, Uesugi and Murakami 2007). Differences in the diet composition (*i.e.*, proportion of aquatic vs. terrestrial prey) of birds using riparian habitats dominated by either native or introduced plants may reflect invasion-mediated diet shifts, with potential consequences for the health and persistence of diverse riparian bird communities (Murakami and Nakano 2001). Yet, despite the susceptibility of riparian areas to invasive species (Richardson et al. 2007), few studies have

addressed the potential effects of plant invasion on arthropod subsidies provided to avian consumers.

This study evaluates the contribution of aquatic-derived carbon to insectivorous songbird diets, and how an introduced plant, New Mexico locust (*Robinia neomexicana* A. Gray; family: Fabaceae), might alter the aquatic- and terrestrial-derived diet components of these riparian birds. New Mexico locust is native to the southwestern United States and extends into portions of southern Colorado (Little 1976, Pavek 1993). This species was introduced >100 years ago to an area north of its native range in the Clear Creek drainage of the Piceance Basin of northwestern Colorado, USA (Fig 2.1), where it has become well-established and dominant in some reaches of the watershed. Functional traits, such as rhizomatous growth and the ability to fix nitrogen, likely make New Mexico locust a successful pioneer species. Landowners have attempted to remove the plant, with no sustained success in limiting or reducing spread (C. Tysse, personal communication).

To determine whether plant invasion affected riparian consumers via altered resource subsidies, we compared the diets of insectivorous songbirds captured from reference sites and sites invaded by New Mexico locust using stable isotope analysis (SIA) of fecal samples. We predicted that aquatic insects would contribute to the diet of riparian songbirds, but that reliance on this resource subsidy would vary among species (Murakami and Nakano 2001, Yard et al. 2004, Uesugi and Murakami 2007). Specifically, we hypothesized that diets of strict insectivores would have higher proportions of aquatic-derived prey than more omnivorous species. Non-native vegetation often supports depauperate terrestrial arthropod communities compared to native plants (Riedl et al. *in prep*, Simao et al. 2010, Litt et al. 2014), and songbirds

often forage in proportion to prey availability (Busby and Sealy 1979, Howe et al. 2000). Therefore, we predicted that bird fecal samples in invaded sites would have $\delta^{13}\text{C}$ more similar to aquatic-derived isotope signatures, indicating increased reliance on aquatic insects. In addition, non-native vegetation invasions often disproportionately reduce higher trophic level arthropods compared to lower trophic level taxa (Simao et al. 2010, Ballard et al. 2013). Thus, we hypothesized that fecal samples of birds in invaded sites would be less enriched in $\delta^{15}\text{N}$ because of reduced populations of predaceous or parasitic arthropods.

METHODS

Study Area

This study took place in the Clear Creek drainage of northwestern Colorado, USA, located in Garfield County ~60 km northeast of Grand Junction on privately owned lands. The area has undergone oil and gas development, with oil pads and other infrastructure near the riparian zone, including a gravel access road paralleling the main stem of Clear Creek. The landscape is topographically diverse (1500-2700 m elevation) and is characterized by high mesas and steep canyons surrounding the 1st and 2nd order streams of Clear Creek and tributaries. The riparian corridor within the study area averaged 49 ± 8 m SE in width and was dominated by native trees including boxelder (*Acer negundo*), cottonwood (*Populus angustifolia*), and Gambel oak (*Quercus gambelii*), as well as New Mexico locust in invaded areas. The shrub layer consisted of Saskatoon serviceberry (*Amelanchier alnifolia* (Nutt.) Nutt. Ex M. Roem.), big sagebrush (*Artemisia tridentata*), rabbitbrush (*Chrysothamnus* sp.), snowberry (*Symphoricarpos* sp.), chokecherry (*Prunus virginiana*), currant (*Ribes* sp.), and rose (*Rosa woodsii* Lindl.).

Sampling Design

After reconnaissance mapping of stream reaches uninvaded and invaded by New Mexico locust (hereafter, “reference” and “locust”), we established eight 180 m sampling sites within reference and locust reaches, spaced >300 m apart and paired by elevation (Fig. 2.1). Streams within study reaches are relatively narrow (2.3 ± 1.1 m SE), of moderate gradient ($2.2 \pm 1.7\%$ SE, measured from four GPS coordinate measurements), with fine silts and small gravel typical in oil shale geology of the region.

Insect Sampling

Aquatic and terrestrial insects in reference and locust sites were sampled by deploying emergence and pan traps on the upstream, middle, and downstream transect of each sampling reach. We placed floating emergence nets (0.3 m^2) on the water surface to capture adult aquatic insects emerging from the stream channel (Cadmus et al. 2016). We placed pan traps (0.4 m^2) over the water surface to capture adult aquatic insects and terrestrial arthropods falling into the stream. The pan traps were filled 5 cm deep with stream water and approximately 5 mL of biodegradable surfactant was added to reduce surface tension (Wipfli 1997). Both trap types were deployed simultaneously for 48 hr (2015: 21 – 22 July; 2016: 30 – 31 July) and collected insects were preserved in 70% ethanol.

We enumerated and identified all insects collected to family using taxonomic keys (Merritt et al. 1996, Triplehorn and Johnson 2005). We selected dominant herbivorous terrestrial and aquatic insects based on mean percent of individuals in pan traps or emergence nets pooled across years. Dominant herbivorous terrestrial taxa included leafhoppers (Cicadellidae; 9.6%), caterpillars (Lepidopteran larvae; 5.8%) and aphids (Aphididae; 0.44%).

Emergent aquatic insect richness averaged only 2 taxa per sample (a maximum of 6 taxa in one sample) throughout the study, so we considered all functional feeding groups except shredders as candidates for isotope analysis (*i.e.*, filter feeders, collector-gatherers, and algae grazers). Shredders were excluded because they feed on terrestrial leaf litter inputs, and thus would have similar $\delta^{13}\text{C}$ signatures to terrestrial-derived insects. Dominant aquatic taxa in emergence nets included midges (Chironomidae: 57.5%), black flies (Simuliidae: 7.7%, 2015), heptageniid mayflies (7.5%, 2016), and baetid mayflies (6.1%). Heptageniid mayflies were substituted for black flies in 2016 because only one black fly occurred in all samples in 2016.

Songbird Fecal Sampling

Mist-netting of insectivorous songbirds to collect fecal samples for SIA was undertaken in late summer of each year (2015: 11 July–5 August; 2016: 17 July–6 August), corresponding with the time that insects were sampled. We sampled late in the songbird breeding season to minimize disturbance to nesting pairs, and allow capture of adults and fledged juveniles prior to migration. Within each of the eight sampling sites, we set up 4-7 mist nets (38-mm mesh, 6-12 m), placed along habitat edges and bisecting the riparian corridor. We opened nets for 2-4 days per site during the morning hours when weather conditions allowed safe capture and extraction of songbirds; nets were closed during times of rain or high wind. For each captured bird, we recorded the species, mass (g), fat score (0 to 3), and age class (hatch-year (HY) or after-hatch-year (AHY)). We used passive methods (placement in cloth bags) to collect fecal samples and stored samples in 70% alcohol. Bird feces contain insects ingested within a few hours before capture, making them ideal for examining diet changes over small spatial and short time scales (Salvarina et al. 2013). Using feces for dietary analysis is also a less invasive

alternative to stomach lavage or tissue sampling (Podlesak et al. 2005, Painter et al. 2009, Salvarina et al. 2013).

Stable Isotope Processing

Naturally abundant isotopes of carbon (^{13}C) can be used to track time-integrated contributions of aquatic- and terrestrial-derived energy through food webs (Ben-David and Flaherty 2012). Primary producers in aquatic and terrestrial ecosystems often have distinct $\delta^{13}\text{C}$ values because of variation in plant physiology and resource availability, and these tracers exhibit little isotopic fractionation during trophic transfer (DeNiro and Epstein 1978, Rounick and Winterbourn 1986). Additionally, isotopes of nitrogen (^{15}N) can be used to track differences in food web structure because consumers typically become enriched in ^{15}N with increasing trophic position (DeNiro and Epstein 1981, Vander Zanden et al. 1999).

Fecal and insect samples were dried at 60°C for 48 hr, homogenized, and weighed to a precision of 0.001 mg into 4 x 6 mm cylindrical tin capsules. Stable isotopes were measured at the Natural Resource Ecology Laboratory (Colorado State University) using a Carlo Erba NA 1500 (Milan, Italy) coupled with a VG Isochrom continuous flow isotope ratio mass spectrometer (Isoprime Inc., Manchester, UK) to simultaneously determine nitrogen and carbon isotope composition. Ratios of the heavy isotope to its common lighter counterpart (*i.e.*, $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) were expressed in standard δ -notation relative to international standards (Vienna Pee Dee Belemnite and atmospheric nitrogen, respectively) in parts per mil (‰). For instance, $\delta^{13}\text{C}_{\text{sample}} = [({}^{13}\text{C}_{\text{sample}}/{}^{12}\text{C}_{\text{sample}})/({}^{13}\text{C}_{\text{standard}}/{}^{12}\text{C}_{\text{standard}})-1] \times 1000$, and likewise for $\delta^{15}\text{N}$. Analytical precision from multiple in-house runs was 0.2 ‰ for $\delta^{13}\text{C}$ and 0.3 ‰ for $\delta^{15}\text{N}$ (Fry 2007).

Statistical Analyses

Aquatic-Derived Carbon in Songbird Diets. – To identify the relative contributions of aquatic- and terrestrial-derived prey in the diets of songbirds, we used $\delta^{13}\text{C}$ of fecal and insect samples in a single-isotope mixing formula (Fry 2007). SIA of insect samples provide context for $\delta^{13}\text{C}$ shifts in bird diets, and provide terms in the mixing formula used to calculate the proportions of aquatic and terrestrial diet components for each fecal sample. First, we tested for differences in $\delta^{13}\text{C}$ of insect samples to determine appropriate groupings for calculation of diet sources. $\delta^{13}\text{C}$ of insect samples were evaluated using two-way analysis of variance (ANOVA) by year with $\delta^{13}\text{C}$ as the dependent variable, and site type (two levels, fixed effect), species (six levels, fixed effect), and the interaction as independent factors in the model. ANOVA was conducted using SAS v9.3 (SAS Institute, Cary, North Carolina) PROC MIXED. For both 2015 and 2016, $\delta^{13}\text{C}$ isotopic signatures of insect samples were not statistically different between site types ($p > 0.15$), although there was significant species-specific variation ($p < 0.02$, Appendix 2.1). Therefore, $\delta^{13}\text{C}$ signatures of aquatic and terrestrial insects were determined as an average of the three dominant aquatic and terrestrial taxa, respectively, across all sites for each year.

Next, we used a mixing formula to identify the relative proportions of aquatic- and terrestrial-derived insects in songbird diets (Fry 2007):

$$p_1 = (\delta_{\text{sample}} - \delta_{\text{source},2}) / (\delta_{\text{source},1} - \delta_{\text{source},2}) \text{ and}$$

$$p_2 = 1 - p_1$$

where δ_{sample} is the $\delta^{13}\text{C}$ value of each fecal sample, p_i is the proportion of aquatic or terrestrial diet sources, and $\delta_{\text{source},i}$ is the average $\delta^{13}\text{C}$ for each diet source (Fry 2007). In instances of a

“mixing muddle” (*sensu* Fry 2007), where the fecal sample occurred outside the range characterized by aquatic and terrestrial insect isotope signatures, we classified the sample as composed entirely of the diet source the sample most closely resembled.

Because we selected dominant insects feeding primarily on aquatic- and terrestrial-derived primary producers, our source samples did not reflect signatures of higher trophic level arthropods (*e.g.*, predaceous spiders, parasitic wasps). Additionally, inclusion of filter-feeding aquatic insects as candidates for SIA could bias the aquatic-derived signature towards $\delta^{15}\text{N}$ enrichment since this feeding guild incidentally ingests animal parts. Thus, we did not use $\delta^{15}\text{N}$ of insect samples to make inference about trophic position of songbird diets (Appendix 2.1).

Invasion-Mediated Diet Shifts. – We conducted species-specific multivariate analyses to examine invasion-mediated diet shifts, which we defined as differences in songbird diet isotope signatures between reference and locust sites. We analyzed fecal samples from seven songbird species with sufficient sample sizes ($n \geq 2$ samples per site type and year), including five strict insectivores and two omnivorous species whose diets are dominated by insects during the breeding season (Table 2.1). We considered the two species of flycatchers, Cordilleran flycatcher (*Empidonax occidentalis* Nelson) and dusky flycatcher (*Empidonax oberholseri* Phillips), as a single unit (*Empidonax* flycatcher). Analyses were conducted separately for each year to account for known annual variation in arthropod communities (Riedl et al. *in prep*).

We tested for songbird diet shifts between reference and locust sites using one-way MANOVAs with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as dependent variables and site type as the independent variable. All MANOVAs were conducted with SAS PROC GLM. Isotope data were normally distributed, and Satterthwaite degrees of freedom were used to correct for unequal variance

where necessary. F-values from MANOVAs are reported from Wilks' Lambda criteria. ANOVAs were considered to determine whether differences were driven by $\delta^{13}\text{C}$ (diet source) or $\delta^{15}\text{N}$ (diet position). We considered MANOVA or ANOVA model results statistically significant for $p < 0.05$.

We used songbird characteristics associated with the individual birds that provided each fecal sample, including mass, fat score, and age class, to assess potential correlations with diet shifts. For species with sufficient sample sizes ($n \geq 2$ per site and year combination), we tested for differences in mean mass and fat scores between site types using two-tailed Welch's t-tests and non-parametric Wilcoxon rank sum tests, respectively. In addition, where there were significant differences in these body condition measures between site types ($p < 0.05$), we examined the age class composition of samples (HY: AHY ratio).

RESULTS

Aquatic-Derived Carbon in Songbird Diets

We collected and analyzed isotopic signatures of 133 fecal samples from seven species of insectivorous songbirds in reference and locust sites (Table 2.1). Overall, the songbird community consumed $34 \pm 3\%$ SE aquatic-derived carbon throughout the study, with no difference between years or site types (Fig. 2.2, Appendix 2.2). The total contribution (across both years) of aquatic-derived carbon to insectivore diets varied among species, ranging from 18% for MacGillivray's warblers (*Geothlypis tolmiei* Townsend) to 64% for green-tailed towhees (*Pipilo chlorurus* Audubon) (Appendix 2.2).

Insects collected from aquatic systems were more enriched in $\delta^{13}\text{C}$ than their terrestrial counterparts (*i.e.*, less negative $\delta^{13}\text{C}$ isotopic signatures). While the overall contribution of

aquatic-derived insects to the diet of the songbird assemblage was similar between years, species-specific values were frequently different (Fig. 2.2, Appendix 2.2). For example, black-capped chickadees (*Poecile atricapillus* L.) consumed mostly aquatic carbon in 2015, and mostly terrestrial carbon in 2016, and these trends were opposite for MacGillivray's warblers (*Geothlypis tolmiei* Townsend). During both years, however, *Empidonax* flycatchers, warbling vireos (*Vireo gilvus* Vieillot), and yellow warblers (*Setophaga petechia* L.) consumed more terrestrial carbon, and green-tailed towhees consumed more aquatic carbon.

Invasion-Mediated Diet Shifts

General diet shift patterns varied among songbird species and between years, but all significant diet shifts were driven by diet source ($\delta^{13}\text{C}$), which reflects differences in the relative reliance on aquatic- and terrestrial-derived prey resources in reference and locust sites. No differences in $\delta^{15}\text{N}$ signatures were detected for any species evaluated, indicating diet shifts were not driven by trophic position or altered populations of predaceous or parasitic arthropods in locust sites. Of the seven species evaluated, Virginia's warblers (*Leiothlypis virginiae* Baird) showed a significant diet shift towards aquatic-derived carbon in 2015 ($p = 0.021$, Appendix 2.3), and warbling vireos showed a significant diet shift towards aquatic-derived carbon in 2016 ($p = 0.023$; Fig. 2.3, Appendix 2.3). In contrast, *Empidonax* flycatchers showed a significant diet shift that trended towards terrestrially-derived carbon and less enriched $\delta^{15}\text{N}$ in 2016 ($p = 0.002$, Fig. 2.3, Appendix 2.3), but it's unclear if this diet shift was driven by $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$. Analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ independently revealed diet shifts were not statistically different. This discrepancy is likely because multivariate approaches test for differences in the combined effect of dependent variables and therefore can detect differences

too slight for univariate analyses. Yellow warblers and green-tailed towhees showed no diet shifts consistently across years, and MacGillivray's warblers and black-capped chickadees showed no diet shifts in the single years they were evaluated (Appendix 2.3).

Of the seven species for which we investigated body condition differences between reference and locust sites, there were no significant differences in fat scores but body mass differed for two species (Appendix 2.4). Yellow warblers and *Empidonax* flycatchers in reference sites had lower body mass than birds using locust sites, but these patterns were only found in one year for each species (Welch's t-tests, 2015 yellow warblers: $p = 0.036$; 2016 *Empidonax* flycatchers: $p = 0.030$; Appendix 2.4). Examination of age class ratios uncovered that mist nets captured only hatch-year Yellow warblers from reference sites in 2015 (6HY:0AHY), and only after-hatch-year *Empidonax* flycatchers from locust sites in 2016 (0HY:4AHY).

DISCUSSION

Aquatic-derived prey contributed substantially to the diets of avian insectivores in our study, yet reliance on this subsidy varied greatly among species. New Mexico locust invasion was associated with diet shifts toward aquatic-derived insects for Virginia's warblers and warbling vireos. Diet patterns varied among other species, and for species with sufficient sample sizes in both years, the invasion did not appear to have consistent effects across years. Thus, we found mixed support for our hypothesis that insectivores using locust habitats would rely more on aquatic resources, with no evidence that birds using locust habitats would have fecal signatures less enriched in $\delta^{15}\text{N}$ due to reduced abundances of predacious and parasitic arthropods.

Our results suggest the insectivorous bird assemblage in the Clear Creek drainage relies on approximately one-third aquatic insects and two-thirds terrestrial insects during summer months. This finding highlights the importance of maintaining aquatic ecosystem functioning when making land management decisions. Our estimate of the aquatic insect contribution to bird diets is somewhat higher than other published estimates. Along the Colorado River in Arizona, the insectivorous bird community consumed only 9% aquatic insects during summer months (Yard et al. 2004). In riparian forests in Japan, aquatic prey consumed in summer by a diverse bird assemblage averaged 6%, with flycatchers and warblers consuming the highest percentages of aquatic arthropods (range: 0 – 29%, n=18 species; Uesugi and Murakami, 2007). However, community-level estimates are strongly influenced by the species compositions and foraging habits of songbirds included in an assemblage.

We found large variation in diet compositions among insectivorous bird species as well as between years for individual species. However, in both years, green-tailed towhees consumed more aquatic carbon than other species in our assemblage (Fig. 2.2). This is surprising considering their ground foraging behavior and more omnivorous feeding habits compared to the other stricter insectivores (Dobbs et al. 2012). If green-tailed towhees were transitioning to a more omnivorous diet at the end of their breeding season, we likely did not include all potential food resources (*i.e.*, seeds and fruits) for isotopic analysis. Black-capped chickadee, the other species that transitions to an omnivorous diet after the breeding season, displayed higher aquatic prey consumption in 2015, but higher terrestrial prey consumption in 2016. Differences in timing of shifts to omnivory in late summer could help explain inter-annual variation in diet compositions for this species. Similar to our findings, utilization of aquatic

subsidies has been found to vary greatly among species in other studies (Nakano and Murakami, 2001; Uesugi and Murakami, 2007; Yard et al., 2004). For example, yellow warbler diets examined by Yard et al. (2004) contained a higher mean percentage of aquatic arthropods (16%) than the other five insectivores examined, and reliance on different insect taxa among species suggested resource partitioning within the insectivore assemblage.

Significant shifts in Virginia's warbler and warbling vireo diets, driven by $\delta^{13}\text{C}$, support our hypothesis that birds would rely more on aquatic insects at locust sites (Appendix 2.3, Fig. 2.3). These species are foliage gleaners that might be more impacted by the reductions in terrestrial insects often associated with invasion (DeGraaf et al. 1985, Simao et al. 2010, Riedl et al. *in prep*, Litt et al. 2014). We found a significant shift in *Empidonax* flycatcher diets that trended towards more terrestrial-derived and lower trophic level prey; however, analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ independently could not account for this difference. Regardless, diet shifts for this feeding guild are consistent with previous research. In a meta-analysis evaluating the effects of subsidy alteration on riparian birds, Marczak et al. (2007) found aerial insectivores displayed the largest mean effect sizes compared to other foraging guilds (*e.g.*, foliage and ground insectivores). Although we detected no significant diet shifts associated with trophic position (*i.e.*, driven by $\delta^{15}\text{N}$) for all species evaluated, this is consistent with findings of Riedl et al. (*in prep*), which found no significant difference in the abundance of predaceous or parasitic arthropods between sites.

Our analysis of body condition showed evidence of higher body mass of yellow warblers and *Empidonax* flycatchers foraging in locust habitats (Appendix 2.4), suggesting that differences in diet do not necessarily translate into fitness consequences. However, age class

compositions of songbirds sampled from reference and locust sites may provide some explanation for the body condition patterns found in our study. Additionally, differences in songbird reproductive success in reference and locust habitats might better indicate potential fitness effects of invasion (Stinson and Pejchar, *in prep*), but these metrics were beyond the scope of our study. Previous work comparing 12 measures of body condition in southwestern willow flycatchers (*Empidonax traillii extimus* Phillips) using native and non-native *Tamarisk* habitats similarly found little support for negative physiological effects associated with invasion (Owen et al. 2005). In general, few studies have rigorously addressed whether invasion-mediated diet shifts affect fitness and further research is warranted to better understand potential fitness consequences of invasion across different species and ecological contexts (Stinson and Pejchar, *in review*).

We assumed that insectivorous songbirds consume prey in proportion to what is available, exhibiting prey switching in response to reductions in preferred prey resources (Busby and Sealy 1979, Howe et al. 2000, Uesugi and Murakami 2007). Therefore, alterations to the availability of insects from different sources or trophic levels should translate into shifts in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively. Because we did not detect differences in insect $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ signatures between sites, diet shifts likely did not result from invasion-mediated changes to prey signatures. It is possible, however, that birds modify foraging strategies in response to altered resource subsidies (Yard et al. 2004). Studies evaluating food web impacts on other consumer taxa do not always reveal diet patterns parallel to subsidy alterations (*e.g.*, Saunders and Fausch 2007, Roon et al. 2016). For example, Roon et al. (2016) found that compared to native deciduous trees, the invasive European bird cherry (*Prunus padus* L.) was associated with

4 to 6 times less terrestrial arthropod biomass on foliage and 2 to 3 times less biomass falling into streams. Despite the temporally consistent differences in resource availability reported, there was no difference in the proportion of terrestrial insects detected in the diets of juvenile coho salmon (*Oncorhynchus kisutch* Walbaum).

Despite the diverse arthropod prey communities at our study sites, we are confident our selection of dominant low trophic level insects sufficiently describes source origin of diets. Multiple studies have reported that midges (or other Diptera), leafhoppers, and caterpillars comprise the majority of prey in insectivorous riparian bird diets, including for upper-canopy gleaners like yellow warblers (Busby and Sealy 1979, Yard et al. 2004, Durst et al. 2008). However, our inability to measure isotopic signatures of all available prey items limits interpretation of consumer isotope data in relation to prey items. Pan trap sampling may not have captured a representative sample of the prey items available to avian insectivores. Other studies have used Malaise traps to capture flying insects and sweep-nets or beat-sheets to sample at a variety of vegetative height classes (Yard et al. 2004, Wiesenborn and Heydon 2007), potentially providing a more accurate characterization of prey availability for assemblages with diverse foraging strategies. Additionally, because richness in pan trap samples averaged 18 taxa, there were more than three “dominant” herbivorous terrestrial taxa. Our pan traps and emergence nets captured 139 and 23 different arthropod taxa, respectively (Riedl et al., *in prep*), and incorporating this level of isotopic resolution is rarely feasible. Furthermore, large isotopic variation of fecal samples may be indicative of more generalist foraging strategies (*i.e.*, feeding on a wide range of potentially unmeasured diet sources). Thus,

our estimates of aquatic- and terrestrial-derived diet proportions should be considered to provide a general index rather than an exact proportion.

Our study system experienced differences in precipitation between years that may have contributed to inconsistencies in diet shifts. Higher spring and summer precipitation resulted in approximately 3 times faster stream discharges in 2016 compared to 2015, corresponding with more apparent invasion effects on arthropod resources in 2015 (Riedl et al., *unpublished data*). Likewise, Durst et al. (2008) detected invasion-mediated food web impacts only during the one drought year in their five year study. In addition, the timing of our sampling in the late summer likely influenced our findings (Uesugi and Murakami 2007). Future work may find more pronounced invasion-mediated diet shifts in spring for systems invaded by plants with a later leaf-out phenology than native vegetation. In our system, delayed timing of New Mexico locust leaf-out likely provides less foliage to support arthropod production in late spring.

Future studies could employ SIA of blood or tissue samples, which incorporate diet components ingested over days to weeks (Podlesak et al. 2005, Wolf et al. 2009), and could be used in study systems with greater spatial separation between site types to eliminate random effects of the most recently ingested items. Unlike fecal SIA, blood/tissue SIA incorporates diet components assimilated into the consumers' tissues, which may better reflect utilized resources (Salvarina et al. 2013). Varying digestibility of diet components may also hinder the ability to detect diet shifts using fecal samples (Salvarina et al. 2013). Modern molecular genetic techniques, such as non-invasive molecular scatology and DNA barcoding methods, can also be used to obtain more precise taxonomic identification of prey items (Carreon-Martinez and Heath 2010, Wong et al. 2015).

CONCLUSIONS

Using isotopic signatures of insects and bird feces, we estimated the riparian bird community consumed 34% aquatic carbon, which highlights the importance of aquatic resource subsidies to riparian consumers and the need to consider aquatic ecosystem functions when managing riparian habitats. Diet shifts between reference and locust habitats were highly species-specific and inconsistent across the two years of the study, although we found some support for our hypothesis that New Mexico locust invasion was associated with diet shifts towards aquatic-derived carbon for two insectivorous bird species. We detected this alteration associated with an invasion near the plant's native range, which suggests that species introduced from more geographically disparate areas could have even more pronounced impacts on riparian food webs. An increased focus on resource subsidies will provide a more mechanistic understanding of the consequences of anthropogenic change by examining interacting processes across ecosystems.

TABLES

Table 2.1 Number of fecal samples collected from songbird species at uninvaded (reference) and invaded (locust) sites in the Clear Creek drainage of northwest Colorado. Common names of songbird species are listed in taxonomic order. Cordilleran flycatcher and dusky flycatcher were analyzed together as *Empidonax* flycatchers. Black-capped chickadees and green-tailed towhees are omnivores that are insectivorous during the breeding season, while the other five species are strict insectivores year-round.

Songbird Species	Reference			Locust		
	2015	2016	Total	2015	2016	Total
<i>Empidonax</i> Flycatcher	6	4	10	12	4	16
Warbling Vireo	1	4	5	2	2	4
Black-capped Chickadee	0	8	8	8	4	12
MacGillivray's Warbler	3	2	5	8	1	9
Virginia's Warbler	2	1	3	7	0	7
Yellow Warbler	6	7	13	10	6	16
Green-tailed Towhee	2	7	9	11	5	16
All Species Combined	20	33	53	58	22	80

FIGURES

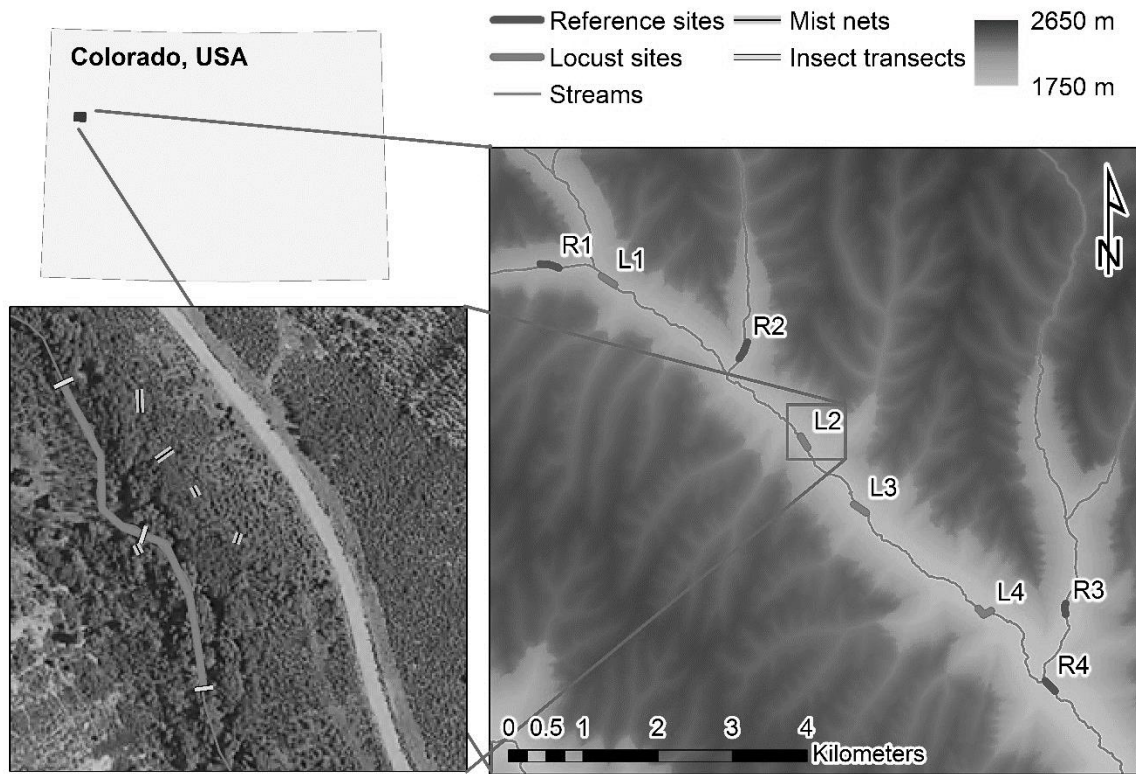


Figure 2.1 Insect and bird fecal sampling sites along stream reaches along uninvasive (reference) and invasive (locust) stream reaches in the Clear Creek drainage of northwestern Colorado. The lower left inset provides an example of mist net and vegetation transect locations at a single site, where fecal and insect samples were collected, respectively.

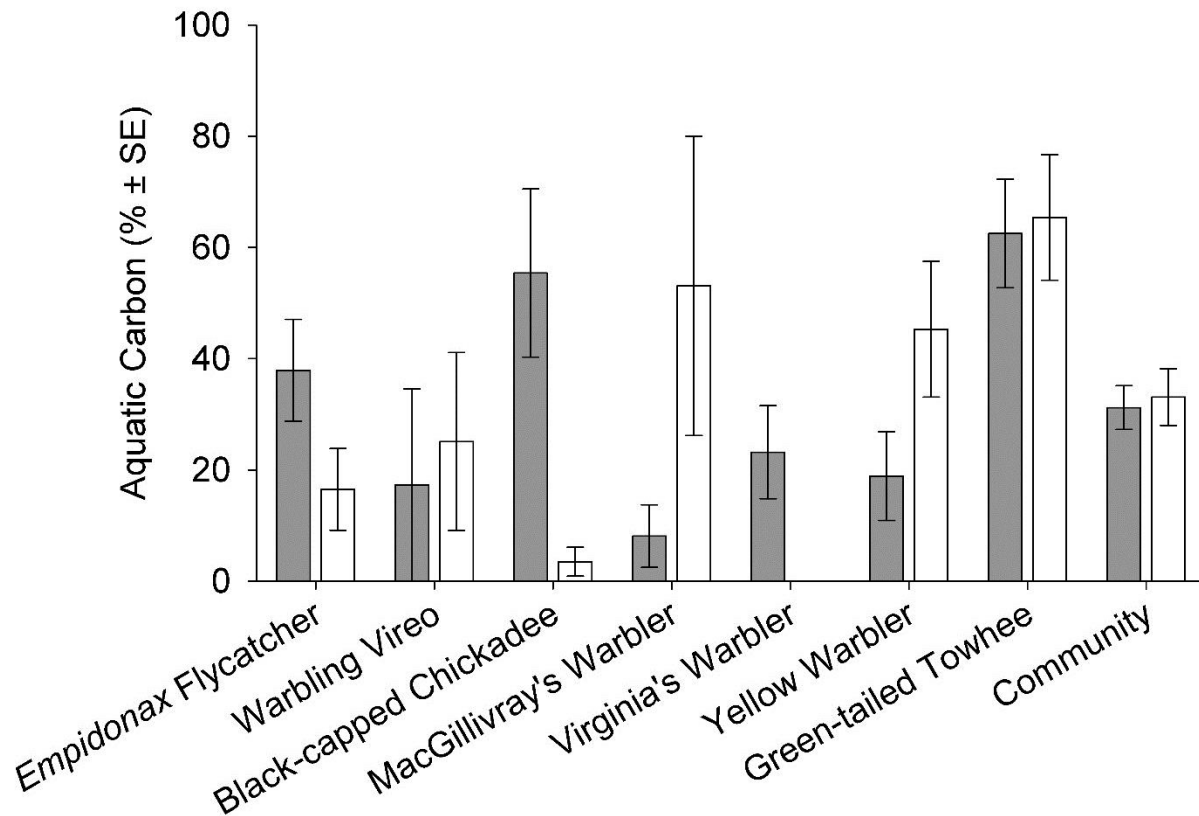


Figure 2.2 Contributions of aquatic-derived insect carbon (mean % $\pm$ SE) to the diets of songbird species, individually and overall (*i.e.*, “community”) for 2015 (grey) and 2016 (white). Results were determined using a single isotope mixing formula and $\delta^{13}\text{C}$ signatures of insect and fecal samples, pooled across reference and locust sites. There were insufficient sample sizes ($n < 2$ per year) to calculate means for Virginia’s warbler in 2016.

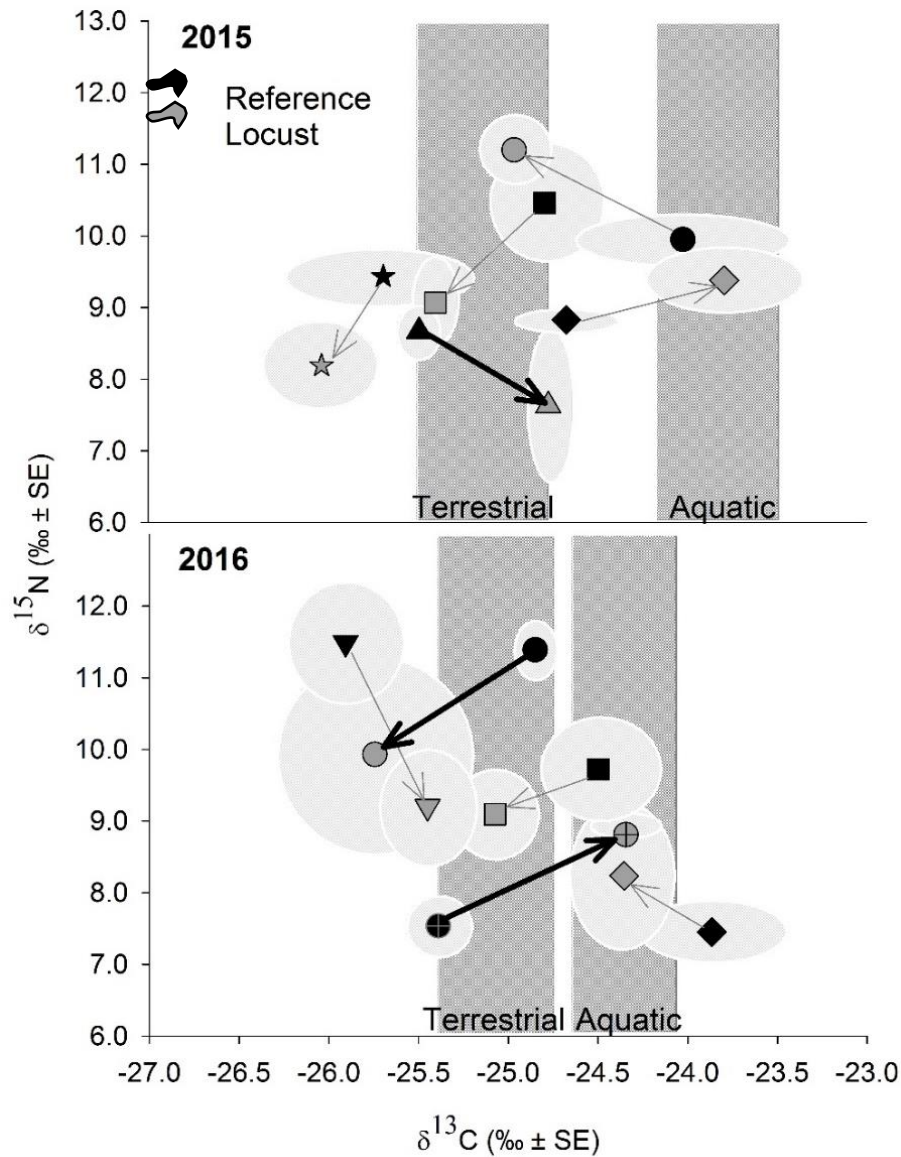


Figure 2.3 Biplots show $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of songbird fecal samples, illustrating 3 significant invasion-mediated diet shifts (bold arrows; see Appendix 2.3). Arrows connect reference to locust sites for each bird species sampled in the Clear Creek drainage of northwest Colorado. Dark gray shading shows the standard error of the $\delta^{13}\text{C}$ signatures of terrestrial and aquatic insect samples. Light gray ellipses represent the standard error encompassed by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of songbird fecal samples. *Empidonax* flycatchers (○), yellow warblers (□), and green-tailed towhees (◇) were evaluated in both years. MacGillivray's warblers (☆) and Virginia's warblers (△) were only evaluated in 2015, and warbling vireos (⊕) and black-capped chickadees (▽) were only evaluated in 2016.

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CHAPTER THREE

FIELD AND MESOCOSM APPROACHES TO EVALUATE NATIVE AND NON-NATIVE LEAF LITTER DECOMPOSITION AND MACROINVERTEBRATE GROWTH RATES

INTRODUCTION

Deciduous riparian vegetation plays a direct role in headwater stream ecosystems by determining the timing, quality, quantity, and diversity of leaf litter inputs. Decomposition of leaf material fuels local instream production, as evidenced by leaf litter exclusion experiments that caused secondary production to decrease 10-35% (Wallace et al. 1997). Additionally, processing of leaf litter is essential for delivery of organic matter to consumers downstream (Vannote et al. 1980). Beyond aquatic ecosystem processes, riparian vegetation can also provide important resources for terrestrial consumers. Slow decomposing narrowleaf cottonwood (*Populus angustifolia* James) leaves, contrasted with faster decomposing Fremont cottonwood (*Populus fremontii* S. Watson), have been found to support 25% more emergent insects (Compson et al. 2013), an important source of nutrients for riparian consumers (Baxter et al. 2005).

Dynamics of leaf litter inputs can be altered by non-native vegetation invasions (Graca 2001, Kennedy and Hobbie 2004, Hladyz et al. 2011, Mineau et al. 2012), and riparian systems are particularly prone to non-native vegetation invasion (Richardson et al. 2007). However, the native or non-native status of plant species is usually a poor predictor of ecosystem function alterations. Differences in macroinvertebrate growth or leaf decomposition rates are often due to leaf stoichiometry, with high C:N ratios typically indicating a recalcitrant and poor quality

resource (Going and Dudley 2008). Regardless, non-native species management decisions should be based on ecosystem functioning of the novel species assemblage, not the origin or stoichiometry of the invading species (Hladyz et al. 2009, Davis et al. 2011).

I used a series of field and mesocosm experiments to determine the dynamics of leaf litter decomposition and macroinvertebrate growth in response to a non-native riparian plant invasion. New Mexico locust (*Robinia neomexicana* A. Gray; hereafter “locust”) was introduced to the study system over 100 years ago, and landowners have attempted unsuccessfully to remove it using three different methods (C. Tysse, Chevron, personal communication). I first determined whether the locust invasion translated to impacts on the benthic aquatic insect community and aquatic ecosystem function in the field. I compared leaf decomposition rates and community composition of macroinvertebrates colonizing leaf litter packs in reaches uninvaded and invaded by locust (hereafter, “reference” and “locust,” respectively). Next, in a mesocosm experiment, I tested whether locust leaf litter altered growth rates of the least salmonfly (*Pteronarcella badia* Hagen), a common stonefly (Plecoptera) shredder, compared to leaves from 2 dominant native trees: boxelder (*Acer negundo* L.) and Gambel oak (*Quercus gambelii* Nutt.). Boxelder and Gambel oak are common riparian and upland tree species, respectively, at the study location in the Clear Creek drainage of northwest Colorado (Fig. 1.1). In this experiment, I also determined if the non-native leaves decomposed at a different rate compared to the 2 native leaf species.

METHODS

Field Experiment

To assess potential differences in leaf decomposition between locust and reference sites, I identified aquatic insects colonizing leaf packs and measured leaf decomposition and microbial respiration rates. Fresh Gambel oak leaves were collected from the Clear Creek drainage in early July 2015 and air-dried in the laboratory prior to enclosure in mesh bags (10 x 10 cm, 1 cm² mesh size, 2 g leaf litter/bag). On 19 July 2015, leaf packs were soaked in stream water for approximately 2 hr before deployment. Three leaf packs were processed immediately after deployment to determine mass lost due to handling. Nine leaf packs were anchored with steel rebar to the streambed in riffles at the downstream transect of the eight study reaches (Fig. 1.1). I randomly sampled 3 replicates from each reach after 5, 24, and 48 days in the stream. Some sampling days resulted in 2 replicates because leaf packs detached from rebar. If this left only 1 leaf pack to sample by day 48, I removed 4 replicate leaf packs.

On each sampling date, leaf packs were transported to the laboratory in coolers and processed within 24 hours. Samples were rinsed over a 350 µm sieve to separate insects from leaf material. After identification, I sorted shredding insects from other functional groups and dried insects at 60°C for 48 hr to obtain biomass. I obtained AFDM of leaf litter remaining by drying at 60°C for 24 hr and combusting at 500°C for 1 hr. Microbial respiration was determined according to Graça et al. (2005). Briefly, I measured the change in DO of an airtight 60 mL centrifuge tube filled with water and containing a subsample of leaf material from each litter pack. Measurements were taken before and after 24 h of dark storage at a constant 4°C. Microbial respiration rates were determined using linear regressions of oxygen consumption

per hr and expressed per g AFDM of leaf disks. Decay coefficients, k (d^{-1}), of leaf litter were calculated for each leaf pack. Decay coefficients represent the linearized form of the simple exponential decay model $M_t = M_0 e^{-kt}$, where M_0 and M_t are the AFDM of leaves at the beginning and end of each sampling period, t is time spent in the stream, and k is the decay rate coefficient (Graça et al. 2005).

Analysis of leaf litter decomposition and microbial respiration rates were conducted in SAS PROC MIXED using analysis of covariance (ANCOVA) with riparian forest type as the independent variable and day as the covariate (v9.3, SAS Institute, Cary, North Carolina). Respiration rate was $\ln(x+1)$ transformed to meet ANCOVA assumptions. The insect community composition was evaluated in the PERMANOVA+ add-on package for PRIMER v7 (Plymouth, United Kingdom, Anderson et al. 2008) with the same ANCOVA structure. I used the PERMDISP function to ensure homogeneity of variance for models with a significant forest type effect, and similarity percentage analysis (SIMPER) to identify taxa that contributed most to community composition differences.

Mesocosm Experiment

I conducted a mesocosm experiment at the Colorado State University Stream Research Laboratory in Fort Collins, Colorado, to compare decomposition and growth rates elicited by non-native and native leaf litter. The 18 stream mesocosms in the greenhouse hold a total of 72 one L mesh baskets (500 μm). Flow-through mesocosms received 20 L of natural water directly from a mesotrophic reservoir fed by the Poudre River watershed. Paddlewheels maintain current at 0.45 m/s, and water is delivered at a rate of 0.3 L/min (turning over approximately every hr).

Fresh Gambel oak, boxelder, and locust leaves were collected in July 2016 and air dried in the laboratory. Approximately 3 g of leaf litter were weighed into each mesh basket and randomly assigned to a stream. Leaves were conditioned for 2 weeks in flow-through systems prior to adding the study organism.

I collected least salmonflies from a nearby Poudre River tributary, Elkhorn Creek. Clear Creek and Elkhorn Creek are both small, 1st-2nd order streams within approximately 100 m elevation of each other. Least salmonflies were collected in September 2016 when water temperature and pH were within the range of physicochemical measurements throughout our study sites in Clear Creek (although conductivity was an order of magnitude lower at Elkhorn Creek; see Riedl et al. chapter 1).

Shredding insects contribute to 20-30% of leaf litter decomposition (Petersen et al. 1989), and least salmonflies are shredding stoneflies common in small to large streams throughout western North America (Merritt et al. 1996). They are the smallest species of the family Pteronarcyidae, have 1-2 year life cycles, and typically emerge in May or June. I used a small seine to collect organisms and transported them in coolers. Using an ocular micrometer under a standard dissecting microscope, I measured individual head capsule widths and then randomly assigned organisms to a mesh basket. I determined head capsule width and mortality weekly, including on the final (20th) day when I preserved organisms in 80% ethanol prior to head capsule measurement.

On day 0 of the growth experiment, 3 replicates of each litter type were stored in Ziploc® bags and frozen to determine mass lost due to handling. An additional 3 replicates of each litter type did not receive a least salmonfly and these were frozen on day 20 of the growth

experiment to determine if consumption from 1 individual could be detected. AFDM of leaf matter was processed according to the field experiment.

Growth rates were calculated with the equation $g = (\ln W_i - \ln W_0)/t$, where W_0 and W_i represent head capsule widths or leaf litter mass and the beginning and end of each measurement period, and t corresponds to experiment day. Growth rates were analyzed using SAS PROC MIXED repeated measures ANCOVA, with leaf species as the main factor, day as a covariate (3 levels, fixed effects), stream ID (18 levels, random effect) as a blocking factor, and basket ID as the repeated measure. Growth rate was square root transformed to meet ANCOVA assumptions. Decomposition rates were calculated using the equation $k = \ln(M_t - M_0)/t$, where M_0 and M_t represent leaf mass at the beginning and end of the experiment, and t corresponds to the 20 days in the experiment. Decomposition rates were analyzed using SAS PROC MIXED ANOVA, with leaf species (3 levels, fixed effect), treatment (2 levels, fixed effect), and stream ID as a blocking factor (18 levels, random effect). Treatment refers to baskets that did or did not receive a least salmonfly.

RESULTS & DISCUSSION

Field Experiment

I found higher shredder biomass and faster leaf decomposition rates in uninvaded reaches, although these differences were not significant due to high variability within reference and locust sites. Gambel oak leaf packs decomposed at similar rates in reference and locust sites (Fig. 3.1). The magnitude of this difference in decomposition rate was similar to Kominoski et al. (2012) and Hladyz et al. (2011), and the overall leaf decomposition rate in our study was comparable to previous research (Webster et al. 1999). Microbial respiration rates significantly

increased from $0.43 \pm 0.038 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1} \text{ AFDM}^{-1}$ on day 5 to 1.70 ± 0.270 on day 48 ($F = 29.36$, $p < 0.0001$), although microbial respiration did not differ between reference and locust sites ($F = 0.01$, $p = 0.94$). There was no significant difference in the abundance or biomass of shredders on leaf packs ($p > 0.65$), and multivariate analyses showed community composition of insects on leaf packs did not differ between reference and locust sites ($pseudo-F = 1.92$, $p(MC) = 0.072$). The dominant shredder (*Amphinemura banksi* Baumann & Gaufin), was two times more abundant on leaf packs at reference sites compared to locust sites and comprised $52 \pm 1\%$ of shredder abundance and $46 \pm 5\%$ of shredder biomass in all leaf packs. Additionally, biomass of shredders collected in benthic samples during the summer 2015, coinciding with this decomposition study, was nearly 6 times greater at reference sites compared to locust sites (Fig. 3.1).

While our results showed no significant trends in total benthic insect biomass, Australian streams invaded by *Salix* sp. had approximately 25% more benthic insects in reference reaches (McInerney et al. 2016). Our findings contribute to a gap in knowledge concerning impacts of exotic woody species on decomposition rates. A meta-analysis by Ferreira et al. (2016) showed that although impacts of riparian forest change on decomposition rates are well studied, the majority (59%) of these studies involved replacements by *Eucalyptus* sp. plantations, and only 11% of studies involved replacement by exotic woody species.

Gambel oak is a common tree in upland zones of our study sites. It is possible that a less recalcitrant leaf species common to the riparian zone, like boxelder or locust, would display even greater differences in decomposition rates between locust and reference sites. Attempts to measure locust decomposition in the field suggest that these leaves are poorly retentive

(Riedl, personal observation). Reaches invaded by locust may receive less organic matter because of lower retention, and this discrepancy in resource availability may account for slight differences in decomposition and community composition (Dobson and Hildrew 1992, Moline and Poff 2008).

Mesocosm Experiment

Growth rate was not significantly affected by leaf species (Fig. 3.2). Other studies evaluating a widespread riparian invader, salt cedar (*Tamarix* sp.), reported contrary results. In a 7 week feeding experiment, crane flies (*Tipula* sp.) grew more than 2 times faster when fed native cottonwood leaves compared to salt cedar leaves (Moline and Poff 2008). In a feeding trial of similar duration as ours, caddisfly (*Lepidostoma* sp.) biomass was nearly 3 times higher when fed salt cedar leaves, but more than 2 times lower when fed non-native cane (*Arundo* sp.) leaves, compared to willow (Salaceae) leaves (Going and Dudley 2008). Our inability to detect differences may be due to high initial mortality that resulted from handling stress in boxelder and Gambel oak treatments (5 out of 18 individuals per treatment). After d 7 measurements, mortality occurred less frequently—only 1 or 2 individuals per treatment.

Leaf litter decomposition rate was significantly different among leaf, but there was no effect of least salmonfly on decomposition rate (Fig. 3.3). Boxelder and locust both decomposed faster than Gambel oak ($p < 0.0001$). Studies of native versus non-native litter decomposition rates reveal consistent trends related to leaf stoichiometry, but not origin status (Hladyz et al. 2009). Nonetheless, context specific results about non-native species are important for informing local management decisions. These results suggest locust should not be removed if management concern centers on instream food web functions. Locust leaves

supported rates of macroinvertebrate growth and decomposition that were similar to leaves from boxelder, the dominant riparian tree. However, the two leaf species likely have different retention potential.

It is interesting to note that there was no effect of least salmonfly treatment on leaf decomposition rate, suggesting that the consumption of leaf material by 1 individual is negligible over a 20 day period. Also, decomposition of Gambel oak was an order of magnitude slower than decomposition rates detected in the field experiment, highlighting the importance of physical processes and a robust macroinvertebrate community.

FIGURES

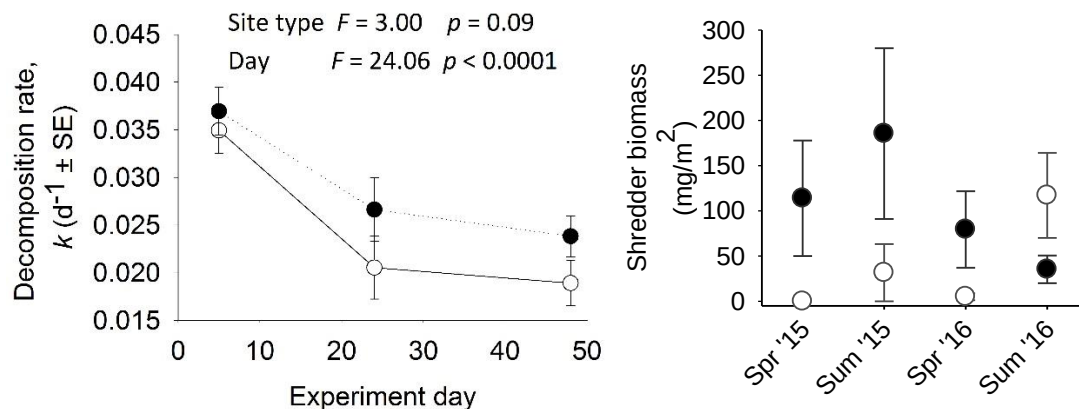


Figure 3.1 Left panel: decomposition rate ($\pm$ SE) of Gambel oak leaves placed in locust (open circles) and reference (filled circles) stream reaches in the Clear Creek drainage of northwestern Colorado in summer 2015. Right panel: biomass ($\pm$ SE) of shredders collected in Surber samples in spring and summer of 2015 and 2016. See Riedl et al., chapter 1 for study site and Surber sampling method descriptions.

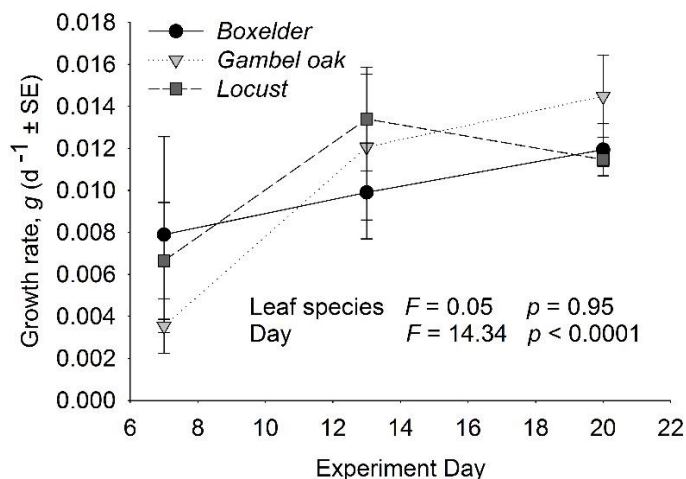


Figure 3.2 Growth rates of least salmonfly throughout the feeding trial, determined from weekly head capsule width measurements.

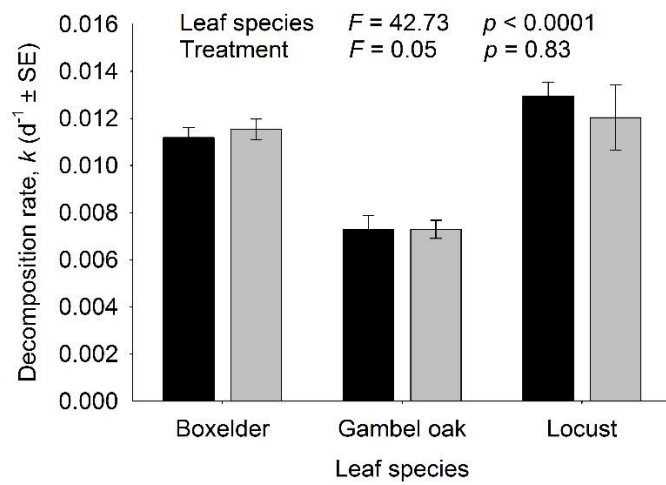


Figure 3.3 Decomposition rates of leaf litter species as determined from mass lost at the end of the feeding trial. Locust decomposed faster than Gambel oak but equally as fast as the dominant native tree, boxelder. There was no difference in decomposition rate if treatments included a least salmonfly (black bars) or not (gray bars).

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APPENDICES

Appendix 1.1 Justification for candidate vegetation characteristics used in model selection to determine what environmental factors best described arthropod community and subsidy patterns. Percent ground cover and vertical structure of specific tree and shrub species were also included in order to compare their effects with locust, and stream characteristics were included for models explaining benthic or emerging aquatic insects. Many variables were removed after a variable reduction process (see methods).

Variable	Justification	Citations
<i>Vertical vegetation structure</i>		
Cover (%)	Provides food and habitat for terrestrial arthropods	Munne et al. 2003
	Organic matter inputs to streams provide food and substrate for benthic and emerging aquatic insects	Carline and Walsh 2007, Merten et al. 2014
Richness	Drives patterns in arthropod richness	Cardinale et al. 2006
<i>Ground cover</i>		
Cover (%)	Provides food and habitat for terrestrial arthropods	Munne et al. 2003
	Organic matter inputs to streams provide food and substrate for benthic and emerging aquatic insects	Carline and Walsh 2007, Merten et al. 2014
Richness	Drives patterns in arthropod richness	Cardinale et al. 2006
Non-native (%) ^b	Can decrease abundance and richness of terrestrial arthropods	Simao et al. 2010, Litt et al. 2014
Bareground (%)	Can increase erosion into stream channel and correspond with decreases in vegetative structure or ground cover	Munne et al. 2003, Carline and Walsh 2007
Grasses (%)	Support different arthropod communities than other vegetative growth forms	Kawaguchi and Nakano 2001
Forbs (%)	Attract pollinators and can increase food web complexity	Burkle et al. 2012
Shrubs/trees (%)	Used as index of riparian health	Munne et al. 2003

^b See Appendix 1.2 for listing information at the time of research.

Appendix 1.2 United States Department of Agriculture's Plants Database (plants.usda.gov/java/) classification of surveyed non-native species in the Clear Creek drainage, Colorado, at the time of research.

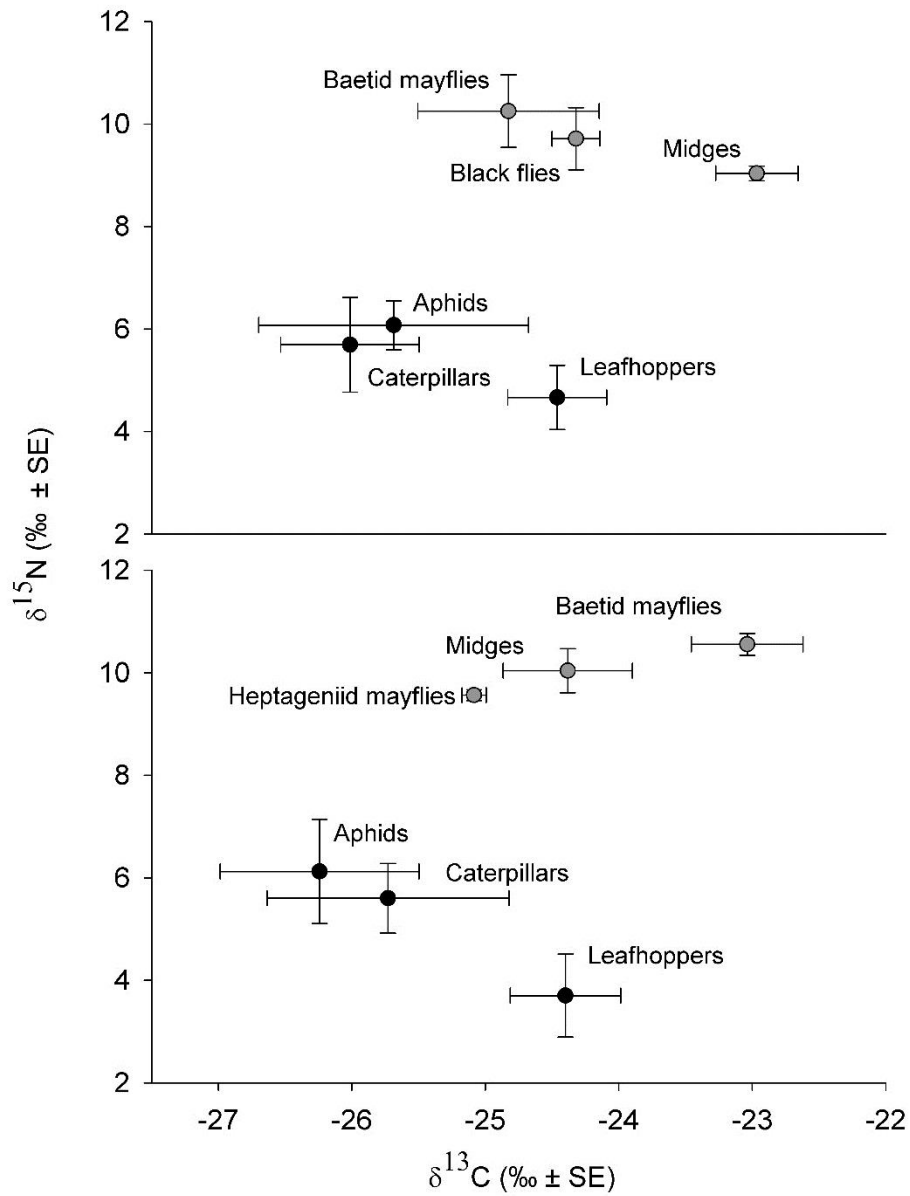
Common name	Scientific name	Status
Greater burdock	<i>Arctium lappa</i> L.	Introduced
Shepherd's purse	<i>Capsella bursa-pastoris</i> (L.) Medik.	Introduced
Canada thistle	<i>Cirsium arvense</i> (L.) Scop.	B List
Bull thistle	<i>Cirsium vulgare</i> (Savi) Ten.	B List
Gypsyflower	<i>Cynoglossum officinale</i> L.	B List
Common dandelion	<i>Taraxacum officinale</i> F.H. Wigg.	Introduced
Common mullein	<i>Verbascum thapsus</i> L.	C List
Cheatgrass	<i>Bromus tectorum</i> L.	A List
Small tumbleweed mustard	<i>Sisymbrium loeselii</i> L.	Introduced
Common plantain	<i>Plantago major</i> L.	Introduced
Catnip	<i>Nepeta cataria</i> L.	Introduced
Field pennycress	<i>Thlapsi arvense</i> L.	Introduced
Red clover	<i>Trifolium pretense</i> L.	Introduced
Crested wheatgrass	<i>Agropyron cristatum</i> (L.) Gaertn.	Introduced

Appendix 1.3 Models selected under AICc model selection to explain biomass and richness of each arthropod sampling method conducted in the Clear Creek drainage. Models within 2 Δ AICc of the top model were evaluated and are shown here, excluding models with pretending variables. “K” indicates number of parameters in each model and includes the intercept. Model weight describes each model’s weight of evidence, where the larger model weight indicates lowest uncertainty, and the sum of weights for each model set cannot exceed 1.

AICc	K	Model weight	Adj. R ²	Variables
<i>Pan trap biomass</i>				
546.73	5	0.16	0.15	grass cover, vegetation cover, vegetation richness, boxelder structure
547.18	5	0.13	0.15	vegetation cover, vegetation richness, boxelder structure, cottonwood structure
547.79	5	0.093	0.15	bare ground cover, grass cover, vegetation cover, boxelder structure
548.17	6	0.077	0.15	non-native cover, vegetation cover, vegetation richness, structural richness, locust structure
548.18	6	0.076	0.15	grass cover, vegetation cover, vegetation richness, boxelder structure, chokecherry structure
548.20	6	0.076	0.15	grass cover, forb cover, boxelder structure, cottonwood structure, chokecherry structure
548.28	6	0.072	0.15	grass cover, forb cover, vegetation cover, boxelder structure, cottonwood structure
548.30	6	0.072	0.15	bare ground cover, grass cover, vegetation cover, vegetation richness, boxelder structure
548.32	6	0.071	0.15	vegetation cover, vegetation richness, locust structure, boxelder structure, cottonwood structure
548.64	6	0.061	0.15	vegetation cover, vegetation richness, boxelder structure, cottonwood structure, chokecherry structure
548.66	6	0.060	0.15	vegetation cover, vegetation richness, structural richness, locust structure, cottonwood structure
548.69	6	0.059	0.15	grass cover, vegetation cover, vegetation richness, locust structure, boxelder structure
<i>Pan trap richness</i>				
-142.88	4	0.46	0.26	non-native cover, forb cover, vegetation cover
-141.15	6	0.19	0.27	non-native cover, forb cover, vegetation cover, boxelder structure, cottonwood structure
-140.91	6	0.17	0.27	non-native cover, forb cover, vegetation cover, vegetation richness, cottonwood structure
-140.90	6	0.17	0.27	non-native cover, forb cover, vegetation cover, vegetation richness, boxelder structure

(Appendix 1.3 continued from previous page.)

<i>Beat sheet abundance</i>				
82.39	6	0.16	0.26	forb cover, vegetation cover, rabbitbrush cover, locust structure, boxelder structure
82.40	7	0.16	0.27	non-native cover, forb cover, vegetation cover, rabbitbrush cover, structural richness, boxelder structure
82.86	7	0.13	0.26	grass cover, forb cover, rabbitbrush cover, locust structure, boxelder structure, cottonwood structure
83.42	7	0.10	0.26	forb cover, rabbitbrush cover, structural richness, locust structure, boxelder structure, cottonwood structure
83.82	7	0.078	0.26	forb cover, vegetation cover, rabbitbrush cover, structural richness, boxelder structure, cottonwood structure
83.83	7	0.078	0.26	non-native cover, grass cover, forb cover, rabbitbrush cover, locust structure, boxelder structure
83.83	7	0.078	0.26	bare ground cover, forb cover, rabbitbrush cover, structural richness, boxelder structure, cottonwood structure
83.85	7	0.077	0.26	bare ground cover, forb cover, rabbitbrush cover, structural richness, locust structure, boxelder structure
83.86	7	0.077	0.26	non-native cover, forb cover, vegetation cover, rabbitbrush cover, locust structure, boxelder structure
83.96	7	0.073	0.26	grass cover, forb cover, vegetation cover, rabbitbrush cover, locust structure, boxelder structure
<i>Emergence richness</i>				
-235.70	9	0.27	0.14	velocity, thalweg depth, wetted width, grass cover, forb cover, vegetation cover, vegetation richness, chokecherry structure
-235.52	9	0.25	0.14	velocity, thalweg depth, wetted width, non-native cover, grass cover, forb cover, vegetation cover, vegetation richness
-234.86	9	0.18	0.14	velocity, thalweg depth, wetted width, bare ground cover, grass cover, forb cover, vegetation cover, vegetation richness
-234.51	9	0.15	0.13	velocity, thalweg depth, wetted width, bare ground cover, grass cover, forb cover, vegetation cover, chokecherry structure
-234.44	9	0.15	0.13	velocity, thalweg depth, wetted width, grass cover, forb cover, vegetation cover, rabbitbrush cover, chokecherry structure
<i>Benthic biomass</i>				
-297.70	3	0.27	0.13	bankfull width, litter storage
<i>Benthic richness</i>				
265.02	6	0.69	0.32	velocity, water temperature, bankfull width, litter storage, bare ground cover
266.58	7	0.31	0.32	velocity, water temperature, bankfull width, litter storage, grass cover, vegetation richness



Appendix 2.1 Biplots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of aquatic (gray) and terrestrial insect taxa (black) used as an index of songbird diet sources in 2015 (top) and 2016 (bottom). Common names of taxa are displayed near the mean.

Appendix 2.2 Comparison of aquatic carbon contributions to songbird diets across species and years, based on data pooled across reference and locust sites. Mean estimates of % aquatic carbon $\pm$ SE were calculated using a single isotope mixing formula and $\delta^{13}\text{C}$ signatures of insect and fecal samples. The terrestrial-derived diet component is the remaining percentage (1 – aquatic percentage).

Songbird Species	2015		2016		Total	
	<i>n</i>	Mean % Aq. $\pm$ SE	<i>n</i>	Mean % Aq. $\pm$ SE	<i>n</i>	Mean % Aq. $\pm$ SE
<i>Empidonax</i> Flycatcher	18	38 $\pm$ 9	8	17 $\pm$ 7	26	31 $\pm$ 7
Warbling Vireo	3	17 $\pm$ 17	6	25 $\pm$ 16	9	22 $\pm$ 12
Black-capped Chickadee	8	55 $\pm$ 15	12	3 $\pm$ 3	20	24 $\pm$ 8
MacGillivray's Warbler	11	8 $\pm$ 6	3	53 $\pm$ 27	14	18 $\pm$ 8
Virginia's Warbler	9	23 $\pm$ 8	1	-	10	24 $\pm$ 8
Yellow Warbler	16	19 $\pm$ 8	13	45 $\pm$ 12	29	31 $\pm$ 7
Green-tailed Towhee	13	62 $\pm$ 10	12	65 $\pm$ 11	25	64 $\pm$ 7
Community	78	33 $\pm$ 4	55	34 $\pm$ 5	133	34 $\pm$ 3

Appendix 2.3 One-way MANOVAs and ANOVAs by year, testing for differences in songbird diet $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between reference and locust sites. Only species with multiple samples per site/year combination were evaluated (dashes in place otherwise). Significant differences ($p < 0.05$) are shown in bold. Fig. 2.3 shows directionality of significant diet shifts with bolded arrows.

Songbird Species	$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (MANOVA)				$\delta^{13}\text{C}$ (ANOVA)				$\delta^{15}\text{N}$ (ANOVA)			
	2015		2016		2015		2016		2015		2016	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
<i>Empidonax</i> Flycatcher	1.28	0.32	28.2	0.002	0.81	0.38	2.52	0.16	2.13	0.17	1.08	0.34
Warbling Vireo	-		19.4	0.019	-		13	0.023	-		4.37	0.10
Black-capped Chickadee	-		1.43	0.29	-		0.8	0.38	-		2.9	0.12
MacGillivray's Warbler	0.64	0.55	-		0.31	0.59	-		1.41	0.27	-	
Virginia's Warbler	4.41	0.067	-		8.79	0.021	-		0.23	0.64	-	
Yellow Warbler	2.72	0.10	1.64	0.24	3.89	0.069	1.7	0.22	1.85	0.19	0.42	0.53
Green-tailed Towhee	0.38	0.70	0.54	0.60	0.51	0.49	0.79	0.40	0.24	0.63	0.61	0.45

Appendix 2.4 Summary of body condition measurements for songbird species with sufficient sample sizes ($n \geq 2$ samples per site type and year; dashes in place otherwise). Sample sizes (n) and mean measurements of mass (g $\pm$ SE) and fat (score $\pm$ SE) are summarized by year and site type (reference and locust). Significant differences ($p < 0.05$) between site types are shown in bold.

Songbird Species	2015 Reference			2015 Locust			2016 Reference			2016 Locust		
	n	mass	fat	n	mass	fat	n	mass	fat	n	mass	fat
<i>Empidonax</i> Flycatcher	4	12.38 $\pm$ 0.94	0.50 $\pm$ 0.20	10	11.4 $\pm$ 0.49	0.50 $\pm$ 0.11	4	10.88 $\pm$ 0.13	0.75 $\pm$ 0.25	4	11.38 $\pm$ 0.13	0.75 $\pm$ 0.48
Warbling Vireo	1	-	-	2	-	-	4	12.00 $\pm$ 0.20	1.00 $\pm$ 0.00	2	11.50 $\pm$ 0.50	1.50 $\pm$ 0.50
Black-capped Chickadee	0	-	-	8	-	-	8	11.06 $\pm$ 0.20	0.75 $\pm$ 0.25	4	11.13 $\pm$ 0.66	1.00 $\pm$ 0.00
MacGillivray's Warbler	3	10.50 $\pm$ 0.29	0.00 $\pm$ 0.00	8	10.81 $\pm$ 0.28	0.25 $\pm$ 0.16	2	-	-	1	-	-
Virginia's Warbler	2	8.25 $\pm$ 0.25	-	7	7.71 $\pm$ 0.26	-	0	-	-	1	-	-
Yellow Warbler	6	8.33 $\pm$ 0.38	0.50 $\pm$ 0.18	10	9.45 $\pm$ 0.25	0.33 $\pm$ 0.22	7	9.14 $\pm$ 0.14	0.71 $\pm$ 0.29	6	8.75 $\pm$ 0.21	0.50 $\pm$ 0.22
Green-tailed Towhee	2	28.00 $\pm$ 2.00	1.00 $\pm$ 1.00	10	25.25 $\pm$ 0.55	1.60 $\pm$ 0.30	7	28.86 $\pm$ 0.96	1.29 $\pm$ 0.29	5	27.00 $\pm$ 1.64	0.80 $\pm$ 0.37