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## Abstract

Headwater streams in the midwestern United States are an important ecosystem because they are home to key macroinvertebrate species and serve as the starting point for river systems. According to the river continuum concept, any terrestrial inputs to headwater streams can influence biotic communities and abiotic conditions downstream. Amur honeysuckle (*Lonicera maackii*) is an invasive shrub species prominent in the midwestern U.S. whose allelopathic properties have proven detrimental to local biodiversity. We hypothesized that increasing *L. maackii* density along headwater streams would alter the diversity of aquatic macroinvertebrate communities. Five sites located in Montgomery and Miami Counties, Ohio were chosen to represent varying *L. maackii* density along the banks of headwater streams: one heavily invaded site, two moderately invaded sites, and two reference sites (little to no invasion). This study focused on the EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera), which represent orders of insects whose larvae are known to be sensitive to poor stream water quality. The presence of *L. maackii* had a significant negative impact on the percentage of EPT individuals. There was no significant effect of *L. maackii* invasion on the number of families within EPT taxa (EPT Index). We noticed the greatest separation of these metrics as well as macroinvertebrate relative abundance between the heavily invaded site and one reference site. Seasonal effects do not seem to be a significant driver of alterations in EPT assemblages. This study hopes to show that the intensity of *L. maackii* has effects on macroinvertebrate health, carrying management implications. The results of this study suggest that land managers effectively manage *L. maackii* invasion so as not to significantly disrupt macroinvertebrate diversity and abundance.

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## Introduction

The structure and biology of riparian vegetation is tightly connected to the ecological function and biota of headwater streams. Light availability, water chemistry and temperature, and metabolism of streams are all strongly connected to the riparian zone (Clinton 2011; Roth et al. 2010, Tank et al. 2010, Polis and Strong 1996). Deposition of detrital materials from terrestrial plants provides energy and habitat resources for aquatic organisms (Baxter et al. 2005, Wallace et al. 2015), and the metabolic activity and biological diversity of headwater streams are linked to the functioning of larger rivers and water bodies (Vannote et al. 1980, Tank et al. 2010). Changes to the riparian zone have strong potential to influence the aquatic biota due to the importance of these terrestrial-to-aquatic linkages (Wallace et al. 1997, Gregory et al. 2001). Indeed, timber harvesting (Likens et al. 1978), mining (Pond et al. 2008), agriculture (Moore and Palmer 2005), and other anthropogenic activities in riparian habitats are well-known to drive direct, measurable effects on stream biota (Swank et al. 2001, Wallace et al. 2015). Exotic plant invasions that alter the plant community in riparian forests have the potential to influence aquatic organisms and alter stream ecosystem function (e.g., Richardson et al. 2007). For example, invasive plant leaves may have different decay rates than native species altering in-stream organic matter cycling (Swan et al. 2008, Mineau et al. 2012). Some work suggests that riparian invasion has the potential to alter stream geomorphology (Fei et al. 2014) or contribute woody materials with unique characteristics into aquatic systems (Tabacchi and Planty-Tabacchi 2003). Riparian invasion has been associated with alterations to in-stream biota (Bailey et al. 2001, Reinhart and VandeVoort 2006). Due to the potential for negative effects, invasive

plant removal has become a restoration objective of riparian zone management in some ecosystems (Richardson et al. 2007, Hultine et al. 2010)

Macroinvertebrate communities are strong indicators of changing environmental conditions in the riparian habitat and play an important role in the aquatic foodweb. Macroinvertebrates are standard aquatic bioindicators (Smith and Voshell 1997, Ohio EPA 2018) and have been used to assess stream biology in a variety of settings (Moore and Palmer 2005). Sensitivity to disturbance is variable among macroinvertebrate taxa (Wallace 1996; Hilsenhoff 1988; Smith and Voshell 1997). For example, Psephenidae in the Coleoptera are tolerant of metal pollution, yet sensitive to habitats with high algal and inorganic sediment presence (Voshell 2002). Variation in sensitivity has allowed for the establishment of macroinvertebrate metrics to assess stream quality. A standard macroinvertebrate-based assessment technique for streams focuses on the Ephemeroptera, Plecoptera, and Trichoptera (*i.e.*, EPT taxa) which are insect orders that are sensitive to environmental perturbations (Voshell 2002). Assessment of aquatic macroinvertebrates in the order Chironomidae has also provided a useful tool for assessing factors that may impair the biology of streams (Lenat 1983, Rae 1989, Janssens De Bisthoven et al. 2005). Chironomids are more tolerant than many other macroinvertebrate taxa and, thus, abundance of organisms in this order may be an indication of impairments to stream biology. Changes to the macroinvertebrate community have strong potential to manifest in higher trophic levels as they are prey for other invertebrates and fish (Vannote et al. 1980; Wallace 1996).

*Lonicera maackii* (Amur honeysuckle) is an exotic shrub that has established in forests throughout much of the American Midwest and invasion by this species has

strong potential to alter the biology of streams that meander through invaded habitats (McNeish and McEwan 2016). Originally brought to North America for horticultural purposes, this species has become a prolific invader of temperate deciduous forests (Luken and Thieret 1996). Invasion by *L. maackii* is facilitated by a complex profile of traits (McNeish and McEwan 2016) including rapid and plastic growth (Luken and Mattimiro 1991), extended phenology (McEwan et al. 2009a), herbivore resistance (McEwan et al. 2009b, Lieurance and Cipollini 2012) and allelopathy (Dorning and Cipollini 2006, McEwan et al. 2010). Invasion by *L. maackii* has been associated with negative effects on native plants (Gould and Gorchoy 2000, Collier et al. 2002, Gorchoy and Trisel 2003, Miller and Gorchoy 2004) and removal is known to create positive effects on understory vegetation (Hartman and McCarthy 2004). *Lonicera maackii* invasion is associated with changes to terrestrial ecosystem processes including acceleration of leaf litter decomposition (Arthur et al. 2012; Poulette and Arthur 2012) and increased microbial activity associated with rapid C and N cycling (Kolbe et al. 2015, Woods et al. 2019).

*Lonicera maackii* has strong potential to alter the aquatic biota in streams where it is a significant component of the riparian vegetation. Invasion of *L. maackii* in riparian areas is regionally extensive (R.W. McEwan, *pers. observation*), likely due to the edge characteristics of this habitat type (Bartuszevige et al. 2006), and a variety of effects on streams have been discovered. For instance, a unique macroinvertebrate community colonized experimental leaf packs in which *L. maackii* was present, as compared to leaf packs with native riparian trees present (McNeish et al. 2012). In particular, Chironomidae was more prevalent in experimental units containing *L. maackii* and this

contributed to overall dominance by the gathering-collector functional feeding group in those leaf packs. In a restoration experiment, when *L. maackii* was removed from riparian zones, there was increased macroinvertebrate density, diversity, and functional richness, suggesting that riparian invasion alters the functional composition of the benthic macroinvertebrate community (McNeish et al. 2017). Custer et al. (2017) found that *L. maackii* flowers and fruits created both lethal and sub-lethal effects on a set of model aquatic macroinvertebrates using both *in-situ* and *ex-situ* microcosms. In a microcosm-based dilution-series experiment using *L. maackii* leaf leachate, *Hyaella azteca* experienced strong toxic effects from materials collected in spring and fall with no significant effects related to materials collected during other seasons (Borth et al. 2018). These results suggest potential biochemical changes related to the phenology of the plant; however, the implications of seasonality in leaf toxicity have not been fully explored. Indeed, although this series of studies provides clear evidence of *L. maackii* effects on aquatic macroinvertebrates, no stream-scale experiments have been conducted to verify the field relevance of these discoveries.

In this study, we assessed macroinvertebrate communities from headwater streams with varying densities of *L. maackii* invasion. We hypothesized ( $H_1$ ) that macroinvertebrate abundance would be lower in streams with increased *L. maackii* invasion intensity. We also hypothesized that the macroinvertebrate community composition would be influenced by the present of *L. maackii* ( $H_2$ ), and, specifically, that more sensitive insect taxa will be negatively impacted by increasing *L. maackii* invasion ( $H_3$ ).



## Methods

### *Site Selection and Plot Design*

We conducted this study within Miami and Montgomery counties in Southwestern Ohio, USA. Regional climate is temperate with an average high temperature of 16.4 C, an average low temperature of 5.9 and an average of 102.7 cm of rainfall annually (Cox Dayton International Airport Weather Station). We selected five different 1<sup>st</sup> and 2<sup>nd</sup> order headwater streams spanning a gradient of *L. maackii* invasion intensity (Supplemental Document 1). We selected two streams with minimal *L. maackii* invasion which served as reference sites (REF.E & REF.A). Two stream sites were selected that were moderately invaded by *L. maackii* based on visual inspection (MOD.E & MOD.C). Finally, we selected a heavily invaded site to represent a site where *L. maackii* had established dominance in the shrub layer (HVY.B; Supplemental Document 1). Sites were similar in terms of sinuosity, discharge, size, and surrounding land-use and all streams meandered through forests that were free from obvious, recent, anthropogenic influence from agriculture or land development (Supplemental Table 1). The sites all had relatively similar forest canopy compositions, and were indicative of typical secondary forests in the region. Prominent tree species included *Acer negundo*, *Celtis occidentalis*, *Gleditsia triacanthos*, *Platanus occidentalis*, *Populus deltoides* and *Tilia americana*, among others. Riparian zone sites were scouted through visual inspection and selected for being similar in all other aspects but varying in *L. maackii* invasion (Supplemental Table 1).

At each stream, a set of five (6 m long) permanent instream plots were established along a 30 m reach following the thalweg. The 30 m reach was established by incorporating a multiplier of ~20-30× the wetted width of the stream (Peck et al. 2006; USEPA 2006). Streams were sampled seasonally for a total of five times between 2015-16 (August 2015, October 2015, February 2016, April 2016, and August 2016). Three plots from each stream were sampled at each time-point, and each plot was randomly selected using a random number table.

### *Sample collection*

A D-frame dipnet (500 µm mesh) was used to sample benthic macroinvertebrates from multiple habitats within each stream plot. Each plot was sampled for 60 seconds moving in a zig-zag (right, center, and left) motion going from downstream to upstream (Peck et al. 2006; Ferreira et al. 2014; Silva et al. 2014). Once time expired, the sampling stopped, and benthic macroinvertebrates were processed in the field. Prior to sampling, two large buckets were filled with filtered stream water (500 µm sieve). Only contents from inside the dipnet were rinsed into one bucket, and the net was visually inspected to remove all invertebrates with forceps. Any invertebrates on the outside of the net were excluded from the sample. Large leaves, twigs, and cobbles were rinsed in the bucket, and visually inspected before discarding. The benthos contents in the bucket were then swirled and poured through a 500 µm sieve. The other bucket with filtered stream water was used to rinse the bucket 3×, and the bucket was visually inspected for remaining

invertebrates. The contents in sieve were then placed into a bottle and filled with 95% ETOH.

### *Sample processing*

All samples were subsampled to 200 organisms following recommendations outlined in Barbour et al. (1999). To accomplish this, a pan with 30 squares was used to subsample, and a random number set of 30 numbers was generated. Four squares were sampled initially, and then any subsequent squares were sampled until  $200 \pm 40$  organisms were sampled. The sampled organisms and ETOH were poured through a 500  $\mu\text{m}$  sieve and rinsed with water. All debris was rinsed in the sieve, visually inspected, and removed. The contents from the sieve were spread evenly in the pan, and then squares randomly sampled. All organisms were then sorted under a dissecting microscope, and enumerated. Subsequently, all sorted organisms were stored in scintillation vials with freshly made 70% ETOH. Organisms were counted and taxonomically identified to order. Additionally, individuals within Ephemeroptera, Trichoptera, and Plecoptera were identified down to family to describe the number of distinct families within these respective orders and to allow for the calculation of an EPT Index. A few replicates were voided due to desiccated conditions in some of the vials (MOD.C Fall, 2 plots, and REF.A Spring, 1 plot), respectively.

### *Data Analysis*

Total macroinvertebrate abundance, and relative abundances at the Order level were calculated for each site and season. The percentage of EPT taxa were calculated by summing all individuals within the orders Ephemeroptera, Plecoptera, and Trichoptera, and then dividing by the total macroinvertebrates by each site and season, for a total of  $n = 25$  samples. An EPT index was also created for each plot for each sampling event. The same was done for the family Chironomidae, dividing the abundance of individuals by the total number of macroinvertebrates for each site and season. For % EPT, % Chironomidae, and EPT index, we applied linear mixed-effects model to determine if they were dependent on site or season, using the *lme* function in the *nlme* package (Pinheiro et al. 2019). We used site as a random effect where we were testing the effect of season to reduce variation driven by site, and where we were testing the effect of site, we used season as a random effect. We used Tukey post-hoc analysis to determine if there were statistical differences between the means of these parameters based on levels of *L. maackii* invasion using the *lsmeans* function from *emmeans* package (Lenth et al. 2018).

Non-metric multidimensional scaling (NMDS) were also completed using the *vegan* package (Okasanen et al. 2019), utilizing relative abundance data at the order level. We used 95% confidence intervals to group data by season or site. We conducted an analysis of similarity (ANOSIM) with 999 permutations to see how different the sites or seasons were from each other using the *anosim* function. Similarity were also calculated using the *simper* function with Bray-Curtis dissimilarities to determine which taxa had the most influential contributions to differences found between either site or season in the

NMDS. All analyses were conducted in R v. 3.6.0 and data were visualized using *ggplot2* (Wickham 2016).

## Results

There was variation in the relative abundances of macroinvertebrate taxa by *L. maackii* invasion intensity and season of sampling among the various treatments (Figure 1). Specifically, one site without *L. maackii* invasion (REF.E) often had different macroinvertebrate communities than did the heavily invaded site (HVY.B). Trichoptera were present in a lower abundance at the heavily invaded sites compared to the moderately invaded and uninvaded sites. Macroinvertebrate abundance was relatively similar in fall and spring, but the second summer had lower abundances than other seasons and there was a spike in abundance in the winter at a non-invaded site (Supplemental Figure 1).

The overall abundance of EPT taxa decreased in association with increasing *L. maackii* invasion intensity (Percent EPT; Figure 2). Specifically, there was a stepwise decrease in the amount of EPT taxa, where uninvaded Englewood had the highest %EPT taxa prevalent, followed by the uninvaded Aullwood site and moderately invaded sites, and then the heavily invaded Buckeye site ( $t_{5,16} = 2.66$ ,  $P < 0.001$ ). There was a very large difference in percent EPT between heavily invaded Buckeye site (median ~15% EPT individuals) and uninvaded Englewood Reference (median ~70% EPT individuals). There was no impact of season on % EPT ( $P > 0.05$ ; Supplemental Figure 3).

The EPT Index scores demonstrated a similar pattern to % EPT where heavily invaded sites had lower EPT scores than non-invaded sites. There is a stepwise pattern of median scores through the *L. maackii* invasion gradient, though the overall EPT index scores are not significantly different from each other ( $P > 0.05$ ). Buckeye heavy site had the lowest score (median = 2.67), followed by moderately invaded Charleston Falls (median = 4), uninvaded Aullwood site (median = 5.67), and moderately invaded Englewood (median = 5), and uninvaded Englewood reference site had the highest EPT score (median = 5.33; Figure 3). There was a significant seasonal effect on the EPT index, where Summer 1 ( $t_{5,16} = -2.16$ ,  $P = 0.0465$ ) and Summer 2 ( $t_{5,16} = -1.06$ ,  $P = 0.0219$ ) had lower EPT scores than the Spring and Winter samplings (Supplemental Figure 3).

In this study, *L. maackii* invasion did not alter the percentage of Chironomidae individuals ( $P > 0.05$ ; Figure 4), but Chironomidae varied by sampling season ( $F = 3.33$ ,  $P < 0.03$ ; Supplemental Figure 4). There was a higher relative abundance of Chironomidae in the first summer sampling compared to the second summer sampling. There were no significant differences of percent Chironomidae when comparing any of the other season samplings to each other or to the summer samplings.

The NMDS ordinations show significant grouping of macroinvertebrate communities when categorized by both site (ANOSIM  $R=0.215$ ,  $P = 0.004$ ; Figure 5) and season (ANOSIM  $R = 0.337$ ,  $P < 0.001$ ; Supplemental Figure 5). For the site treatments, accounting for variations in honeysuckle invasion, the most influential taxa were Oligochaeta, Ephemeroptera, and Trichoptera. The ordination shows a complete separation of Englewood Reference and Buckeye Heavy, meaning these treatments have

different relative abundance makeups of macroinvertebrate communities (Figure 5). Additionally, Plecoptera, Isopoda, Ephemeroptera, and Trichoptera were most influential in discerning communities by sampling season. Communities collected in the fall and spring were very similar, and the two summer samplings were also similar to each other (Supplemental Figure 5).

## Discussion

Headwater streams are strongly influenced by the structure and biology of riparian plant communities, which can be substantially altered by invasive species. *Lonicera maackii* is one of the most significant invasive plants in North America and is well-known to influence the biodiversity and ecosystem function of invaded plant communities and a growing body of evidence suggests that terrestrial invasion of this species can alter aquatic biology in streams (McNeish et al. 2016). Across a gradient of invasion intensity, we hypothesized (H1) that increasing invasion of *L. maackii* would be associated with decreasing abundance of macroinvertebrates; however, we found no evidence for this. Abundance varied substantially both within and across sites and throughout the growing season. Even so, our data indicate that the macroinvertebrate composition is altered by the presence of *L. maackii* (supporting  $H_2$ ) and we specifically note that more sensitive taxa were less frequent in streams with a higher riparian dominance of the invasive shrub (confirming  $H_2$ ). McNeish et al. (2017) found that removal of *L. maackii* from headwater stream riparian areas resulted in an increase in macroinvertebrate density. Taken together, these results may suggest that the abundance of macroinvertebrates is affected by *L. maackii* invasion, and specifically, that a decrease

in the prevalence of EPT taxa was associated with an increase in *L. maackii* invasion intensity. Additionally, *L. maackii* invasion influenced overall community composition (as indicated by NMDS analysis) with a particularly clear separation between one of the reference sites and the stream site with the heaviest invasion.

Effects of terrestrial invasion on aquatic biota may be related to the deposition of materials with chemical or physical characteristics that are unique to the system. Invasive species success has been related to “distinctiveness” of the species within the invaded habitat (Ricciardi and Atkinson 2004). The ecological uniqueness of the species may provide advantages in the invaded range (Strauss et al. 2006), and specifically, the Novel Weapons Hypothesis posits biochemical uniqueness as a fundamental feature of plant invasion biology (Callaway and Ridenour 2004). *Lonicera maackii* leaf materials contain phenolic compounds which have been implicated in negative effects on insect herbivores in the invaded range (Lieurance et al. 2015). Deposition of leaf materials with novel, toxic, phytochemistry into headwater streams could partly explain the patterns we discovered of less sensitive taxa in sites with the heaviest invasion. Toxic effects of “novel subsidies” (*sensu* Custer et al. 2017) from *L. maackii* have been detected in both *in-situ* and *ex-situ* microcosms. Borth et al. (2018) discovered variation in mortality rate of target organisms from leaves collected at different times during the growing season suggesting phytochemical variation related to plant phenology. Lieurance et al. (2015) identified secondary metabolites in the chemistry of *L. maackii* leaves, including iridoid glycosides and phenolics, as potentially important sources of anti-herbivore effects. Future work that focuses on the effects of foliar materials containing anti-herbivory



phytochemistry on aquatic macroinvertebrates may offer key insights into the invasion biology of *L. maackii* and other problematic invasive species of riparian habitats.

In addition to novel chemistry, shifts in leaf decay rates and shading may be mechanisms by which riparian invasion alters stream biology. *Lonicera maackii* leaves that are deposited into streams have an extremely rapid decay rate, which may also influence aquatic macroinvertebrates as leaf material. Rapid decay could influence aquatic macroinvertebrates in a variety of ways including reduced permanence of leaf structures that serve as habitat and a shift from CPOM to FPOM in streams. Bailey et al. (2001) found a 2× decrease in aquatic macroinvertebrate richness associated with rapid decay of foliage from salt cedar (*Tamarix ramosissima*) which is invasive in riparian zones in the American Southwest. Similarly, McNeish et al. (2015) found rapid decay in *L. maackii* leaves and that the macroinvertebrate community colonizing these leaves was lower in density. Russian olive (*Eleagnus angustifolia*) is an invasive woody shrub that is problematic in riparian areas and exhibited more rapid decay and release of nitrogen in terrestrial habitats but was indistinguishable when submerged (Harner et al. 2009). *Acer plantanoides* decomposition was similar to native species; however, strong effects were found on colonizing macroinvertebrate composition (Reinhart and VandeVoort 2006).

Inclusion of plant community composition into management of riparian habitats could improve outcomes for aquatic biota. Terrestrial-aquatic connections are fundamental (Vannote et al. 1980) and alterations to stream biota from changes to terrestrial habitats are seen in a lot of different systems (Wallace et al. 1997, Gregory et al. 2001, Richardson et al. 2007, Moore and Palmer 2005). In recognition of the importance of terrestrial-aquatic linkages, many agencies have adopted rules about

activity in areas adjacent to streams and these “best management practices” are enforced to protect stream health (Moore et al. 2005, Phillips et al. 2000). Though quantifiably effective, these management practices rarely consider species *composition* of the riparian forest and, instead, focus largely on the width and structural integrity of the protective buffer. Our findings, and those of others (McNeish et al. 2012, McNeish et al. 2017, Reinhart and VandeVoort 2006), indicate that compositional shifts associated with terrestrial plant invasion are an important consideration. Terrestrial subsidies are most important in smaller streams and the influence of terrestrial plant invasions on aquatic systems is likely much reduced in larger streams, rivers or larger lakes. Flow is likely an important determinant of exposure to phytochemical toxins and, thus, we hypothesize that stagnant waters are more likely to be influenced by the deposition novel subsidies from invasive species than during high flow conditions. Thus, the importance of riparian invasion as a structuring factor for aquatic macroinvertebrates may be the highest in small, stagnant, habitats such as small ponds are low-flow headwater streams, and relatively insignificant in large flowing rivers. Future work testing this hypothesis would advance understanding of invasion biology and riparian forest management.

In summary, we implemented an *in-situ* experiment that provided evidence that riparian zone invasion by the shrub *L. maackii* alters aquatic macroinvertebrate communities. Our stream-scale results indicate that *L. maackii* invasion altered the overall macroinvertebrate community where there is a dense invasion and sensitive species may be especially susceptible to negative impacts of this invasion. *Lonicera maackii* has rapid decomposition and alters organic matter availability and structure throughout the stream and may release toxic novel subsidies, directly impacting local

communities. Our data add to a growing body of evidence that suggests this species has the potential to fundamentally alter headwater streams that meander through invaded forests. To our knowledge, these results provide the first stream-scale observational results to support a series of experiments and more reductionist microcosm projects that indicated *L. maackii* materials influenced the biology of aquatic systems. These results provide a strong impetus that managing riparian invasion of *L. maackii* would increase overall stream health by conserving healthy macroinvertebrate communities within headwater streams.

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## Figure Legends

Figure 1: Relative abundance of sampled macroinvertebrate taxa by site and season in streams characterized by a gradient of riparian *L. maackii* density in temperate forests in southwest Ohio. The codes include the treatment followed by the first letter of the site name: REF = little to no *L. maackii* invasion, MOD = moderate invasion, and HVY = heavy invasion. Taxa are sorted down to Order, excluding the family Chironomidae in the Order Diptera, classes Gastropoda and Bivalvia, and the subclasses Oligochaeta, Hirudinea, and Acari.

Figure 2: Percentage of EPT individuals (%EPT) in streams characterized by a gradient of riparian *L. maackii* density in temperate forests in southwest Ohio. This metric is calculated by summing all individuals in the Orders Ephemeroptera, Plecoptera, and Trichoptera and dividing by the total number of macroinvertebrates in the sample. Letters represent statistically significant differences ( $P < 0.05$ ) revealed by Tukey's post hoc comparisons.

Figure 3: EPT Index along a gradient of riparian *L. maackii* density in streams characterized by a gradient of riparian *L. maackii* density in temperate forests in southwest Ohio. An EPT Index is the sum of all distinct families identified within the orders Ephemeroptera, Plecoptera, and Trichoptera. There were no statistically significant differences between groups ( $P > 0.05$ )

Figure 4: Percentage of Chironomidae individuals in samples collected in streams characterized by a gradient of riparian *L. maackii* density in temperate forests in southwest Ohio. This metric is calculated by summing all individuals in the family Chironomidae and dividing by the total number of macroinvertebrates in the sample. There were no significant differences of percent Chironomidae by invasion of *L. maackii* ( $P > 0.05$ )

Figure 5: NMDS ordination plots of macroinvertebrate samples collected in streams characterized by a gradient of riparian *L. maackii* density in temperate forests in southwest Ohio. (ANOSIM  $R = 0.215$ ,  $P = 0.004$ ). The codes include the treatment followed by the first letter of the site name: REF = little to no *L. maackii* invasion, MOD = moderate invasion and HVY = heavy invasion. Plots are comparing relative abundance data at the order level.





Figure 2

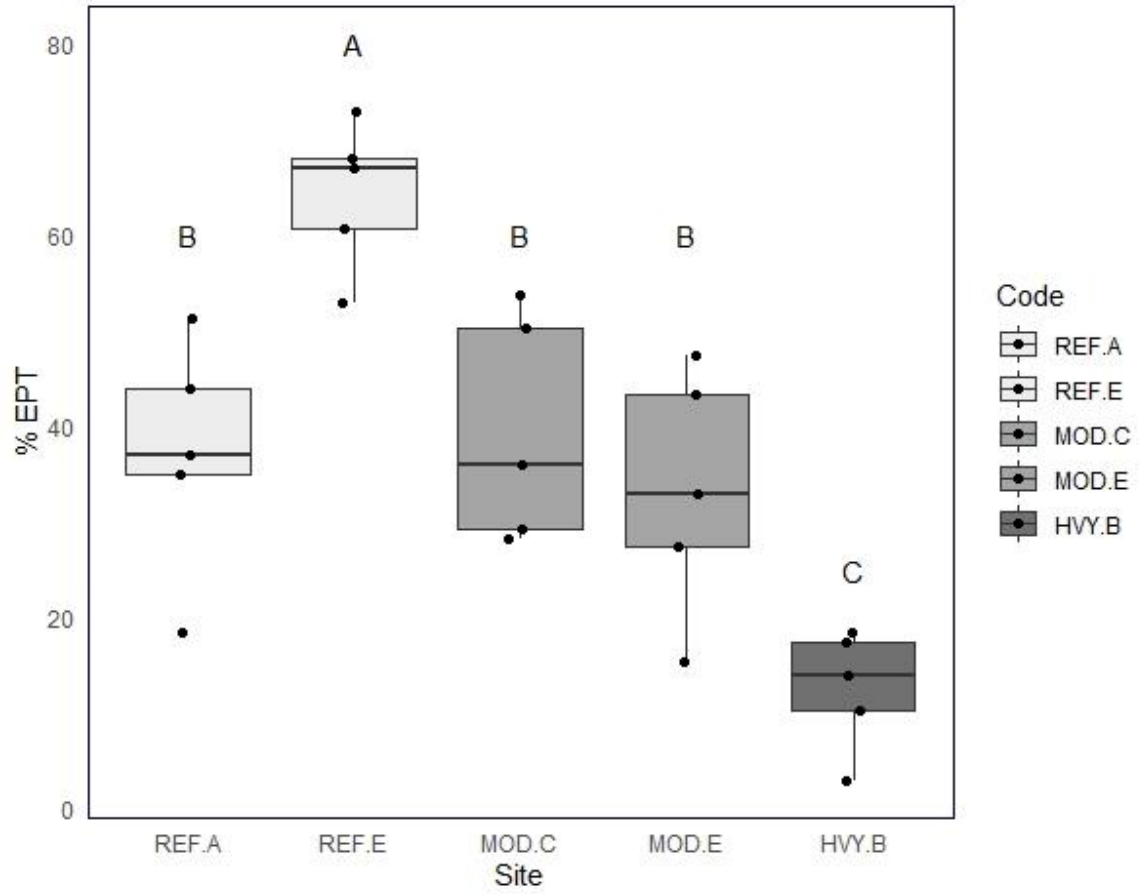


Figure 3

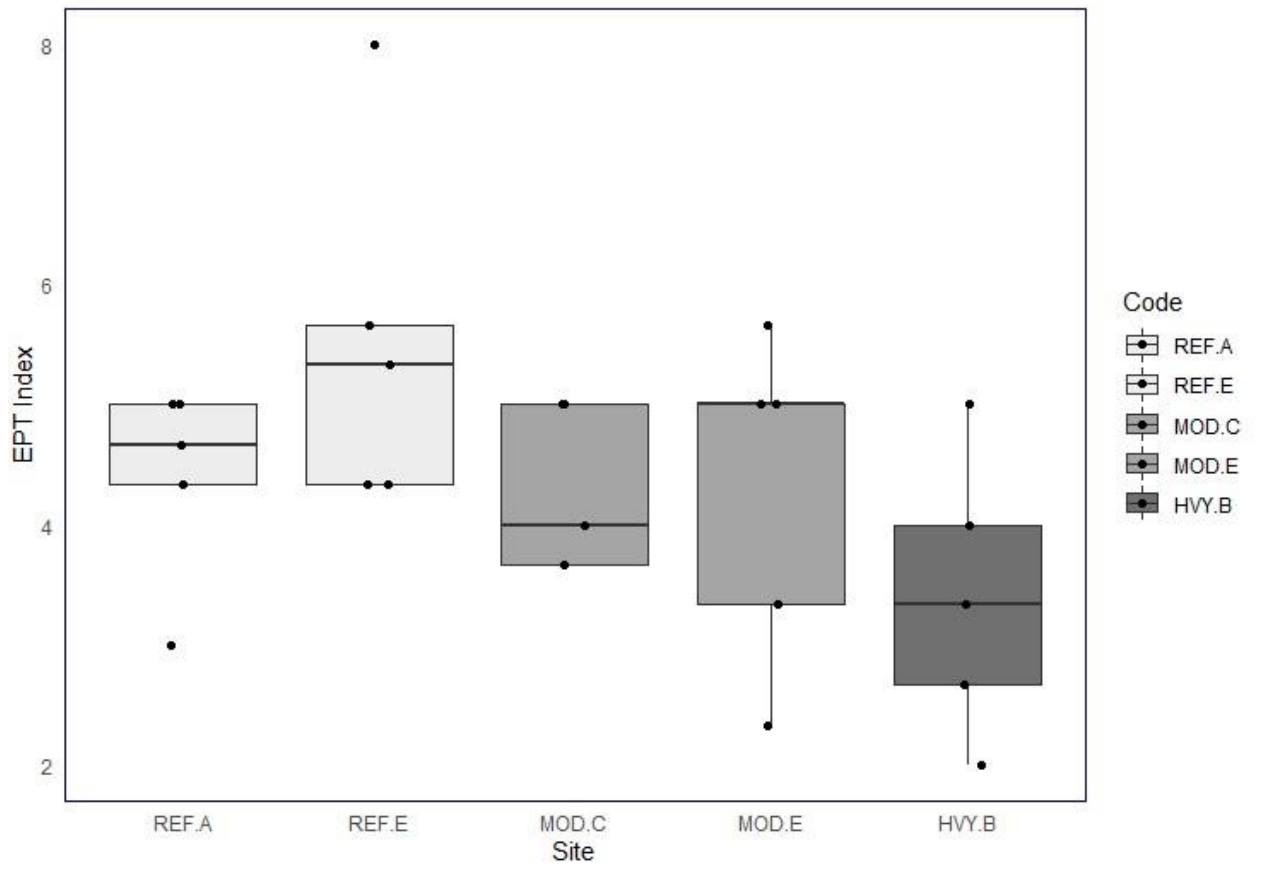


Figure 4

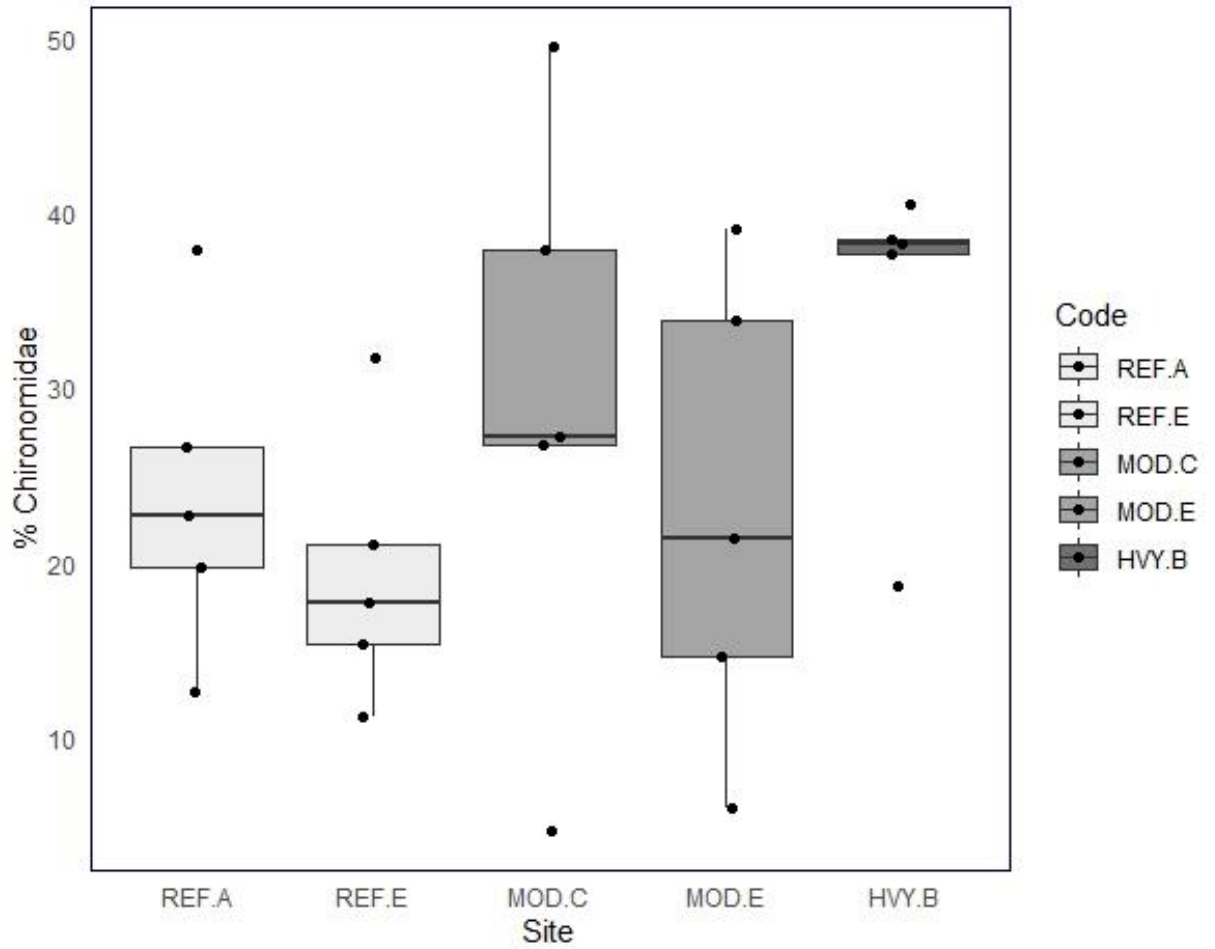
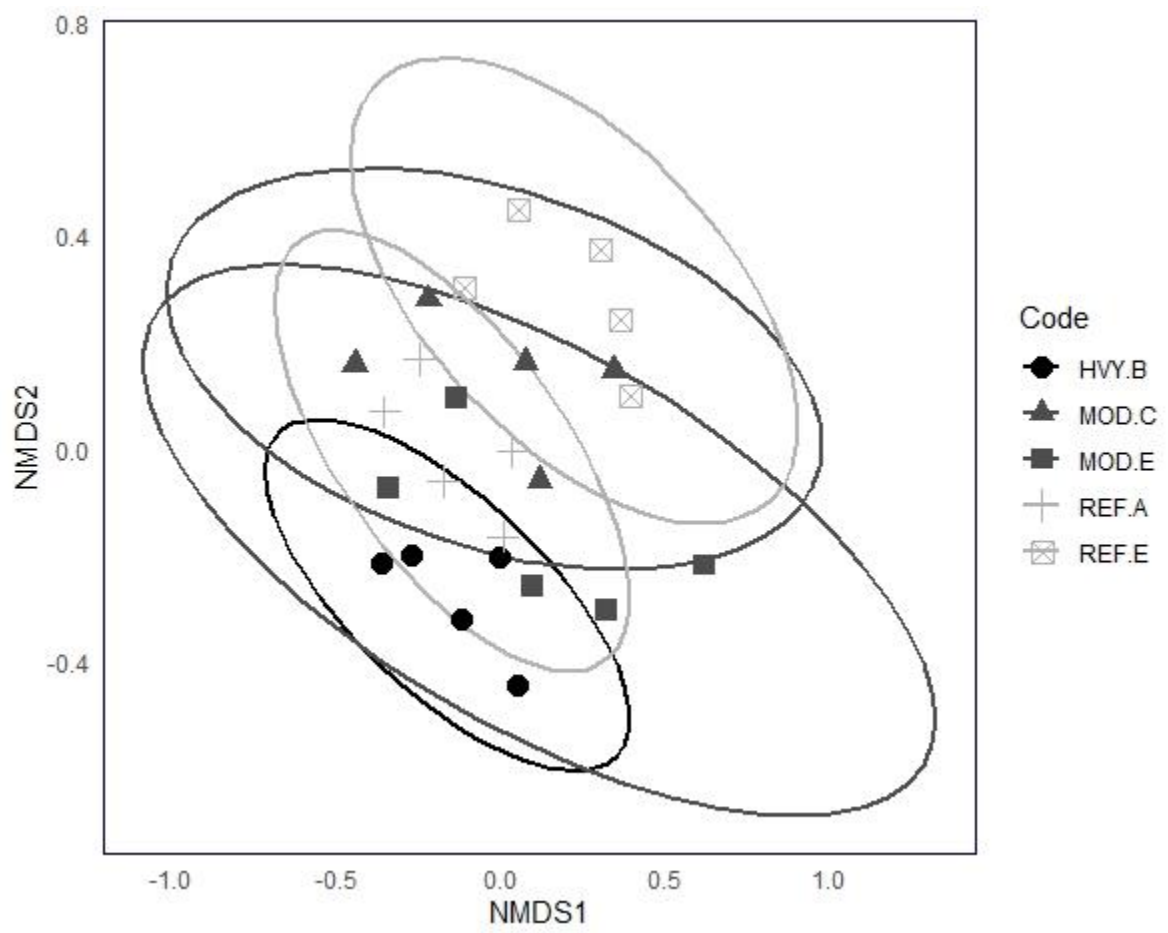


Figure 5



## Supplemental Materials

**Supplemental Table 1.** Five 1<sup>st</sup> and 2<sup>nd</sup> order streams in Southwestern Ohio, USA were selected based on their variation in *Lonicera maackii* invasion density. We selected two streams with minimal *L. maackii* invasion to serve as reference sites: (REF.E) located in Five Rivers Metroparks, Englewood, Ohio, USA, and Wiles Creek - Aullwood Reference (REF.A) located in Aullwood Audubon Center, Dayton, Ohio, USA. Two moderately invaded sites were 1st order headwater streams: Englewood Moderate (MOD.E) located in Five Rivers MetroParks, Englewood, Ohio, USA, Charleston Falls Moderate (MOD.C) located in Miami County Park District, Tipp City, Ohio, USA. A heavily invaded site was Buckeye Trail Heavy (HVY.B) located in Five Rivers MetroParks, Vandalia, Ohio, USA. Mean ( $\pm$ SE) basal area ( $\text{m}^2 \text{ha}^{-1}$ ) and density (stems  $\text{ha}^{-1}$ ) and of total, live, and dead Amur honeysuckle (*L. maackii*) in five headwater stream riparian forests in southwestern Ohio experiencing differing levels of invasion by the exotic shrub *Lonicera maackii*.

Site	Total	Live	Dead
<b>Basal Area</b>			
HVY.B	7.09 $\pm$ 1.44	3.80 $\pm$ 1.11	3.29 $\pm$ 1.45
MOD.C	4.50 $\pm$ 0.58	2.38 $\pm$ 0.72	2.12 $\pm$ 1.00
MOD.E	5.48 $\pm$ 0.66	3.08 $\pm$ 0.93	2.40 $\pm$ 1.07
REF.A	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00
REF.E	0.16 $\pm$ 0.14	0.01 $\pm$ 0.01	0.15 $\pm$ 0.14

<b>Density</b>				
HVY.B	10648.15 ± 1964.08	5833.33 ± 1491.14	4814.81 ± 914.75	
MOD.C	6666.67 ± 804.28	3634.26 ± 1104.97	3032.41 ± 1023.40	
MOD.E	5787.04 ± 793.55	2893.52 ± 801.41	2893.52 ± 980.40	
REF.A	23.15 ± 23.15	23.15 ± 23.15	0.00 ± 0.00	
REF.E	231.48 ± 137.34	23.15 ± 23.15	208.33 ± 142.32	

**Supplemental Table 2:** Site characterization for five headwater streams in southwestern Ohio used to assess the influence of *Lonicera maackii* on stream biology. The Headwater Habitat Evaluation Index (HHEI; OEPA 2012) was performed at all sites in 2015. In addition, all sites were characterized for physical (instream habitat, watershed area, and % forest cover), water-only metal concentrations, sediment toxicity (USEPA 2000), water-only toxicity (USEPA 2002), and sediment total organic carbon (TOC). Bank full width, wetted width, and maximum pool depth was measured within each experimental stream reach (30 m). All sediments were measured for % moisture and TOC (Heiri et al. 2001; Santisteban et al. 2004). Sediments were dried at 105 °C for 24 ± 2 h, and TOC was calculated based on loss on ignition (LOI; (550 °C at 4 ± 0.5 h) using a correction factor of 0.38 to convert LOI to organic carbon (Redfield 1934). Watershed area and % forest cover was calculated using USGS Stream Stats 4.0 program (<http://streamstatsags.cr.usgs.gov/streamstats/>; Supplemental Table 2).

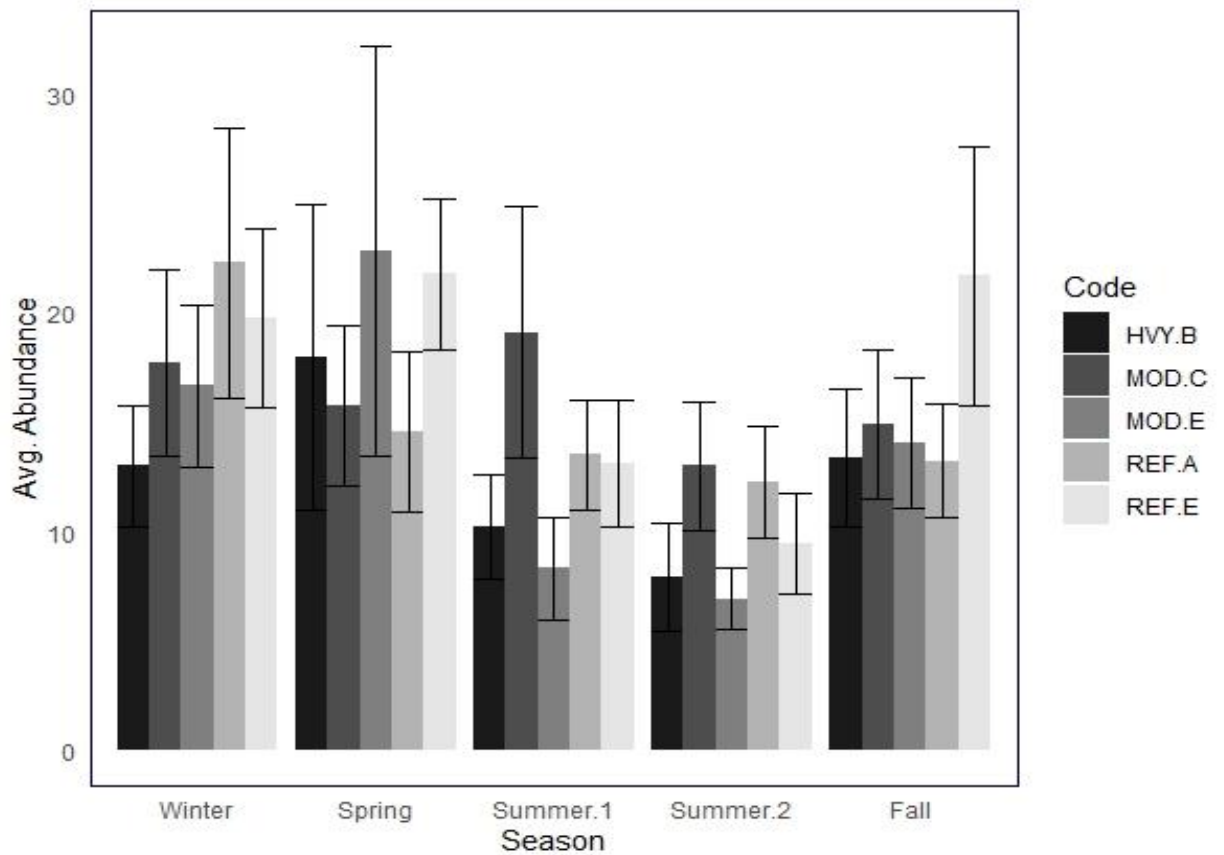
	REF.A	REF.E	MOD.E	MOD.C	HVY.B
Stream Order	2nd	2nd	1st	1st	1st
HHEI	79	83	79	78	74
Watershed* (mile <sup>2</sup> )	0.32	0.22	0.04	0.07	0.1
Sediment % Solids	81.7 ± 2.0	84.7 ± 1.3	81.3 ± 1.0	80.5 ± 1.8	81.6 ± 0.9
Sediment % TOC	0.6 ± 0.3	1.8 ± 0.08	1.7 ± 0.5	1.1 ± 0.2	0.8 ± 0.04
Bankfull Width (m)	3.6 ± 0.2	5.0 ± 0.3	3.7 ± 0.7	4.0 ± 0.5	2.6 ± 0.8
Wetted Width (m)	2.7 ± 0.6	1.8 ± 1.2	1.6 ± 1.0	1.8 ± 0.7	2.6 ± 0.8

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Maximum Pool Depth (cm)	20.5	11	12	18.5	12
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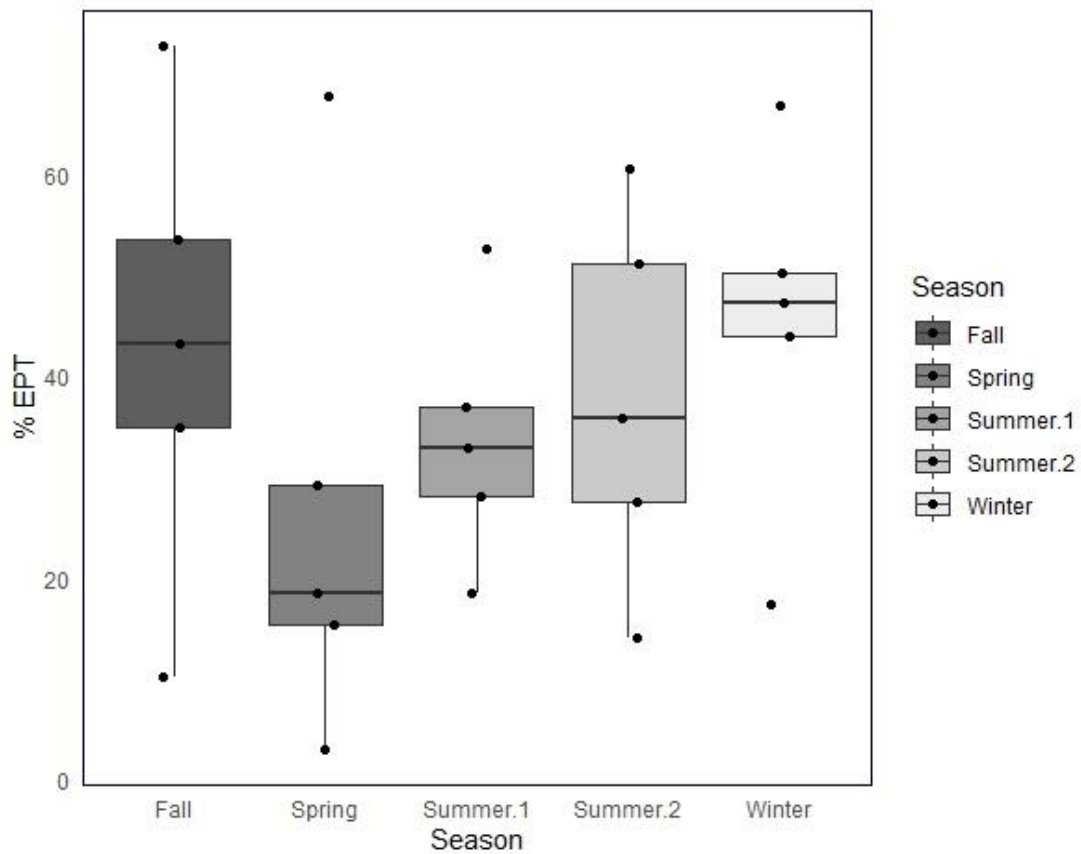
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**Supplemental Figure 1:** Average number of individual macroinvertebrates counted at each sampling event along a gradient of riparian *L. maackii* density in temperate forests in southwest Ohio.



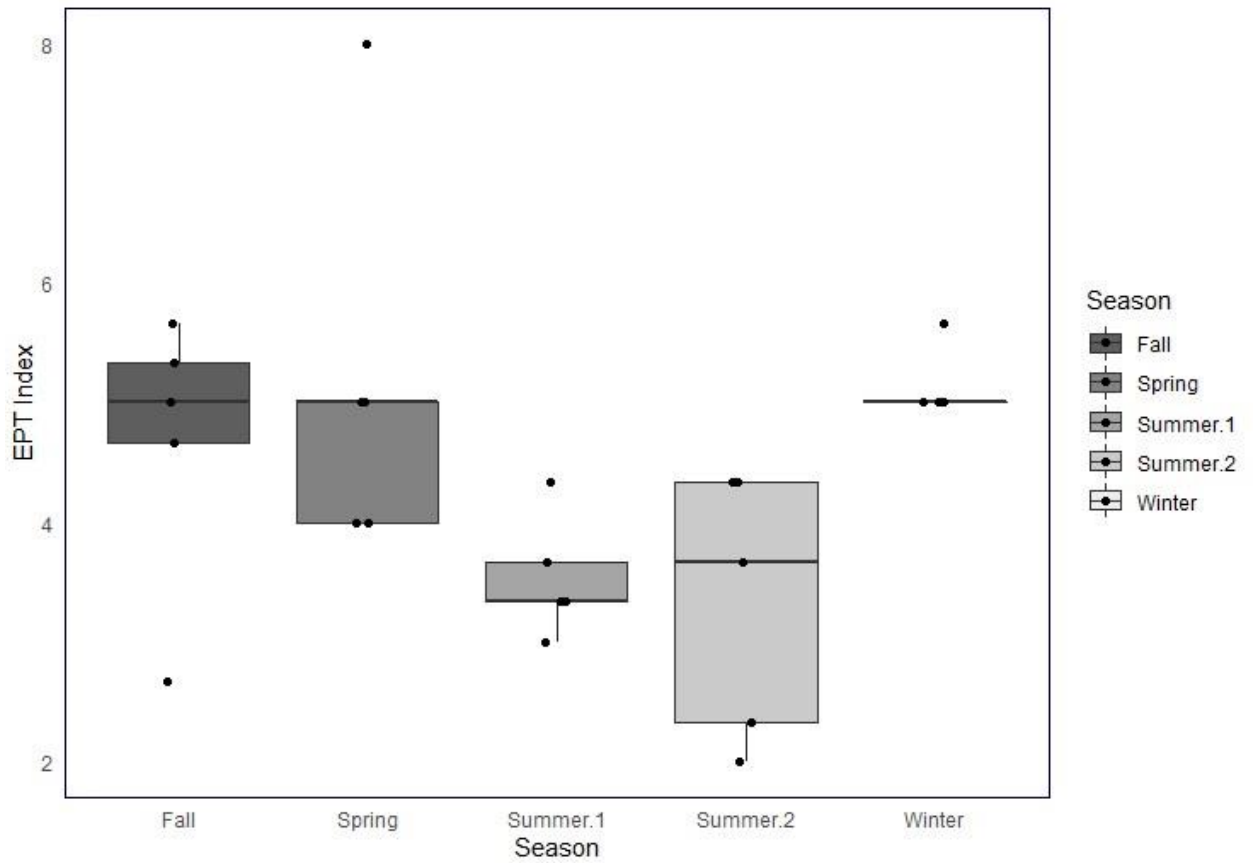
**Supplemental Figure 2:** Percentage of EPT individuals (%EPT) by seasonal sampling.

This metric is calculated by summing all individuals in the Orders Ephemeroptera, Plecoptera, and Trichoptera and dividing by the total number of macroinvertebrates in the sample. This metric was calculated using either treatment of season as a random effect to look at *L. maackii* effects and seasonal effects separately.



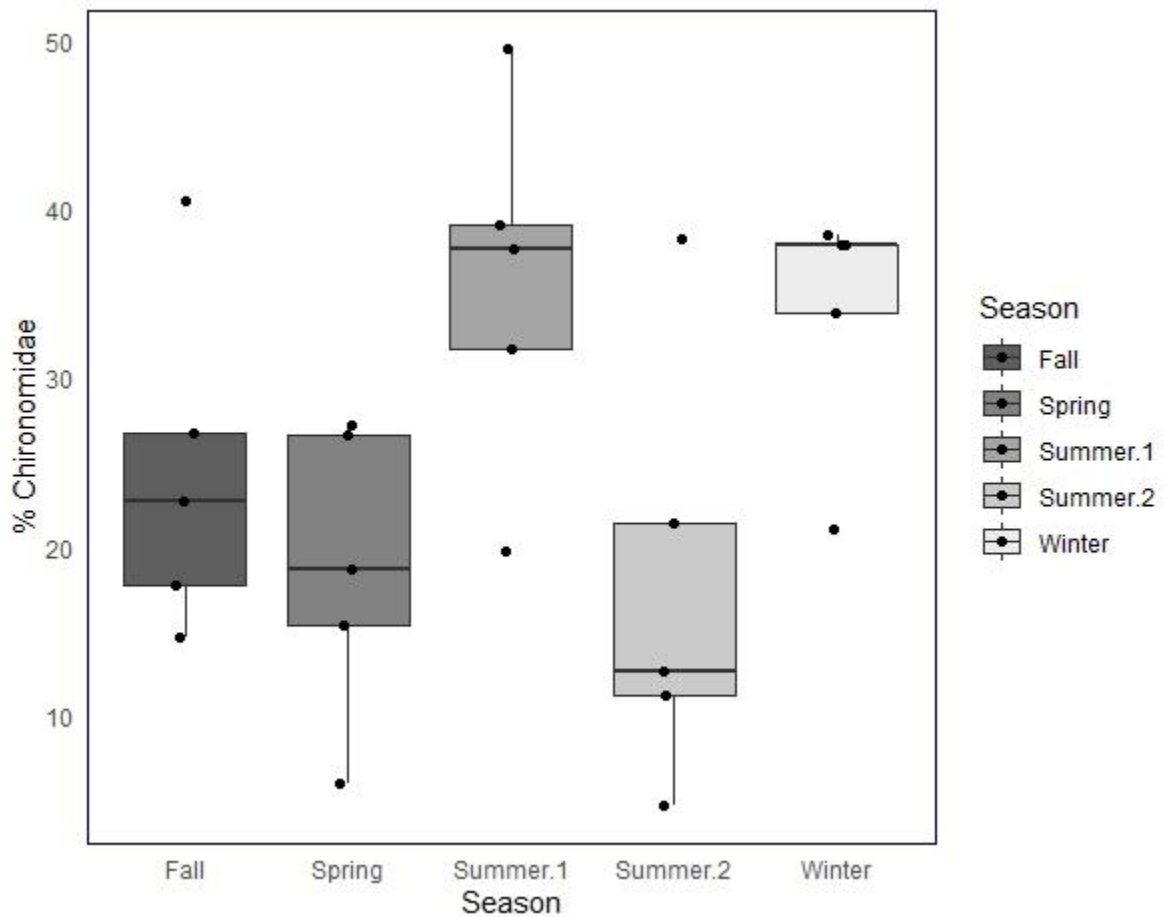


**Supplemental Figure 3:** EPT Index by seasonal sampling. An EPT Index is the sum of all distinct families identified within the Orders Ephemeroptera, Plecoptera, and Trichoptera. This metric was calculated using either treatment or season as a random effect to look at *L. maackii* effects and seasonal effects separately.



**Supplemental Figure 4:** Percentage of Chironomidae individuals by seasonal sampling.

This metric is calculated by summing all individuals in the family Chironomidae and dividing by the total number of macroinvertebrates in the sample. This metric was calculated using either treatment of season as a random effect to look at *L. maackii* effects and seasonal effects separately.



**Supplemental Figure 5:** NMDS ordination plots by seasonal sampling. Plots are comparing relative abundance data at the Order level (see Figure 1).

