BOTTOM-UP EFFECTS ALTER HEMIPTERAN ABUNDANCE AND DIVERSITY

by

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ABSTRACT

Phytophagous insects, specifically Hemiptera, are an integral part of the natural environment, serving as prey for a wide variety of predators and playing significant roles as herbivores. I examined top-down and bottom-up factors affecting hemipteran communities along the Potomac River in Washington, Frederick, and Montgomery Counties in Maryland. Insects were collected by vacuum sampling and in leaf litter collections and analyzed with simple linear regression. Greater vegetation diversity was related to a decreased hemipteran abundance, indicating that plant composition in riparian forests influences hemipteran life histories. The increased volume of coarse woody debris was negatively correlated with hemipteran abundance and diversity, which may be due to changes in vegetation and insect community composition in areas of increased woody debris. Top-down effects did not influence hemipteran abundance or diversity within this study system. These multi-trophic relationships are important to examine as they provide a greater understanding about the overall true bug community structure and functionality as it relates to conserving biodiversity.

ACKNOWLEDGEMENTS

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INTRODUCTION

Insects are an incredibly abundant group and are the most diverse group of organisms throughout the world (Pinedo-Escatel and Moya-Raygoza 2018). Their small size, rapid reproductive cycles, and ability to survive in almost any habitat allow them to occupy diverse niches in the environment (Moir et al. 2011). The true bugs (Order Hemiptera), specifically, fill multiple functional groups as herbivores, prey, and predators, and as such, can be attributed with significant functional importance to the natural ecosystem (Moir et al. 2005). Within the environment, herbivorous hemipterans have been found to affect vegetation through herbivory and by transporting plant pathogens (Purcell and Frazier 1985, Pinedo-Escatel and Moya-Raygoza 2018). Hemipterans are incredibly speciose and include groups such as the leafhoppers (Cicadellidae), planthoppers (Fulgoridae), cicadas (Cicadidae), aphids (Aphididae), and shield bugs (Pentatomidae). There are roughly 80,000 species of Hemiptera within 37 different families throughout the world, with approximately 11,000 of these species inhabiting North America (Price et al. 2011). This order of insects inhabits very diverse environments and includes specialists, generalists, herbivores, predators, plant parasites, and ecologically important species.

Herbivorous hemipterans serve as intermediate taxa between predators and vegetation; therefore, both top-down and bottom-up effects have the capacity to influence hemipterans in the environment (Purcell and Frazier 1985, Heong et al. 1992, Hunter and Price 1992, Narhardiyati and Bailey 2005, Kos et al. 2011, Moreira et al. 2012). Top-down effects occur when a predator influences prey taxa which can impact their population dynamics (Purcell and Frazier 1985, Heong et al. 1992, Narhardiyati and Bailey 2005, Kos et al. 2011, Moreira et al. 2012). For herbivores, these top-down effects indirectly alter plant communities by decreasing the extent of herbivory on plants (Costamagna and Landis 2006, Kos et al. 2011). Bottom-up effects occur as traits of the

plant community, such as species composition and relative abundance, influence higher trophic levels, such as herbivorous insects. These plants cause changes to herbaceous insect groups by altering the extent and availability of food, shelter, and substrate, ultimately impacting hemipteran diversity and population dynamics (Hunter and Price 1992, Kos et al. 2011). Top-down and bottom-up factors interact with each other throughout the environment, collectively exerting influence over hemipteran communities. In addition to bottom-up and top-down impacts from vegetation and predators, environmental and climatic variables may also influence hemipteran diversity and abundance (Moir et al. 2005, Moreira et al. 2012, Virla et al. 2015, Meneses et al. 2016). Hemipteran abundance may be influenced by many factors including humidity, rainfall, temperature, predator presence, and biodiversity of native and non-native vegetation.

A prevalent top-down effect influencing hemipteran biodiversity and abundance is predation by other insects and larger organisms, such as birds and mammals. Insect predators of herbivorous hemipterans are known to include ladybird beetles (Coleoptera: Coccinellidae), damselbugs (Hemiptera: Nabidae), lacewings and allies (Neuroptera), assassin bugs (Hemiptera: Reduviidae), and spiders (Araneae); however, little is known about their impact on herbivorous hemipteran populations and overall diversity (Narhardiyati and Bailey 2005, Virla et al. 2015, Weiser-Erlandson and Obrycki 2015). Such predatory insects have been shown to affect leafhopper mortality in alfalfa fields (Weiser-Erlandson and Obrycki 2015). Unfortunately, little is known about predator impacts on herbivorous hemipteran and leafhopper populations in other ecosystems (Virla et al. 2015). It has been suggested that the presence of predators may decrease the abundance and diversity of Hemiptera (Heong et al. 1992). These predator-prey interactions can have significant effects on the abundance and distribution of prey species by influencing their movement patterns, oviposition sites, reproduction, and mating strategies. Therefore, it is

imperative to study the interrelations of hemipteran prey and their predators more closely as they relate to true bug biodiversity and abundance from a top-down perspective.

While top-down effects are present, herbivores can also be influenced by bottom-up processes through the vegetation (Hunter and Price 1992, Denno et al. 2003, Moreira et al. 2012). Bottom-up effects resulting from plant-herbivore interactions greatly influence the population dynamics of phytophagous insects by affecting where and in what densities they may colonize an area. Specifically, plant nutrient composition, location, neighboring plants, and chemical defenses have been shown to affect hemipteran diversity and behavior (Rebar and Rodriguez 2015, Pan et al. 2016). Availability of nutrients such as nitrogen and phosphorous, which are essential for exoskeleton and egg development, can have significant effects on herbivorous insect diversity as some insects are better adapted to survive with limited nutrients (Wetzel et al. 2016). Therefore, variability in nutrients throughout the environment can alter insect diversity and spatial distribution (Wetzel et al. 2016). The ratios and presence of nitrogen, phosphorous, and other nutrients within palatable vegetation can impact insect development throughout instar stages, site selection for oviposition, geographic distribution, and host plant preference due to chemical plant defenses (Silberbush et al. 2014, Chen et al. 2016, Wetzel et al. 2016). Vegetation location and neighboring plant composition have also been shown to alter hemipteran diversity as some insects may prefer to feed on one host plant but oviposit on a neighboring plant (Moreira et al. 2012). This may influence the insect community within the area as the offspring will now have different interspecific interactions with residents of the neighboring plant (Pan et al. 2016). In addition, more diverse plant communities will promote a greater diversity of food sources, particularly for specialists, thereby producing more diverse insect communities (Moir et al. 2011, Moreira et al. 2012). Specialist herbivores may choose to inhabit a particular area when their food source is

readily available and abundant. This becomes possible with an increase in vegetation diversity. These factors can greatly influence hemipteran diversity and abundance by influencing their behavior, growth, and survival.

As both top-down and bottom-up effects have been shown to impact herbivorous true bug communities, it is critical to further understand hemipteran diversity and abundance and their relation to predators and plant composition. I aim to determine whether top-down or bottom-up effects are driving changes in the diversity and abundance of hemipterans in riparian forests in Maryland's Potomac River Valley. The objective of this study is to understand relationships between Hemiptera and their top-down and bottom-up influences. I hypothesize that vegetation diversity will have a positive effect on hemipteran diversity while predator density will be inversely correlated with true bug abundance. These hypotheses are motivated by previous findings in agricultural and grassland studies, which suggest that predator presence is negatively correlated with hemipteran prey abundance and that hemipteran diversity increases with an increase in vegetation biodiversity (Lewinsohn et al. 2005, Sandom et al. 2014, Weiser-Erlandson and Obrycki 2015). This work will identify whether these patterns are applicable to riparian forested ecosystems in the eastern US. Additionally, much of the literature focuses solely on either top-down or bottom-up effects, where I hypothesize that both effects are prevalent and influential. This work expands current understanding of tri-trophic interactions with hemipteran insects as the study will provide a comprehensive lens into how both top-down and bottom-up effects influence hemipteran diversity and abundance in riparian forests of eastern North America.

MATERIALS AND METHODS

The United States National Park Service (NPS) maintains 425 forest vegetation monitoring plots throughout the national parks in Maryland, Washington D. C., and Northern Virginia. These plots were designed to monitor the long-term changes in forest vegetation within the parks and have been selected from a generalized random tessellation stratified design. Of these, a subset of 30 plots was chosen for sampling within a 120-km stretch of the Chesapeake and Ohio Canal National Historical Park (C&O Canal) in Washington, Frederick, and Montgomery Counties, Maryland. These areas are representative of floodplains in the Piedmont and Valley and Ridge physiographic provinces (Figure 1). Each plot was 30 m in diameter and previously subdivided for the NPS to monitor trees, shrubs, saplings, and seedlings.
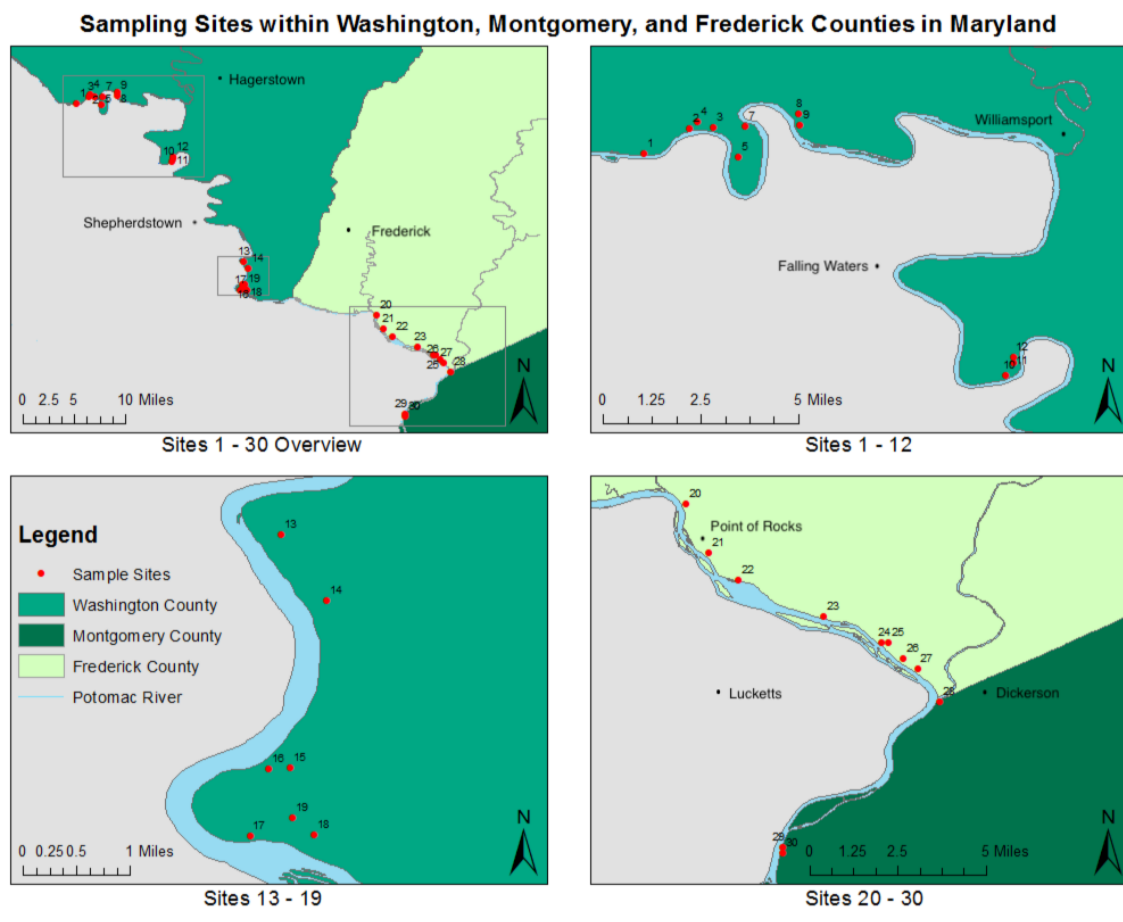
Figure 1: Map of Field Site Locations throughout the C&O Canal in Washington, Frederick, and Montgomery Counties, Maryland.

Forest vegetation data are collected by NPS specialists trained to identify invasive and native vegetation within the plots. These data include trees, saplings, shrubs, seedlings, and coarse woody debris that are sampled in each plot within a four-year panel design. The majority of study plots contain maples (*Acer negundo* and *A. saccharinum*) and black locust (*Robinia pseudoacacia*) in the canopy, as well as paw-paw (*Asimina triloba*) and northern spicebush (*Lindera benzoin*) in the understory. Several plots also contain invasive species such as Japanese stiltgrass (*Microstegium vimineum*) and multiflora rose (*Rosa multiflora*) on the forest floor. I defined vegetation abundance as the abundance of tree seedlings in three subplots, each approximately 47-m^2 in size, which is referred to as seedling abundance.

Each plot was sampled with standard field collection techniques for invertebrates in the forest understory through the collection of vacuum and leaf litter samples (Moir et al. 2005, 2011). To avoid disturbing vegetation within the long-term monitoring plots, I sampled within a 2-m circular buffer along the perimeter of the 30-m diameter plot (Figure 2). I conducted vacuum sampling twice in 2018, once in late June and again in early September, to account for seasonal variation in insect life histories. I collected samples by strapping a paint strainer bag to the vacuum (Black + Decker LSWV36, Connecticut, USA), ensuring that the bag would not be sucked into the mulcher, and vacuumed plant material and spaces between plants within a 2-m wide circular buffer around the plot for a standardized time of 8 minutes. Larger insects and spiders in the sample area were collected by hand if the vacuum sampling was not effective in capturing the larger specimens. Once the 8 minutes was complete, the vacuum was left on while the bag was removed and tied off. The bag was then labeled and placed in a container with ethyl acetate to asphyxiate collected insects for at least 15 minutes. Although 30 plots were identified, one plot was completely inaccessible during the second round of vacuum sampling so was removed from analyses.
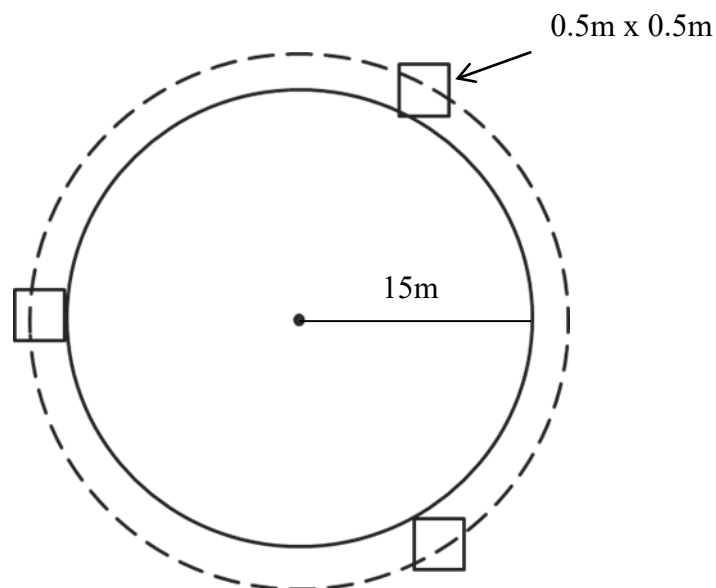
Figure 2: Diagram for sampling of study sites. Each plot is 30 m in diameter, vacuum sampling was conducted in a 2 m buffer, and leaf litter collections occurred at 120, 240, and 360 degrees from magnetic north using a 0.5 x 0.5 m PVC square.

I also collected leaf litter samples once, in early September 2018, as sampling the same location twice did not allow adequate leaf litter to accumulate in the area. To collect leaf litter, I located the center of each plot, and sampling locations were determined at 120, 240, and 360 degrees from magnetic north at the perimeter of the circular plot. A 0.5 x 0.5 m PVC sampling square was placed upon the ground, avoiding live trees or shrubs, and all of the leaf litter and woody debris within the plot was collected and placed into a bucket (Figure 2). The buckets were then taken to the lab and invertebrates were collected manually by hand. To preserve the insects for later identification, all collected invertebrates were placed into marked sample containers with 95% ethanol to preserve genetic material for a concurrent NPS study.

Invertebrates from both sampling periods and methods were identified using current taxonomy and available identification keys with a stereo microscope within the laboratory (Ubick

et al. 2005, Triplehorn et al. 2017). Hexapods and spiders were first identified to family, with further classification to morphospecies level. I conducted all analyses with Program R, including the vegan, ggplot2, and biodiversityR packages (Kindt and Coe 2005, Wickham 2016, R Core Team 2018, Oksanen et al. 2019). I used morphospecies or recognizable taxonomic units (RTUs) to calculate predator and hemipteran diversity. Hemipteran insect predators and herbivorous hemipteran abundances were defined as the sum of individuals per morphospecies per sampling plot. Seedling abundance was defined as the sum of individuals per species per plot. I used the Shannon-Weiner Diversity Index (*H*) to determine predator diversity, plant diversity, and hemipteran diversity within each site. Species richness was estimated with species accumulation curves for hemipteran predators, hemipterans, and vegetation. Rarefaction curves were created to plot the number of families as a function of the number of sites sampled within the study. These figures are designed to compare the species richness of each group accounting for different sample sizes. Rarefied species richness was used for regression analyses to account for varying sample sizes. I used simple linear regression to identify correlations between vegetation diversity and herbivorous true bug diversity, as well as herbivorous true bug diversity and predator diversity and abundance. I used similar methods to identify correlations between the abundance and diversity of Hemiptera and the percent of non-native vegetation within each plot, as well as coarse woody debris, percent of native ground cover, and proximity to the river. In addition, multidimensional scaling (MDS) was used to visualize similarity in hemipteran and vegetation communities across the study area. MDS is a robust technique for visualizing similarity in ecological data (Schiffman et al. 1983, Minchin 1987, Williams et al. 2002).

The linear regression analysis examined the relationships between hemipteran abundance and diversity and hemipteran insect predator abundance, insect predator diversity, seedling

abundance and diversity, proximity to the Potomac River (m), percent of native and non-native

ground cover, and volume of coarse woody debris (Table 1). Models were considered significant

at α=0.05. All data were tested for normality using the Shapiro-Wilks test. For those covariates

that exhibited non-normality (vegetation seedling abundance, proximity to river, percent native

and non-native vegetation, and coarse woody debris), I used Tukey's Ladder of Powers

transformation to normalize the observed data (Pek et al. 2017).

Table 1: Results of Simple Linear Regression analyses.
*Indicates significance (α = 0.05)

Dependent Variable	Independent Variable	SE	t	P	R^2
Hemipteran diversity	Predator diversity	0.124	0.961	0.345	0.033
	Predator abundance	0.004	0.113	0.911	0.001
	Vegetation diversity	0.126	-0.594	0.558	0.013
	Seedling abundance	8.92	-0.080	0.937	0.000
	Proximity to river (m)	0.132	1.07	0.296	0.005
	% Native ground cover	2.59×10^{-7}	0.257	0.799	0.003
	% Non-native ground cover	0.021	-0.546	0.589	0.011
	Coarse woody debris (m^3)	0.034	-3.46	0.001*	0.309
Hemipteran abundance	Predator diversity	6.51	1.42	0.166	0.070
	Predator abundance	0.202	1.73	0.095	0.100
	Seedling abundance	474.	0.773	0.446	0.022
	Vegetation diversity	6.31	-2.07	0.048*	0.137
	Proximity to river (m)	7.76	0.854	0.401	0.026
	% Native ground cover	1.33×10^{-5}	1.55	0.132	0.082
	% Non-native ground cover	1.06	-1.75	0.092	0.102
	Coarse woody debris (m^3)	1.77	-3.85	<0.001*	0.355
Predator diversity	Coarse woody debris (m^3)	0.063	-0.462	0.648	0.008
Predator abundance	Coarse woody debris (m^3)	2.00	0.132	0.896	0.001
Non-Hemipteran diversity	Coarse woody debris (m^3)	0.052	1.92	0.065	0.121
Non-Hemipteran abundance	Coarse woody debris (m^3)	10.5	-1.11	0.275	0.044

ArcGIS (ArcMap 10.7; ESRI, Redlands, California, USA) was used to create a family distribution map across the selected sites. This family distribution map included vegetation and habitat data for leafhoppers (Cicadellidae) which are found throughout all of the sampled sites. As global climates warm and impact herbivorous insects by altering the synchronization of phenological events as well as altering their host plants with shifts in distribution (Lewinsohn et al. 2005), this map helps predict possible shifts in family distribution in relation to future climate change events. I used shapefiles from the Maryland GIS Catalog (2010, 2015, 2017) and the U.S. Census Bureau Department of Commerce (2016). These shapefiles include Maryland cities, county boundaries, and national land cover. I then added leafhopper diversity data as graduated symbols for each site that was sampled.

ArcGIS was also used to analyze potential relationships between land cover data and hemipteran diversity and abundance as well as predator diversity and abundance. Shapefiles from Maryland's GIS Catalog (2010, 2015, 2017), the U.S. Census Bureau Department of Commerce (2016), and the Chesapeake Conservancy (2014) were used. I created a 150-m buffer surrounding the sample sites to incorporate greater amounts of land cover. I then added hemipteran abundance as graduated color symbols for each site. I used Ordinary Least Squares simple linear regression models to analyze possible relationships between hemipterans and the percent of different land cover types in each plot. Land cover types included water, tree canopy, low vegetation, as well as shrubland.

RESULTS

I collected a total of 5,692 insects and related arthropods belonging to a total of 161 identified morphospecies. These included 21 distinct morphospecies of Hemiptera and 108 morphospecies of their predators, including morphospecies in orders such as Hymenoptera and Araneae. The remaining 32 morphospecies identified included arthropods and arachnids that were not Hemiptera or their respective predators, therefore not included in the analyses. These included insects such as pseudoscorpions, mites, flies, and collembola among others. I found an average of 26.4 ± 2.97 (mean $\pm$ SE) hemipteran insects per plot with an average of 38.0 ± 2.69 predators per plot (Table 2). Predator species accumulation curves were steeper and peaked higher than herbivorous species (Figure 3). Total insect species diversity ranged from 1.23–2.81. Plot 2 had the highest insect diversity within this range (H=2.81) while Plot 30 had the lowest diversity (H=1.23, Table 3).

Table 2: Covariates with mean and standard error.

Variables	Mean ± SE
Hemipteran diversity (H)	1.26 ± 0.055
Hemipteran abundance	26.4 ± 2.97
Predator diversity (H)	2.23 ± 0.085
Predator abundance	38.0 ± 2.69
Vegetation diversity (H)	1.77 ± 0.084
Vegetation seedling abundance	66.5 ± 6.13
Proximity to river (m)	204 ± 22.4
% Native ground cover	88.9 ± 1.81
% Non-native ground cover	11.0 ± 1.81
Coarse woody debris (m^3)	6.32 ± 1.22
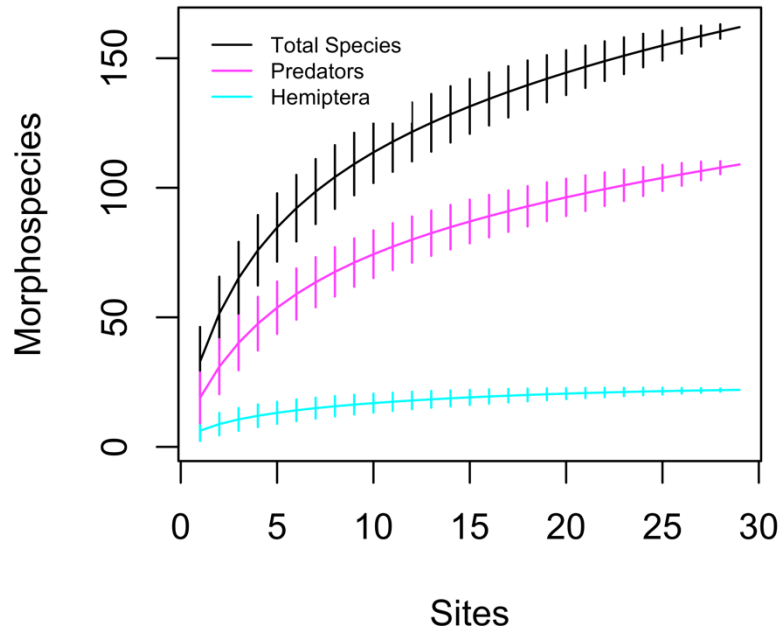
Figure 3: Comparison of morphospecies accumulation curves (*n*=29, CI=95%).

Table 3: Shannon-Weiner Diversity Index (*H)* values for all insect morphospecies sampled across 29 plots (*H*=1.227–2.806).

Plot #	H	Plot #	H	Plot #	H	Plot #	H	Plot #	H	Plot #	H
1	2.554	7	2.641	12	1.988	17	2.603	22	2.384	27	1.988
2	2.806	8	2.074	13	2.390	18	1.565	23	2.059	28	1.900
3	2.491	9	1.617	14	1.872	19	2.169	24	2.525	29	2.252
4	2.565	10	2.022	15	2.125	20	2.463	25	1.732	30	1.227
5	1.378	11	2.359	16	2.496	21	2.483	26	1.960		

The results of the linear regression analysis revealed that there were no significant relationships between hemipteran abundance and diversity and predator abundance and diversity (Table 1). Hemipteran diversity was not significantly related to their predator diversity (t=0.961, P=0.345, Figure 4A) throughout the study area. Predator diversity within the area did not have a significant effect on hemipteran abundance (t=1.42, P=0.166, Figure 4B). Similarly, predator abundance was not significantly related to hemipteran diversity (t=0.113, P=0.911, Figure 4C) and hemipteran abundance was not significantly affected by predator abundance throughout all of the sites (t=1.73, P=0.095, Figure 4D). Hemipteran diversity was not significantly affected by vegetation diversity (Figure 5A). However, hemipteran abundance was significantly related to vegetation diversity (t=-2.07, P=0.048, R^2=0.137, Figure 5B). Vegetation abundance did not significantly impact hemipteran abundance (t=0.773, P=0.446, R^2=0.022, Figure 5C) nor diversity (t=-0.080, P=0.937, R^2=0.000, Figure 5D). The relationship between the distance from the Potomac River and hemipteran diversity was not significant (t=1.07, P=0.296, Figure 6A) and neither was hemipteran abundance (P=0.401, t=0.854, Figure 6B). However, both hemipteran diversity (t=-3.68, P=0.001, R^2=0.309, Figure 6C) and abundance (t=-3.851, P<0.001, R^2=0.355, Figure 6D) were significantly related to the volume of coarse woody debris in each plot. Hemipteran abundance was also not significantly related to native (t=1.55, P=0.132, Figure 7A) or non-native (t=-1.75, P=0.092, Figure 7B) vegetation within the sampled sites. The percent of native (t=0.257, P=0.799, Figure 7C) and non-native (t=-0.546, P=0.589, Figure 7D) vegetation similarly did not affect hemipteran diversity. Ordination plots indicate that hemipteran community composition between plots was relatively similar to each other; however, plots 5, 14, and 28 were most dissimilar (Figure 8A). Plots 5, 14, and 28 did not contain many Cicadellidae morphospecies 3 (mean=0) or Psyllidae (mean=1.7) while other plots contained several individuals from both

families (mean=4.5 and 3.0, respectively). Vegetation community structure was relatively consistent throughout all of the plots, but plot 26 was very dissimilar from the majority (Figure 8B). Plot 26 may differ due to a low abundance of American pawpaw (*Asimina triloba*, mean=0) and spicebush (*Lindera benzoin*, mean=0) compared to other plots (mean=11.6 and 2.4, respectively).
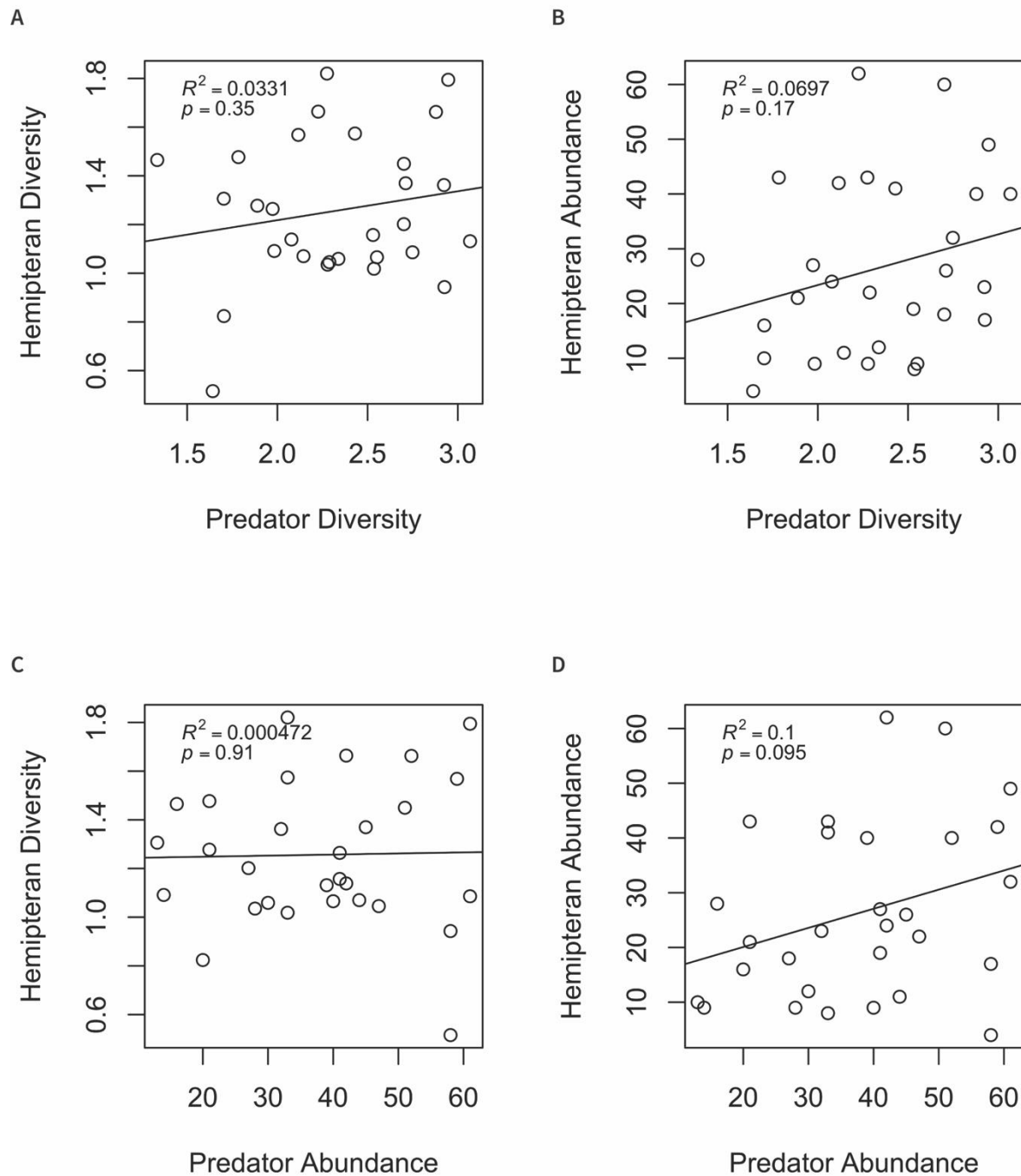
Figure 4: Simple linear regression analysis examining hemipteran abundance and diversity as it is affected by hemipteran insect predator abundance and diversity throughout the C&O Canal. (A) Hemipteran diversity affected by predator diversity (P=0.35, R^2=0.0331). (B) Hemipteran abundance affected by predator diversity (P=0.170, R^2=0.070). (C) Hemipteran diversity affected by predator abundance (P=0.910, R^2=0.001). (D) Hemipteran abundance affected by predator abundance (P=0.095, R^2=0.100).
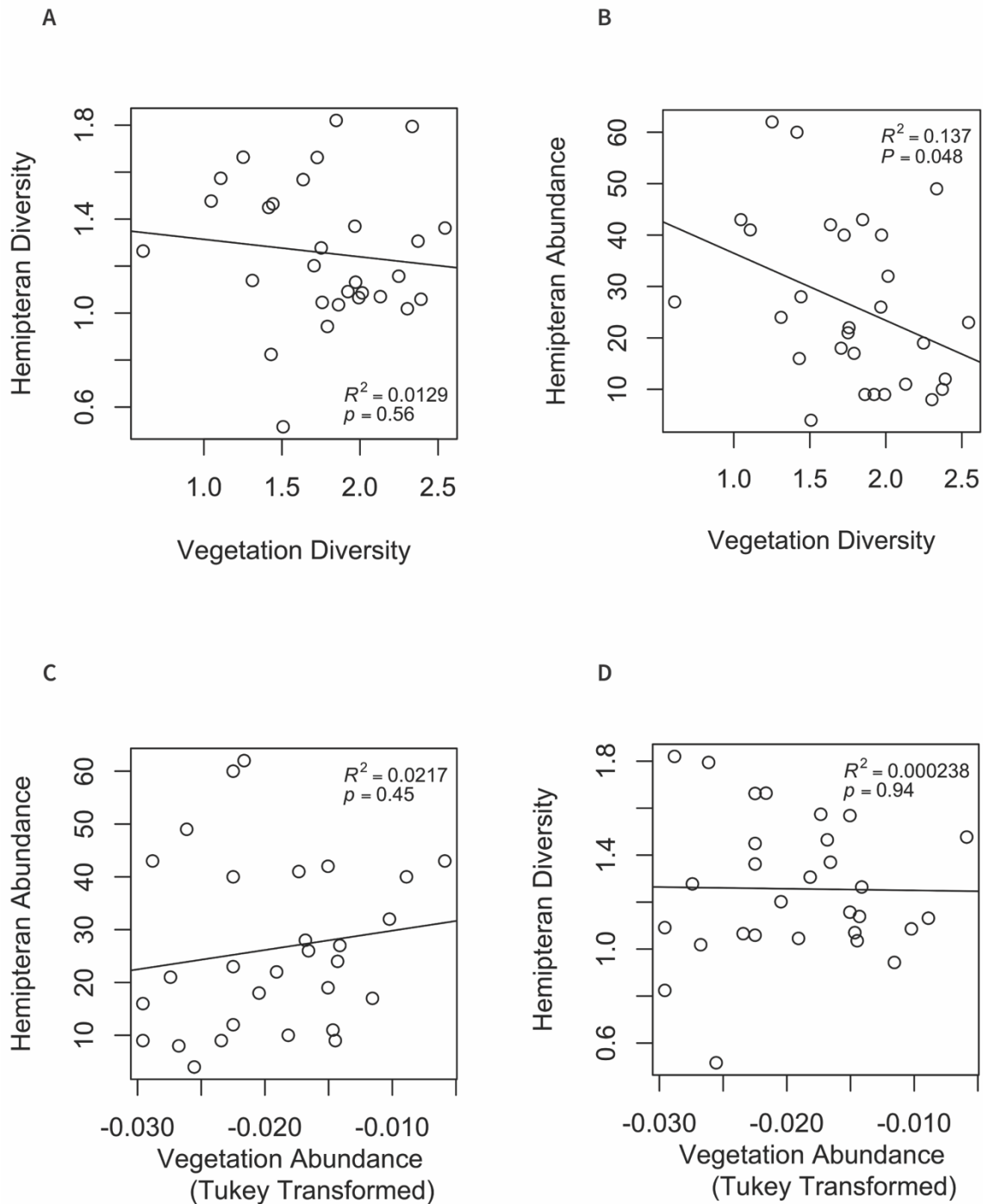
Figure 5: Simple linear regression analysis examining hemipteran diversity and abundance affected by total vegetation diversity and seedling abundance across Maryland (n=29). (A) Hemipteran diversity affected by total vegetation diversity (P=0.560, R^2=0.013). (B) Hemipteran abundance affected by vegetation diversity (P=0.048, R^2=0.137). (C) Hemipteran abundance affected by vegetation seedling abundance (Tukey Transformed) (P=0.450, R^2=0.022). (D) Hemipteran diversity affected by vegetation seedling abundance (Tukey Transformed) (P=0.940, R^2=0.000).
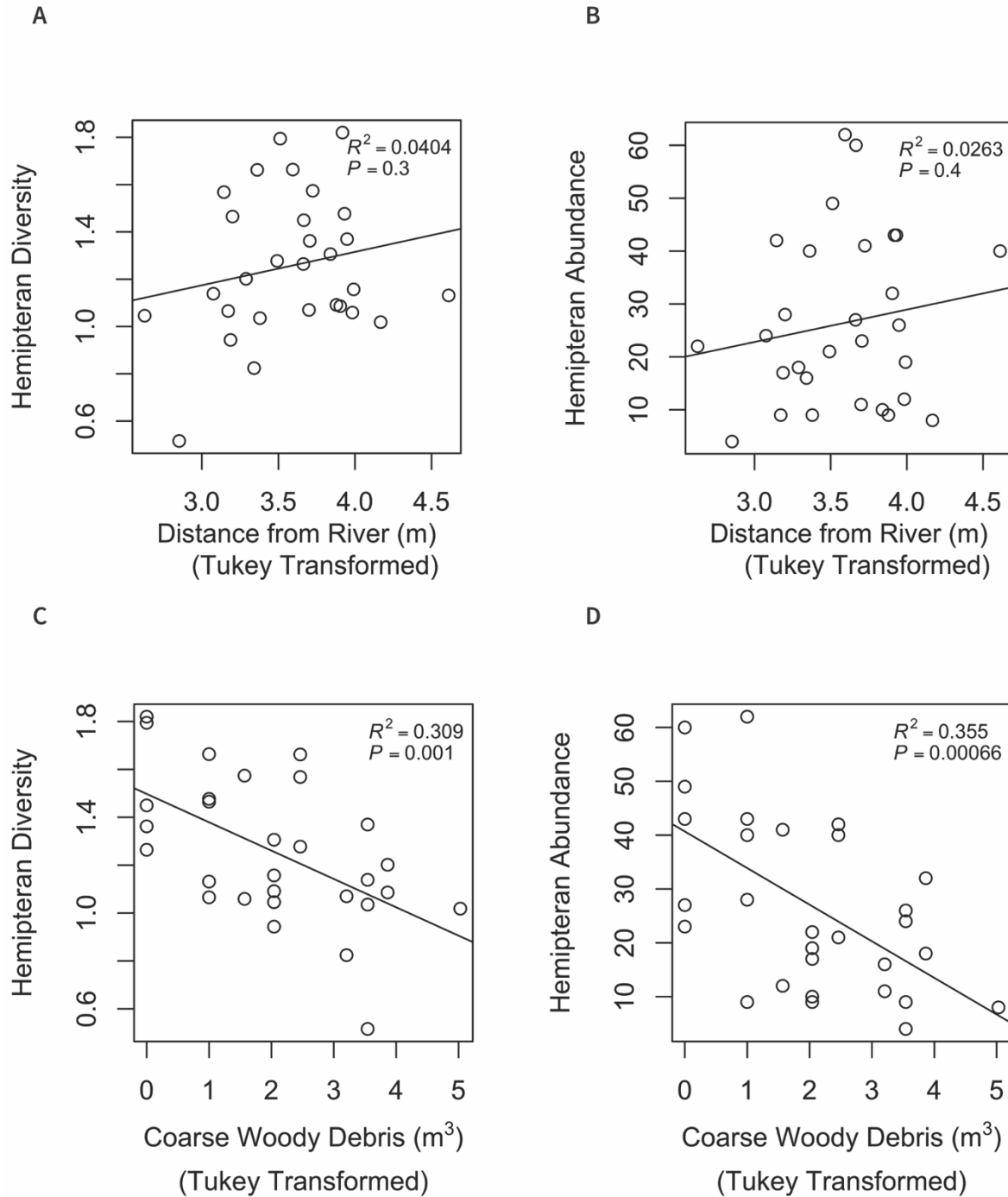
Figure 6: Simple linear regression analysis of hemipteran abundance and diversity in relation to the distance from the Potomac River (m) (Tukey Transformed) and the percent of coarse woody debris (Tukey Transformed). (A) Hemipteran diversity affected by the distance from the river (P=0.300, R^2=0.040). (B) Hemipteran abundance affected by the distance from the river (P=0.400, R^2=0.026). (C) Hemipteran diversity affected by coarse woody debris (P=0.001, R^2=0.309). (D) Hemipteran abundance affected by coarse woody debris (P<0.001, R^2=0.335).
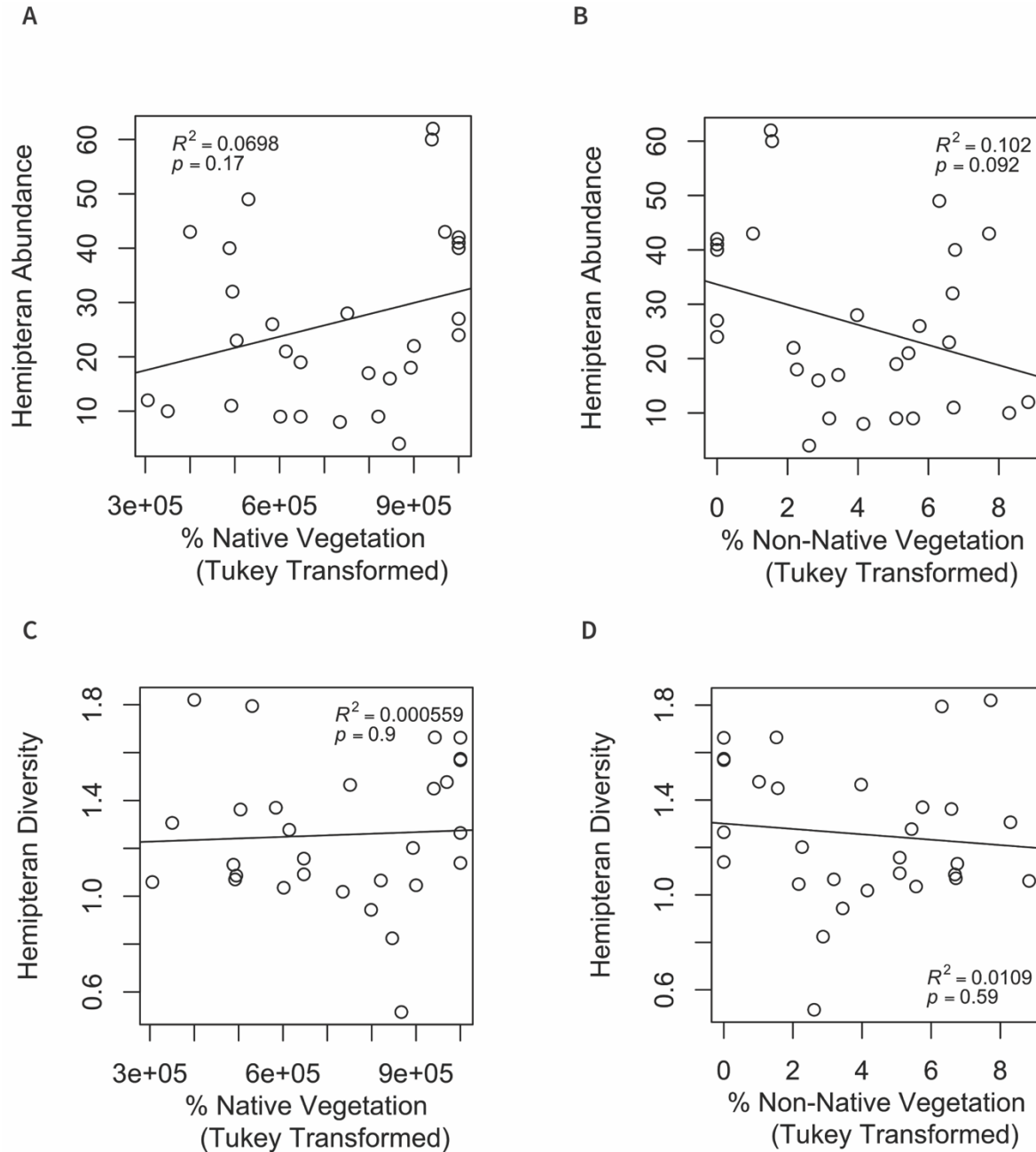
Figure 7: Simple linear regression analysis examining how hemipteran abundance and diversity is affected by the percent of native and non-native vegetation present throughout all sites sampled (n=29). (A) Hemipteran abundance affected by percent of native vegetation (P=0.132, R^2=0.082). (B) Hemipteran abundance affected by percent of non-native vegetation (P=0.092, R^2=0.102). (C) Hemipteran diversity affected by percent of native vegetation (P=0.799, R^2=0.003). (D) Hemipteran diversity affected by percent of non-native vegetation (P=0.589, R^2=0.011).
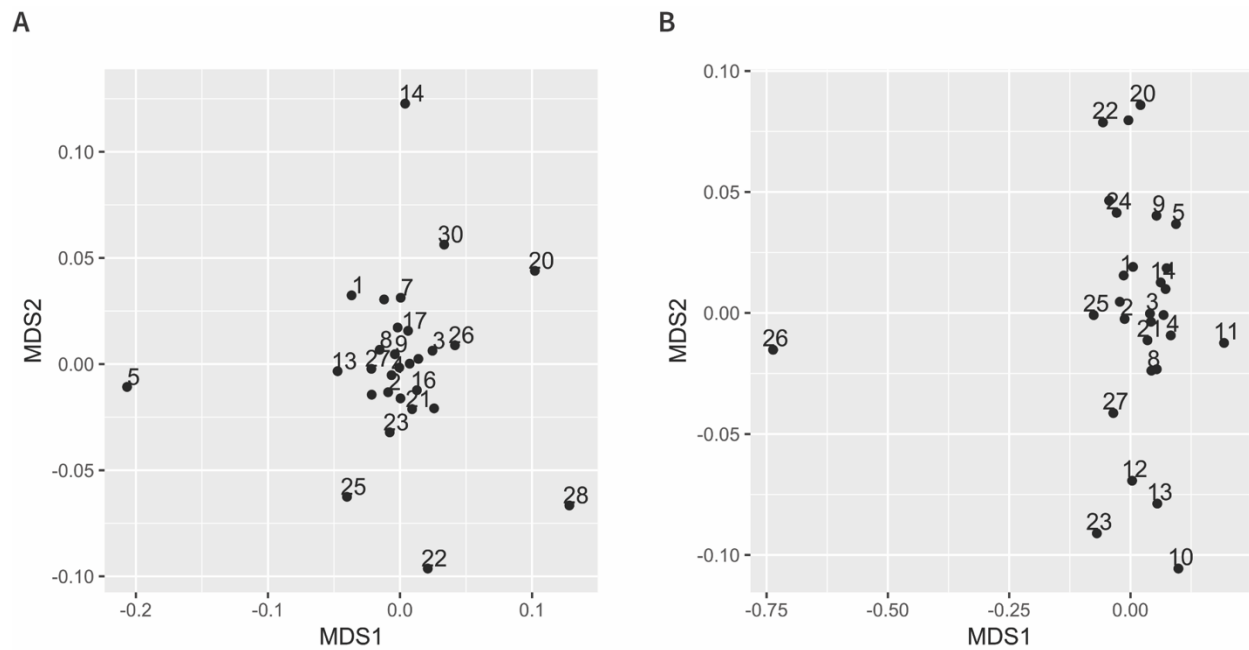
Figure 8: Ordination plots showing MDS results of hemipteran diversity (A) and vegetation diversity (B).

Hemipteran abundance and diversity, predator abundance and diversity, and leafhopper abundance and diversity were not significantly related to the percent of different land cover types according to geoanalysis (Table 4).

Table 4: Results of Ordinary Least Squares Simple Linear Regression Geoanalyses.
*Indicates significance ($\alpha = 0.05$)

| Dependent Variables	Independent Variables	SE	t	P	R²
Predator abundance	% Water	0.001	-0.361	0.721	-0.032
	% Tree canopy	0.046	0.378	0.708	-0.032
	% Shrubland	0.006	0.086	0.932	-0.037
	% Low vegetation	0.051	0.500	0.621	-0.028
Predator diversity	% Water	0.000	0.312	0.757	-0.033
	% Tree canopy	0.002	-0.213	0.833	-0.035
	% Shrubland	0.000	-0.794	0.434	-0.013
	% Low vegetation	0.002	-0.223	0.826	-0.035
Hemipteran abundance	% Water	0.002	-0.299	0.767	-0.034
	% Tree canopy	0.051	0.145	0.886	-0.036
	% Shrubland	0.006	0.978	0.337	-0.002
	% Low vegetation	0.057	0.235	0.816	-0.035
Hemipteran diversity	% Water	0.000	-0.218	0.829	-0.035
	% Tree canopy	0.001	0.375	0.711	-0.032
	% Shrubland	0.000	-0.338	0.738	-0.033
	% Low vegetation	0.001	0.343	0.734	-0.033
Leafhopper abundance	% Water	0.000	-0.511	0.614	-0.027
	% Tree canopy	0.004	0.379	0.707	-0.032
	% Shrubland	0.000	0.774	0.446	-0.015
	% Low vegetation	0.004	0.466	0.645	-0.029
Leafhopper diversity	% Water	0.000	-0.526	0.603	-0.027
	% Tree canopy	0.001	0.387	0.702	-0.031
	% Shrubland	0.000	0.954	0.349	-0.003
	% Low vegetation	0.001	0.452	0.655	-0.029

DISCUSSION

I found that while bottom-up influences from local vegetation affect Hemiptera, top-down effects from the predatory community are not detectable in our study area. These results support my hypothesis that bottom-up forces have greater impact on the intermediate trophic level, specifically herbivorous hemipterans, in a riparian forest.

My original hypothesis, that greater vegetation diversity will positively affect hemipteran diversity, is not supported. Instead, I found that increased vegetation diversity is correlated with a reduction in hemipteran abundance. My results also show that hemipteran abundance and diversity are both negatively related to an increased volume of CWD. Additionally, predator density showed no effect on hemipteran abundance or diversity, contrary to my second hypothesis.

Greater vegetation diversity was significantly related to a decreased hemipteran abundance. This was unexpected as I had predicted that greater plant diversity would lead to greater herbivore diversity and abundance. I predicted this as previous studies report increased insect diversity and abundance with greater plant diversity (Moir et al. 2011, Moreira et al. 2012). This negative relationship may be explained by plant characteristics such as spatial placement within sites, leaf waxiness, or plant nutrient composition. The location and density of vegetation within sampling sites can impact herbivorous insect dispersal, leaf wax can influence phytophagous insects' ability to feed, and plant nutrient composition including nitrogen and phosphorous concentrations are well-known to impact insect herbivory (Purcell and Frazier 1985, Denno et al. 2003, Meneses et al. 2016, Nobre et al. 2016, Pan et al. 2016). Increased vegetation diversity is also known to decrease herbivorous insect abundance due to increased plant competition in grassland, agricultural, and laboratory settings (Mattson 1980, Awmack & Leather 2002, Kigathi et al. 2013, Wetzel et al. 2016, Nitschke et al. 2017). Plants can compete over access to sunlight, nutrients,

and space which lowers individual plant performance (Hirose & Werger 1995, van Ruijven & Berendse 2005, Abass et al. 2013). This decline in plant quality could decrease the likelihood of being chosen by herbivorous insects (including Hemiptera) as sites for feeding, oviposition, and mating. Additionally, increased plant competition can alter the chemical defenses that plants emit, making them less palatable and indirectly decreasing hemipteran abundance (Kigathi et al. 2013, Nitschke et al. 2017).

Another potential reason for decreased hemipteran abundance is that specialist herbivores may not be as prevalent in the study area. Many of the hemipterans collected are part of the Cicadellidae, which are known to be generalist feeders and several species can coexist on the same host by dividing available resources (Poos and Wheeler 1943, Logarzo et al. 2002, Bentz and Townsend 2005). Individual species of Cicadellidae also use multiple plant types as host plants for oviposition and development (Poos and Wheeler 1943, Logarzo et al. 2002, Bentz and Townsend 2005). Therefore, it is likely that I mostly collected generalist hemipterans that would not be as tightly correlated with a diverse plant community. Several of the sites also contain exotic plant species such as multiflora rose (*Rosa multiflora*) and Japanese stiltgrass (*Microstegium vimineum*) which may be a potential confounding factor as non-native plants can decrease hemipteran abundance (Wu et al. 2009, Elleriis et al. 2015).

Hemipteran abundance and diversity were also negatively affected by a greater volume of CWD within each plot. This negative relationship may be due to changes in vegetation community structure. Areas with large amounts of CWD may have contained more down trees and woody debris with less live vegetation. This may have changed the herbivorous insect community structure and hemipteran abundance and diversity may not actually be as reduced as I found. In

this case, there would be more saprophytic and detrital insects in the environment and less of the hemipterans that I was interested in.

Several covariates related to bottom-up forces did not have significant effects on hemipteran abundance nor diversity. No relationships were found between hemipteran abundance and diversity and seedling abundance or the percent of native vegetation, contrary to my expectations. These relationships may not have been present due to the possibility that hemipterans prefer to feed on herbaceous vegetation, which was not incorporated into seedling abundance. Young seedlings do not have nearly as much palatable plant biomass. Hemipterans may also prefer non-native plants over native plants for conducting their life processes: generalist herbivorous insects may choose to feed on non-native plants that have similar characteristics to their native host plants (Morrison and Hay 2011, Pearson and Hipp 2014). Generalists vary their host plant utilization due to seasonal patterns as well as changes in plant nutrients and chemical defenses (Loganzo et al. 2002, Nobre et al. 2016). This provides further evidence that the hemipterans found in this area could include mostly generalist feeders.

I also found no relationship between hemipteran abundance and diversity as they relate to the percent of non-native vegetation ground cover. Initially, I predicted a negative relationship to exist between this covariate and the herbivorous hemipteran community. My results may be due to a low mean percent of non-native ground cover in each plot (Table 2) as well as increased vegetation diversity. Although much of the park contains invasive species, current study plots are dominated by diverse native vegetation throughout, which decreases potential for invasive plant species to colonize and directly influence Hemiptera. Thus, the hemipterans within this area may be dominated by generalist feeders that could prefer a range of native and non-native plants (Lenhart et al. 2015).

Results from this study show that the predator community does not significantly affect hemipteran communities in this riparian forest. This is contrary to my original hypothesis expecting hemipteran diversity and abundance to be inversely correlated with predator abundance and diversity. These relationships may not have been found due to predator-predator interactions that have been shown to decrease the potential for top-down control (Montoya et al. 2003, Finke and Denno 2004, Schuldt et al. 2011). Intraguild predation is common in natural ecosystems and is known to reduce predation risk (Sih et al. 1998, Frago and Godfray 2014). Specifically, intraguild predation is strong in spider communities which are common hemipteran predators in this area. In addition, the greater diversity of vegetation in mesic and riparian forests may overpower any effect of predators within this community. This may occur as bottom-up forces can cascade up to higher trophic levels and impact top-down effects by altering species interactions and predator's abilities to suppress the intermediate trophic level (Denno et al. 2003, Moreira et al. 2012). Other potential reasons may include the increased rainfall throughout this year as predator and prey behaviors are known to be altered during times of increased precipitation (Tauber et al. 1998). Furthermore, predator abundance throughout the sample sites was low (Figure 9).
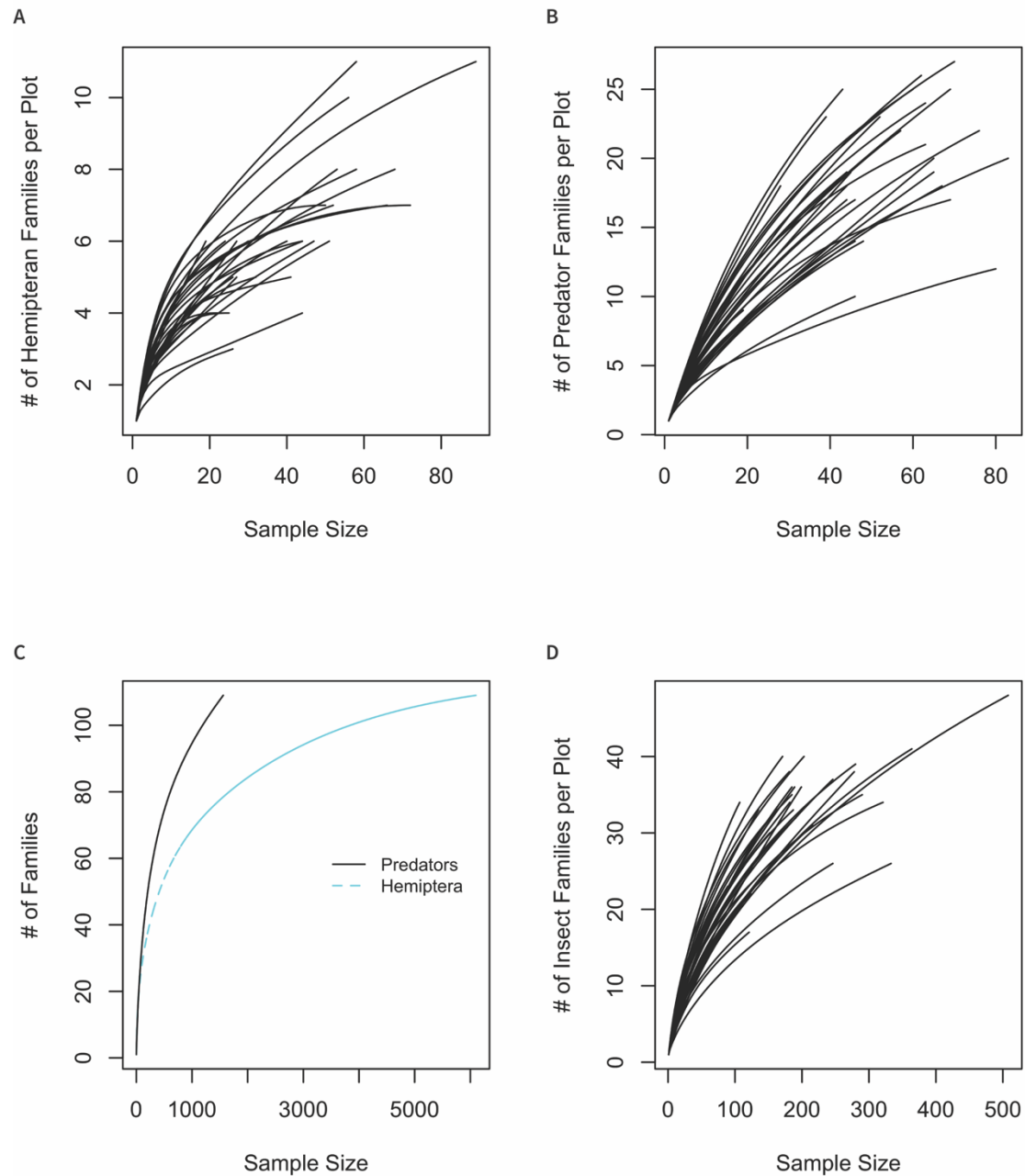
Figure 9: Rarefaction curves of a comparison of Hemiptera and their insect predators, Hemiptera, hemipteran predators, and total morphospecies sampled. Plot (A) compares the sum of all Hemiptera (*n*=21) vs the sum of all predators (*n*=108) sampled. Plot (B) shows Hemiptera sampled throughout 29 sites. Plot (C) compares all of the morphospecies (*n*=161) to Hemiptera (*n*=21) sampled throughout all 29 sites. Plot (D) shows the sampling sizes of all insects collected.

Leafhoppers are an important hemipteran family in the intermediate trophic level and have the potential to be used as indicators of forest health (Pinedo-Escatel and Moya-Raygoza 2018). Changes in vegetation complexes are expected due to global warming events such as increases in temperature and rainfall and will likely affect many organisms and insects, including Hemiptera (Chen et al. 2019). However, leafhopper morphospecies are able to live in a variety of land cover types (Figure 10) and could be expected to have ample habitat to migrate to in response to predicted warming events in this region. Additionally, at the scale at which land cover type was assessed, hemipterans and their predators were not affected by different land cover types in this area, but they may respond differently in other ecosystems (Figure 11).
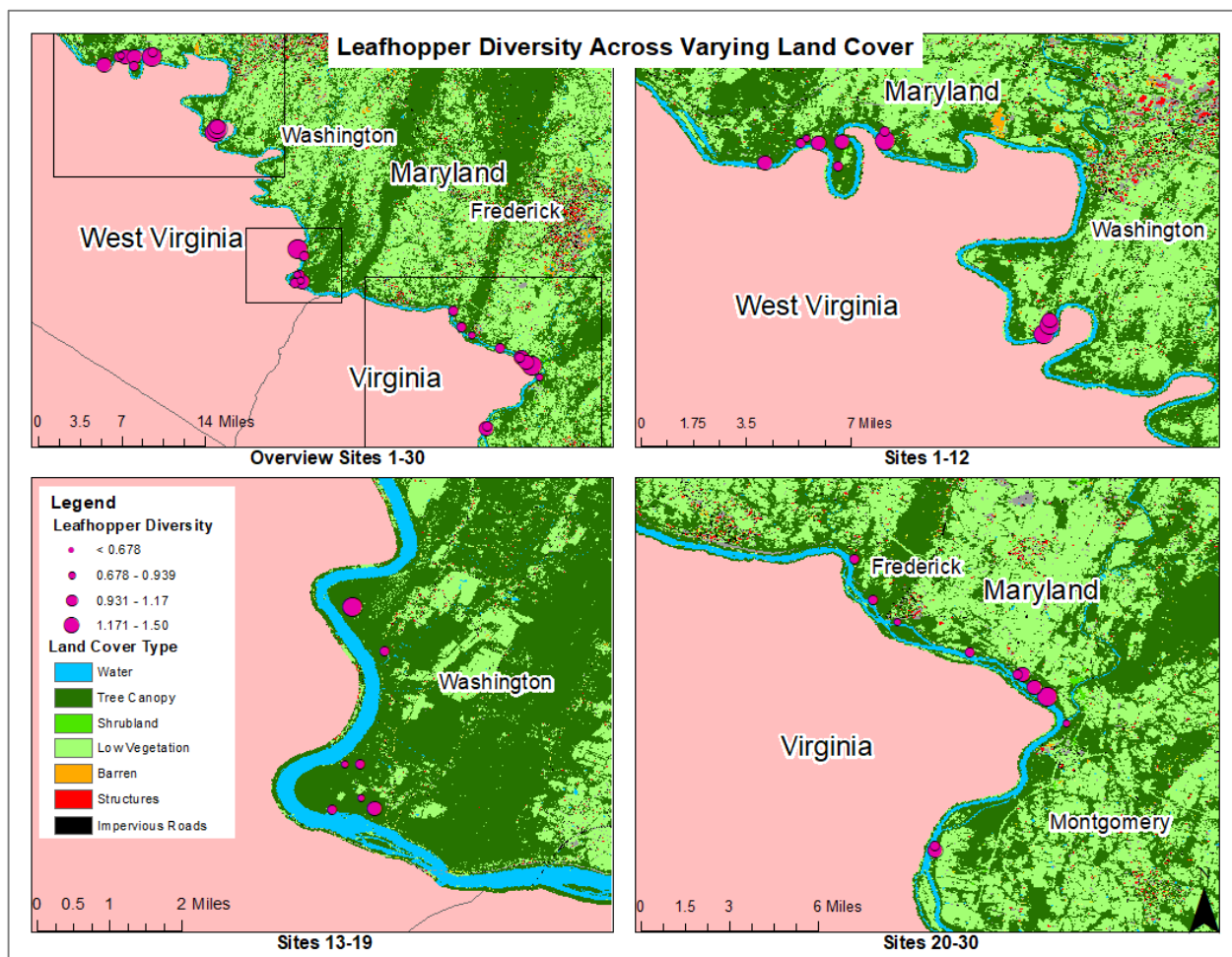
Figure 10: Leafhopper (Family: Cicadellidae) morphospecies distribution across varying landcover types in Washington, Frederick, and Montgomery Counties, Maryland.
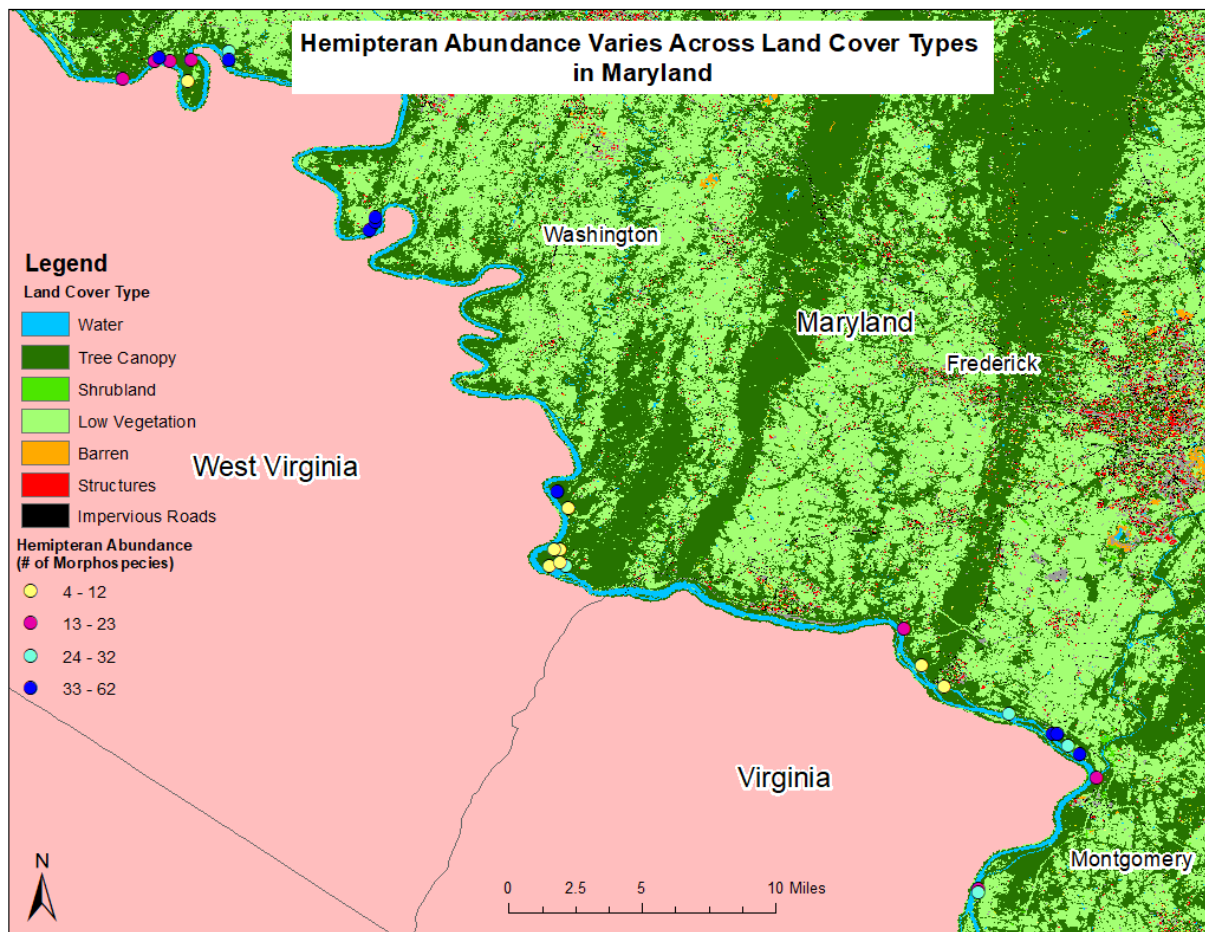
Figure 11: Hemipteran abundance across varying land cover types in Maryland.

In acknowledgement of these results, several limitations exist within the current study. Although vacuum sampling is an effective strategy to collect small insects throughout the air and on the surfaces of vegetation, it may miss some larger predators. This may limit the number of invertebrates collected even with manual sampling of larger predatory insects. Manual sorting of specimens from the collected detritus may also allow small or flying insects to be inadvertently missed. Additionally, it may also be beneficial to sample abiotic factors including rainfall and temperature as these have been shown to affect hemipteran and predator behavior in agricultural, tropical, and estuarine environments (Wolda 1978, Heong et al. 1992, Pinedo-Escatel and Moya-Raygoza 2018). Moving forward, one potential improvement would be to categorize specific hemipteran and leafhopper species instead of broader morphospecies. This will allow for a more detailed representation of specific life histories and a greater potential for species comparisons. Overall, my results may differ from other studies due to differences in ecosystem interactions between both biotic and abiotic factors such as rainfall, temperature, and insect behavior and availability in a riparian environment. The relationships I uncovered between Hemiptera and vegetation differed from much of the literature; however, previous studies occurred in very different systems including wetlands, tropical forests, and agricultural areas.

Bottom-up effects are the primary factors influencing herbivorous Hemiptera in this riparian environment. My results indicate that bottom-up effects have greater influence on hemipteran communities in this study area. Specifically, vegetation diversity and coarse woody debris have a significant negative impact on hemipteran abundance and diversity in my sample sites. Although I did not find top-down effects, hemipteran life histories may be more strongly influenced by predators in other environments. Hemipterans and phytophagous insects are fundamental components within forest ecosystems and are known to be impacted by small

environmental changes. With changing climates, changes in hemipteran abundance and diversity may be able to provide insight into altering invertebrate community structure. Studying these multi-trophic interactions remains important to understand complex functions and relationships in natural ecosystems.

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