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Evaluation of the Relationship between Stream Habitat Quality and Taxa and Trait Richness and Diversity in Piedmont Streams in North Carolina

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Abstract: As impervious cover increases with urban development, stream channels are degraded by increased stormwater runoff, which negatively impacts stream habitat quality and benthic macroinvertebrate diversity. We examined the relationship between stream habitat diversity and aquatic insect taxa and trait richness and diversity at the watershed scale in 30 streams, covering a gradient of stream habitat quality. We then quantified the relationship between taxa and trait richness and diversity and seven microhabitats at the reach scale in ten streams with high habitat quality. We found that both taxa richness and diversity declined at a greater rate than trait richness and diversity along an in-stream habitat diversity gradient. Taxa richness was positively correlated with pools, runs, backwater, leaf packs, and riffles, while trait richness was positively correlated with runs, small wood, and riffles. Taxa diversity was positively correlated with pools and leaf packs while trait diversity was positively correlated with runs, leaf packs, small wood, and riffles. An indicator species analysis revealed that specific taxa and traits were associated with specific microhabitats or combinations of microhabitats. By correlating the taxa in urban streams with specific microhabitats, we can better evaluate the success of stream restoration in restoring stream function and in stimulating benthic macroinvertebrate recovery.

Keywords: benthic macroinvertebrates; habitat diversity; taxa richness and diversity; trait richness and diversity; microhabitats; urban stream; restoration

1. Introduction

A healthy stream ecosystem has been described as having high taxa diversity and function [1,2]. A diversity of habitats with heterogeneous substrates is important for a stream to support a high diversity of benthic macroinvertebrate species and their associated functional traits [3–10]. This is because each species possesses traits that reflect adaptations to the specific microhabitats where they are found [8]. Species' traits have been used to characterize the functional composition of aquatic insect communities [11], including life history (rate of development), mobility (ability to fly, crawling rate), morphology (size, shape, armoring), and ecology (habitat preference, thermal preference, feeding preference).

Biodiversity has been shown to be correlated with changes in ecosystem function in both terrestrial [12–18] and aquatic [8,19,20] communities. Functional diversity is the diversity of organismal traits that impact ecosystem functioning [21,22]. Tilman [21] further defined functional diversity as the component of biodiversity that drives ecosystem dynamics such as productivity, nutrient cycling, stability, and community composition. The habitat template theory states that species found within a specific habitat or even

smaller microhabitats are those species that have been filtered from a larger regional species pool based on the functional traits that enable them to survive and reproduce in that habitat [23–25]. Lamouroux et al. [8] supported the habitat template theory's proposition that habitats with similar characteristics should support species with similar functional traits. This suggests that similar microhabitats should be able to support different species with similar functional traits.

Stream habitat quality and benthic macroinvertebrate diversity are negatively impacted by urbanization. Walsh et al. [26] described the urban stream syndrome as a predictable collection of the negative impacts of urbanization that alter natural hydrology and subsequently impact channel geomorphology and stream biota. As the percent of impervious cover (%IC) increases with urban development, stream channels are degraded by an increased stormwater runoff volume and intensity [27–32]. This degradation can be seen in changes to particle size, stream flow, and silt deposition, which impacts the aquatic insects' habitat [4].

The response of watershed managers to urban stream degradation is to return the stream to a more natural condition through stream restoration techniques such as the natural channel design method [33,34]. A general assumption for stream restoration projects has been that restoring channel geomorphology to resemble a reference stream would result in the recovery of benthic macroinvertebrate assemblages [35]. Unfortunately, most natural channel design approaches do not result in improvements to benthic macroinvertebrate diversity and function [36–40]. Several hypotheses have been suggested to explain why macroinvertebrate communities do not improve, including (1) urban infrastructure constraints limiting restoration options [37], (2) the restoration scale being too small to have an impact [37–39,41,42], and (3) relatively little attention being given to managing upstream influences from the watershed, including increases in stormwater volume and intensity [26,43–46].

Numerous researchers have shown that stream restorations that only address geomorphological stream channel characteristics without considering stream functional traits and the ecological requirements of benthic macroinvertebrates fail to stimulate the recovery of benthic macroinvertebrate assemblages [35,36,47,48]. To inform restoration design and implementation more fully, we examined the relationship between stream habitat quality and aquatic insect assemblage diversity and function with two overall objectives. Our first objective was to examine the relationship between stream habitat diversity and aquatic insect taxa and trait richness and diversity by evaluating 30 streams in Piedmont, North Carolina, spanning a gradient of good to poor habitat quality according to the watershed scale. Our second objective was to quantify the relationship between taxa and trait richness and diversity and microhabitats at the reach level in 10 streams with high habitat quality. While several taxa traits may be found commonly throughout the various stream microhabitats, adaptations of species to specific microhabitats may result in the community becoming susceptible to the loss of taxa traits with the loss of microhabitats. The redundancy of functional traits among microhabitats will make stream ecosystems more resilient to change when a few species with redundant functions are lost due to some disturbance.

2. Materials and Methods

2.1. Study Sites

Thirty streams near Mecklenburg County, North Carolina, spanning a gradient of good to poor habitat quality and exhibiting a percentage of impervious cover ranging from 4.8–78.5% were identified (Figure 1; Table S1) to answer the watershed level questions. Ten of these streams with good to excellent North Carolina Biotic Index (NCBI) scores [49,50], good habitat conditions, and impervious cover ranging from 4.8% to 10.9% were selected for the reach level study (Table S1). The annual precipitation in Mecklenburg County in 2015 was 125.7 cm, which was 19.9 cm above normal. However, this rainfall total was not very different from the average rainfall of 112.4 inches for the previous 3 years. The annual average temperature in Mecklenburg County in 2015 was 17.0 °C which was approximately

normal (15.5 °C) [51]. Watershed size, percent of impervious cover, Enhanced Mecklenburg Habitat Assessment Protocol (EMHAP) scores, and location are summarized in Table S1. A summary of the environmental data for each site is presented in Table S2.
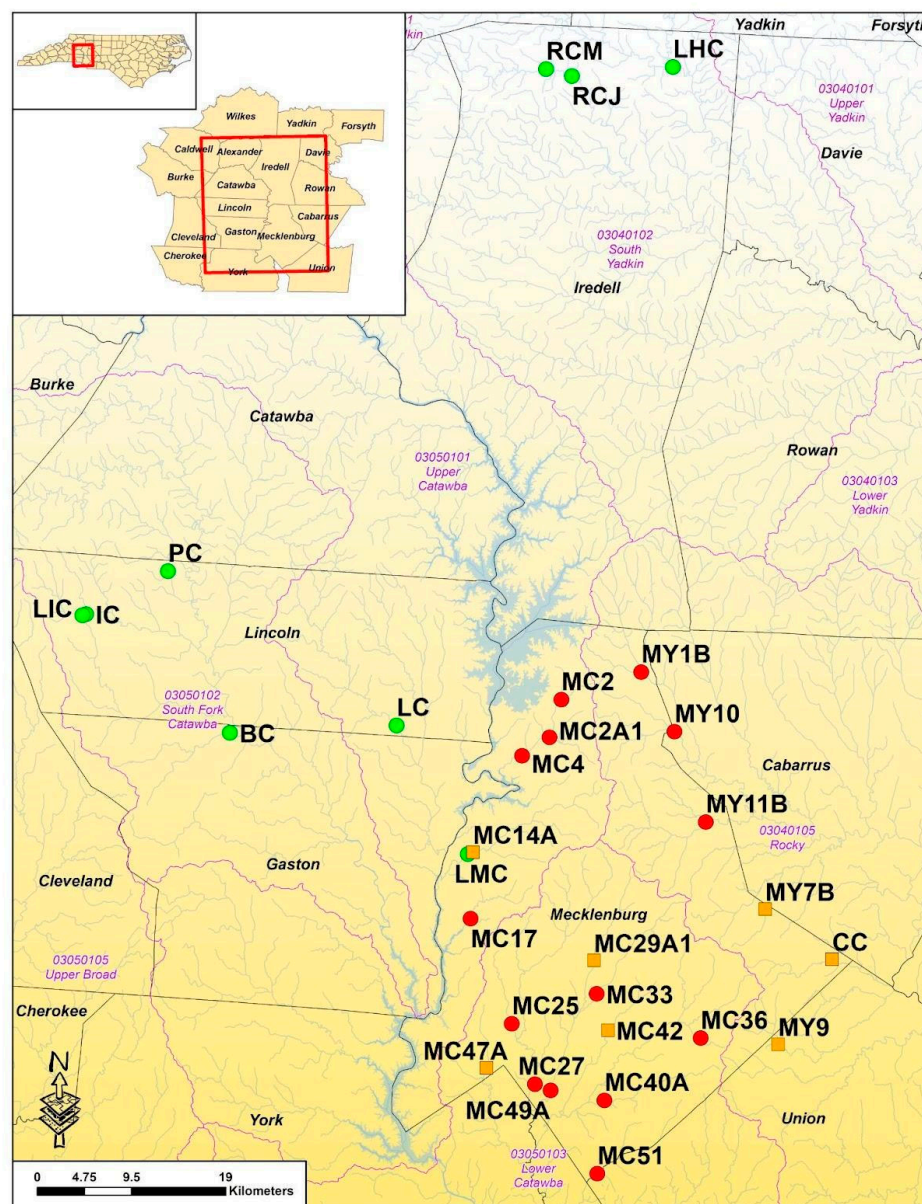
Figure 1. Map of rural and urban stream study sites in Mecklenburg, Lincoln, and Iredell Counties in Piedmont, North Carolina. Stream Habitat Conditions: supporting (green); partially supporting (orange); impaired (red).

Stream habitat conditions were assessed at all 30 sites using the EMHAP, which is based on the USEPA Rapid Bioassessment Protocols [52]. EMHAP evaluates 10 habitat condition parameters, including instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, frequency of riffles, channel flow status, bank vegetation protection, bank stability, and vegetative riparian zone width [53,54]. EMHAP scores have been associated with stream habitat conditions ranging from degraded (<60) to fully supporting (≥160). EMHAP scores ranged from 58.3–169.7 across all sites and 137.3–169.7 for the 10 reach scale sites (Table S1). Each site was assigned as Supporting (≥140), Partially Supporting (100–139.99), or Impaired (<100) based on its EMHAP score. These EMHAP

groups were selected based on the correlation of EMHAP scores with %IC and EPA rapid bioassessment ratings [52].

The taxa richness in Piedmont, North Carolina, streams was negatively correlated with an increasing watershed % of impervious cover (%IC). Likewise, the stream habitat condition, as measured using the Enhanced Mecklenburg Habitat Assessment Protocols (EMHAP), declined with an increasing %IC. Therefore, it is not surprising to see that taxa richness increases with EMHAP scores [55]. The list of taxa collected from all sites is summarized in Table S3.

2.2. Aquatic Insects

Aquatic insects were collected July–October 2015 from a 100-m segment at 20 of the 30 sites by Charlotte-Mecklenburg Storm Water Services (CMSWS) personnel using the standard qualitative method developed by the North Carolina Department of Environmental Quality (NCDEQ) Biological Assessment Unit [49] and described in the Mecklenburg County Bioassessment Standard Operating Procedures [50]. This is the normal sampling period for CMSWS bioassessment sampling. A review of the previous 5 years of CMSWS data at each site showed that the 2015 sample results were typical for these sites. The standard qualitative method collections consists of 2 kick-net (500 μm) riffle samples; 3 sweep-net (500 μm) samples from microhabitats found within runs and pools, such as root wads, soft sediment in undercut bank areas, woody debris, macrophyte beds, and overhanging vegetation; 1 leaf-pack sample; 2 rock and/or log wash samples; and visual collections. The aquatic insects in these samples were sorted in the field and preserved in glass vials containing 95% ethanol. All aquatic insects were identified to the lowest possible taxonomic level (genus or species) using North American and Southeastern USA regional taxonomic keys [56–58].

At the 10 rural sites, aquatic insects were collected quantitatively from 8 microhabitats, including riffles, root wads, undercut banks, woody debris, leaf packs, backwater, macrophyte beds, and sandy areas found in the 100-m study reach. A *riffle* is an area in a stream where water moves quickly over hard substrates (boulders, cobble, gravel, bedrock, and woody debris) resulting in a rippling effect on the water surface. *Root wads* are tree or other plant roots exposed along the stream channel edges, providing a habitat for benthic macroinvertebrates and fish. An *undercut bank* area is where the wetted channel of a stream flows underneath the stream bank itself due to erosion. The stream bank forms a roof ledge over the channel itself. *Woody debris* are any stable branches, fallen trees, logs, stumps, or log jams that are present in a stream and could be used as a habitat by fish or benthic macroinvertebrates. A *leaf pack* is a decomposing clump of leaves caught behind an obstruction such as a rock or piece of woody debris in the stream channel. Only seasoned leaves that have a slimy layer of bacteria on their surface are suitable habitats, for it is the bacterial growth on the leaves that attract benthic macroinvertebrate shredders. A *backwater area* is a pool adjacent to one of the banks and is located behind an obstruction, such as a sand bar, woody debris, or a topographical feature, such as bedrock, where water accumulates and does not follow the natural flow of the stream. *Macrophyte beds* are areas where aquatic vegetation grows in the stream substrate and is submerged or emergent, often floating on the surface of a stream or extending above a stream. *Sandy areas* are located along the margins of a stream where slower currents allow silt and soft sediment to accumulate.

Quantitative samples of the aquatic insects found in each microhabitat were collected July–August 2015. Within a representative riffle in the study reach at each site, a 1- by 3-m area was sampled using a kick net. The substrate within this area was thoroughly disturbed, with each rock rubbed to dislodge aquatic insects. The substrate was also visually inspected for organisms still attached. The root wads, undercut banks, leaf packs, backwater, macrophyte beds, and sandy areas were sampled using a Surber sampler, through which a 0.25 m^2 area can be sampled. The substrate within the area isolated using the Surber sampler (500 μm) was thoroughly disturbed. A visual inspection of the substrate was conducted to collect organisms still attached. Three Surber samples were taken from

each microhabitat for a total of 0.75 m^2 per microhabitat. Each of the Surber samples for each microhabitat was taken from a different area within the study reach to eliminate the effects of patchy distributions of aquatic insects. Woody debris was visually examined using a flexible square made from string the same size as the Surber sampler that could be draped over woody debris of various diameters. Woody debris of various sizes and diameters were sampled for a total of 0.75 m^2.

The 3 samples for each microhabitat were composited into 1 sample per microhabitat. Large debris and leaves were removed from each sample in the field. The remaining debris and organisms were preserved in 95% ethanol and sorted in a lab. All organisms were identified to the lowest taxa possible (genus or species). The 10 rural sites were added to the overall data set (20 other sites) by converting the data from quantitative to qualitative using the same protocol as described above. For a detailed analysis on the impact of microhabitat on aquatic insect diversity and function, the quantitative 10-site data were analyzed independently of the 20-site data. After collection was completed, macrophyte beds were removed from the analysis since they were found in only 4 sites with 8 to 27 taxa and 14 to 75 total organisms collected.

We assigned macroinvertebrate traits using the categories described by Poff et al. [11]. Poff et al. [11] described 20 benthic macroinvertebrate traits grouped into 4 major categories: life history (rate of development), mobility (ability to fly, crawling rate), morphology (size, shape, armoring), and ecology (habitat preference, thermal preference, feeding preference) (Table S4). The Poff dataset contains taxa traits for all benthic macroinvertebrates at either the genus or species level except for Bivalvia (clams and mussels), Crustacea (amphipods and crayfish), Gastropoda (snails), Hirudinea (leeches), Oligochaeta (worms), three families of Coleoptera (Gyrinidae, Hydrophilidae, and Ptilodactylidae; beetles), and three families of Diptera (Culicidae, Dixidae, and Tabanidae; flies). We excluded from the analysis taxa that did not have traits associated with them.

2.3. Habitat Diversity

At each of the sites, the number of microhabitats, including pools, runs, root wads, undercut banks, woody debris, leaf packs, backwater, and macrophyte beds found in the 100-m study reach were counted. Because riffles are among the most productive habitats found in streams [1,59] the length of each riffle was also measured. An index was developed by CMSWS based on EPA stream habitat assessment protocols [52] to incorporate length into the count of riffles [54]. This index was used in the calculation of each habitat's Shannon–Wiener diversity (H′) index. The number of transitions between major habitat types, such as riffles, runs, pools, and backwater areas, was calculated.

2.4. Data Analysis

All data analyses were conducted in R, version 4.0.3 [60]. Taxa and trait diversity were calculated as Shannon–Wiener diversity (H′) indices at the 30 sites (diversity function in the 'vegan' package; [61]). We tested all data for normality using the Shapiro–Wilks normality test and found that both the watershed and the microhabitat scale macroinvertebrate data were significantly different from normal distribution. Therefore, we chose non-parametric statistics to evaluate differences among groups of parameters. We used a non-metric multidimensional scaling (NMDS) analysis to evaluate the relationship between taxa and trait distribution among streams with varying EMHAP scores and among microhabitats (metaMDS function in the 'vegan' package; [61]).

To investigate the relationship between taxa and trait metrics and habitat at both scales, we used the Kendall rank correlation test (cor.test function in the 'stats' package in R [60]). To investigate how these metrics varied with habitat diversity, they were plotted as a linear

regression, and their slopes were compared. To further determine which components of habitat diversity had the greatest contribution to these metrics, we used multiple linear regression. The initial set of parameters tested included the number of pool, run, backwater, root wad, undercut bank, leaf pack, small wood (3–8 cm), large wood (>8 cm), and riffle microhabitats and habitat diversity (H′). A stepwise approach was used using the *lm* function in the 'stats' package in R [60]. At the local scale, we tested differences among these metrics across microhabitats using the nonparametric Kruskal–Wallis rank sum test with the Dunn Test (dunnTest function in 'FSA' package in R [62]).

To further investigate the impact of microhabitats on the distribution of taxa and traits within a stream, the aquatic insects in the 10 rural sites with better habitat quality were collected quantitatively. However, the aquatic insects in the urban streams were collected qualitatively. We tested for significant differences between aquatic insect Shannon–Wiener diversity (H′) of abundance scores per each microhabitat sampling result reported as diversity of total number of organisms per taxa and diversity of number of organisms per m^2 per taxa using the Student's *t*-Test (*t*-test function in the 'stats' package R [60]). The results indicate that there was no statistical difference between the 2 data sets. To keep the data analysis consistent with the microhabitat and reach scales, we decided that the total abundance data would be used in the analysis of the macroinvertebrate data as the differences in the total abundance and the abundance per m^2 data sets were not significant.

To calculate the percent occurrence of microhabitats each trait was found in, the total number of microhabitat samples a trait was found in was divided by the total number of microhabitats sampled in the study. We used the Kruskal–Wallis rank sum test to test the null hypothesis that the percent occurrence of each trait was the same in each microhabitat. The Dunn Test for multiple comparisons was used to determine which traits had significantly different percent occurrences among the microhabitats.

To determine if there are specific taxa associated with specific microhabitats, an indicator species analysis was conducted using the multipatt function of the indicspecies R package (multipatt function in the indicspecies package in R [63]).

3. Results

3.1. Taxa and Trait Richness and Diversity

Similar taxa and traits are found in streams with similar stream habitat quality. NMDS plots of both taxa and trait abundance show that the taxa and traits found in streams with better stream habitat quality (supporting) are less similar than those found in the streams with poorer habitat quality (partially supporting and impaired) (Figure 2a,b).

Taxa richness and diversity increased more dramatically in response to increases in stream habitat condition than trait richness and diversity. Taxa richness increased from 18 to 94 taxa with increasing habitat diversity while trait richness did not vary as strongly (48 to 58 traits) in the same streams (Figure 3a). Both taxa and trait diversity did not vary strongly with increasing habitat diversity (Figure 3b) where taxa diversity increased (2.2 to 3.8) and trait diversity only increased slightly (3.5 to 3.8). Taxa and trait richness are positively correlated with stream habitat condition. Streams with high EMHAP scores have high habitat diversity; however, non-supporting streams can also have high diversity indicating that other factors, such as watershed and riparian conditions, can impact streams even though they have a good habitat. There were significant positive correlations between taxa richness and Shannon-Wiener diversity (H′) and habitat Shannon–Wiener diversity (H′) (Table 1; Kendall's rank correlation tau = 0.3828 and 0.3314 respectively; α < 0.01 and < 0.05). The correlations between trait richness and diversity and habitat Shannon–Wiener diversity (H′) were not significant.

(**a**)

(**b**)
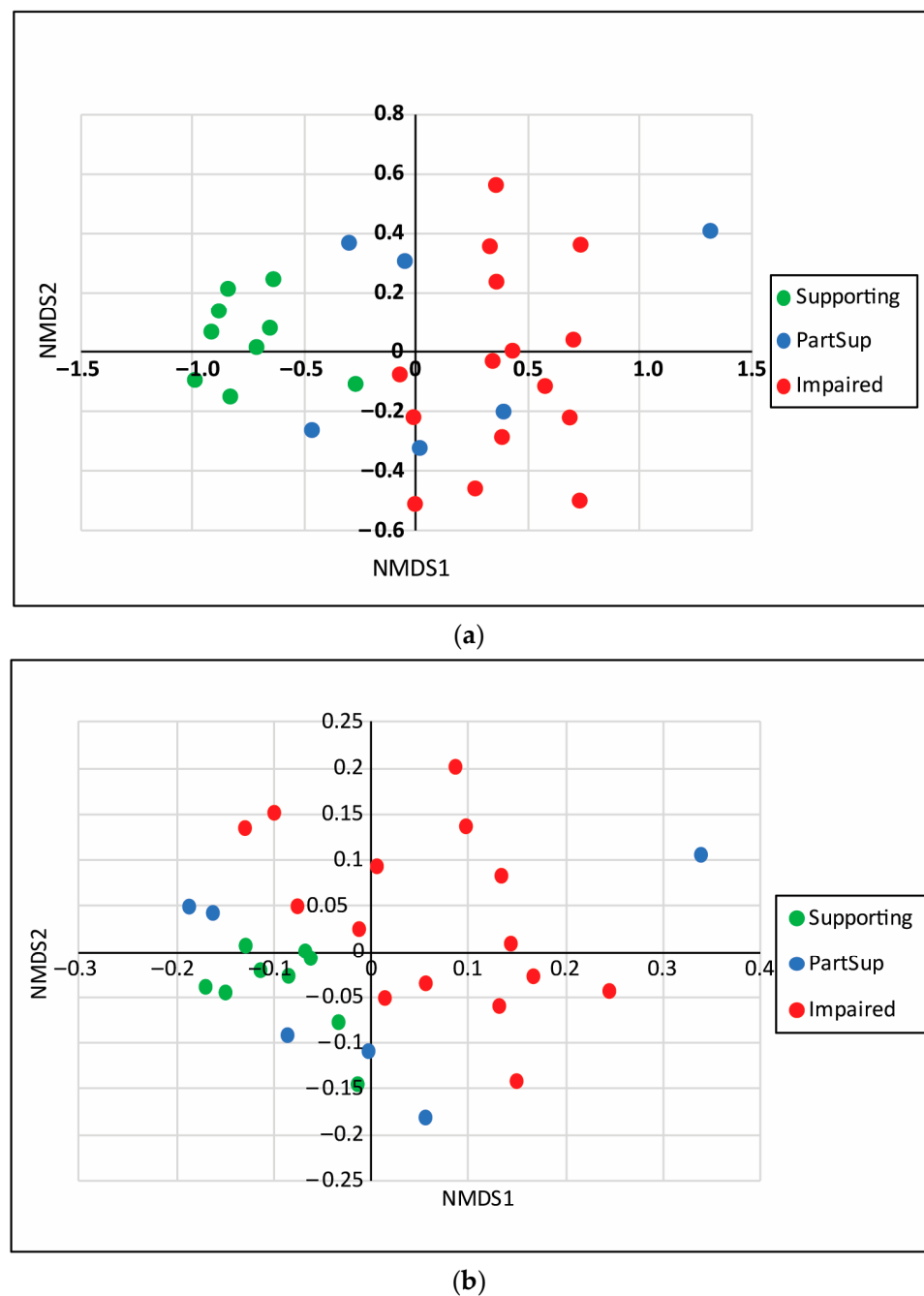
Figure 2. (**a**) NMDS plot of total taxa abundance (total number of organisms per taxon) found in streams spanning a gradient of stream habitat condition ranging from good (supporting) to poor (impaired) EMHAP scores. Taxa found in the streams with better habitat conditions are less like the taxa found in the streams with poor habitat conditions (N = 30 stream samples). (**b**) NMDS plot of total trait abundance (total number of organisms per trait) found in streams spanning a gradient of stream habitat condition ranging from good (supporting) to poor (impaired) EMHAP scores. The traits found in the streams with better habitat conditions are less like the traits found in the streams with poor habitat conditions (N = 30 stream samples).
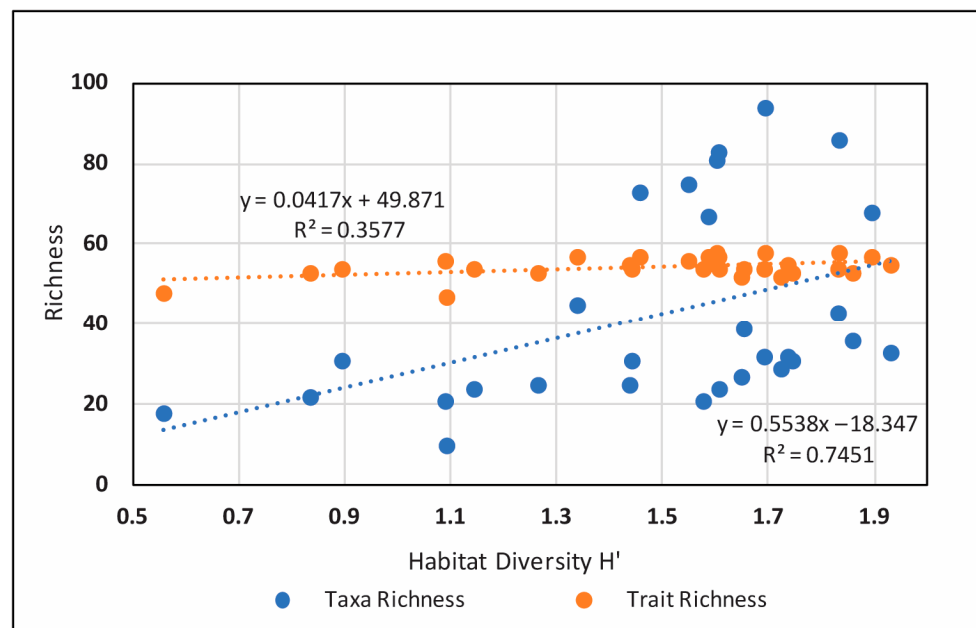
(**a**)
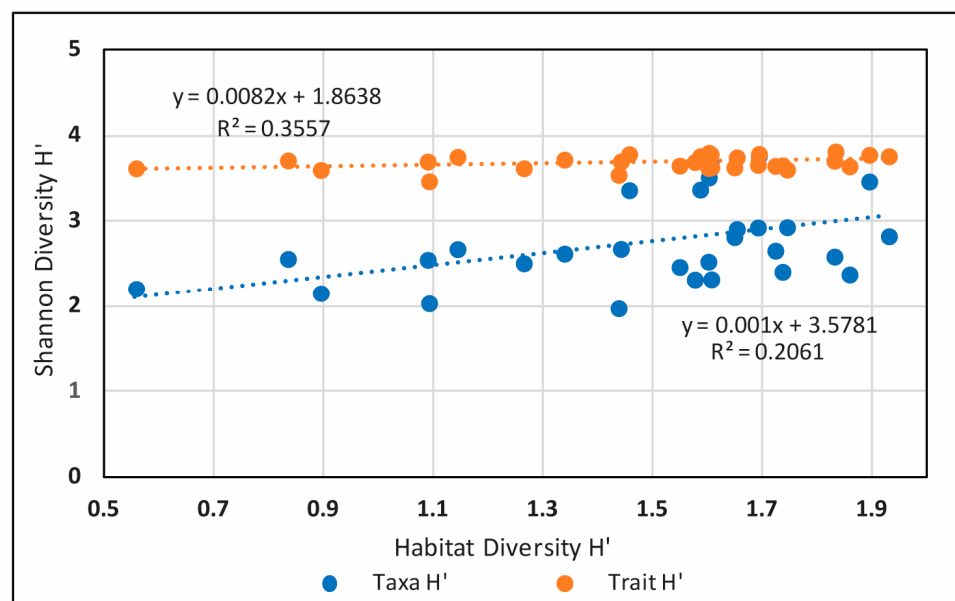
(**b**)

Figure 3. (**a**) Taxa and trait richness by stream habitat diversity (H′). Taxa richness increased at a greater rate than trait richness with stream habitat quality (N = 30 stream samples). (**b**) Taxa and trait diversity (H′) by stream habitat diversity (H′). Taxa diversity increased at a greater rate than trait diversity with stream habitat quality (N = 30 stream sites).

Riffles are important for taxa and trait richness, but other microhabitats are important as well. There were significant positive correlations between taxa richness and five microhabitats including pools, runs, backwater, leaf packs, and riffles (Table 1). There were significant positive correlations between taxa diversity (H′) and pools and leaf packs. There were significant positive correlations between trait richness and runs, small wood, and riffles. There were significant positive correlations between trait diversity and runs, leaf packs, small wood, and riffles.

Table 1. The Kendall rank correlation test shows the correlation between taxa and trait richness and diversity (H′) with microhabitats. Habitat diversity is calculated as the habitat Shannon–Wiener diversity H′.

Taxa Metric	Kendall's Rank Correlation Tau			
Microhabitats	Taxa Richness (S)	Taxa Shannon Diversity (H′)	Trait Richness (S)	Trait Shannon Diversity (H′)
Pool	0.363 **	0.277 *	0.285	0.270
Run	0.423 **	0.250	0.446 **	0.312 *
Backwater	0.359 **	0.117	0.172	0.032
Root Wad	0.186	0.118	0.089	0.069
Undercut Bank	0.071	0.241	0.028	0.136
Leaf Pack	0.345 **	0.357 **	0.229	0.29 *
Small Wood	0.117	0.188	0.290 *	0.274 *
Large Wood	0.228	0.210	0.185	0.171
Riffle Index	0.489 **	0.261	0.558 ***	0.341 **
Habitat Diversity	0.383 **	0.331 *	0.155	0.210

Correlation coefficients significant p-values—* $\alpha < 0.05$, ** $\alpha < 0.01$, and *** $\alpha < 0.001$.

Regression models identified critical habitats for taxa and trait richness and diversity. A multiple linear regression analysis developed models that relate taxa richness, trait richness, taxa diversity, and trait diversity to microhabitats observed in streams (Table 2). The regression model for taxa richness includes the riffle, large wood, run, and leaf pack microhabitats. The model for taxa diversity includes the leaf pack, riffle, and undercut bank habitats. The model for trait richness includes the riffle and small wood microhabitats and habitat diversity while the model for trait diversity includes the leaf pack and riffle microhabitats. All the models include riffles, reflecting the importance of riffles in developing and sustaining the aquatic insect assemblages in streams. Leaf packs were also shown to be important microhabitats as they were included in three of the models.

Table 2. Multiple linear regression models for taxa and trait richness and diversity (H′). The initial set of parameters tested included: number of pool, run, backwater, root wad, undercut bank, leaf pack, small wood, large wood, riffle microhabitats, and habitat diversity (H′).

	Goodness of Fit		Regression Model
	Adj. R²	p	
Taxa Richness	0.674	1.3×10^{-6}	22.443 + 2.942 (RiffleInd) + 0.486 (LgWood) − 6.728 (Run) + 0.697 (LeafPack)
Taxa Diversity (H′)	0.4144	6.9×10^{-4}	2.248 + 0.026 (LeafPack) + 0.024 (RiffleInd) + 0.010 (UndercutBank)
Trait Richness	0.410	7.6×10^{-4}	48.736 + 0.137 (RiffleInd) + 2.568 (HabitatH′) + 0.014 (SmWood)
Trait Diversity (H′)	0.261	6.4×10^{-3}	3.636 + 0.003 (LeafPack) + 0.003 (RiffleInd)

3.2. Microhabitats

Taxa and trait diversity vary among microhabitats. The highest numbers of total taxa and total organisms were collected from riffles while the lowest numbers were found in sand and undercut banks. The list of taxa collected from all sites is summarized in

Table S3. Taxa diversity was lowest in sand compared to the other microhabitats, although not significantly lower than taxa diversity in undercut banks and wood (Kruskal–Wallis $p < 0.05$; Dunn test $p < 0.05$; Figure S1). Trait diversity did not follow the same pattern as taxa diversity across microhabitats. Trait diversity was similar in the leaf pack, riffle, root wad, undercut bank, and woody debris microhabitats and higher than trait diversity in backwater and sand microhabitats (Kruskal–Wallis $p < 0.05$; Dunn Test $p < 0.05$; Figure S2).

Taxa and traits differ across microhabitats. The NMDS plots for taxa by microhabitat indicate that taxa found in riffles are closer in composition to the taxa in leaf pack and woody debris. The taxa compositions found in the root wad, undercut bank and backwater microhabitats were similar but differed from those in the riffle, leaf pack, and woody debris microhabitats. The taxa found in the sand overlapped with the undercut bank and backwater microhabitats (Figure 4a). The distribution of traits among the microhabitats followed a pattern similar to the distribution of the taxa. The NMDS plots for traits by microhabitat showed that the macroinvertebrate assemblages found in the riffle were similar to the traits found in the leaf pack and woody debris microhabitats. In contrast, the traits found in the undercut bank, backwater, and root wad microhabitats were less like the traits found in the riffles, leaf packs, and on woody debris (Figure 4b).

There were specific taxa and traits associated with specific microhabitats. Each taxon possesses a unique combination of traits, some of which can be shared with other taxa found in different microhabitats. However, no one taxon can possess all traits as sub-traits within each trait group describe different strategies to survive in the stream. For example, some taxa, such as the Simuliidae, physically attach themselves to the substrate to filter food particles from areas with faster flow rates while other taxa swim from rock to rock in the same microhabitat looking for food.

The percentage of traits found in each microhabitat ranged from 0 to 16%, indicating that the taxa traits found in each microhabitat reflect the adaptations that taxa commonly possess in each microhabitat. Some of the traits were found in multiple microhabitats, providing multiple locations where similar traits and the corresponding ecosystem function can be found.

Out of 156 taxa collected from the 10 sites, the indicator species analysis identified ($p < 0.05$) 10 taxa (20.4%) associated with backwater, 2 taxa (4.1%) with leaf packs, 33 taxa (67.4%) with riffles, 3 taxa (6.1%) with root wads, and 1 taxon (2.0%) with woody debris (Figure 5; Table S5). While certain species were associated with a specific habitat, they were also found in other microhabitats as well (Table S5). The indicator species analysis also associated 13 taxa with combinations of microhabitats, such as leaf packs and riffles. Additional associations include backwater and leaf packs, backwater and riffles, and root wads and woody debris (Table S5).

While most traits were found in each microhabitat, there were some traits that were more commonly found in one habitat than in the other microhabitats. Out of 58 taxa traits possessed by the taxa collected from the 10 sites (Table S4), the indicator species analysis identified the majority of traits (46; 79.3%) were associated with taxa collected from riffles (Table S6). One trait (1.7%), respiration through the plastron, was associated with leaf packs and one trait (1.7%), climber, was associated with root wads. The remaining percentage of traits was either associated with more than one microhabitat or not associated with any microhabitats (Table S6). Three traits—abundant in drift (ADRF), multivoltine (MV), and cold stenothermal (CLD)—were associated with both leaf packs and riffles. Four traits were associated with combinations of three microhabitats, such as burrower (BRW) and depositional only (DEP) with backwater, leaf packs, and riffles; sprawler (SPL) with backwater, riffles, and root wads; and nonseasonal development (NSE) with riffles, root wads, and woody debris.
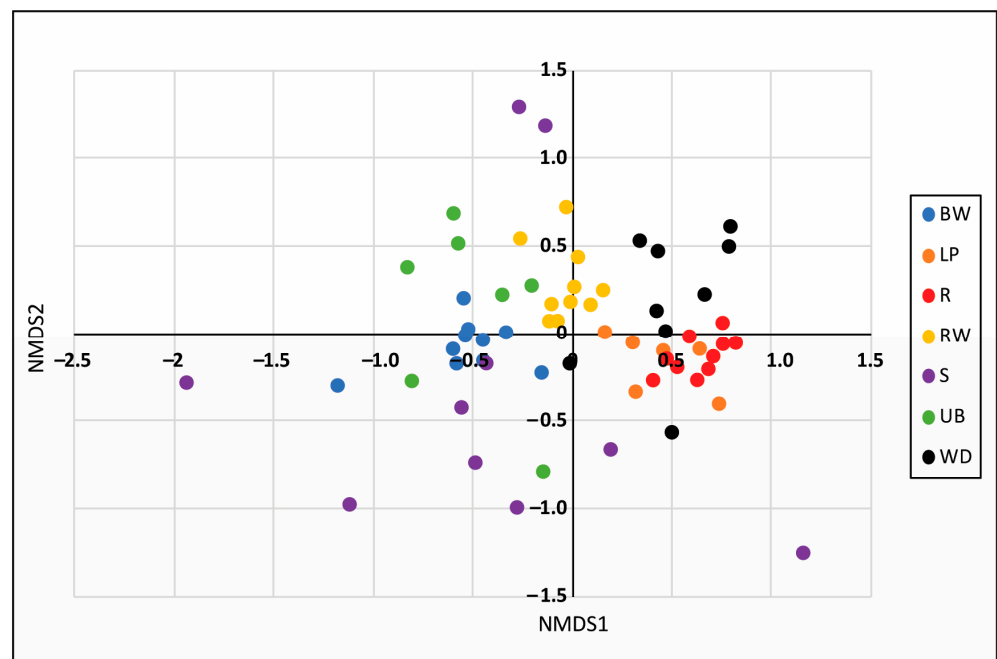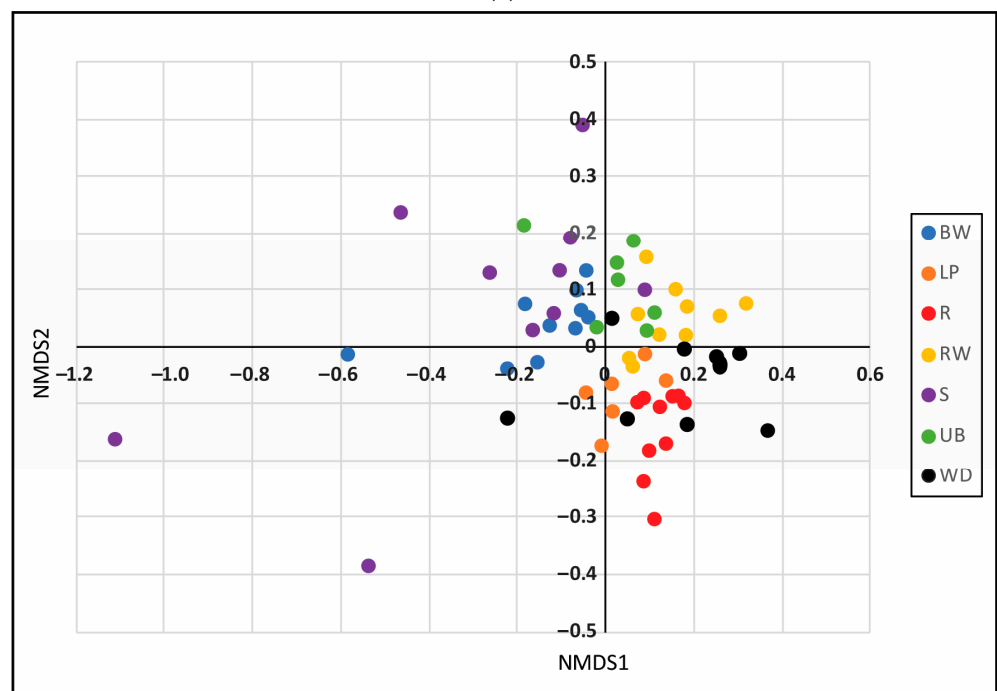
Figure 4. (**a**) NMDS plot of taxa abundance across all microhabitats. Taxa found in riffles are more similar to the taxa found in leaf pack and woody debris microhabitats and are less similar to the taxa found in root wad, undercut bank, and backwater microhabitats. (**b**) NMDS plot of trait abundance across all microhabitats. Traits found in riffles are more similar to the traits found in leaf pack and woody debris microhabitats and are less similar to the traits found in root wad, undercut bank, and backwater microhabitats. The number of samples for each microhabitat: backwater (BW, 10), leaf pack (LP, 6), riffle (R, 10), root wad (RW, 10), sand (S, 10), undercut bank (UB, 7), and woody debris (WD, 9).
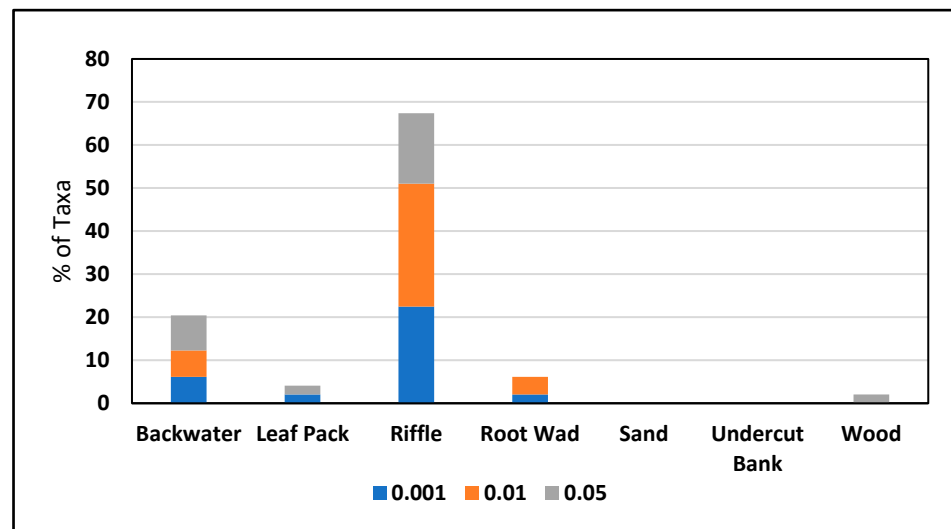
Figure 5. The indicator species analysis identified 10 taxa significantly associated with backwater, 2 taxa with leaf packs, 33 taxa with riffles, 3 taxa with root wads, and 1 taxa with wood. A total of 67.35% of the taxa were significantly associated with riffles, 20.41% with backwaters, 6.12% with root wads. The number of samples for each microhabitat = 10 except for leaf pack (6), undercut bank (7), and wood (woody debris; 9).

4. Discussion

4.1. Taxa and Trait Patterns at the Watershed Scale

When comparing the 30 sites across a habitat gradient, supporting streams were more similar to each other than to partially supporting and impaired streams when described in terms of taxa abundance; however, supporting and partially supporting streams were more similar when described in terms of trait abundance. In our study, habitat diversity did not change systematically with watershed size. We found that the impact of urbanization on habitat diversity and aquatic insect taxa richness was greater than the watershed drainage area [55]. As EMHAP scores declined, habitat diversity had greater variability in the partially supporting and impaired sites. Some streams that were identified as having low EMHAP scores had diverse habitats. This variability may be due to different types of stressors across these watersheds, including percent of impervious cover, presence or absence of stormwater control measures, and pollution being sourced from different land use, such as commercial and industrial versus residential [26,64–67].

Urbanization results in degraded stream channels, reduced stream habitat diversity, and impaired benthic macroinvertebrate communities [26,30,65,66,68–71]. We found that taxa richness and diversity were significantly correlated with habitat diversity. The impaired streams had lower habitat diversity than both the partially supporting and supporting streams. The supporting streams' habitat were more heterogeneous, sustaining a more complex benthic macroinvertebrate assemblage. Wang et al. [72] found that stream habitat quality was negatively correlated with percent of urban land area. Gage et al. [73] found that benthic macroinvertebrate communities were less diverse in streams in urbanized watersheds than in streams in rural watersheds. In this study, we found little to no change in trait richness and diversity as the habitat diversity improved, indicating that there was no relationship between trait and habitat diversity. Similar to our study, Peru and Doledec [74] found that, while taxa richness varied greatly in response to natural environmental gradients, trait diversity was fairly stable. Bêche et al. [75] found that benthic macroinvertebrate trait composition changed very little during the different seasons of the year while both taxa assemblages and abundances changed significantly.

We found that both taxa richness and diversity increased at a greater rate than trait richness and diversity along an in-stream habitat diversity gradient. Functional redundancy, an important component of ecosystem resilience, occurs when more than one species shares

one or more functional traits [76]. In our study, functional redundancy was higher in urban streams with a lower habitat diversity [55], indicating that trait composition did not change with habitat condition, reflecting the resiliency of the community trait composition [74]. Rosenfeld [77] found that functional redundancy was higher in ecosystems in which taxa niches overlapped. Streams with a lower habitat diversity would have more overlap of niches that are inhabited by species with similar traits [76–78].

We found that most urban streams that have been studied have poor habitat conditions. which is typical of streams in watersheds with high impervious cover. The US Geological Survey conducted a series of studies on the impact of urbanization on streams and found that most urban streams had severely degraded channels and low habitat diversities [64–66,68,70]. The habitat in these streams has homogenized, reducing the diversity of habitats available to benthic macroinvertebrate assemblages. As a result, streams that do not have a high diversity of habitats have a high redundancy of traits since the benthic macroinvertebrates are living in similar types of habitats that favor similar traits [77–81]. Streams with a higher habitat diversity are generally found in watersheds with a lower development and lower percent of impervious cover. These streams have more trait specialization, lower redundancy, and greater resiliency against disturbances associated with urbanization [9].

Our regression models identified riffles as a key microhabitat for diversity and richness (taxa and trait), reflecting the importance of riffles for developing and sustaining the aquatic insect assemblages in streams. Leaf packs were also shown to be important microhabitats, as they were included in three of the models. Several of the partially supporting and impaired streams in our study lacked riffles and woody debris while others lacked undercut banks and root wads, limiting the diversity of both taxa and trait richness and diversity.

High habitat diversity and complexity is important for a healthy stream benthic macroinvertebrate community [1,59]. We examined the relationship between microhabitats and the aquatic insect assemblages at the watershed scale and found several different habitats significantly positively correlated with taxa and trait richness and diversity (Table 1). Taxa richness was positively correlated with pools, runs, backwater, leaf packs, and riffles, while trait richness was positively correlated with runs, small wood, and riffles. Taxa diversity was positively correlated with pools and leaf packs, while trait diversity was positively correlated with runs, leaf packs, small wood, and riffles. Taxa richness was correlated with more microhabitats than trait richness, reflecting the redundancy of traits that are shared by taxa living in similar microhabitats. The multiple linear regression models showed that riffles and leaf packs are particularly important microhabitats in the development of the macroinvertebrate assemblages found in streams. In addition to riffles and leaf pack microhabitats, large wood and runs were important for taxa richness, undercut banks were important for taxa diversity, and habitat diversity and small wood were important for trait richness. A watershed-level analysis may not explain the taxa and trait variations in the aquatic insect assemblages as a result of microhabitat preferences compared to reach scale analysis [82–85]. The aquatic insect assemblages within each microhabitat should be expected to differ, as the assemblages are the result of environmental filters, such as food resources, hydrology, and structural features of the microhabitat [8,9,78].

4.2. Taxa and Trait Patterns at the Reach Scale

In our 30-site study, we correlated taxa and trait diversity and richness to habitat parameters that led to the observation that there were microhabitats that were more important than others in contributing to diversity and richness. Thus, we specifically sampled multiple microhabitats in the nine supporting and one partially supporting streams to determine the relationship between microhabitats and aquatic insect taxa and trait richness and diversity.

Using NMDS, we examined the impact that individual microhabitats had on the distribution of taxa and traits and found similar taxa and traits in similar microhabitats. For example, the taxa and traits found in riffles and leaf packs were more similar than taxa

and traits found in undercut banks and root wads. This may be due to differences observed in the stream flow velocities within the microhabitats. While we did not quantify water velocity, riffles and leaf packs are generally found in stream reaches with faster stream velocities, and root wads and undercut bank areas are found along stream edges where the stream velocities are often slower [86]. Flow conditions have been identified as one of the environmental filters, along with substrate particle size and water depth, determining the composition of benthic macroinvertebrate assemblages within microhabitats [6,8,87–89]. Scotti et al. [90] found similar taxa and trait compositions in streams in grasslands and pastures where habitats were also similar. These results support our hypothesis that similar traits are found among the aquatic insect taxa residing in similar microhabitats within the same stream.

Taxa and trait diversity and richness generally followed a similar pattern to their distribution across microhabitats where the highest taxa diversity and richness were found in riffles while the lowest were found in sand and undercut banks. While we found practically every trait in the microhabitats sampled, the traits were not evenly distributed among the microhabitats. Higher percentages of most traits were found in riffles. However, there were higher percentages of a small number of traits, such as good armoring, plastron respiration, and climber, found in other microhabitats, such as woody debris, root wads, and leaf packs.

At the habitat scale, we found that the highest taxa richness and diversity were in riffles. Gregory [91] and Wang et al. [92] found that taxa richness of benthic macroinvertebrates was slightly higher on woody debris than in riffles but had lower densities of organisms. Large woody debris can increase stream habitat complexity by altering the flow path in a stream, resulting in the formation of pools, exposure of gravel and cobble substrates, enhancement of sediment deposition, an increase in the retention of leaves and other organic debris, and the provision of stable substrates for benthic macroinvertebrates and periphyton [93–98]. Large woody debris serves as refugia for fish and benthic macroinvertebrates during high flows as well as a stable substrate for both benthic macroinvertebrates and periphyton [88,93,99]. Coe et al. [95] reported that woody debris supports a unique community of benthic macroinvertebrates.

An indicator species analysis revealed that there were specific taxa and traits that were associated with specific microhabitats or combinations of microhabitats (Tables S5 and S6). For example, *Triaenodes ignitus* (Walker 1852), *Calopteryx* (Leach 1815), and *Labrundinia pilosella* (Loew 1866) were associated with root wads, while *Isonychia* (Eaton 1871), *Stenacron interpunctatum* (Say 1839), and *Corydalus cornutus* (Linnaeus 1758) were associated with riffles, and *Rheocricotopus robacki* (Beck and Beck 1964), *Acroneuria abnormis* (Newman 1838), and *Perlesta* (Banks 1906) were associated with both leaf packs and riffles. Taxa that were associated with specific microhabitats were not limited to utilizing that microhabitat alone. For example, *Gomphus* (Leach 1815), which was associated with the backwater microhabitat, was found in all microhabitats except riffles, and *Polypedilum illinoense* (Malloch 1915), which was associated with leaf packs, was found in all microhabitats except sand. Gregory [91] found specific taxa associated with either woody debris or riffle microhabitats. Other studies showed that some taxa preferred specific substrates [4,100] or combinations of substrates, water velocities, water depths, or benthic coarse particulate organic matter [8,87,88,101,102]. While most traits were associated with riffles, several traits were significantly associated with other microhabitats, such as root wads (e.g., CLB) and leaf packs (e.g., PLA). Taxa and traits found in a specific stream reach or microhabitat are the result of numerous environmental filters, such as physical (water velocity and habitat diversity), environmental (water chemistry, pollution, and temperature), and biological (food types, mobility, and life cycle requirements) [8,88,89,103–105].

5. Application to Stream Restorations

Stream restoration has become a multimillion-dollar industry as watershed managers respond to the degradation of urban stream channels and stream biota by restoring streams to a more natural state. Unfortunately, the biological community enhancements expected through urban stream restoration have not occurred in most cases, which may be due to the general focus on stream channel stabilization more than instream habitat restoration [39–41,106,107].

Our results show that all microhabitats contribute to the overall ecosystem function by providing habitats for a diverse range of aquatic insect assemblages. We show that woody debris, leaf packs, undercut banks, and root wads support species that would otherwise not be found in the stream. Woody debris has been shown to have an important role in creating and maintaining diverse flow patterns and heterogeneous habitats [93,94,97,108,109] as well as maintaining healthy food webs and promoting carbon assimilation [95,98,108,109]. Our results show that there are specific taxa that are associated with microhabitats such as root wads, backwater, and leaf packs, and traits associated with root wads and leaf packs. These taxa and traits would add to stream biodiversity and ecosystem function.

Insect reproduction and adult emergence, two important stages of the aquatic insect life cycle, are often overlooked when stream restoration projects are planned [105,110]. Merten et al. [110] showed that the abundance of substrates such as wood and boulders that have surfaces exposed to the air that allow adult aquatic insects to exit the stream are correlated with an emerging insect biomass. They recommended that stream restoration designers should include habitats for aquatic insect emergence. Jordt and Taylor [105] found a shortage of rocks suitable for oviposition in restored stream segments. Most boulders in restored stream segments are not stable and often roll during storm events, making them unsuitable for successful oviposition and limiting reproductive success.

If we want to improve biodiversity and ecosystem function in degraded urban streams, we recommend that stream restoration engineers broaden the types of microhabitats included in restoration design. Increasing the diversity of microhabitats could increase the biodiversity of taxa and traits, resulting in a more resilient ecosystem. It is possible that the retention of woody debris and leaf packs and the occurrence of undercut banks and root wads would increase with restoration age. However, these microhabitats would surely develop if the restoration design encouraged these microhabitats.

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