Channel type and substrate influence on aquatic macroinvertebrate abundance and diversity in a montane stream

Honors Thesis

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Abstract

Aquatic macroinvertebrate communities respond strongly to changes in their habitat, making them useful indicators of conditions in streams. It is important to understand the relationship between habitat complexity and macroinvertebrate communities, especially as streams become more affected by anthropogenic influences. This study investigates the differences in macroinvertebrate abundance and diversity between primary and secondary channels, as well as cobble and wood substrates. The study took place at Little Beaver Creek, a montane stream in northern Colorado that is relatively undisturbed by humans. Multiple cobble and wood samples were selected from both the main and side channels, and the macroinvertebrates present on each sample were identified to order. These results were analyzed, along with data related to the decay class of the wood samples and the substrate size, using single and multiple linear regression models and the Shannon-Wiener Species Diversity Index. The results indicate that substrate was a significant predictor of total macroinvertebrate density ($p = 0.001$), while channel location was not ($p = 0.9$). There were some variations in this result when considering specific taxa. Cobbles had a significantly higher density of individuals than wood, and the side channel had a higher density of individuals than the main channel, although this result was not statistically significant. Shannon index values for the main channel were higher than the side channel (1.48 vs. 1.24). Although some trends were not statistically significant, the results highlight the value of habitat complexity. Maintaining diverse substrate types and multi-channel morphology is necessary to support more robust macroinvertebrate communities.

Introduction

Aquatic macroinvertebrates play a vital role in freshwater ecosystems by serving as a primary food source and aiding nutrient cycling through decomposition of organic matter (Wallace & Webster, 1996). A diverse macroinvertebrate population is essential to maintain a resilient stream ecosystem, as these organisms support food web stability (Wallace & Webster, 1996). Macroinvertebrates are also valuable bioindicators that reflect water quality and overall stream conditions, and assessment of macroinvertebrate presence, diversity, and abundance is a tool for ecological monitoring in freshwater systems (Sallenave, 2023; Xu et al., 2014). Previous research suggests that macroinvertebrate density is highly correlated with greater levels of habitat complexity within the environment, such as the presence of large wood, diverse substrates, and varied flow patterns (O'Connor, 1991). Understanding how habitat diversity influences macroinvertebrate populations is vital in the face of increasing anthropogenic pressures and climate change. Human activity such as channelization and wood removal reduces spatial heterogeneity throughout the river corridor, resulting in a decreased variety of substrates and fewer segments with multiple channels. These alterations can lead to a decline in macroinvertebrate abundance and diversity, ultimately compromising the health of the ecosystem as a whole (Chen et al., 2025; Wallace & Webster, 1996). This research focuses on characterizing macroinvertebrate populations in a natural stream with few direct human impacts. The goal is to observe how macroinvertebrate populations vary between primary and secondary channels and wood and cobble substrates within the same montane stream in northern Colorado.

Background

Aquatic macroinvertebrates are valuable for assessing water quality because of their sensitivity to changes within their environment. Macroinvertebrates respond to stress from a variety of spatial or temporal scales, providing useful information about the overall health of an aquatic ecosystem (Xu et al., 2014). Using macroinvertebrates to monitor water quality through bioassessments has numerous advantages over chemical monitoring. Bioassessments provide integrative responses over time and are convenient due to the abundance of macroinvertebrates in most aquatic habitats, even low-order streams that may not support fish (Sallenave, 2023). Macroinvertebrates can be easily collected and identified using minimal equipment with methods that do not adversely affect other organisms (Sallenave, 2023). They also cannot escape pollution events due to limited mobility, and they bioaccumulate toxic substances, allowing for the detection of pollutants that may be undetectable in the water source (Sallenave, 2023). Some species are more tolerant of pollution than others, so the presence or absence of certain species reflects environmental conditions. A well-known group of indicator species are the EPT taxa. This includes individuals in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (Kenney et al., 2009). Their increased sensitivity to dissolved oxygen levels, pH, and sedimentation cause them to be present only in streams with high water quality (Hamid & Rawi, 2017).

Stream ecologists also categorize macroinvertebrates by functional feeding groups. This is a classification based on the primary method used to acquire food. It allows similar morphologies and behaviors to be grouped together despite taxonomic differences (Merritt et al., 2017). The five major functional feeding groups are collector/gatherers, collector/filterers, scrapers, predators, and shredders (Gholizadeh & Heydarzadeh, 2020). Gatherers feed primarily on fine particulate organic matter (FPOM, < 1 mm diameter); filterers have anatomical structures or silk-like secretions that allow them to remove particulate matter from the water column; scrapers have specialized mouthparts adapted for scraping periphyton from minerals and organic substrates; shredders feed on decomposing vascular plant tissue, living macrophytes, or decomposing wood; and predators feed primarily on animal tissue (Wallace & Webster, 1996). The distribution of functional feeding groups within a stream provides insight into how energy is being transferred through the system, and its overall condition (Merritt et al., 2017). There are often shifts in dominant functional feeding groups in a stream under disturbance or impairment. Collector/gatherers and tegument-breathing taxa are more prevalent in impacted systems due to their generalist nature, while specialists like scrapers and shredders are less common where ecosystem impairment has occurred (Williams-Subiza et al., 2024).

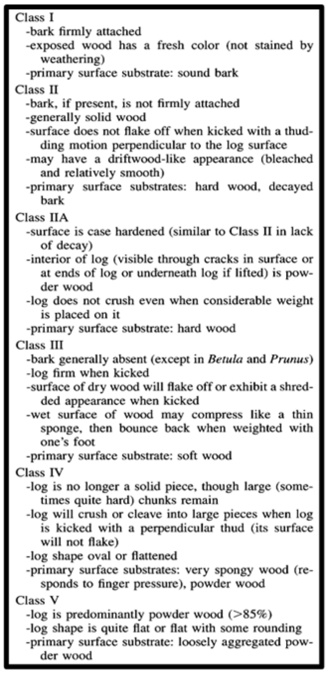
As consumers at intermediate trophic levels in lotic food webs, macroinvertebrates play an important and multifaceted role in the ecology of a stream. They are influenced by both bottom-up (resource-driven) and top-down (predator-driven) trophic forces and act as a link between producers and higher trophic levels (Wallace & Webster, 1996). They are a vital food source for fish and maintain and modify key ecosystem processes such as nutrient cycles, primary productivity, and decomposition (Baxter et al., 2004; Wallace & Webster, 1996). These additional functions often align with functional feeding groups. For example, shredders are essential for decomposition, converting coarse particulate organic matter (CPOM, > 1mm diameter) into FPOM that can more easily be transported; grazers regulate algal standing crop and productivity; and filterers remove suspended particles that would have otherwise passed through the stream segment unused (Wallace & Webster, 1996).

Existing research suggests that habitat complexity influences macroinvertebrate species distributions. Habitat complexity can be described as complexity in both the composition (element types and proportion of each type present) and configuration (arrangement, shape, neighboring elements) of the substrate mosaic (Beisel et al., 2000). Examples of elements include morphological units such as pools and riffles, and different substrates such as wood, sand, cobbles, and boulders. These factors impact biotic diversity, nutrient dynamics, retention and distribution of organic matter (OM), and predator-prey interactions (Wallace & Webster, 1996). As the habitat becomes more structurally complex, macroinvertebrate species diversity and abundance tend to increase as more microhabitats become available. (O'Connor, 1991). Furthermore, physical heterogeneity provides refugia for macroinvertebrates during times of disturbance (Beisel et al., 2000).

The existence of multiple stream channels is another aspect of habitat complexity. Research suggests that side channels differ from main channels by a variety of metrics. They tend to be more spatially and temporally variable and provide a greater range of physical conditions throughout the year (Sobotka & Phelps, 2017). Side channels also generally have a smaller width, depth, and flow velocity compared to main channels (Sobotka & Phelps, 2017). Hydraulic parameters, part of the physical heterogeneity of a stream, influence the distribution of macroinvertebrate functional feeding groups, as does the macroinvertebrates' relationship to resource availability (Wallace & Webster, 1996). Filterers generally thrive in high velocity, low-retention habitats, or habitats with concentrated food resources such as suspended OM, while gatherers and shredders prefer low-velocity, high-retention pools (Sobotka & Phelps, 2017; Wallace & Webster, 1996). The existence of multiple channels creates opportunities for organisms with specific habitat preferences to thrive.

The role of wood, including large wood (at least 1 m long and 10 cm in diameter), smaller wood pieces, and living wood, in stream ecosystems is also important to understand in the context of macroinvertebrate communities and habitat complexity. Large wood plays a major role in increasing the retention time of nutrients and detritus and influences the physical structure of the stream through the dissipation of flow (Wohl, 2024). Large wood accumulations and beaver dams that span most or all of a stream channel aid in the creation of step and pool patterns (Benke & Wallace 2003). This results in differing habitats upstream and downstream of the wood accumulation or dam because increased water retention creates slower flow upstream and faster flow downstream. (Benke & Wallace, 2003). The increase in spatial heterogeneity caused by wood creates greater habitat diversity and refugia for organisms (Benke & Wallace, 2003). Assemblages of large wood in the channel have been found to provide shelter and food and improve oxygen conditions, subsequently enhancing macroinvertebrate diversity (Verdonschot & Verdonschot, 2023). Decades of intentional wood removal and deforestation have caused a dramatic decline of wood loads in streams and rivers globally (Wohl, 2024). In the US alone, from 1867-1912, at least 1.5 million snags were removed from major rivers in the Southeast, Central, Southwest, and West Coast regions of the country for navigation improvement, but modification and removal of wood in streams and rivers began long before 1867 and continues today (Wohl, 2014).

Macroinvertebrates use wood as habitat and a food source. The distribution of macroinvertebrates on wood greatly depends on the characteristics of the wood in the system. Loose wood is preferred by shredders while more stable snags tend to be dominated by filterers and gatherers (Benke & Wallace, 2003). Research on macroinvertebrates has historically focused primarily on mineral substrates, but wood has the potential to provide macroinvertebrates with superior habitat. Large wood can provide a variety of surface textures that make it an ideal site for macroinvertebrate attachment. Unlike mineral substrates, wood fosters the colonization of biofilms and plants, it can be eaten, and is soft enough to be gouged to create refuges (Benke & Wallace, 2003). Its effectiveness at trapping other OM with its branching structure also benefits macroinvertebrates. However, in some cases, smaller, more mobile pieces of wood may not provide a habitat as stable as mineral substrates. The decay stage of wood is another important element that factors into its suitability as a potential habitat. Aspects such as permeability, structural integrity, and surface irregularity impact its suitability as macroinvertebrate habitat (Pyle & Brown, 1998). Pyle & Brown have devised a useful wood decay classification system (Figure 1).



Class I
-bark firmly attached
-exposed wood has a fresh color (not stained by weathering)
-primary surface substrate: sound bark
Class II
-bark, if present, is not firmly attached
-generally solid wood
-surface does not flake off when kicked with a thudding motion perpendicular to the log surface
-may have a driftwood-like appearance (bleached and relatively smooth)
-primary surface substrates: hard wood, decayed bark
Class IIA
-surface is case hardened (similar to Class II in lack of decay)
-interior of log (visible through cracks in surface or at ends of log or underneath log if lifted) is powder wood
-log does not crush even when considerable weight is placed on it
-primary surface substrate: hard wood
Class III
-bark generally absent (except in *Betula* and *Prunus*)
-log firm when kicked
-surface of dry wood will flake off or exhibit a shredded appearance when kicked
-wet surface of wood may compress like a thin sponge, then bounce back when weighted with one's foot
-primary surface substrate: soft wood
Class IV
-log is no longer a solid piece, though large (sometimes quite hard) chunks remain
-log will crush or cleave into large pieces when log is kicked with a perpendicular thud (its surface will not flake)
-log shape oval or flattened
-primary surface substrates: very spongy wood (responds to finger pressure), powder wood
Class V
-log is predominantly powder wood (>85%)
-log shape is quite flat or flat with some rounding
-primary surface substrate: loosely aggregated powder wood

Figure 1. Wood decay classes (Pyle & Brown, Table 1, p. 239, 1998)

Objectives

The objective of this research is to observe how macroinvertebrate populations vary between primary and secondary channels within the same river and compare macroinvertebrate population densities between wood and cobble substrates. I hypothesize that:

(1) The main channel will have a greater abundance of macroinvertebrates because it provides a more stable habitat. The main channel typically maintains continuous flow whereas side channels often experience greater temporal variability, such as drying during low flow or stagnation during baseflow, which may limit the persistence of macroinvertebrate populations. The stability of flow in the main channel likely supports a more continuous food supply and refuge availability, which might contribute to higher abundance.

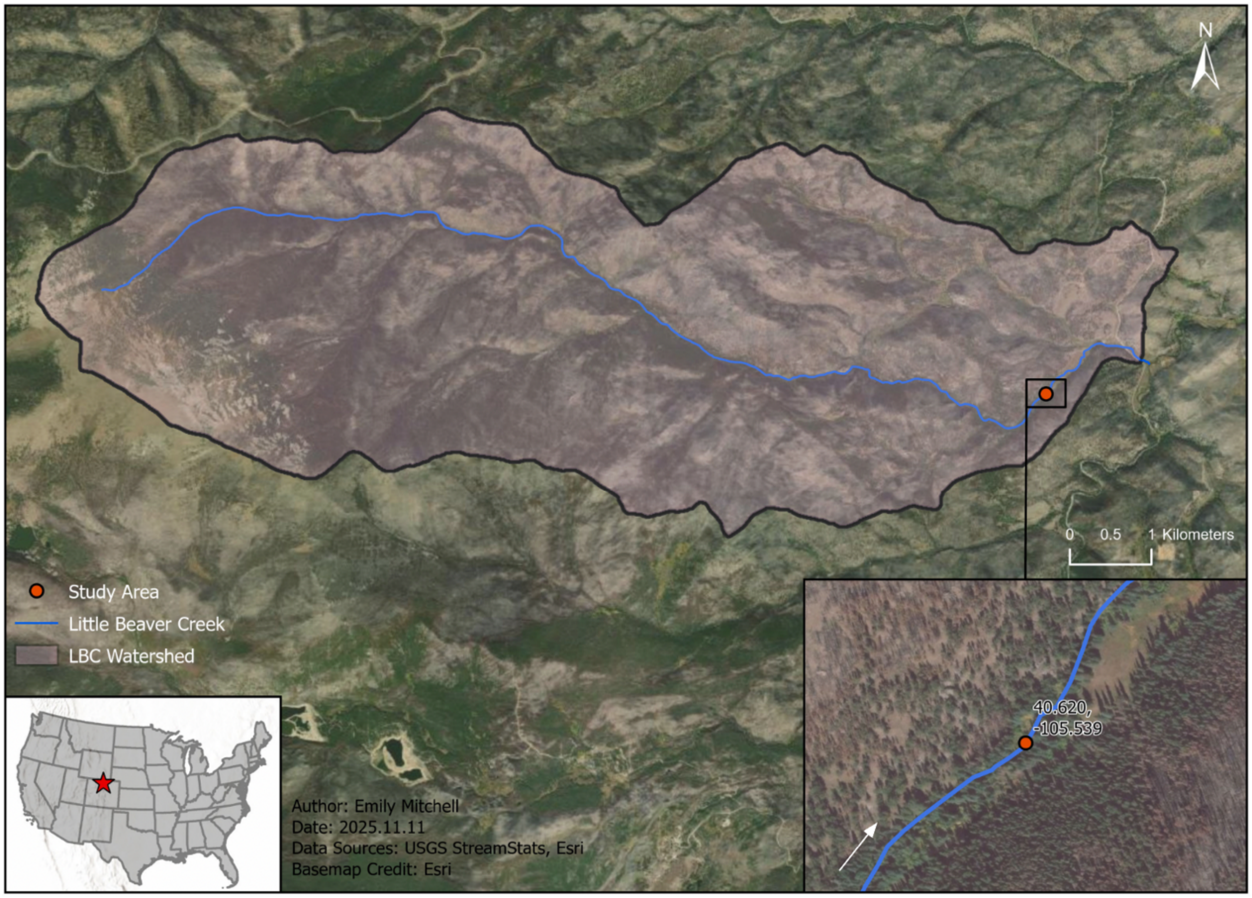
(2) The macroinvertebrate community composition will differ between wood and cobble substrates. Different substrates provide distinct physical structures and food resources that might attract macroinvertebrates in varying functional feeding groups and contribute to differences in overall community composition between substrate types.

(3) Macroinvertebrate abundance on wood will be correlated with the stage of wood decay. As wood decomposes, it undergoes physical and biological changes such as fragmentation and increased colonization of microbes, which influence its suitability as a substrate. Wood in early decay stages may have limited ideal surfaces for colonization while wood at a more advanced decay stage is often softer and supports more diverse

microbial and algal communities, potentially providing both food and shelter for macroinvertebrates.

Study Site

Little Beaver Creek (LBC), a tributary of the South Fork Cache la Poudre River in the Cache la Poudre watershed, is a montane stream located in northern Colorado (Figure 2). It drains approximately 40 km^2 and is primarily fed by snowmelt. Heavy spring runoff causes a streamflow peak in late spring or early summer that averages 1.26 m^3/s (Wohl et al., 2022). The watershed receives an average of 55 cm of precipitation annually and has a mean annual temperature of 8.3 °C (Wohl et al., 2022). It is a cobble-bed stream located within an old-growth forest, where large wood is recruited through treefall and is found throughout the river corridor including within the active channel. The study area consists of an alternating pattern of narrow, confined segments known as strings and less confined segments known as beads. In the beads, wider floodplains that range from 15 m to 50 m in width allow for the formation of secondary channels (Wohl et al., 2022). Approximately 86% of LBC burned during the Cameron Peak wildfire in 2020 (Wohl et al., 2022). However, this study took place in an unburned reach in the lower forested portion of the river corridor.



Author: Emily Mitchell
Date: 2025.11.11
Data Sources: USGS StreamStats, Esri
Basemap Credit: Esri

Figure 2. Map of the Little Beaver Creek drainage area with an inset showing the coordinates of the study location (40.620, -105.539). The small white arrow indicates flow direction.

Methods

Field Methods

A reach of LBC was selected that has multiple channels and contained wood pieces in both the main and side channel. Site measurements were taken for each channel individually, including the channel length, width, thalweg depth, and discharge. The lengths and widths of the channels were taken at the upstream channel split, and the downstream point of channel reconnection. The main channel segment was 25 m long, and the side channel segment was 17 m long. Width measurements were also taken at a few intervals along the middle of the segment to determine average width. Measurements for width and depth were based on water depth at the time of measurement. A lateral cross section survey at the site of sampling was conducted to determine an average depth. Depths were recorded at intervals of 50 centimeters. I also measured the deepest point of the thalweg for each channel. Velocity data were collected using a Marsh-McBirney flow meter. An average velocity measurement was taken at each interval where depth was measured. The depth of the velocity measurement was 0.8 of the water depth from the surface when possible. Velocity was only measured once during the field season during base flow.

The macroinvertebrate data were collected by randomly selecting three cobbles and three pieces of wood, one at a time, from each channel. Each sample was placed into a small tray, and tweezers and a combination of water and ethanol from squeeze bottles were used to carefully clean and remove all specimens from the substrate. Each specimen was then removed from the tray with tweezers and identified to order. This process was repeated for each sample and channel. Sample dimensions for the cobbles involved measuring the x, y, and z axes, and wood sample dimensions included piece length and approximate average diameter. For each wood sample, the level of decay was also determined using the classification system from Pyle and Brown, 1998 (Figure 1).

Statistical Methods

The collected data were compiled into a spreadsheet, processed for analysis, and imported to R Studio. Total density of specimens per unit area was calculated by dividing total specimens per sample by the surface area of the sample.

$$Density = \frac{n}{SA}$$

n = *number of individuals on the sample*

SA = *surface area of the sample*

Density of specimens was also calculated per order using the same equation. Richness was defined as the total number of different orders observed in a given area and was calculated as the sum of observed orders. Single linear regression and multiple linear regression models were tested to determine the relationship between the predictor (channel, substrate, and surface area) and response variables (total density, Ephemeroptera density, Plecoptera density, Trichoptera density, and richness). These relationships were compared between the various groups (main vs side channel, cobbles vs wood, decay stage). An alpha level of 0.05 was used to determine statistical significance, with p-values derived from t-tests within the regression framework.

The Shannon-Wiener Species Diversity Index was calculated for the major groups of interest (cobbles, wood, main channel, side channel) using the following equation:

$$H = -\sum_{i=1}^{s} p_i \ln(p_i)$$

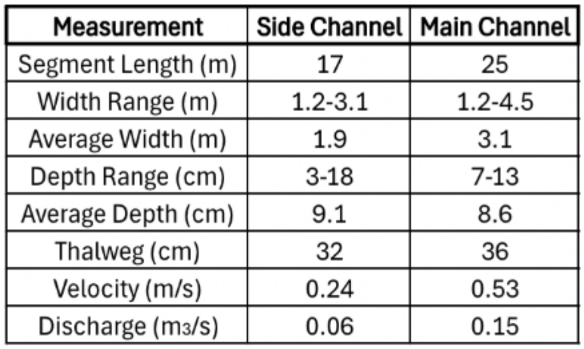
H = Shannon index value
p_i = *proportion of individuals found in the ith species*
s = number of species in the community
ln = natural logarithm

Order level data were used in place of species level data in this calculation. Shannon index values calculated with species level data are typically within the range of 1.5 - 3.5 and rarely surpass 4.5 (Magurran, 1988). Higher Shannon index values indicate higher diversity of species in a particular community.

Results

Study Site

This research was conducted on August 6, 2025. The reach of interest included a main channel directly adjacent to a side channel, which differed in size and flow characteristics. The length of the segments was 25 m and 17 m for the main channel and side channel, respectively. Other measurements indicate that the main channel was wider, deeper, and had a higher discharge than the main channel (Table 1).

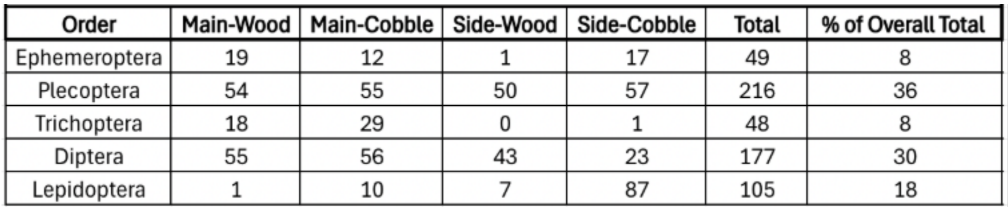


Measurement	Side Channel	Main Channel
Segment Length (m)	17	25
Width Range (m)	1.2-3.1	1.2-4.5
Average Width (m)	1.9	3.1
Depth Range (cm)	3-18	7-13
Average Depth (cm)	9.1	8.6
Thalweg (cm)	32	36
Velocity (m/s)	0.24	0.53
Discharge (m$_3$/s)	0.06	0.15

Table 1. Measurements of the main and side channels.

Specimens Surveyed

A total of 595 macroinvertebrates were surveyed on three cobble samples and three wood samples in both the main channel and the side channel (n = 12) (Table 2). Five orders were observed: Ephemeroptera, Plecoptera, Trichoptera, Diptera, and Lepidoptera (Figure 3).



Order	Main-Wood	Main-Cobble	Side-Wood	Side-Cobble	Total	% of Overall Total
Ephemeroptera	19	12	1	17	49	8
Plecoptera	54	55	50	57	216	36
Trichoptera	18	29	0	1	48	8
Diptera	55	56	43	23	177	30
Lepidoptera	1	10	7	87	105	18

Table 2. Number of individuals surveyed by order for each substrate-channel pairing (n = 3). Total number of individuals surveyed from each order and percentage of overall total for each order are also reported.

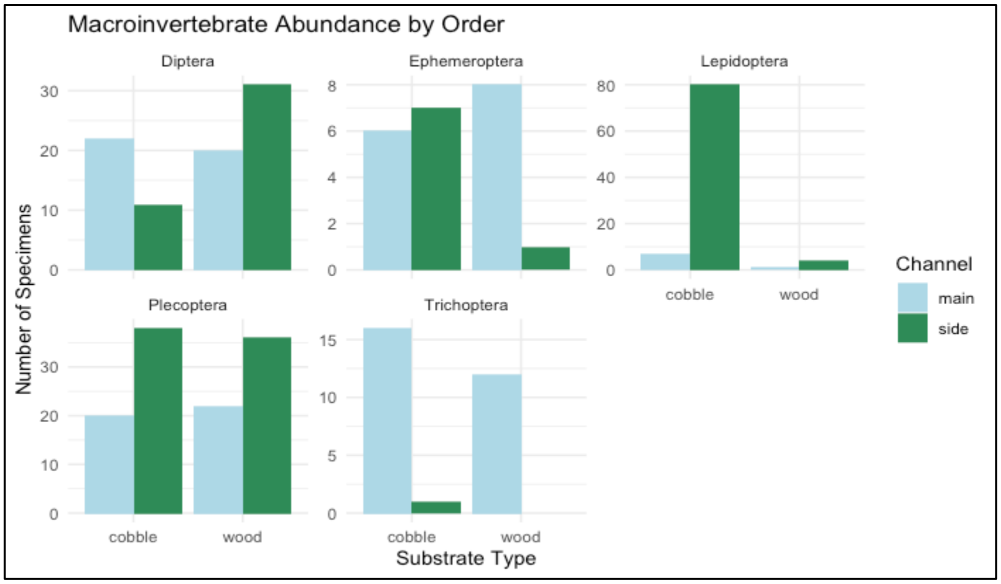


Figure 3. Observed macroinvertebrate abundance by order, substrate, and channel.

Average Density of Individuals

The average density of individuals per square centimeter, including all orders and both substrate types, was 0.062 (0.029 - 0.12) in the main channel and 0.064 (0.016 - 0.14) in the side channel (Table 3). For cobbles, the average density of individuals per square centimeter was 0.095 (0.067 - 0.14), and on wood, the average density was 0.031 (0.016 - 0.059) individuals/cm^2 (Table 4). The substrate density calculations included all orders and both channel types.

Order	Main Channel Average Density	95% CI	Side Channel Average Density	95% CI
Ephemeroptera	0.0055	0.0025 – 0.0086	0.0074	-0.0036 – 0.018
Plecoptera	0.022	0.008 – 0.036	0.023	0.0084 – 0.037
Trichoptera	0.0098	-0.0008 – 0.0205	0.00018	-0.0003 – 0.0006
Lepidoptera	0.0024	-0.0016 – 0.0063	0.018	-0.017 – 0.054
Diptera	0.022	0.0074 – 0.037	0.015	0.005 – 0.026
Total	0.062	0.023 – 0.10	0.064	0.015 – 0.11

Table 3. Average density of individuals per cm^2 for all observed orders in the main channel and the side channel with 95% confidence intervals.

Order	Cobble Average Density	95% CI	Wood Average Density	95% CI
Ephemeroptera	0.01	0.0017 – 0.019	0.0025	-0.0013 – 0.0063
Plecoptera	0.031	0.021 – 0.042	0.013	0.0046 – 0.022
Trichoptera	0.0084	-0.0034 – 0.020	0.0016	-0.0007 – 0.004
Lepidoptera	0.02	-0.015 – 0.054	0.0011	-0.0001 – 0.0023
Diptera	0.025	0.011 – 0.040	0.012	0.0057 – 0.019
Total	0.095	0.067 – 0.12	0.031	0.013 – 0.048

Table 4. Average density of individuals per cm^2 for all observed orders on cobbles and wood with 95% confidence intervals.

Breaking down average density to each cobble-substrate pairing reveals additional patterns in the data (Figure 4, Figure 5). Total density of individuals on wood was higher in the main channel than the side channel (0.035 individuals/cm^2 vs. 0.027 individuals/cm^2), but total density of individuals on cobbles was higher in the side channel than the main channel (0.10 individuals/cm^2 vs. 0.90 individuals/cm^2).

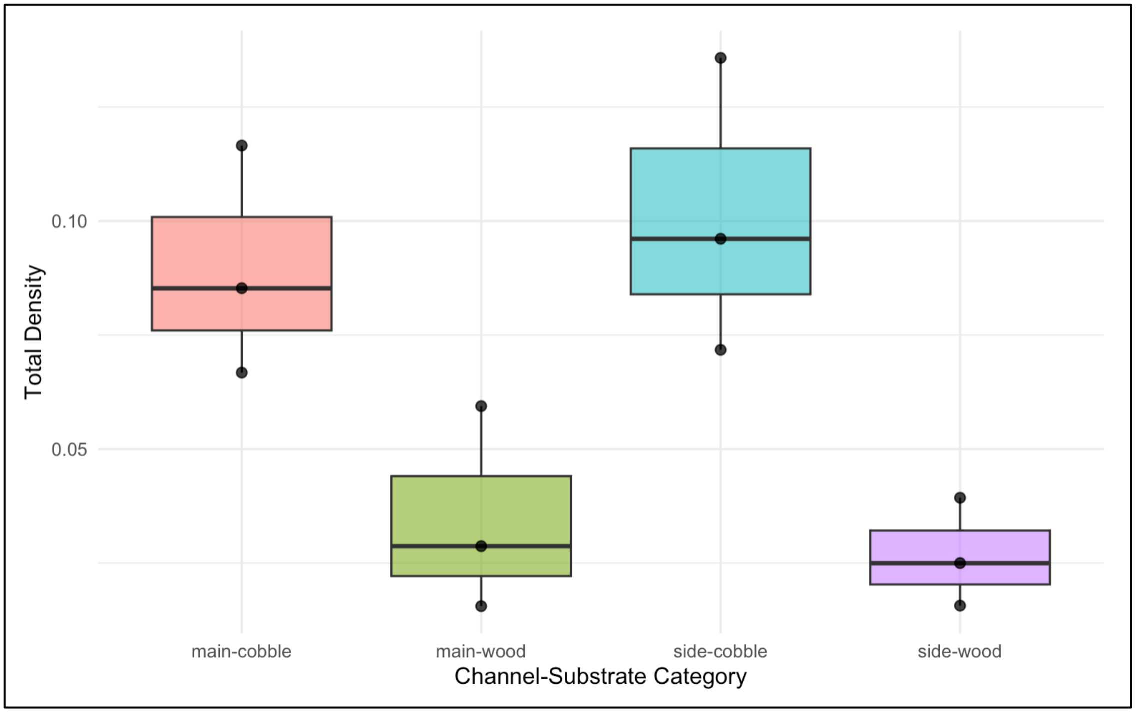


Figure 4. Box plot of total density for each channel-substrate pairing with actual data points for each sample in each category.

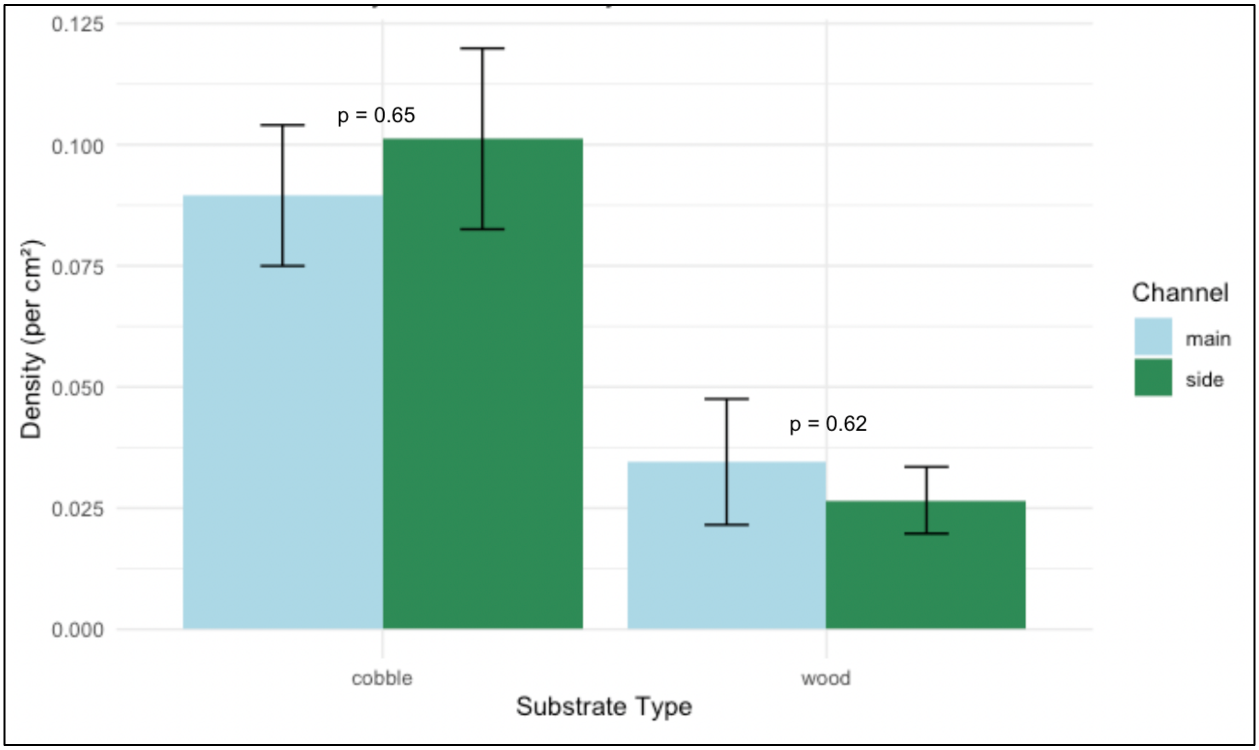
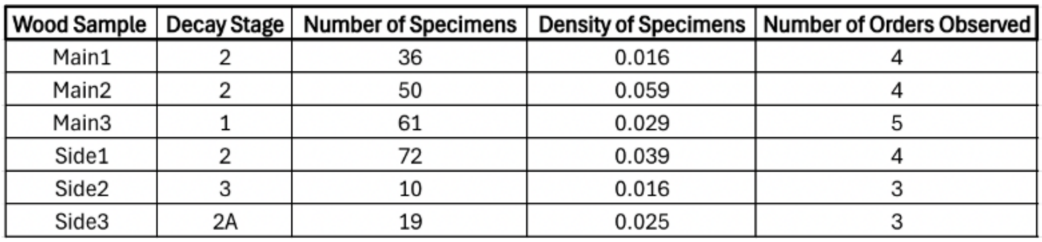


Figure 5. Mean total density of individuals per cm^2 by substrate and channel. Reported p-values indicate that channel is not a significant predictor of total density for wood or cobble substrates.

Decay Stage

The wood pieces observed in this study were mostly intact and in the early stages of decay (Table 5). The pieces were generally solid wood with at least some bark attached. The decay stage of the wood pieces sampled was not significantly correlated with total density of macroinvertebrates and not a significant predictor of total density ($p = 0.58$).



Wood Sample	Decay Stage	Number of Specimens	Density of Specimens	Number of Orders Observed
Main1	2	36	0.016	4
Main2	2	50	0.059	4
Main3	1	61	0.029	5
Side1	2	72	0.039	4
Side2	3	10	0.016	3
Side3	2A	19	0.025	3

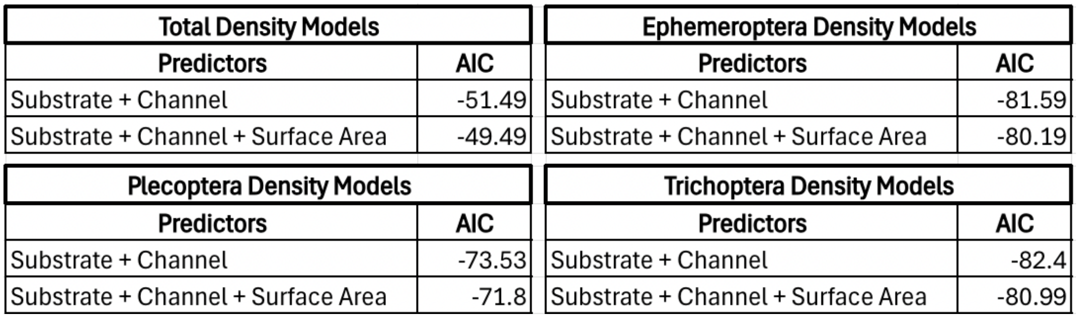
Table 5. Each wood sample, its associated decay stage, abundance, density, and richness.

Diversity Indices

The Shannon-Wiener Species Diversity Index for all samples in the main channel was 1.37. The value for all samples in the side channel was 1.27. For all wood and cobble samples, the values were 1.24 and 1.48, respectively.

Model Results

The multiple linear regression models were compared using Akaike's Information Criterion (AIC), where the lowest AIC value represents the model with the best fit while considering model complexity. Predictor variables for the best fitting multiple linear regression models from AIC for total density and density of each EPT order were substrate and channel (Table 6). The predictor variables for the best fitting model from AIC for richness were substrate, channel, and surface area (Table 7).



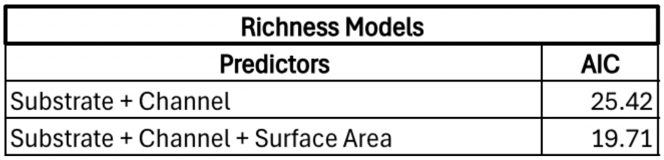
Total Density Models	
Predictors	**AIC**
Substrate + Channel	-51.49
Substrate + Channel + Surface Area	-49.49

Ephemeroptera Density Models	
Predictors	**AIC**
Substrate + Channel	-81.59
Substrate + Channel + Surface Area	-80.19

Plecoptera Density Models	
Predictors	**AIC**
Substrate + Channel	-73.53
Substrate + Channel + Surface Area	-71.8

Trichoptera Density Models	
Predictors	**AIC**
Substrate + Channel	-82.4
Substrate + Channel + Surface Area	-80.99

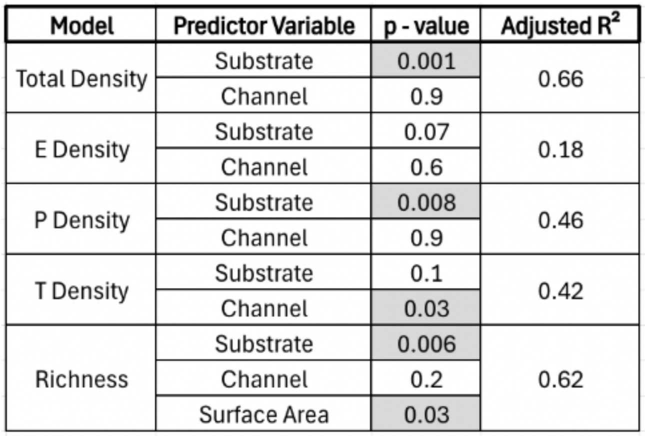
Table 6. Tested multiple linear regression model variations for predicting total density of individuals and density of EPT individuals with associated AIC values.



Richness Models	
Predictors	**AIC**
Substrate + Channel	25.42
Substrate + Channel + Surface Area	19.71

Table 7. Tested multiple linear regression model variations for predicting richness and associated AIC values.

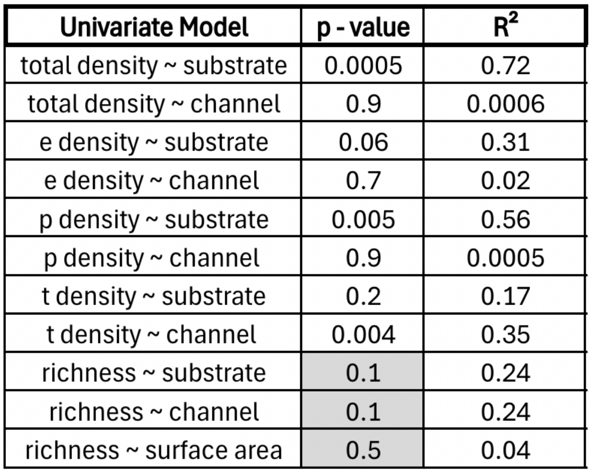
The results of the best fitting total density multiple linear regression model indicate that (i) substrate type is a significant predictor of total density and (ii) the total density of individuals on wood substrates is significantly lower than on cobble when accounting for channel ($p = 0.001$). However, the total density model also indicates that the channel variable is not a significant predictor of total density, and total density in the side channel is not significantly different from the main channel ($p = 0.89$). The Plecoptera density model had similar results, with substrate type being a significant predictor ($p = 0.008$) and channel type being insignificant ($p = 0.92$). The results of the best fitting Ephemeroptera density model indicate that there was not a statistically significant relationship between substrate type or channel location and Ephemeroptera density ($p = 0.07$, $p = 0.64$). The results of the best fitting Trichoptera model indicate that substrate type is not a significant predictor of Trichoptera density when accounting for channel location ($p = 0.1$), but that channel location is a significant predictor of Trichoptera density when accounting for substrate type ($p = 0.03$). The richness model results reveal that there is a statistically significant effect of substrate type on the total number of orders observed when accounting for channel and surface area, and that the average number of orders found on wood substrate is predicted to be lower than on cobble by 1.27 ($p = 0.006$). Furthermore, when considering substrate type and surface area, richness in the main channel is not significantly different from the side channel ($p = 0.2$). Surface area is a significant predictor of richness when accounting for substrate and channel type, with larger surface areas having a greater order-level richness ($p = 0.03$). These results, along with the adjusted R^2 values from each model, are summarized in Table 8.



Model	Predictor Variable	p - value	Adjusted R^2
Total Density	Substrate	0.001	0.66
	Channel	0.9	
E Density	Substrate	0.07	0.18
	Channel	0.6	
P Density	Substrate	0.008	0.46
	Channel	0.9	
T Density	Substrate	0.1	0.42
	Channel	0.03	
Richness	Substrate	0.006	0.62
	Channel	0.2	
	Surface Area	0.03	

Table 8. Results of the best multivariate total density, EPT density, and richness models. Significant results are highlighted.

There is no major difference between p-values for the predictors in the univariate and multivariate models except for models predicting richness (Table 9). This indicates that substrate, channel, or surface area alone are not enough to significantly explain variance in richness.



Univariate Model	p - value	R^2
total density ~ substrate	0.0005	0.72
total density ~ channel	0.9	0.0006
e density ~ substrate	0.06	0.31
e density ~ channel	0.7	0.02
p density ~ substrate	0.005	0.56
p density ~ channel	0.9	0.0005
t density ~ substrate	0.2	0.17
t density ~ channel	0.004	0.35
richness ~ substrate	0.1	0.24
richness ~ channel	0.1	0.24
richness ~ surface area	0.5	0.04

Table 9. Results of the univariate total density, EPT density, and richness models. Results of the richness models that are much different from the multivariate model are highlighted.

Discussion

The purpose of this study was to compare macroinvertebrate populations between main and side channels, as well as wood and cobble substrates. The primary result is that substrate type was a highly significant factor when predicting total density of individuals, while channel location generally was not.

Influence of Channel Type on Abundance and Richness

The first hypothesis, which predicted that the main channel would support a greater total abundance of macroinvertebrates due to its presumed stable habitat, was not supported, as channel type was not found to be a significant predictor of total density ($p = 0.89$, $R^2 = 0.66$). Average densities for the main and side channel were nearly identical (Main: 0.062 individuals/cm²; Side: 0.064 individuals/cm²), and the number of observed orders was the same for each channel. The lack of significant difference between channel types suggests that the expected benefits of main channel habitat stability were potentially offset by the structural complexity or resource availability offered by the side channel. The main channel had greater width, depth, and discharge than the side channel. Even though side channels can be more variable, they can also provide a greater range of physical conditions. So, while the community composition was similar in both channels, perhaps it is being driven by different factors. There was also an outlier sample that could be contributing to the higher average density observed in the side channel. One cobble sample from the side channel contained 80 individuals in the order Lepidoptera, and the average number of Lepidoptera individuals on the other cobble samples in the side channel was 3.5. There was no apparent reason that this cobble hosted such a high density of Lepidoptera. If the total density calculations for the side channel are redone without this outlier, the density of specimens in the side channel becomes 0.05. This is much lower than the original value and the value for the main channel. It also aligns more closely with the original hypothesis. Perhaps if the sample size had been bigger, this outlier would have been smoothed out, or we would have found more evidence that Lepidoptera density in the side channel is truly much higher than the main channel.

An exception to the general lack of channel effect was observed in the order Trichoptera, where channel location was a statistically significant predictor of density ($p = 0.03$, $R^2 = 0.42$). The main channel had a much higher density of Trichoptera individuals. The majority of the observed Trichoptera belong to the collector/filterer functional feeding group. Since filterers often thrive in high-velocity habitats, the higher discharge of the main channel may explain why this order was influenced more strongly by channel location.

The Shannon-Wiener Species Diversity Index, one of the most widely used diversity indices in ecology, allows for comparison of the diversity of macroinvertebrate communities across different channels and substrates examined in this study. The Shannon index values grouped by channel were 1.37 for the main channel and 1.27 for the side channel. Because the calculations for this study were only done with specificity to the order level, the values are lower than if they had been calculated with species information. This is due to the fact that the Shannon index is sensitive to both the number of groups (richness) and the evenness of individuals across groups.

The main channel has a slightly higher diversity index compared to the side channel, suggesting the main channel generally had a more diverse macroinvertebrate community across all sampled substrates. This aligns with the average observed number of orders on each sample separated by channel. The value was higher for the main channel (4.5) than the side channel (3.8). However, the results of the models indicate that this difference is not statistically significant. In addition to the density models discussed above, the best fitting model for richness indicated that richness in the main channel is not significantly different statistically from the side channel ($p = 0.2$, $R^2 = 0.62$). The finding that surface area was a significant predictor of richness ($p = 0.03$, $R^2 = 0.62$), regardless of substrate or channel, supports the ecological principle that greater physical heterogeneity and structural complexity allow for more microhabitats. It also suggests that heterogeneity or the size of the substrate sample may have influenced density and richness more strongly than the channel from which the sample came.

Influence of Substrate Type on Abundance and Richness

The second hypothesis, predicting that macroinvertebrate community composition would differ between wood and cobble substrates, was strongly supported, indicating that substrate type is a significant driver of both total density and richness. Total density on wood was significantly lower than on cobble, and richness was significantly predicted by substrate, with wood samples estimated to have 1.27 fewer orders than cobble ($p = 0.001$, $R^2 = 0.66$; $p = 0.006$, $R^2 = 0.62$). While wood has many established ecological functions and enhances habitat heterogeneity, the significantly lower density and richness observed on wood substrates suggest the existence of potential trade-offs in terms of stability or suitability of the habitat. This could be due to the characteristics of the wood available for sampling. In both channels, the wood pieces present were relatively small and mobile, potentially providing unstable habitat conditions. If the wood had been larger and more stable, it might have had a higher density of macroinvertebrates. Furthermore, sampling for this study took place in a cobble bed stream, so access to cobbles as a potential substrate was far greater than access to wood. The limited access to wood as a potential substrate might help explain differences in density observed between substrates.

Substrate type did influence community structure for the EPT taxa. A single specimen in the order Ephemeroptera was present on wood in the side channel, but more Ephemeroptera specimens were present on wood than on cobble in the main channel (Table 1). This aligns with the results of the Ephemeroptera density model, which indicates that substrate was not a statistically significant predictor of total density ($p = 0.07$, $R^2 = 0.18$). Perhaps in the main channel the wood had more abundant periphyton than in the side channel, causing Ephemeroptera in the scraper functional feeding group to prefer it. The only EPT taxa whose density was significantly predicted by substrate type was Plecoptera ($p = 0.008$, $R^2 = 0.46$).

The cobble substrate had a noticeably higher Shannon diversity index of 1.48 compared to the wood substrate Shannon diversity index value of 1.24. This indicates that across both channels, cobbles generally support a more diverse macroinvertebrate community than wood. This aligns with the average observed number of orders per wood and cobble samples, which were 4.5 and 3.8, respectively, and the statistical findings related to total density and richness.

Wood Decay Stage

The third hypothesis, which predicted that the degree of wood decay would impact total macroinvertebrate density, was not supported. The decay stage of the wood pieces, assessed using the classification system from Pyle and Brown (1998), was not found to be a significant predictor of total macroinvertebrate density ($p = 0.58$, $R^2 = 0.08$). This result could be due to the limited range of decay classes sampled. Most of the samples were in the second decay class, with only one sample in decay class I and one sample in decay class III. The samples all showed minor signs of decay and potentially did not capture the full range of habitats that wood can provide. Furthermore, it is possible that other factors such as the stability of the wood or the flow parameters in the stream masked the influence of the decay stage in this reach. The classification system used in this study was also devised for wood in terrestrial ecosystems, so perhaps a different classification system for wood in aquatic systems would be helpful.

Implications

Overall, substrate type was the primary factor driving differences in macroinvertebrate density and richness in this reach of LBC. The low R^2 values of some of the models (adjusted R^2 values of 0.18, 0.46, and 0.42 for the individual EPT density models) indicate that there is still a substantial amount of variability in density, particularly at the order level, that is unexplained. Even though channel type was not a significant predictor of density or richness, the presence of distinct channels with varying discharges and depths provided a greater range of physical conditions and opportunities for macroinvertebrates in diverse functional feeding groups to thrive and maintain essential ecosystem processes like decomposition and nutrient cycling. Despite total density being much lower on wood substrates than on cobbles, the presence of macroinvertebrates on wood indicates that it is providing habitat and contributing to the heterogeneity of the substrate mosaic.

Limitations and Future Research

The primary limitations of this study were its short duration and small sample size. Conducting the macroinvertebrate survey on one day did not allow me to capture temporal dynamics of the macroinvertebrate communities. I only surveyed three wood samples in each channel because of the limited number of wood pieces present. Future research should address these limitations by increasing the sample size, number of reaches studied, and number of samples taken throughout the field season. It could also expand this study by comparing macroinvertebrate populations across different streams of varying size and location. Going beyond order-level identification would allow for more in-depth analysis of the distribution of functional feeding groups, and research into additional properties of the wood samples beyond decay, such as permeability or mobility, could reveal more information about its suitability as a substrate.

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