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Small but Mighty: Utilization of Macroinvertebrates as Indicator Species of Stream Health Across Different Land Use Areas in Vermont

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Abstract
Anthropogenic activities, including land use changes, exert a significant impact on the physical and chemical characteristics of streams as well as the assemblage of organisms living in and around the stream. Urban streams are especially vulnerable to alterations in nutrient loads, hydrological regimes, and physiochemical variables, which can lead to reduced biodiversity and negative impacts on ecosystem processes (e.g., leaf litter decomposition). This study aimed to assess the health of four streams in Vermont using metrics for macroinvertebrate community health and organic matter processing. Streams were selected to represent different land use practices: two streams were in an urban catchment area (>10% of the catchment area was covered in human development), and two streams were in a forested catchment area (85% of the catchment area was covered by natural vegetation). Stream health was evaluated based on the stream's respective macroinvertebrate species biomass, diversity, abundance, functional feeding group proportions, and organic matter processing. We predicted that streams with higher water quality (expected to be the forested streams) would harbor healthier macroinvertebrate communities (reflected by greater abundance, diversity, and biomass) leading to more organic matter processing compared to streams of lower water quality (expected to be urban streams). Data were gathered by collecting macroinvertebrate samples from the streams through the use of leaf litter bags. Measures of stream physiochemical variables (temperature, conductivity, and pH) were also taken in each stream. The results displayed differences between urban and forested streams in some physiochemical variables, such as higher temperature and conductivity in urban streams compared to the forested streams (PERMANOVA: $p<0.05$). There was no difference between urban and forested streams in terms of macroinvertebrate abundance, richness, biomass, or functional feeding group relative abundance (ANOVA: $p>0.05$). Consequently, no significant differences were observed in remaining litter between land uses or streams (ANOVA: $p>0.05$). Overall, these results suggest that, in the streams studied, different land use practices do not significantly affect their macroinvertebrate communities. The goal of this study was to act as a baseline of stream health in Vermont by exploring the effects of local urbanization on some Vermont streams and their macroinvertebrate assemblages.

Introduction:
Studies assessing the health of urban and forested streams have received increased attention in recent years and provide valuable insights into how human activities impact the physiochemistry and hydrology of these ecosystems, ultimately affecting their biological communities (Hirst, H., et. al. 2002). Most previous research focuses on the assessment of water quality by measuring physical and chemical factors. However, biomonitoring proves to be an incredibly useful measurement that integrates pressures from water quality and habitat conditions (Liu et. al., 2017). Biomonitoring includes considering the organisms living in and around a body of water us(particularly streams) and the functions the organisms play in that environment (e.g., leaf litter decomposition). The presence of aquatic organisms specifically, can be helpful as an indicator of the conditions of the water due to the role they play in organic matter processing (McCabe and Gotelli, 2000). A wide variety of aquatic organisms have been suggested as indicator species of water health, but macroinvertebrates (i.e., insects in their nymph and larval stages, snails, worms, crayfish, and clams that spend at least part of their lives in water) have become the most commonly used of these

organisms to indicate ecosystem alterations, even over fish populations (Ruaro et. al., 2016). Macroinvertebrates prove to be one of the best indicator species because of their inability to easily move out of an area of pollution due to lack of mobility, and because of their differential sensitivity to pollutants of different types. For example, macroinvertebrates can be used to reflect specific pollutants through the variations in their species diversity. Stream health may be reflected in these measurements as greater species richness and specifically richness of more sensitive species generally reflects better water quality (Hirst et. al. 2002).

One of the main roles that macroinvertebrates play in their ecosystems is leaf litter decomposition. Plant litter decomposition is the main pathway for nutrient cycling and transfers of carbon from vegetation into soil and streams and is, therefore, a critical ecosystem process (Paudel et. al., 2015). Leaf litter also provides primary resources for the micro-organisms and detritivores, such as macroinvertebrates, that break down the organic matter. The abundance of these macroinvertebrates is dependent on the amount of leaf litter accumulated in the streams. More riparian vegetation will lead to more leaf litter in the stream which will increase the number of macroinvertebrates in that stream. High levels of macroinvertebrate abundance can lead to higher levels of organic matter processing (Graca 2001). In this way, the riparian vegetation around a stream can control the abundance of macroinvertebrates: streams surrounded by more vegetation will be expected to have higher levels of macroinvertebrate abundance and therefore higher decomposition rates. Landscape urbanization has the potential to affect the assemblages of aquatic macroinvertebrates (and thus the organic matter processes of the streams) by destroying riparian vegetation.

Urbanization's ability to impact stream conditions and affect natural ecosystems is usually driven by increased nutrient and organic matter loads, altered hydrology, and reduced biodiversity in affected urban streams (Classen, R. L., et. al. 2019). Previous studies conducted in Puerto Rico, the U.K., and the US have all found that streams are highly affected by altered land uses and water pollution from domestic and industrial waste and that these effects can have dramatic consequences for species living in these streams (Classen et. al., 2019, Hirst, et. al., 2002, Mahler & Barber, 2017, and Chadwick et. al., 2012). Urbanization as a specific alteration of land use and its negative impact on stream health has been well documented, with the consensus being that compared to natural streams, macroinvertebrate species richness and abundance in urban streams is low, especially for sensitive species (Liu et. al., 2017).

The overall effect of changed stream chemical, physical, and biological attributes (due to urbanization) on macroinvertebrate populations is known as "Urban Stream Syndrome" which proposes, similarly to above, that streams in urban areas typically show a decline in macroinvertebrate richness compared to less urban areas (Chadwick, M. et. al. 2012). Previous works have provided evidence for this syndrome. For example, Classen, R. L., et. al. (2019) found that in less urbanized areas, there was a high abundance and functional diversity of insects, whereas in streams in more urbanized areas, there were fewer insects, and less functional diversity (i.e., the range of roles performed by different species within an ecosystem).

Previous papers have also found negative relationships between urbanization and macroinvertebrate diversity and abundance. Research carried out by McCabe, D. J. et. al. (2000) showed that macroinvertebrate abundance and species density were lower in streams that had been disturbed (due to urbanization). Goodnight (1973) argued that a particular change in stream condition (reduced levels of dissolved oxygen in water) which can be caused by urbanization, affected gill-breathing aquatic insects in the stream: In low-quality streams, there were fewer of these aquatic insects (such as mayflies, stoneflies, caddis flies) and more oxygen deprivation tolerant species (like leeches). In Gál, B. et. al. (2020), researchers focused on a specific component of urbanization, road crossings, and their effect on the richness and abundance of native macroinvertebrates, finding that the road crossings had a negative effect on these metrics. The paper suggested that roads and road crossings can modify and degrade the natural flow and biodiversity of streams by increasing the extent of impermeable surfaces which reduces water infiltration into soil and increases surface-run-off. The run-off can carry pollutants like heavy metals, nutrients, pesticides, and alien species into the streams which may kill sensitive species like Ephemeroptera and Plecopteran.

Despite all this research, there is still uncertainty surrounding the mechanisms of how urbanization alters stream ecosystem function (Bellucci, C., et. al. 2013). Some aspects of macroinvertebrate responses, like their response to metals in streams, have only just begun to be studied (Hirst, H., et. al. 2002). And even in studies that analyze the well-researched effects of urbanization on streams, sometimes the outcomes contradict what is expected. In the results of research conducted on St. Johns River in Florida by Chadwick, M. et. al. (2012), urban developments appeared to increase rather than decrease macroinvertebrate richness. In addition, an increase in more pollutant-tolerant taxa was not observed in the urban streams. These contrasting results propose the possibility that we do not know for certain how urbanization is affecting macroinvertebrate communities. Therefore, more research must be conducted comparing macroinvertebrate species richness, diversity, and abundance in forested and urban streams.

Here, we hypothesized that the urban streams would have less macroinvertebrate abundance and diversity and a lower distribution of functional groups when compared to the forested streams and therefore experience more leaf litter remaining in the leaf packs following the days of exposure in streams (as less macroinvertebrates would be present to perform litter decomposition). We based this hypothesis upon the "urban stream syndrome" conceptual framework and previous research work that has shown evidence of this result in their respective experiments. We expected to find statistically significant differences in macroinvertebrate communities between the streams of the two land uses, which would support the notion that land use practices affect stream health, as macroinvertebrates are indicators of water quality.

Our research provides evidence that contributes to the long-standing debate on whether all urban streams experience urban stream syndrome. Regardless of how unexpected they are, the results still highlight how land use affects its surrounding environment, specifically in Vermont, a location where few projects have conducted their research. Figuring out the health of different streams, and more importantly, the reason for varied health, can help support water management policies under the Federal Clean Water Act (Bellucci, C., et. al., 2013). Understanding why a stream is experiencing poor water quality could help us to implement local changes to improve stream health. Through this research, we were able to provide evidence for the effect (or lack thereof) of land use on stream water quality. Understanding these effects could influence the formation and implementation of state/county regulations on infrastructure development and urbanization near natural water sources.

Materials and Methods:
In this experiment, we examined how land use in stream catchment areas affected the communities of aquatic macroinvertebrates in those streams. To do so, we collected macroinvertebrates from leaf packs placed in streams that differed by land use and then quantified the macroinvertebrate communities through measures of diversity, abundance, biomass, and proportion of functional feeding groups. Data were also gathered on stream physiochemical characteristics to analyze measurable differences between the urban and forested streams.

Site selection:
To begin the experiment, we selected four streams that are tributaries to the Winooski River in Vermont (Figure 1). Streams selected were of first or second order characterized by their small sizes, shallow depths, permanence and stability, and maximum widths of 10 m. Two streams drained through an urban catchment, where at least 10% of the catchment area was covered in human settlement (or other urban infrastructure) and were referred to as urban streams (Potash Brook and Muddy Brook, Figure 2). The other two streams drained through a forested catchment where at least 85% of the catchment area was covered by natural vegetation and were referred to as forested streams (Brown Brook and Stevensville Brook, Figure 3). A catchment is an area of land where water precipitation is collected by a natural area and channeled into a stream or water source.

Figure 1. Relative location of the urban streams (red pin on left side of figure) and the forested streams (red pin on right side of figure).

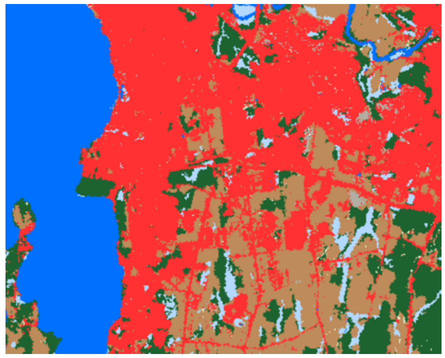

Figure 2. From USGS LCMAP (LCMAP Viewer, USGS, 2021). Land use in the catchment area of the urban streams. Red denotes developed land, light brown cropland, and green tree cover.

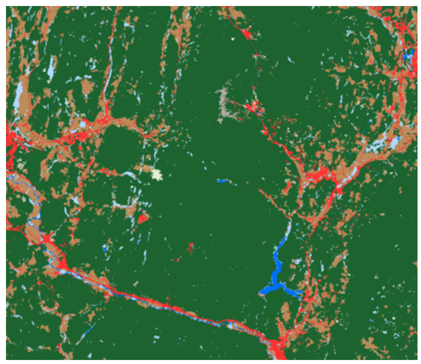

Figure 3. From USGS LCMAP (LCMAP Viewer, USGS, 2021). Land use in the catchment area of the forested streams. Red denotes developed land, light brown cropland, and green tree cover.

Experimental Design:

In this experiment, we employed leaf litter bags to collect the macroinvertebrates. The leaf bags were filled with the leaves of the Norway Maple tree (*Acer platanoides*, Sapindaceae), which was introduced to Vermont in 1762. Leaves were gathered either just before their senescence or shortly after they fell into the forest ground. Once collected, leaves were dried through air drying and fluffing for 5-8 days until they reached a constant dry mass. The leaf packs were constructed from two layers of coarse mesh. We measured out approximately 3 g of leaves and placed each portion onto the layers of mesh which were then folded up and zip-tied shut at the opening to secure the leaves inside. Each pack was labeled with a number 1-32 using a piece of tape.

A total of eight randomly chosen leaf packs were deployed into each stream. These leaf packs were placed in locations with minimal human impact to reduce the risk of disturbance. In each stream, the eight litter bags were split evenly between four pools which were selected to be at least 5 m apart from each other along a distance 10 times the width of the stream. To secure the leaf packs, a rebar pipe was placed into each pool, and nylon rope was used to anchor two randomly chosen bags to the rebar (Figure 4). At each site, we recorded the water temperature, pH, and conductivity of the stream as well as the depth of each pool. The bags were placed on May 27th, 2023, and collected on June 24^{th,} 2023 for a total of 28 days, similar to the exposure time of previous works by Hepp et. al. (2016) and Iniguez-Armijos et al. (2016). Collection of the bags consisted of using a 0.5

mm sieve to lift the bags out of the water and scissors to cut them loose from the nylon rope. Leaf bags were then placed into labeled Ziplock bags into which about 10 mL of 90% ethanol was poured to euthanize and preserve the macroinvertebrates.

Figure 4: Two leaf bags attached to a piece of rebar in a pool of one of the forested streams (Stevensville Brook).

Laboratory work:

In the laboratory, leaf bags were placed onto white trays, removed from the Ziplock bags, and cut open. The contents of each bag were washed into the tray with distilled water to remove as much inorganic matter as possible. Macroinvertebrates were then separated from the organic matter via tweezers and placed into ethanol-filled vials. Each leaf pack had a separate vial for macroinvertebrates found within.

Organic matter measuring:

Once macroinvertebrates were separated from the leaves, the leaves were allowed to completely dry until they reached a constant mass before being weighed again.

Macroinvertebrate identification:

Each vial of macroinvertebrates was poured onto a petri dish and examined under a microscope. Individual macroinvertebrates were identified to the family taxon using the taxonomic keys of Merritt et al. (2018) as well as the ID key of macroinvertebrates.org. Once identified, the macroinvertebrates were classified into functional feeding group categories (shredder, collector, predator, or scraper) using “An Introduction to the Aquatic Insects of North America.” Functional feeding group classification is an organization of macroinvertebrates based on the behavioral mechanisms of food acquisition. To estimate biomass, macroinvertebrate length was measured through a microscope using a piece of 1 mm grid paper placed under the petri dish (Figure 5).

Figure 5a: *Baetidae* Ephemeroptera, a member of the mayfly species under a microscope and atop grid paper.

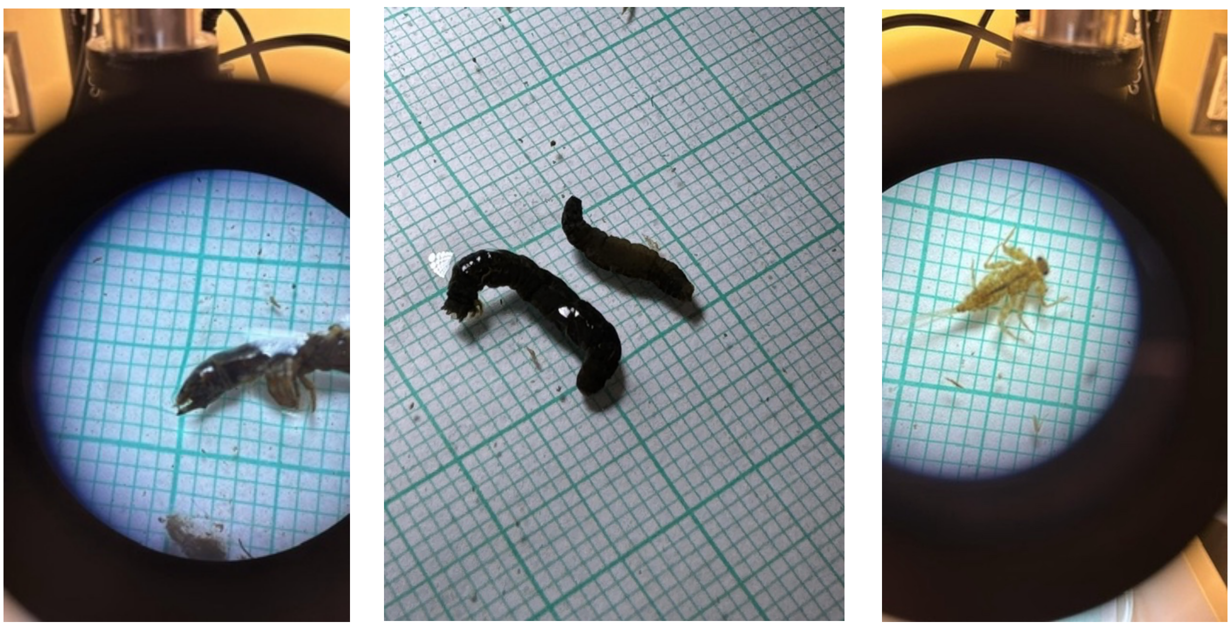

Figure 5b: Macroinvertebrates from the families Megaloptera, Tipulidae, and Leptohyphidae in order from left to right.

<u>Statistical analysis:</u>

To determine if land use (urban vs. forested) in stream catchment areas affected organic matter processing, we used a two-way ANOVA with land use type and stream as the main effects and organic matter remaining as the response variable. Differences among physiochemical variables (pH, temperature, conductivity, and pool depth) were examined using a non-parametric PERMANOVA. Average macroinvertebrate richness and abundances per gram of organic matter remaining were calculated for each stream and another two-way ANOVA was run. The composition of functional feeding groups of macroinvertebrates in each stream was calculated into relative abundances and analyzed for significant differences using a two-way ANOVA. Lastly, the biomass of each stream was calculated using the length measurements of each macroinvertebrate. Biomass was reported in units of mg AFDM/g and was calculated using the equation mg x 0.9, with mg = aL^b where L is the length of each macroinvertebrate in mm and "a" and "b" are constants dependent on the family of the macroinvertebrate. All statistical analyses were conducted using R program version 2023.12.1+402.

Results:

Physiochemical Variables of Streams:

Urban streams were significantly warmer and had higher conductivity values than forested streams (Fig. 1; pseudo F = 5678.17, p = 0.002; pseudo F = 3481.71, p = 0.006). Significant temperature differences (Fig. 1; pseudo F = 222.37, p = 0.001) and conductivity (Fig. 1; pseudo F = 356.15, p=0.001) also occurred between individual streams, with Muddy brook having significantly higher temperature values (mean = 23.7 °C) than Brown brook (mean = 14.4 °C), and Potash brook having significantly higher conductivity values (mean = 1675 μS/cm) than Stevensville brook (mean = 18 μS/cm). Stream temperature varied from 14.2°C in Brown brook to 23.8°C in Muddy brook. Conductivity values ranged from 17.1μS cm^{-1} in Stevensville brook to 1738μS cm^{-1} in Potash brook.

pH values did not differ between land uses (Fig. 1; pseudo F = 3.9265, p > 0.05), but did differ significantly between individual streams (Fig. 1; pseudo F = 9.7371, p = 0.008), with Muddy brook having a significantly higher pH value (mean = 8.2) than Stevensville brook (mean = 7.5).

We found no statistical differences in the average depth of the pools (where the leaf packs were placed) between streams or between land use types (p>0.05).

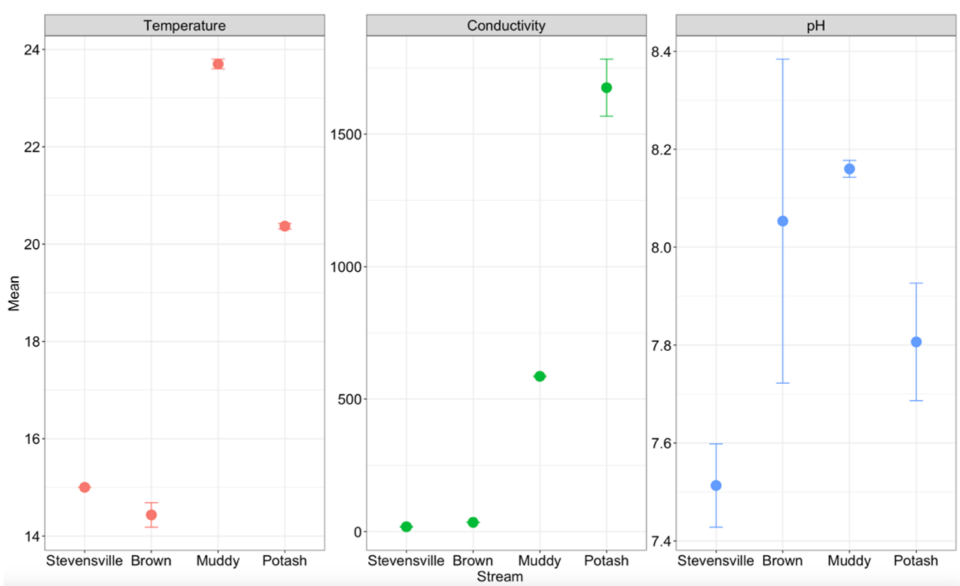

Figure 1: Values for stream temperature (°C), conductivity (μS cm^{-1}), and pH for each stream (n=4). Circles represent the mean of the data with vertical bars representing the standard deviations. Land use on temperature (p=0.002), land use on conductivity (p=0.006), and land use on pH (p=0.093).

Stream on temperature (p=0.001), stream on conductivity (p=0.001), stream on pH (p=0.008) from a PERMANOVA.

<u>Relationship Between Organic Matter Remaining and Land Uses:</u>
There was no significant difference in the grams of remaining litter in the leaf packs between land uses (Fig. 2; $F = 0.471$ $p = 0.471$). However, we found significant differences between the individual streams (Fig. 2; $F = 6.874$, $p = 0.00373$). A Tukey test showed that leaf packs from Potash brook had a significantly greater mass of organic matter reaming than those from Muddy brook (Fig. 2; $p = 0.0047$).

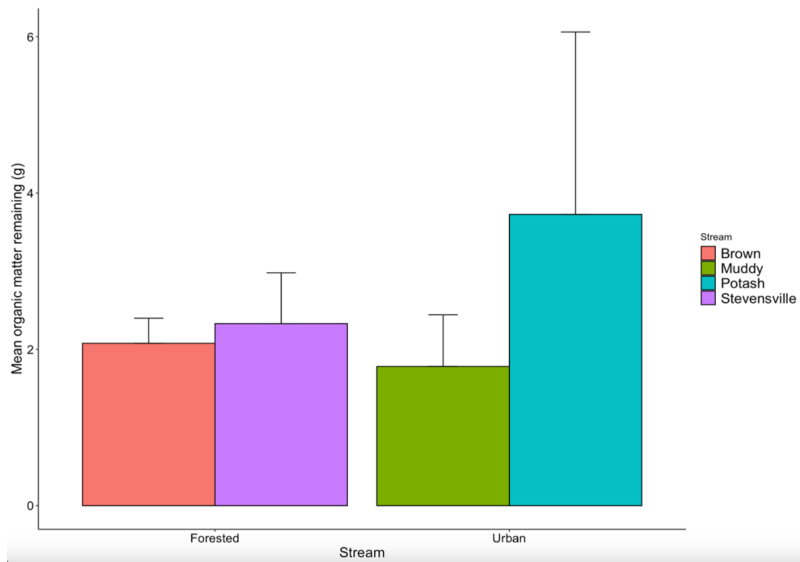

Figure 2. Mean and standard deviation of organic matter remaining in leaf packs from Vermont streams, 8 samples taken from each stream (n=32). Different colors denote different streams. Bars reach up to the height of the mean value for each stream with standard error bars atop. Land use on organic matter remaining (p=0.471), Stream on organic matter remaining (p=0.00373) from a two-way ANOVA.

<u>Macroinvertebrate Assemblages in Leaf Packs in Four Streams Along an Urban Gradient:</u>
The mean abundance of invertebrates did not differ between streams of different land use types (Fig. 3; $F = 0.093$, $p > 0.05$). There was a significant difference however, in the mean abundance of macroinvertebrates between two of the streams (Fig. 3; $F = 3.947$, $p = 0.0309$), with Muddy Brook having a significantly higher abundance of macroinvertebrates found in its leaf packs (21 individuals/g) than Potash brook (9 individuals/g) ($p = 0.0444$).

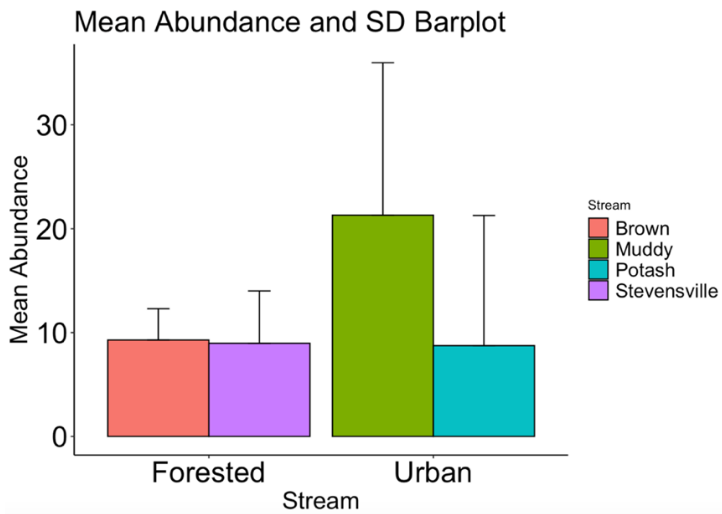

Figure 3: Mean abundance (number of individuals per gram of organic matter remaining) and standard deviation of macroinvertebrates from the streams leaf packs, 8 samples taken from each stream (n=32). Different colors denote different streams. Bars reach up to the height of the mean value for each stream with standard error bars atop. Land use on abundance (p=0.7625), Stream on abundance (p=0.0309) from a two-way ANOVA.

<u>Land Use and Stream Identity on Macroinvertebrate Richness:</u>
There was no significant difference in the richness of macroinvertebrates found between streams of different land uses (Fig. 4; F = 0.02, p > 0.05) or between any of the individual streams (Fig. 4; F = 2.034, p > 0.05).

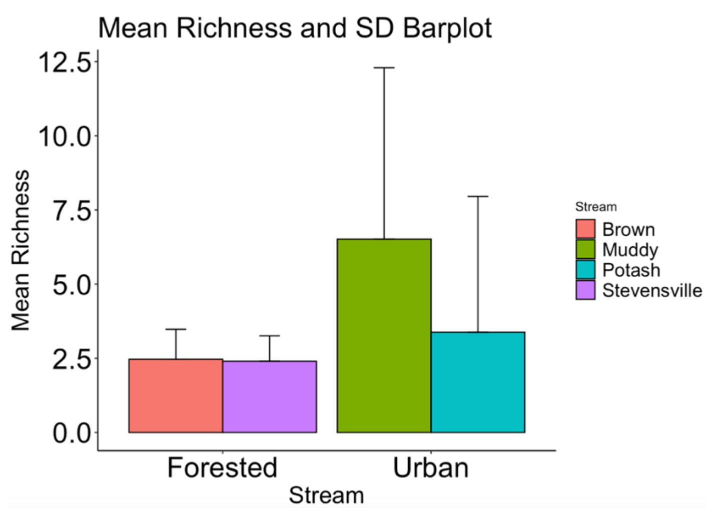

Figure 4: Mean richness (number of distinct macroinvertebrate families per gram of organic matter remaining) and standard deviation of macroinvertebrates from the streams leaf packs, 8 samples taken from each stream (n=32). Different colors denote different streams. Bars reach up to the height of the mean value for each stream with standard error bars atop. Land use on richness (p=0.89), Stream on richness (p=0.15) from a two-way ANOVA.

Land Use and Stream Identity on Macroinvertebrate Biomass:

Average macroinvertebrate biomass was similar between streams of different land uses (Fig. 5; F = 2.354, p > 0.05). However, there was a marginally significant difference in the biomass values between individual streams (Fig. 5; F = 3.112, p = 0.0602), with Muddy brook having a significantly higher mean biomass value (mean= 199 mgAFDM/g) than Stevensville brook (mean= 12 mgAFDM/g).

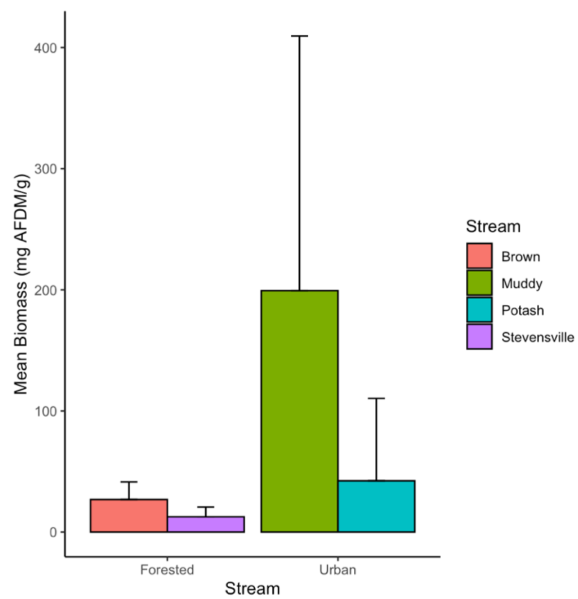

Figure 5: Mean biomass and standard deviation of macroinvertebrates (mg AFDM/g) per gram of organic matter remaining from the streams leaf packs, 8 samples taken from each stream (n=32). Different colors denote different streams. Bars reach up to the height of the mean value for each stream with standard error bars atop. Land use on biomass (p=0.1362), Stream on biomass (p=0.0602) from a two-way ANOVA.

The Proportion of Functional Feeding Groups in Each Stream:

Forested streams had a greater richness of shredder macroinvertebrates while urban streams had a greater richness of collector macroinvertebrates. Both streams had similar proportions of scraper macroinvertebrates, but Muddy Brook seemed to have less richness of predator macroinvertebrates than the other three streams (Figure 6).

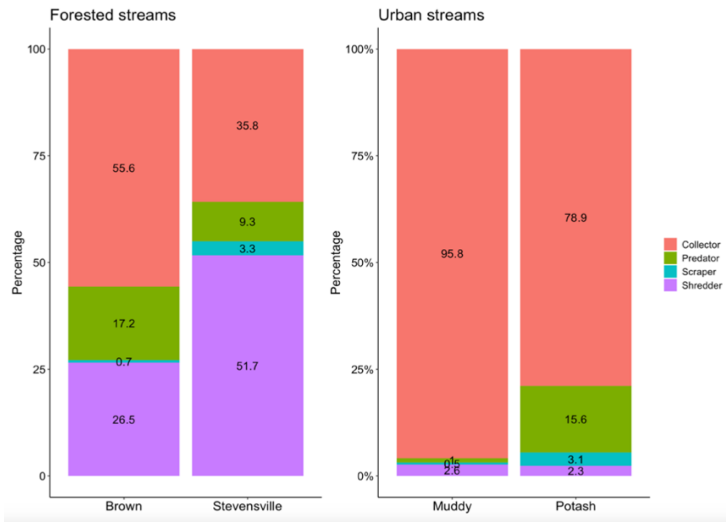

Figure 6: Richness of each macroinvertebrate functional feeding group in proportions for each stream, data collected from 32 samples (n=32). Different colors denote different functional feeding groups, and numbers on bars are values for the percentage of stream richness made up of that functional group.

Our results similarly show that forested streams had a greater abundance of shredder macroinvertebrates while urban streams had a greater abundance of collector macroinvertebrates. Both streams had similar values for the abundance of scraper macroinvertebrates, but Muddy Brook seemed to have a lower abundance of predator macroinvertebrates than the other three streams (Figure 7).

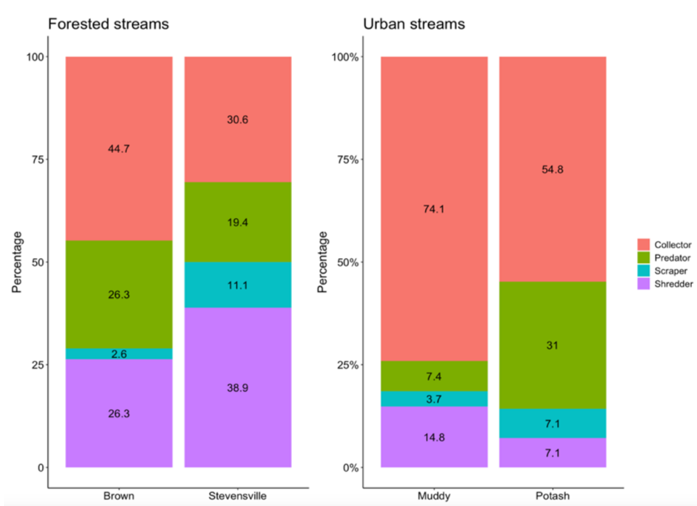

Figure 7: Abundance of each macroinvertebrate functional feeding group in proportions for each stream, data collected from 32 samples (n=32). Different colors denote different functional feeding groups, and the numbers on the bars are values for the percentage of stream abundance made up of that functional group.

<u>Relative Abundance of Functional Feeding groups in each stream:</u>

Contrary to the apparent results of the proportionality of macroinvertebrates, forested streams did not have a significantly greater relative abundance of shredders than urban streams (Fig. 8; $F = 0.01$, $p > 0.05$), but there was a significant difference in shredder abundance between individual streams (Fig. 8; $F = 5.187$, $p > 0.0184$), with Stevensville brook having a greater abundance of shredders (51 individuals per gram of organic matter remaining) than Muddy brook (15 individuals per gram)(Fig. 8; $p = 0.059$).

Similarly, there was no significant difference in collector relative abundance between land uses (Fig. 8; $F = 1.777$, $p > 0.05$). However, between streams, there were significant differences (Fig. 8; $F = 20.517$, $p < 0.002$): Stevensville brook had a significantly lower relative abundance (30 ind/g) of collectors than Potash (74 ind/g, $p=0.0002$), Muddy brook (71 ind/g, $p=0.000$), and Brown brook (57 ind/g, $p=0.0037$).

There was no significant difference in relative scraper abundance between land uses (Fig. 8; $F = 1.606$, $p > 0.05$) or between any of the streams (Fig. 8; $F = 2.270$, $p > 0.05$). The same was true for relative abundance of predators (Fig. 8; $F = 0.057$, $p > 0.05$; $F = 1.888$, $p > 0.05$).

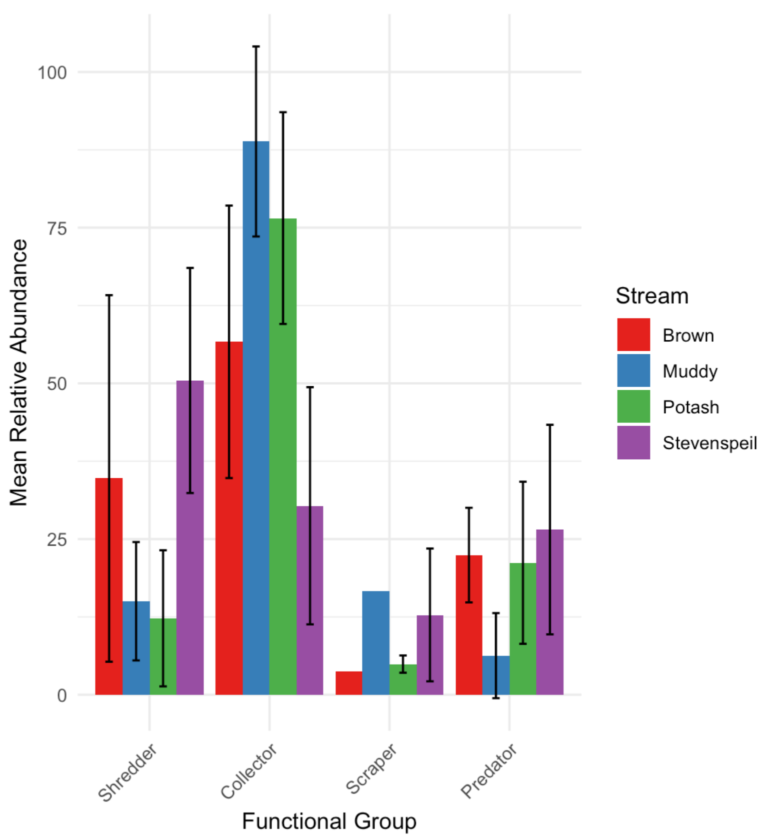

Figure 8: Relative abundance of each functional feeding group for each stream. Data collected from 32 samples (n=32). Different colors denote different streams. Bars reach up to the height of the mean value for each stream with standard error bars atop. Land use on shredder abundance ($p=0.9886$), stream on shredder abundance ($p=0.0184$), Land use on collector abundance

(p=0.194), stream on collector abundance (p=3.82e-06), Land use on scraper abundance (p=0.261), stream on scraper abundance (p=0.199), Land use on predator abundance (p=0.814), stream on predator abundance (p=0.184) from two-way ANOVA.

Discussion:
One of the main factors impacting stream conditions is human development which can lead to the deterioration of the natural ecosystem. Urbanization as a specific land use change has had a large impact on stream ecosystems by increasing nutrient and organic matter loads and altering physiochemical and hydrological conditions (Classen R. L et. al., 2019). The impact of urbanization has been shown to be even greater than the impact of agricultural practices (Chen et. al., 2023). Catchment area urbanization can also impact stream aquatic communities consequently altering the ecosystem function and processes such as leaf litter breakdown (Iñiguez-Armijos et. al., 2016). Using the “urban stream syndrome” framework (Walsh et al. 2005), we hypothesized that the urban streams would have lower levels of organic matter processing and macroinvertebrate abundance, diversity, biomass, and distribution of functional feeding groups when compared to the forested streams.

The hypothesized lower levels of organic matter processing were expected to lead to a marked difference between streams with different land uses, with natural streams showing less remaining organic matter. Contrary to this prediction, we found no difference in the organic matter remaining in the leaf packs between the streams of the land uses (Fig. 2). Previous studies have found that the decreased leaf litter breakdown rates along an urban gradient are usually due to changes in pH, water temperature, and shredder invertebrates (Iñiguez-Armijos et. al., 2016). Although we observed a significant difference in water temperatures between our urban and forested streams, there was no significant difference in pH levels or shredder invertebrate communities. The lack of variation specifically in macroinvertebrate assemblages (in the form of shredder communities) may explain why we found no difference in organic matter processing: all streams had similar abundances of shredders available to decompose the leaf litter. As such, the leaves were broken down at similar rates among the streams.

The shredders were not the only functional feeding group to experience a lack of change in assemblages based on land use. Our results found that although it initially seemed that the forested streams had greater richness and abundance of shredders and that urban streams had greater richness and abundance of collectors (Fig 6 and Fig 7), in reality, there were no statistically significant differences in the shredder or collector community compositions between streams of different land uses (Fig 8). The same was true of scraper and predator compositions. This was an unexpected result as the majority of studies (including work by Paul et. al., 2006 and Hepp et. al., 2016) have found a greater abundance of shredders in more forested streams since shredder macroinvertebrates feed on leaf litter and so high levels of organic matter accumulating in these streams (due to surrounding vegetation) correlates with high levels shredder densities (Graca, 2001). Other papers have found more generally, significantly higher levels of functional feeding group diversity in forested streams. In Classen, R. L., et. al. (2019), researchers found that in less urbanized areas, there was a higher functional diversity of macroinvertebrates, whereas in streams in more urbanized areas, where the community was dominated by more pollutant-tolerant species (midges, bloodworms, and leeches) there was less functional diversity.

Although we found no significant difference between streams of different land uses, we did observe a significant difference in the relative abundance of functional feeding groups between some of the individual streams that should not be ignored. One of the forested streams (Stevensville) had a greater abundance of shredders than one of the urban streams (Muddy). It is therefore important to note that if our sample size had been smaller and had only included Stevensville brook as our forested sample and Muddy brook as our urban sample, our results would have mimicked what was expected: with the forested stream having a greater abundance if shredders than the urban stream.

Outside of functional feeding groups, other metrics of macroinvertebrate communities were equally unaffected by the differences in land use. Our results found no difference in the mean macroinvertebrate abundance (Fig. 3) or richness (Fig. 4) between land uses. While similar studies have found the same result (Danger et. al., 2004), these findings contradicted our hypothesis, as we expected forested streams to exhibit a higher abundance of insects and greater species diversity, while streams in more urbanized areas were anticipated to have fewer insects and less diversity, mainly due to pollution and habitat loss (Classen R. L et. al., 2019). We hypothesized that water pollution from road runoff and urban development would lead to a reduction in at least taxa richness, although it might not affect abundance (since tolerant organisms could potentially increase in density) (Couceiro et. al., 2007). However, that was not the case as richness did not differ significantly.

Though our results are not in line with what we predicted using the urban stream syndrome framework, they do not completely contradict the framework, unlike some studies that have found that urban streams have higher levels of macroinvertebrate richness and abundance due to more acidic water in forested sites. These papers argue that pH is the environmental parameter most closely related to the variation in community composition observed among sites (Bücker et. al., 2010). As our study did not yield these results, the lack of difference in pH of the streams may be the cause of the complementary lack of difference in macroinvertebrate richness and abundance. Perhaps, had our streams differed significantly in pH we would’ve found that the streams with higher pH values (theoretically the urban streams) had greater macroinvertebrate richness and abundances. Still, other papers have found that it is stream temperature that explains the variability in composition, with the number of insect orders and families increasing linearly with maximum stream temperature (Jacobsen et. al., 1997). However, if this held for our study, we would’ve expected to see the urban streams (which were warmer) having higher levels of richness and abundance, which was not the case.

There was also no significant difference between land uses in the biomass of the macroinvertebrates found (Fig. 5). A paper by Sterling et. al. (2016) found that a decrease in macroinvertebrate biomass in urbanized watersheds was mostly influenced by conductivity and nutrient concentrations. Although we know that conductivity values were higher in the urban streams perhaps these differences were not great enough to affect the biomass of the macroinvertebrates.

The lack of significant differences in macroinvertebrate communities between the streams of different land uses is unexpected and could be the result of many different possibilities. One explanation for these results is that the level of urbanization around the “urban” streams was not great enough to impact the macroinvertebrates living within the streams. Perhaps a lack of prominent development in these urban areas manifests itself in the form of low levels of impervious surface cover in the urban catchment areas. Even though the urban stream catchment areas have more impervious surface cover than the forested streams, it is possible that the percentage of impervious surface in Vermont is not pronounced enough to affect the communities of aquatic organisms significantly. In a study completed in Maine by Morse et. al. (2003), researchers found that the taxonomic richness of stream insect communities showed an abrupt decline as the percent impervious surfaces of the catchment area increased above 6%. Although the urban cover of the urban streams’ catchment area is above 6%, the impervious surface cover may be less than that, and thus macroinvertebrates may be unaffected.

Another explanation for the lack of urbanization effect on the streams could be based on the “type” of urbanization occurring in these catchment areas. Perhaps the type of urban development around these Vermont urban streams was not “industrial” enough to affect the streams. For example, in urban areas where factories and other manufacturing activities are occurring, the streams may be more impacted by that activity compared to urban areas that are housing sites or similar areas with low levels of development and pollution. While the percent catchment area that was developed was similar in this study to previous research, it is a difference in the type of development that may explain the differing results.

Insignificant effects of urbanization on the streams were reflected in the physiochemical measurements. While our findings revealed a quantitative distinction between some of the physiochemical variables of urban and forested streams, these differences seemingly did not impact the macroinvertebrate assemblages, perhaps because they were too marginal. Our results show that temperature values were higher in the urban streams than the forested streams (Fig. 1). This was in line with our expectations as streams draining through urban areas tend to be warmer than forested streams due to urban air quality, temperature of impervious surfaces, and decreased canopy cover. Urban streams receive their runoff from water that flows through hot storm drains and over paved roads which can lead to dramatic increases in temperature (Somers et. al., 2013). Other papers have similarly found that the water temperature of streams increases as catchment areas become more developed due to run-off (Iñiguez-Armijos et. al., 2016). Runoff is caused by an increased extent of impermeable surfaces that reduces water infiltration into the soil. The run-off can carry pollutants like heavy metals, nutrients, pesticides, and alien species into the streams which may kill sensitive species. Even though we found a significant difference in temperature between the streams of different land uses, this difference would’ve been larger, and urban streams even hotter had the % impervious surface cover of the urban catchment areas been greater.

In terms of conductivity, our results also aligned with our expectations showing higher conductivity levels in the urban streams (Fig. 1). Previous studies have found that urban streams which are more at risk of pollutants have higher concentrations of electrical conductivity (Daniel et. al., 2002). Road salting practices could have also contributed to this difference as urban streams can be subjected to the salinization of their water due to the influx of road salt (Daley et. al., 2009). Previous studies have also found that an increase in impervious surfaces around streams leads to higher levels of stream conductivity and chloride (Morgan et. al., 2012). Similarly to temperature, even though we found a significant difference in this metric, the difference would've been greater had there been a higher % impervious surface cover. Had this been the case, the differences in conductivity, and also in temperature, may have been enough to impact the macroinvertebrate communities.

Finally, our results showed that there was no statistically significant difference in pH between the land uses (Fig. 1). Previous studies on streams in/near major cities have found low pH levels (more acidic) due to road salt applications which cause increased salinity and mobility of H^+ ions and trace metals such as Zn and Cd (Löfgren, S., 2001). However, since most of these studies were completed in very urbanized areas, it is possible that the urban streams used in this experiment did not have a developed enough catchment area to experience these effects. As such, the low levels of urbanization did not have a great enough effect to impact the pH of the urban streams or the macroinvertebrates living in them.

Conclusions:

This study demonstrates that the low levels of urbanization around the studied streams are not great enough to impact the macroinvertebrate communities within them. The lack of impervious surface cover or inadequate type of urbanization in the stream catchment areas means that any physiochemical differences that exist between the streams studied were not great enough to affect the macroinvertebrate community assemblages in the streams and thus the organic matter processes were not significantly different. This research provides evidence regarding the influence (or lack thereof) of local Vermont land uses on the streams in the area. Serving as a baseline for stream health, this study is among the first to evaluate macroinvertebrate assemblages in the state of Vermont. The findings presented may offer insights into potential outcomes by expanding the sample size to include more streams in each land use category or different land uses. Furthermore, incorporating more stream reaches could provide a better understanding of the impact of land uses at the watershed level. However, all of these considerations must be weighed against the associated time, effort, and findings.

Further research will be needed to assess the impacts of specific changes to stream variables and their effects on the invertebrate communities to gain a full understanding of the vulnerability of Vermont aquatic macroinvertebrates. Research could also evaluate other functional groups (diet, dispersal, reproduction, etc.) of macroinvertebrates (not just functional feeding groups) and see if a significant difference in assemblage exists in that sphere. Previous research by Irons III et. al. (1994) found that leaf litter decomposition rates decreased in forested streams due to the low temperatures of forested streams which inhibited the activity of microbial populations. The elevated temperatures and high nutrient loads in urban streams stimulated microbial activity which accelerated the leaf decomposition. Future research should explore the contribution of microbial assemblages to litter breakdown rates.

Figuring out the health of different streams, and more importantly, the reason for varied healthiness, can help support water management. Understanding why a stream may be experiencing unhealthy community assemblages or ineffective organic matter processing will help communities implement local changes. Or, in this case, obtaining knowledge on the baseline of a healthy stream's measurements will help to prevent future urbanization from affecting it. Prevention is easier than restoration so continuing to study healthy streams is important if we want to maintain ecosystem vitality.

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