## ARTICLE



# Patterns of diversity in stream macroinvertebrate communities in a low-gradient agricultural stream (Sydenham River, Ontario, Canada) were driven by surficial geology and catchment position

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# Abstract

Freshwater biodiversity is increasingly at risk wherever land uses such as agriculture exert multiple stressors that degrade habitat quality. Although stream macroinvertebrates act as bioindicators for monitoring these impacts, their responses are context-specific: examining drivers of community composition is therefore important to understand the results of monitoring efforts. In a primarily agricultural landscape, 15 sites across the Sydenham River watershed, Ontario, Canada, were assessed for in-stream habitat quality and stream macroinvertebrate diversity. We predicted that community assemblage would be driven by differences in surficial geology across branch (east branch *versus* north branch) and catchment position (main stem *versus* tributary). We found that the main stem of the east branch was characterised by significantly higher proportions (P = 0.053) and abundance (P = 0.038) of Ephemeroptera–Plecoptera–Trichoptera (EPT) taxa than north branch sites were, and sites in the east and north branch tributaries were characterised by significantly lower Hilsenhoff Biotic Index (HBI) scores (P = 0.088). Redundancy analysis found that substrate size was the main driver of community composition, generating a model that described these patterns across branch and catchment position. Our findings suggest that EPT abundance and HBI scores were the variables that were most effective at revealing differences in stream communities due to agricultural impacts and that sediment size is an important driver of these patterns.

# Policy highlights

Tributaries of the Sydenham River watershed, Ontario, Canada, present an opportunity for restoration, particularly in the river's north branch.

In this study, geological differences (sediment size) contributed to differences in stream macroinvertebrate communities, where percentages of gravel, bedrock, and boulders best predicted community composition.

Sites characterised by finer sediment were least biodiverse and dominated by tolerant taxa such as Oligochaeta, Corixidae, and Caenidae. Diversity of sediment grain size (*i.e.*, sand, silt, and clay) was linked to diversity of stream macroinvertebrate communities, most notably Ephemeroptera, Plecoptera, and Trichoptera taxa.

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The Hilsenhoff Biotic Index (HBI) scores and Ephemeroptera–Plecoptera–Trichoptera (EPT) abundance captured differences across branch and catchment position most, likely indicating higher levels of impact in smaller streams. Family richness, EPT richness and Shannon–Wiener diversity index scores did not vary significantly. The results suggest that biomonitoring efforts can benefit not only from examining biodiversity metrics but also from looking at whole communities and individual taxa to identify and evaluate specific parameters.

#### Introduction

Rivers and streams are complex ecosystems that support diverse communities, including the human communities that depend on them for food, water, and other resources. Despite that, nearly half of riverine ecosystems worldwide are impacted by human activities (Su *et al.* 2021), and up to 80% of the world's population face threats to water security (Davies *et al.* 2010). Areas associated with dense settlement and agriculture show significant declines in the biodiversity of species (Davies *et al.* 2010).

Extensive agriculture exerts multiple stressors to in-stream biodiversity, including riparian deforestation (Matthaei *et al.* 2010), nutrient pollution (Elbrecht *et al.* 2016), and fine sediment accumulation (Buendia *et al.* 2013). More specifically, these stressors can change water clarity and temperature, resulting in warmer, hypoxic conditions. This degradation of in-stream habitat selects for certain functional traits and life histories, eventually resulting in predictable changes in community composition (Richards *et al.* 1997). Heavily impacted streams are dominated by taxa that are able to tolerate soft substrates and low oxygen conditions (Burdon *et al.* 2013), while population of sensitive taxa decline as riparian cover is lost and stream temperatures increase (Death and Collier 2010; White *et al.* 2017). As such, impacted streams can be expected to show a lower overall diversity of macroinvertebrates than is found in intact streams.

Benthic biomonitoring relies on these diversity patterns to detect and assess impacts on freshwater ecosystems. Stream macroinvertebrates such as arthropods, crustaceans, molluscs, and annelids are sampled, and overall community composition and diversity can be used to infer ecosystem function (Friberg *et al.* 2011; Buss *et al.* 2015). To determine the state of an ecosystem or assess changes over space and time, biomonitoring uses community-level metrics, including biotic indices (*e.g.*, the HBI; Hilsenhoff 1988) or the proportion of sensitive taxa, to describe the degree to which a given stream has been impacted by environmental stressors. Originally developed to detect the presence of organic pollution (Johnson *et al.* 1993), stream macroinvertebrate indices have also been used to detect impacts of changes in riparian forest cover (Rios and Bailey 2006), fine sediment (Matthaei *et al.* 2010; Buendia *et al.* 2013), and flow (Elbrecht *et al.* 2016).

Stream macroinvertebrate communities respond to factors at both broad and fine spatial scales (Doretto *et al.* 2020). Broad-scale factors such as climate, geomorphology, and dispersal history shape the community composition of stream macroinvertebrates (Gayraud *et al.* 2003). At finer scales (*i.e.*, reach-scale), substrate size, flow (Lamouroux *et al.* 2004), and riparian forest cover (Rios and Bailey 2006; Feld and Hering 2007) have all been found to be important predictors of invertebrate community composition. Buendia *et al.* (2013) describe how fine sediment impacts stream macroinvertebrates through habitat modification, including changes in turbidity, dissolved oxygen (DO), and acidity. Weigel *et al* (2003) describe similar impacts to stream macroinvertebrate composition serves as a habitat characteristic related to relative abundance of invertebrates.

Foundational work such as the river continuum concept (Vannote *et al.* 1980) looks across entire stream systems to emphasise catchment position as influencing communities as they vary along the course of a river. Vannote *et al.* (1980) describe shifts in particulate matter, from coarse, allochthonous matter (such as fallen leaves) to fine particulate matter and periphyton. These shifts in food resources shape stream macroinvertebrate community trends along the course of a river

system. Similarly, Richards *et al.* (1997) describe communities varying according to a range of sediment types, from coarse sediment deposits in headwaters to finer substrate of tributaries and highlight how changes in sediment across a catchment can shape stream macroinvertebrate assemblages. As such, the catchment position at which stream macroinvertebrate communities are monitored could play a role in our understanding of their responses to anthropogenic impacts on streams.

Because stream macroinvertebrate communities respond in a regionally and context-specific way (Norton *et al.* 2000; Bo *et al.* 2020), untangling the influences of large-scale and local processes is important to understanding how stream macroinvertebrate community metrics represent watershed conditions. This is especially important in a biomonitoring context to prevent an assumption that efforts at the reach scale represent the conditions within sub-catchments or at larger scales.

This research is based in the Sydenham River watershed, in southwestern Ontario, Canada, where related work found stream macroinvertebrates could be predictors of the presence of endangered and threatened freshwater mussels (Unionidae; Eveleens *et al.* 2023). The watershed varies in geology and habitat diversity, both of which are thought to lead to differences in biological communities (Osmond 1969; Dextrase 2000; Staton *et al.* 2003), a trend Eveleens *et al.* (2023) also observed in mussel communities. The objective of the present study was to examine the role of catchment position and habitat characteristics in shaping stream macroinvertebrate communities in the Sydenham River watershed. We predicted that the main drivers of stream macroinvertebrate community composition would be due to the local geologic differences between branches (north branch *versus* east branch) and then catchment position (main stem *versus* tributary). We also hypothesised that specific indicators of habitat quality – namely turbidity and inorganic nutrients – would have greater influence than other measures on stream macroinvertebrate diversity.

# Methods

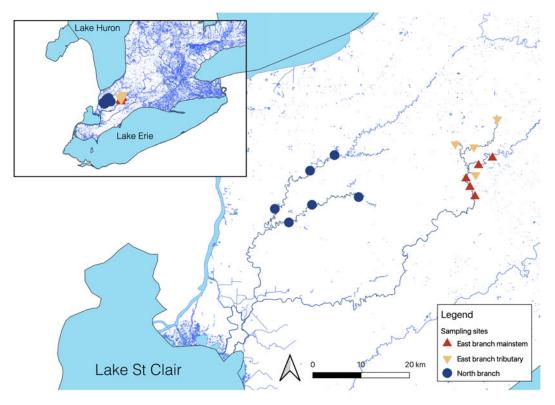
#### Area of study

This study took place in the Sydenham River watershed, a river in southwestern Ontario, near the settlements of Chatham–Kent, Lambton, and Middlesex, within the Traditional Territory of the Anishinaabeg (the Odawa, Ojibwe, and Potawatomi) and the Mississauga and Attawateron (Neutral) nations (see Fig. 1 for a map of the region with sampling sites).

The watershed is located within the Mixedwood Plains ecozone. It was historically covered by forest and swamp but is now occupied by privately owned farms (Staton *et al.* 2003). Despite that, the watershed ecosystem supports a diversity of stream macroinvertebrates, notably freshwater mussels, as well as fish species at risk of extinction (Harris *et al.* 2003; Staton *et al.* 2003).

The river has two main branches, the east branch and the north branch, which meet downstream near the town of Wallaceburg and towards Bkejwanong Territory, towards where the river flows into Lake St. Clair. At 100 km long, the east branch is the longer arm and supports a wider diversity of habitats and species (Osmond 1969; Staton *et al.* 2003). Although both branches flow through glacial till deposits, the east branch is noted for its diverse sediments, including shale and parent materials (Dextrase 2000). The north branch is 70 km long and is less biodiverse as a result of fine sediment accumulation and lower substrate diversity (Osmond 1969; Staton *et al.* 2003). The differences in substrate diversity are thought to shape the river's biological communities, with the east branch, thanks to its more diverse substrate, being more diverse in fish, mussels, and other macroinvertebrates (Osmond 1969; Dextrase 2000; Staton *et al.* 2003). Related research found that mussel species richness was higher in the east branch than in the north branch (Eveleens *et al.* 2023).

A total of 15 sites were randomly selected, using the methodology described in Eveleens *et al.* (2023). We sampled across the Sydenham River's upper east and north branches (Fig. 1).



**Figure 1.** A map of the Lake St. Clair Region watershed, displaying the two main branches of the Sydenham River, Ontario. Site samples are plotted as nodes with colours corresponding to their branch and stream size.

Reaches (sites) ranged from 60 m to 500 m in length and were of third–sixth Strahler stream order. The east branch sampling sites span a range of sizes, with the parts of the channel in contact with water, or average wetted widths, ranging from approximately 3 m to approximately 27 m, and were divided into two categories, main stem (n = 5) and tributaries (n = 4) based on width and Strahler order. The north branch sampling sites (n = 6) mostly comprise narrow tributaries (4-6 m wide on average), with one site being wider (mean wetted width = 15 m). Samples in the north branch were collected in Bear Creek (n = 3) and Black Creek (n = 3). Because sampling took place in autumn 2020, during the COVID-19 pandemic, access to additional sites was limited.

# Site survey procedure

To evaluate habitat conditions, sampling sites were divided into 10 intervals of equal length and assessed using a modified version of Stanfield's (2010) Ontario Stream Assessment Protocol. At each interval, stream morphology variables, including wetted width, bank full width, and maximum depth, were measured. Ten random sediment samples were classified by grain size within each transect, ranging from clay to bedrock according to Wolman's (1954) standard sediment size classification. To ensure randomness, sediment particles were sampled in arbitrarily timed intervals of wandering throughout the transect, with no conscious attention paid to the stream bed (Wolman 1954).

At the most upstream transect of each site, water quality samples and measurements were taken. A YSi ProDSS handheld probe (YSi, Yellow Springs, Ohio, United States of America) was used to measure turbidity, DO, conductivity, pH, and water temperature. Approximately 100 mL

of water was sampled and filtered by syringe onsite using 0.45  $\mu$ m for nitrates (NO<sub>3</sub>–N), nitrites (NO<sub>2</sub>), ammonia (NH<sub>3</sub>), and total dissolved phosphorus. A second sample (~100 mL) was filtered using a 0.22- $\mu$ m filter for nonpurgeable organic carbon. All samples were put on ice in the field and frozen until ready to process.

Water chemistry analyses were carried out by the Organic Analytical and Nutrient Laboratory, a Canadian Association for Laboratory Accreditation-certified laboratory at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, Ontario). Inorganic nutrient analyses were run using a SMARTCHEM<sup>®</sup> 170 Discrete Analyzer (Smartchem Technologies, Pune, India).

## Stream macroinvertebrate sampling and processing

Two riffles and one pool were sampled for stream macroinvertebrates, for a total of three samples per site. Stream macroinvertebrate collection followed the Ontario Benthic Biomonitoring Network stream sampling protocol (Stanfield 2010). Sampling was done using a D-net for three minutes along the width of the sampling site. Samples were stored in room-temperature formal ethanol (15 parts 95% ethanol, three parts 37% formaldehyde, and 7 g Borax per 1-L volume; Krogmann and Holstein 2010; Dumke *et al.* 2013). Samples were washed through a series of sieves (4 mm, 1 mm, and 500  $\mu$ m), and stream macroinvertebrates were separated from detritus under a 10× magnification Leica dissection microscope (Leica Microsystems, Wetzlar, Germany).

Stream macroinvertebrates were identified to at least the family level using Merritt *et al.* (2008) and counted, with the exceptions of Oligochaeta, Nematoda, Collembola, and Hydrachnidia, which were left at their respective taxonomic level. Only segments possessing heads were counted, and terrestrial and planktonic taxa were recorded as present, common, or abundant. Each sample (two riffles and one pool) per site was processed separately.

Total family richness, EPT richness, abundance and percent, and Shannon–Wiener diversity and HBI scores were calculated for each site, combining the invertebrate data from all three samples. Hilsenhoff Biotic Index values were assigned using Hilsenhoff's (1988) assigned tolerance values and HBI values from the New York State Department of Environmental Conservation (Smith and Duffy 2019). Understudied taxa with no assigned tolerance values, such as Pomatiopsidae, Mesoveliidae, Veliidae, and Sciomyzidae, were excluded from HBI calculations: these families were minimally represented in two or fewer samples (0.0017, 0.00072, 0.001, and 0.0019% of abundance in the samples they appeared, respectively).

#### Statistical analyses

Before analysis, habitat data were averaged by site, and variables were standardised (mean = 0, variance = 1). Stream macroinvertebrate samples (riffle 1, riffle 2, and pool) were combined to represent each site (n = 15). To explore differences and relationships between water quality, sediment grain size, and stream macroinvertebrate communities between the north branch and the east branch, we applied a suite of multivariate statistical approaches. Principal component analyses were used to characterise water quality and sediment data across sites and to explain variance with few components. To test the significance of the effects of branch and catchment position on environmental and sediment data, permutational multivariate analyses of the variance of Bray–Curtis distances were performed. Standard student *t*-tests (using a 95% confidence interval) were used to test for significant differences among individual water quality and sediment variables, as well as among diversity metrics across branch (north branch *versus* east branch) and catchment position (main stem *versus* tributaries of the east branch). To compare diversity metrics across three categories (north branch, east branch main stem, and east branch tributaries), analyses of variance were used.

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To identify predictors of community assembly, redundancy analysis was employed. Redundancy analyses are best suited for identifying multiple variables across a short environmental gradient (Legendre and Gallagher 2001). To reduce the effects of overabundant and rare taxa and to optimise for redundancy analysis, Hellinger transformation was applied to abundance data (Legendre and Gallagher 2001). Co-linear variables were screened by a variance inflation factor, and variables that had a variance inflation factor above 5 were removed. A modified stepwise forward selection procedure (Blanchet 2008) was used to identify variables that best explained variance while minimising the saturation of variables in the model. A Monte Carlo permutational test (with 999 permutations) was used to test the significance of the model generated.

All analyses and data manipulation were conducted using R Studio in R (R Studio Team 2022; R Core Team 2021) with base-R and the vegan package (Oksanen *et al.* 2020).

# Results

# Water quality and sediment parameters across branch and catchment position

We found that, of the variables measured, only nonpurgeable organic carbon (P = 0.015), percentage of silt (P = 0.047), and percentage of rubble (P = 0.003) varied significantly (P < 0.05) between branches (east *versus* north); means, variance, and *t*-test results for these parameters are reported in Table 1. Principal component analysis identified two dimensions (PC1 and PC2) that accounted for 53.2% of variance across sites (Fig. 2). Principal component analysis found that the east branch sites were characterised by coarser sediments (mean: 20.9% *versus* 1.3%) and wider reaches (mean: 13.9 m *versus* 6.7 m) than north branch sites were, whereas north branch sites were characterised by finer sediments (38.5% *versus* 12.9% silt) and more turbid waters (72 *versus* 32.8 Nepholometric turbidity units (NTU)) than east branch sites were.

Between the main stem and tributary sites of the east branch, water quality parameters that varied significantly (P < 0.05) included percentage of DO (%; P = 0.023), pH (P = 0.042), nonpurgeable organic carbon (P = 0.043), total bound nitrogen (TN; P = 0.0094), and NOx (nitrates and nitrites; P = 0.0014; Table 2). Principal component analysis identified two dimensions (PC1 and PC2) that accounted for 66.6% of variation (Fig. 3). Principal component analysis revealed that tributary sites were characterised by more turbid waters (64.3 versus 7.76 NTU) and finer sediments (22.2% versus 5.6% clay) than the main stem sites were. Main stem sites had coarser sediments and were characterised by higher loads of DO and nitrogenous compounds than tributary sites were (Table 2).

#### Variation in stream macroinvertebrate communities

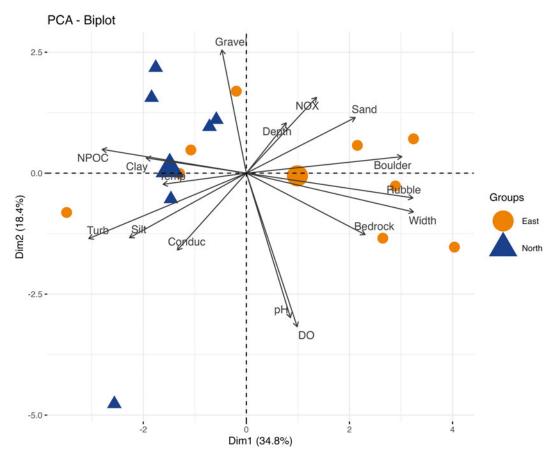
Each sample (n = 45) across all sites (n = 15) contained more than 700 individual stream macroinvertebrates, with no significant differences across branches or other groupings. The number of families identified ranged from 18 (SRE-67 and SRN-100) to 41 (SRE-08). Four taxa were found at every site: Caenidae (Ephemeroptera), Chironomidae (Diptera), Elmidae (Coleoptera), and Hydrachnidia (Trombidiformes). Common taxa (observed at 13 of 15 sites) included Nematoda, molluscs such as Planorbidae spp. and Sphaeriidae spp., and insects in the families Ceratopogonidae (Diptera), Corixidae (Hemiptera), and Coenagrionidae (Odonata). A dozen families were found at only one site, and most of these were found in the east branch's main stem (Gomphidae, Hydrobiidae, Lepidostomatidae, Limoniidae, Pomatiopsidae, Potamanthidae, Psychomyiidae, and Sciomyzidae). Three taxa (Dryopidae, Stratiomyidae, and Phryganeidae) were unique to a single site in an east branch tributary. One taxon (Amnicolidae) was recorded at only a single site in the north branch.

Table 1. Comparison of water quality and sediment size variables that varied significantly across branches (east *versus* north branch of the Sydenham River, Ontario).

| Variable    | Mean (north branch, $n = 6$ ) | Mean (east branch, n = 9) | Degrees of<br>freedom | t-Value | <i>P</i> -value<br>(* <i>P</i> < 0.05) |
|-------------|-------------------------------|---------------------------|-----------------------|---------|--|
| NPOC (mg/L) | 12.30 ± 1.32                  | 6.43 ± 0.90               | 9.39                  | -2.96   | 0.015*                                 |
| Silt (%)    | 38.5 ± 9.75                   | 12.89 ± 4.35              | 7.02                  | -2.40   | 0.047*                                 |
| Rubble (%)  | 1.33 ± 0.66                   | 20.89 ± 4.68              | 8.32                  | 4.13    | 0.003*                                 |

NPOC, nonpurgeable organic carbon.

Asterisks indicate significance.



**Figure 2.** Principal component analysis for sites as they relate to water quality and sediment grain size across east and north branches of the Sydenham River, Ontario. Principal components 1 and 2 (PC1 and PC2) explained 34.8% and 18.4% of variance, respectively.

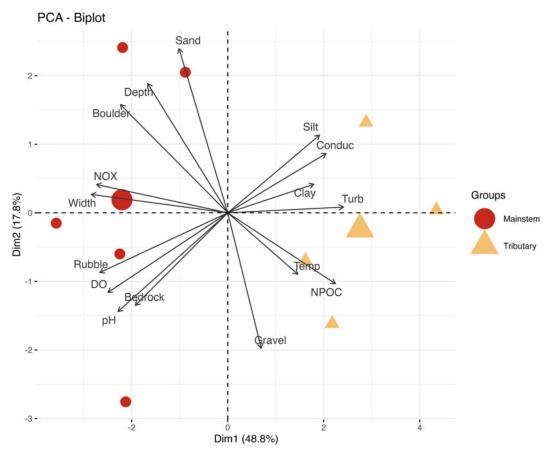
Although samples from the east branch (especially main stem sites) included more taxa than those of the north branch, none of the diversity metrics measured varied significantly across and between branches (east *versus* north). Only comparisons of metrics across three categories (north branch, east branch main stem, and east branch tributaries) differed significantly. Only EPT richness (P = 0.038) varied significantly across branches, with a 95% confidence interval

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| -                        |                            | •                          |      |         |                                     |
|--------------------------|----------------------------|----------------------------|------|---------|-------------------------------------|
| Variable                 | Mean (main stem, $n = 5$ ) | Mean (tributary, $n = 4$ ) | df   | t-value | <i>P</i> -value (* <i>P</i> < 0.05) |
| Wetted width (m)         | 21.84 ± 1.39               | 4.01 ± 0.66                |      | 11.62   | 0.000036*                           |
| DO% (percent saturation) | 125.96 ± 12.53             | 82.53 ± 4.05               | 4.80 | 3.30    | 0.023*                              |
| рН                       | 8.15 ± 0.09                | 7.76 ± 0.12                | 5.61 | 2.62    | 0.042*                              |
| NPOC (mg/L)              | 5.70 ± 0.09                | 9.89 ± 1.25                | 3.03 | -3.35   | 0.043*                              |
| TN (mg/L)                | 3.13 ± 0.14                | 1.27 ± 0.37                | 3.92 | 4.76    | 0.0094*                             |
| NO <sub>X</sub> (mg/L)   | 3.00 ± 0.16                | 0.62 ± 0.31                | 4.64 | 6.86    | 0.0014*                             |
| Rubble (%)               | 29.8 ± 4.92                | 9.75 ± 4.09                | 6.99 | 3.13    | 0.017*                              |

**Table 2.** Summary table of water quality and sediment variables that vary significantly across stream sizes (main stem *versus* tributary reaches in the east branch of Sydenham River only).

D0%, dissolved oxygen percentage;  $NO_x$ , nitrates and nitrites; NPOC, nonpurgeable organic carbon; TN, total bound nitrogen. Asterisks indicate significance.



**Figure 3.** Principal component analysis relating water quality and sediment grain size across main stem and tributary sites of the east branch of Sydenham River, Ontario. Principal components 1 and 2 (PC1 and PC2) explained 48.8% and 17.8% of variance, respectively. Large shapes represent the centroids of their respective groups.

|  |                      |              |                            |                     | East                       |                            |    |                    |                                       |
|--|----------------------|--------------|----------------------------|---------------------|----------------------------|----------------------------|----|--------------------|---------------------------------------|
| Metric   | Minimum              | Maximum      | Mean                       | East main<br>(mean) | tributaries<br>(mean)      | North<br>(mean)            | df | <i>F-</i><br>value | <i>P</i> -value<br>(* <i>P</i> < 0.05 |
| Family<br>richness                             | 18                   | 41           | 30 ± 2.04                  | 36 ± 1.87           | 27 ± 5.12                  | 27 ± 2.6                   | 2  | 2.80               | 0.101                                 |
| EPT richness                                   | 2                    | 18           | 8.6 ± 1.25                 | 12.8 ± 1.71         | 7.0 ± 2.38                 | 6.0 ± 1.46                 | 2  | 4.37               | 0.038*                                |
| EPT%   | 9.1                  | 45           | 26.58 ± 2.7                | 35.34 ± 4.0         | 23.34 ± 4.87               | 21.06 ± 3.45               | 2  | 3.79               | 0.053                                 |
| Shannon–<br>Wiener<br>diversity<br>index score | 0.93                 | 2.40         | 4.66 ± 0.11                | 2.23 ± 0.82         | 1.85 ± 0.09                | 1.74 ± 0.25                | 22 | 2.08               | 0.168                                 |
| HBI score                                      | 7.56<br>very<br>poor | 4.92<br>good | 5.85 ± 0.20<br>fairly poor | 5.26 ± 0.21<br>fair | 6.04 ± 0.25<br>fairly poor | 6.25 ± 0.36<br>fairly poor | 2  | 2.99               | 0.088                                 |

**Table 3.** Summary table of invertebrate diversity metrics (mean, max, and min) across branches and stream size categories, as well as results from analyses of variance comparing east branch main stem, east branch tributaries, and north branch sites.

HBI, Hilsenhoff Biotic Index score; EPT, Ephemeroptera-Plecoptera-Trichoptera taxa. Asterisk indicates significance.

**Table 4.** Predictor variables loaded in redundancy analysis as selected by stepwise forward selection, values corresponding to resulting redundancy analysis or Monte Carlo permutational test (with 999 permutations).

| Variable     | Adjusted $R_2$ | F-value | <i>P</i> -value (* <i>P</i> < 0.05) | Biplot score: RDA1 | Biplot score: RDA2 |
|--------------|----------------|---------|-------------------------------------|--------------------|--------------------|
| Gravel (%)   | 0.10743        | 2.6851  | 0.030*                              | -0.65              | 0.20               |
| Bedrock (%)  | 0.20343        | 2.5666  | 0.049*                              | -0.36              | -0.52              |
| Conductivity | 0.29313        | 2.5228  | 0.046*                              | -0.05              | 0.56               |
| Boulder (%)  | 0.36437        | 2.2330  | 0.041*                              | -0.11              | -0.87              |

RDA, redundancy analysis.

Asterisks indicate significance.

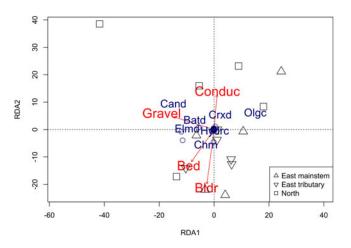
(P < 0.05). The proportion of EPT individuals (P = 0.053) and HBI scores (P = 0.088) varied significantly across the three categories when using a 90% confidence interval (P < 0.1). Table 3 summarises the diversity metrics in greater detail.

## Environmental drivers of stream macroinvertebrate community composition

The global test (with all 11 variables loaded) was statistically significant (F = 2.15, P = 0.033), as compared to a null model, and explained 88.75% of the observed variance. The stepwise forward selection procedure retained four variables (*gravel, bedrock, boulders*, and *conductivity*; Table 4) that explained 54.6% of variance in transformed abundance data across all sites, with the first two constrained axes corresponding to 45.83% of variation. The variables selected were used for our redundancy analysis and plotted (Fig. 4).

The first redundancy analysis axis explained 36.39% of variance and described a gradient where *gravel* and *bedrock* were associated with taxa such as Elmidae, Baetidae, and Sphaeriidae, as well as a few sites in the east branch. In the opposite direction, taxa such as Corixidae and Oligochaeta stood out and were associated with north branch sites.

Figure 4. Redundancy analysis biplot of invertebrate families and sampling sites across variables identified by stepwise forward selection (see Table 4 for variable details). Redundancy analyses 1 and 2 (RDA1 and RDA2) explained 36.39% and 9.44% of variance, respectively. Circles represent invertebrate taxa; other shapes represent sampling sites as identified by the legend. Batd, Baetidae; Cand, Caenidae; Chrn, Chironomidae; Crxd, Corixidae; Elmd, Elmidae; Hydrc, Hydrachnidia; Olgc, Oligochaeta.



The second redundancy analysis axis explained only 9.44% of variance. On one end, *conductivity* and *gravel* were associated with east branch tributaries and taxa such as Caenidae, Corixidae, and Oligochaeta. On the other end, *boulders* and *bedrock* were associated with east branch main stem sites and taxa such as Hydropsychidae and Leptohyphidae.

## Discussion

#### Catchment position and sediment shape stream macroinvertebrate communities

We found that, although there were differences in stream macroinvertebrate communities across the east and north branches of the Sydenham River, these differences were best explained by a combination of catchment position and sediment type. In particular, it appeared that the main stem of the east branch was characterised by greater wetted width and higher loads of nitrogen and DO. The main stem displayed the most diversity in sediment, with sites dominated by bedrock, boulders, and rubble. Sites in the main stem of the east branch displayed the most diversity in stream macroinvertebrate taxa and included the most unique taxa (*i.e.*, taxa found nowhere else in our survey of the watershed). Tributaries of the east branch and north branch appeared more turbid and conductive and were characterised by higher proportions of finer sediments such as clay and silt. Samples from these sites included fewer taxa, and samples had lower proportions of sensitive stream macroinvertebrates (as denoted by EPT abundance).

A closer look at the redundancy analysis axes generated explains how these differences might drive stream macroinvertebrate community assembly through habitat and food resources. Although identifying functional feeding groups for all taxa in this study would not be possible given the taxonomic scope, consideration of the functional feeding groups of taxa identified by the redundancy analysis could explain relationships between substrate and food availability. Functional feeding groups were determined using the Iowa Department of Natural Resources (2024) benthic macroinvertebrate master taxa list.

The east branch, particularly the main stem sites, was associated with larger sediments, such as bedrock and boulders. The redundancy analysis identified taxa such as Elmidae, Baetidae, Hydropsychidae, Sphaeriidae, and Leptohyphidae being associated with these habitats. The presence of collector–filterer taxa (Hydropsychidae, Sphaeriidae) may be related to substrate size because they are predominantly found in areas of high flow, which can be linked to coarse substrate (Szałkiewicz *et al.* 2022). Scrapers like Baetidae and Elmidae would be also expected in the higher reaches of a stream course (Strahler orders 3–4), according to the river continuum concept, where algal and biofilm food sources would be present (Vannote *et al.* 1980). Again, this

could be explained by a larger substrate providing a habitat for this food resource. The overall diversity of functional feeding groups in the species most linked to the east branch main stem sites might best be explained by the type of substrate that is present at those sites and that mediates habitat and food resources.

The north branch, on the other hand, was negatively correlated with large substrates, and north branch sites were associated with taxa such as Corixidae, Oligochaeta, and Caenidae. These taxa are collector–gatherers or predators, which are functional feeding groups primarily associated with finer sediment (Szałkiewicz *et al.* 2022). The lack of scrapers could be related to stream order (as per the river continuum concept; Vannote *et al.* 1980) and to the lack of large substrates available to provide habitat for biofilm and algal food resources. These macroinvertebrate taxa are additionally known to be tolerant of low oxygen and related impacts (Hilsenhoff 1988; Smith and Duffy 2019). Fine substrate in this case provides habitat for sediment-dwelling taxa such as Oligochaeta (Timm 2013) and is tolerated by taxa with adaptations for suspended sediment, such as operculate gills in the case of Caenidae (Notestine 1994). The redundancy analysis also identified conductivity as being associated with these sites, suggesting further evidence of anthropogenic impacts in these sites.

Existing publications on this watershed support the habitat differences found in the present study between branches. Metcalfe-Smith et al. (2003) explain that the Sydenham River's east branch has better water quality and a diversity of habitats that in turn support a more diverse aquatic community. The north branch, on the other hand, has a heavier sediment load and lacks strong flows, which in turn supports fewer predatory taxa and gastropods (Harris et al. 2003). Our analyses of the macroinvertebrate communities, water quality, and sediment in each branch support those earlier findings. Taxa indicative of the presence or absence of threatened and endangered mussel species identified by Eveleens et al. (2023) also match the species identified across the gradient generated by the redundancy analysis in the present study. Leptohyphidae were found to be associated with larger sediment and east branch main stem sites, where previous work suggests the family is positively associated with endangered and threatened mussel species (Eveleens et al. 2023). Corixidae, which we found to be associated with conductive waters and finer sediment, were negatively associated with the presence of several of the species at risk (Eveleens et al. 2023). The congruence of these findings suggests that habitat variables such as sediment size act on both mussel species at risk and the broader macroinvertebrate communities the mussels reside in, a finding that Eveleens et al. (2023) also report.

# Comparing diversity metrics and their implications

The abundance and proportion of EPT taxa and the HBI scores appeared to best capture the differences between the communities of the Sydenham River's north and east branches. Other metrics, including family richness, EPT richness, and Shannon–Wiener diversity, did not differ significantly among the sites or catchments.

The HBI was developed and backed by research in ecotoxicology, with established tolerances noted for several taxa (Hilsenhoff 1988). Although HBI scores are based on tolerance to pollutants and are not factors relating to habitat, they remain a commonly employed metric for biomonitoring across Ontario. Crunkilton and Duchrow (1991) explained that the HBI appears to be less affected by stream order; if this is the case, the tributary sites examined in this research may show increased signs of impact unrelated to stream size, such as pollution or other stressors.

Metrics focusing on EPT taxa may be promising in assessing differences in impact at smaller scales. Weigel *et al.* (2003) describe a positive relationship between EPT taxa and coarse sediments in deep waters. This relationship may explain how EPT abundance and proportion capture differences across stream size and impact.

Although there are ubiquitous, more tolerant Ephemeroptera such as Caenidae, Baetidae, taxa in Plecoptera, and Trichoptera include families that are highly sensitive to impact

(*e.g.*, Isonychiidae, Perlidae, Lepidostomatidae, and Brachycentridae; Hilsenhoff 1988). Our redundancy analysis linked EPT taxa such as Leptohyphidae and Hydropsychidae to coarser sediments. Additionally, rare, sensitive EPT taxa (such as Perlidae, Lepidostomatidae, Brachycentridae, and Isonychiidae) were restricted to the east branch main stem sites. The underrepresentation of these rare taxa in the redundancy analysis is likely due to the impact of Hellinger transformation on rare species (Legendre and Gallagher 2001). Given these relationships, we suggest that metrics relying on the abundance or presence–absence of EPT taxa are appropriate for resolving the impact and habitat diversity of streams in the region at a catchment, reach, or, in this case, site, scale.

Measures such as total family richness and the Shannon–Wiener diversity index may capture general trends in biodiversity, but they do not consider which taxa are present. This further asserts that restoration should be informed by several measures of diversity and by an understanding of which taxa are present and in what proportions. As the results of the present study suggest, an abundance of individual taxa is linked to various abiotic factors: we suggest that conservation practitioners and monitoring programmes not only employ broad measures of diversity but also consider examining stream macroinvertebrate communities as a whole. Looking at biotic factors (*i.e.*, connections among species) can be effective in evaluating the recovery of individual species or entire communities and tends to be ignored in freshwater restoration, where restoring habitat alone often leads to limited success (Eveleens *et al.* 2023).

Conversely, if increasing and maintaining stream macroinvertebrate diversity is the goal (factors that will improve the HBI and EPT% index), reach-scale restoration efforts should focus on preserving the diversity of habitats as it applies to sediment size and flow velocity.

## Headwaters as an opportunity for restoration

The present study found that the East Sydenham River tributaries showed more signs of agricultural impact, as highlighted by lower diversity and higher HBI scores associated with turbidity, conductivity, and fine sediment. Similar patterns were observed for the smaller north branch and its tributaries. Dextrase (2000) reported that headwaters in the region were more degraded and vulnerable to agriculture-linked disturbances than downstream reaches.

More broadly (*i.e.*, in regions outside the watershed), it appears that headwater streams may be more sensitive to these impacts. Weaver *et al.* (2001) found that smaller streams tend to experience more nutrient and sediment pollution associated with runoff. Due to their small size, headwater streams can be more vulnerable to land-based impacts, such as habitat degradation associated with agriculture, and are often ignored in monitoring and restoration practices (Meyer *et al.* 2007). Despite this, headwater streams have the potential to support a diversity of species and can be vital in restoring the ecological health of entire watersheds (Meyer *et al.* 2007). The results of the present study highlight that more habitat diversity (*e.g.*, associated with sediment) can be associated with higher stream macroinvertebrate diversity, and as headwater streams vary at a much finer scale, they may present opportunities to increase habitat diversity and, thus, the diversity of stream macroinvertebrates.

Several previous studies have brought up the role of public perception of headwater streams in explaining why smaller streams may be more degraded or are considered of less concern to restoration efforts (Weaver *et al.* 2001; Armstrong *et al.* 2012). Despite being more vulnerable, small streams have been highlighted as an opportunity for restoring water quality downstream (Lorenz and Feld 2013; Dodds and Oake 2008). These headwater streams process organic matter, cycle nutrients, and foster biodiversity (Clarke *et al.* 2008; Acuña *et al.* 2014), as most streams do, but account for over half of the world's length of river and riparian zones (Downing *et al.* 2012). As such, although numerous and often privately owned and managed, tributaries and small streams where impacts can be most directly and effectively mitigated should be the priorities of local restoration efforts.

## Conclusions

Stream macroinvertebrate communities are shaped by several factors, many of which operate at varying scales within a watershed. Biomonitoring often focuses on a single or a few representative locations in a habitat, with limited insight into the mechanisms and effects of spatial differences. In Ontario's Sydenham River, sediment particle size and stream width are important factors that mediate stream macroinvertebrate community assemblies. Metrics such as EPT abundance, EPT proportions, and the HBI were shown to be effective in detecting differences between the main stem of the river's east branch and the most impacted tributaries of both branches.

These findings add to a body of research that suggests that tributaries and headwater streams need to be considered for restoration due to the impacts they often face from agriculture. Our results suggest that biomonitoring efforts can benefit from not only examining biodiversity metrics but also looking at whole-stream macroinvertebrate communities and individual taxa to identify and evaluate specific parameters.

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