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Anthropogenic land uses influence stream dissolved organic matter quality more than decomposition rates and macroinvertebrate diversity

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ABSTRACT

In the era of the Anthropocene, streams and rivers are among the most heavily impacted ecosystems due to the influence of catchment land use on stream water quality and ecological condition. In practice, structural and functional indicators collected by biomonitoring programs are underused and thus limited in their ability to offer practical insight into functional-based restoration approaches. Here we applied a novel combination of indicators—cotton strip decomposition, benthic invertebrate sampling together with dissolved organic matter (DOM) composition — to determine if streams highly impacted by urbanized and agricultural land use across Windsor-Essex (southwestern Ontario, Canada) were consistent across season, anthropogenic land use or some combination of both. Overall, our results suggest that urbanized and agricultural streams are indeed degraded at a similar level, with high decomposition rates and low levels of macroinvertebrate diversity. Moreover, DOM quality proved to be the most predictable indicator spatially and seasonally, integrating insights from both decomposition and macroinvertebrate indices. Microbial humic-like DOM correlated positively with decomposition rates, and negatively with invertebrate species richness. Our findings show that functional changes in stream ecological condition can be effectively tracked by structural indicators, namely DOM composition. DOM offers a cost-effective approach to assessing ecosystem health and should be explored as a reliable indicator in monitoring programs and to inform functional-based indicators in ecosystem restoration.

1. Introduction

Human activities have altered the earth's surface at such a rapid pace that a new geological period has been proposed – the Anthropocene (Lewis and Maslin, 2015; Zalasiewicz et al., 2010). The scale and impact of human activities are now a dominant force shaping contemporary ecosystems (Foley et al., 2005; Vitousek et al., 1997). Perhaps some of the most vulnerable ecosystems to anthropogenic land use effects are streams and rivers (Allan, 2004). Nested within watersheds, stream ecosystems are hydrologically connected to the landscape that surrounds them (Hynes, 1975), and are thus heavily influenced by activities in their catchment area (Allan et al., 1997). Urbanization and agriculture are the most prolific of human activities, driven by the need to house and feed Earth's growing population. It is estimated that over 50 % of global land is anthropogenically impacted (Ellis et al., 2010), and understanding how these pressures modify stream environments is key to restoring lost ecological integrity in these systems.

Urbanization and agriculture exert many similar and unique stressors on stream ecosystems, including increased nutrient loads, higher water temperatures, and more variable dissolved oxygen levels. The effects of urbanization on streams present as a set of symptoms that are recognizable worldwide, known as the Urban Stream Syndrome (USS). First defined by Meyer et al. (2005), the USS is a common characteristic of streams draining urban land. Symptoms include flashy hydrology, nutrient enrichment, increased temperature, and an increase in eutrophic algae (Walsh et al., 2005). Artificial drainage networks in urban areas increase hydraulic efficiency and the speed at which stormwater runoff enters streams, while headwater burial and channelization reduce habitat complexity (Walsh et al., 2005). Agricultural impacts are similar to the USS in terms of nutrient enrichment and habitat loss (Allan, 2004); however, in agricultural landscapes, streams are subject to runoff from farm fields which often contain high levels of eroded soils, organic pollutants, and pesticides (Allan, 2004). Both agricultural and urban impacts result in degraded ecological conditions.

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Assessing the structural and functional integrity of streams is essential to determine stream health and ecological condition (Palmer and Febria, 2012), and to provide baseline data upon which to develop realistic restoration targets and measure success. Ecological integrity is the ability of ecosystems to support viable native populations and maintain ecological and evolutionary processes (i.e., flow regimes, nutrient cycling) over long time periods (Grumbine, 1994). Biomonitoring and stream assessment protocols typically describe stream condition by measuring structural parameters such as water quality (pH, dissolved oxygen, conductivity, nutrient concentrations), physical characteristics (size, flow, substrate composition), and sampling of stream macroinvertebrates communities (Stanfield, 2010; Jones et al., 2007). These metrics are useful, however routine monitoring of most streams happens at most once or twice a year. At this sampling frequency it is difficult to infer ecological integrity using these physicochemical metrics.

The structure of the stream dissolved organic matter (DOM) pool has also been shown to greatly contribute to ecosystem function while also reflecting watershed-scale biodiversity and land use changes (Jaffé et al., 2008; Wilson and Xenopoulos, 2009). DOM, in constrast to particulate organic matter, is the largest flux of aquatic organic carbon and is an important intermediate in the global carbon cycle (Battin et al., 2008). DOM is a chemically complex mixture of compounds (Sleighter and Hatcher, 2008) whose ecological role includes modulating biogeochemical reactions (Waples et al., 2005) and microbial nutrient cycling (Meyer et al., 1988). The source of DOM in streams can be autochthonous and microbial-derived (Kaplan and Bott, 1982), or allochthonous from terrestrial inputs of plant and soil organic matter (Webster and Meyer, 1997), which has important implications for microbial heterotrophic activity, nutrient cycling and food webs (Kaplan and Cory, 2016). Optical properties of DOM such as fluorescence and absorbance can be used to infer DOM sources and composition (Fellman et al., 2010). Common indices based on optical data are the Humification Index (HIX) which indicates humification extent (Ohno et al., 2002; Zsolnay et al., 1999), the Biological Index (BIX), and indicator of autotrophic productivity (Huguet et al., 2010), and the Fluorescence Index (FI) which varies according to terrestrial and microbial contributions (Cory et al., 2010; McKnight et al., 2001). Parallel factor analysis (PARAFAC) of excitation-emission matrices (EEMs) is a technique that models components of the DOM pool in water samples, allowing researchers to infer potential sources and processing mechanisms (Hosen et al., 2014). Thus, there is good reason to suggest that DOM composition metrics can be used to predict stream ecological integrity and biodiversity whereas other physicochemical metrics cannot.

Ultimately, applying structural measurements to estimate rates of ecosystem processes has limits (Bunn et al., 1999). Instead, these rates can be measured directly by utilizing functional indicators. Organic matter processing in streams, such as the decomposition of plant litter, is a fundamental ecosystem process that can be readily measured using standardized cotton strip assays (Gessner and Chauvet, 2002; Tiegs et al., 2013). Cotton strips are comprised largely of cellulose, the major constituent of plant litter (Egglishaw, 1972). Thus, using standardized strips, decomposition rates can be compared across geographical regions and environmental contexts. Decomposition rates are affected by the activity of microbes, macroinvertebrates, flow, and abrasion (Gessner et al., 1999); and are also sensitive to agriculture (Clapcott et al., 2012) and urbanization (Imberger et al., 2010). This highlights the importance of interpreting functional metrics alongside traditional structural measurements like macroinvertebrate diversity and water physicochemistry.

Together, structural and functional measures allow for a thorough investigation into the ecological integrity of streams with carbon as the key currency for detecting human impacts. Given the widespread deforestation of landscapes for urban and agricultural expansion, common restoration tools aim to boost carbon quality via riparian plantings in a much smaller, concentrated area if at all. As existing stream monitoring protocols indicate similar levels of ecological degredation for both urbanized and agricultural streams, we applied a combination of metrics to explore the extent to which ecological indicators could distinguish seasonal or land use impacts from the other. We tested whether the application of novel structural and functional metrics together might serve as more useful indicators to inform process-based, functional restoration.

Increasingly, human-impacted landscapes are becoming homogenous on the landscape, with fewer reference or unimpacted sites. Such is the case for the most highly populated regions of the Laurentian Great Lakes Basin (*Nayaano-nibiimaang Gichigamin*) of North America (also known as Turtle Island). In this study, we used a modified standard stream assessment protocol including invertebrate samples, optical DOM characterisation, and decomposition rates to assess urban and agricultural streams monthly over the course of a year in the Windsor-Essex region (*Waawiyaatanong*), southwestern Ontario, Canada. Notably, this is a highly developed and homogenized region with no unimpacted reference streams left. Our research goals were to: (1) characterize structural and functional characteristics across a gradient of impacted streams over time, (2) interrogate the effects of land use and time on a suite of stream properties including DOM and decomposition rates. We hypothesized that traditional stream ecological indicators of benthic macroinvertebrate communities and decomposition rates would not be as effective in resolving differences between human-impacted streams. Based on common ecological indicators, we hypothesized that both stream types would be similarly degraded, and thus have comparable decomposition rates and invertebrate biotic index values. We expected to find responses with DOM-based indicators. Specifically, we predicted that DOM in agricultural streams would have higher humic content, and that DOM in urban streams would reflect microbial production. Hence, our final research goal was to (3) infer how the inclusion of DOM-based indicators may be applied as an effective ecological indicator in human-impacted streams for both assessing impact but more importantly to guide future process-based and functional restoration efforts in anthropogenic settings.

2. Materials and methods

2.1. Study design and sample sites

The study was conducted in seven human-impacted streams across the region of Windsor-Essex County (*Waawiyaatanong*) in southwestern Ontario, Canada, part of the Laurentian Great Lakes Basin (*Nayaano-nibiimaang Gichigamin* in Anishnaabemowin, the Ojibwe language) of North America (Turtle Island) (Fig. 1). Streams were selected to represent either dominant agricultural (AG, $n = 4$) and urban (UR, $n = 3$) land use (Site photos in Fig. S1). The urbanized streams in this study were all lined with concrete for a portion of their reaches. Study site characteristics and dominant watershed land use are described in Table 1. Streams were sampled monthly from October 2020 – October 2021. Seasonal descriptions are meteorological: Spring (March, April, May), Summer (June, July, August). Fall (September, October, November), and Winter (December, January, February). Video description of study can be viewed here (https://youtu.be/HDKbPtkkWnM), and all data are available and open access (Nolan et al., 2023).

2.2. Environmental variables

Water temperature (°C), specific conductivity (μS/cm), pH, dissolved oxygen (DO, mg/L), and turbidity (NTU) were measured using a YSI® ProDSS during field sampling each month. Water samples were collected using 150 mL acid-washed HDPE bottles. Samples for DOM qualty analysis were filtered using EZFlow® Membrane 0.22 μm filters to remove prokaryotes and sterilise for longer storage. DOM Samples were stored at 4 °C prior to processing. Samples for nutrient analysis were transported to the laboratory on ice, filtered with VWR® nylon 0.45 μm filters, and stored at −20 °C prior to processing. Nitrate-nitrite (NO_3-

Fig. 1. Site Map. Location of study sites in Windsor – Essex Region (*Waawiyaatanong*), ON, Canada. (Map credit: L.A. Weller).

NO_2; mg/L), ammonia (NH_3; mg/L), and total dissolved phosphorus (TDP; µg/L) concentrations were determined using a SMARTCHEM® 170 Discrete Analyzer. Stream physical characteristics were measured using a modified Ontario Stream Assessment Protocol (OSAP; Stanfield, 2017). Discharge was calculated from flow measurements taken using a Hontzsch® Flowtherm NT in October 2020, and a HACH® FH950 in November 2020-October 2021.

2.3. Stream DOM quality

DOM spectroscopy was conducted following established methods (Cory et al., 2010; Hosen et al., 2021; Weller et al., 2022). Absorbance and fluorescence data were collected on a HORIBA Scientific Aqualog® with a Xe lamp and a quartz cuvette with path length of 1 cm. UV–Vis absorbance measurements from 200 to 800 nm were taken at 1 nm increments. EEMs were taken at excitation wavelengths 240–800 nm at 3 nm increments, and emissions from approximately 244.71–827.32 nm at 2.33 nm increments, with an integration of 4 CCD pixels and CCD gain set to medium. Dissolved organic carbon (DOC; mg/L) and total nitrogen (TN; mg/L) were determined using a Shimadzu® TOC-L/TNM-L. Samples for all sites except MCC in October 2020 were not viable for fluorescence analysis due to improper storage.

Multiple spectroscopic indices were calculated based on EEM scans of water samples ($n = 85$). The humification index (HIX), which indicates the amount of humic content, is calculated as the ratio of the area of the emission spectrum at 435–480 nm to the emission area from 300 to 445 nm at an excitation wavelength of 255 nm (Ohno et al., 2002). The biological index (BIX), which indicates the amount of autotrophic production, is calculated as the ratio of emission intensity at 380 nm divided by 430 nm at excitation 310 nm (Huguet et al., 2009). Higher values of HIX or BIX indicate higher humic or autotrophic DOM, respectively. The fluorescence index (FI), an indicator of terrestrial and microbial contributions to the DOM pool, is calculated as the ratio of emission wavelengths at 450 nm and 500 nm, obtained at an excitation

Table 1
Study site characteristics and dominant watershed land use.

Site	Stream name	Order	Watershed	Watershed Area[1] (km^2)	Dominant land use[1]
FCD	14th Concession Drain	1	River Canard	347.8	Agriculture (88.8 %)
BRD	Branch of Richmond Drain	3	Cedar Creek	128.0	Agriculture (82.1 %)
GIB	Gibbs Drain	1	River Canard	347.8	Agriculture (88.8 %)
LMD	Long Marsh Drain	3	River Canard	347.8	Agriculture (88.8 %)
MMD	Marentette Mangin Drain	1	Turkey Creek	61.1	Urban (83.0 %)
GMD	Grand Marais Drain	3	Turkey Creek	61.1	Urban (83.0 %)
MCC	McKee Creek	2	Windsor Drainage Area	46.8	Urban (93.1 %)

[1] ERCA (2015).

wavelength of 370 nm (McKnight et al., 2001). Higher FI values indicate more microbial contribution (~1.9), and lower values indicate more terrestrial contributions (~1.4). Specific UV absorbance at 254 nm ($SUVA_{254}$) was calculated by dividing the absorbance at 254 nm (A_{254}) by DOC concentration (Weishaar et al., 2003).

Individual DOM components were resolved using PARAFAC analysis and the package *staRdom* (Pucher et al., 2019) in statistical software R 4.0.3. (R Core Team, 2020). Split-half analysis with a Tucker's congruency correlation value above 0.98 was used to validate the final PARAFAC model (Murphy et al., 2013). Following validation, the 4-component PARAFAC model (Table 2) was uploaded to the OpenFluor spectral database (Murphy et al., 2014) for literature comparison and component identification. Raw component values (C1-4) are reported as maximum fluorescence intensity (Fmax). The proportions or relative abundance of each component (Prop C1-4) were calculated by dividing the Fmax of each component by the total Fmax (ex. Prop C1 = C1/C1 + C2 + C3 + C4).

A four component PARAFAC model was validated (n = 84), with three humic-like and one protein-like component (Table 2). C1 was identified as anthropogenic terrestrial humic-like (Liu et al., 2018), associated with nutrient-rich environments (Jutaporn et al., 2020), and similar to coble peak C (Ren et al., 2021). We found C1 was moderately correlated to Coble peak C ($R = 0.42$, $p < 0.0001$) and M ($R = 0.28$, $p = 0.0096$). C2 was identified as ubiquitous terrestrial humic-like (Murphy et al., 2011), recalcitrant (Podgorski et al., 2021), with a low C:N ratio (Kulkarni et al., 2018). We found C2 to have a moderate negative correlation to C:N ratio ($R = -0.47$, $p < 0.0001$). C2 was not correlated with humic Coble peaks A, M, or C. HIX strongly correlated with C1 ($R = 0.69$. $p < 0.0001$) and C2 ($R = 0.75$, $p < 0.0001$) supporting their classification as terrestrial humic-like components. C1 was weakly correlated to $SUVA_{254}$ ($R = 0.25$, $p = 0.022$) and C2 was not. C1 was also the only component correlated with DOC concentration ($R = 0.26$, $p = 0.018$). C3 was identified as microbial humic-like (Kulkarni et al., 2017; Lambert et al., 2017; Williams et al., 2013). Component C3 is similar to Coble peak A (DeFrancesco et al. 2021) but further shown to decompose into peaks B and T (Shutova et al., 2014). There were moderate, significant correlations with peaks B ($R = 0.41$, $p = 0.0001$) and T ($R = 0.39$, $p = 0.0003$). C3 also moderately positively correlated with the C:N ratio ($R = 0.48$, $p < 0.0001$).

2.4. Cotton-strip decomposition

Standardized cotton strips were prepared from bolts of "Artist's Canvas" (Fredrix-brand unprimed 12-oz heavyweight cotton fabric) and deployed according to previously described methods (Tiegs et al., 2013).

Table 2
Description of the four PARAFAC components (n = 84), secondary excitation peaks are given in brackets. Number of OpenFluor matches as of Jan 2022 with high (>95 %) congruence shown. Most similar Coble peak determined by spearman correlation coefficient (R) with component fmax. Description includes literature comparisons of components.

Component	Ex/Em Max (nm)	OpenFluor Matches	Coble Peak[1]	Description
C1	332/411	7	C, M (R = 0.42, p < 0.0001; R = 0.28. p = 0.0096)	**Anthropogenic terrestrial humic-like**[2,3,4] in nutrient rich enviornments[3], similar to Coble peak C[4]
C2	266 (368)/ 472	85	– (all ns)	**Ubiquitous terrestrial humic-like**[5,6,7], recalcitrant[6], has low C:N ratio[7]
C3	<250 (395)/ 386	10	B, T, A (R = 0.41, p = 0.0001; R = 0.39, p = 0.0003; R = 0.21, p = 0.056)	**Microbial humic-like**[8,9,10], similar to Coble peak A[11], shown to further decompose into peaks B and T[12]
C4	281 (<250)/ 336	13	– (all ns)	**Protein-like**[3,12-15], increases during wet seasons[13], highly correlated with urban land[14] and environments with abundant microbial activity[3,15]

ns = not significant, [1]Coble 1996; [2]Liu et al. 2018, [3]Jutaporn et al. 2020, [4]Ren et al. 2021, [5]Murphy et al. 2011, [6]Podgorski et al. 2021 [7]Kulkarni et al. 2018, [8]Kulkarni et al. 2017, [9]Lambert et al. 2017, [10]Williams et al. 2013, [11]DeFrancesco et al. 2021, [12]Shutova et al. 2014, [13]Dalmagro et al. 2019, [14]Du et al. 2021, [15]Chen et al. 2017.

Average deployment period was 30 days for all sites except GMD, where decomposition rates were highest and thus deployment length was approximately 14 days (~50 % decomposition). After removal from field, strips were transported to the laboratory on ice and gently cleaned with 80 % ethanol using a small paintbrush to clear debris. Strips were then oven dried at 40 °C for about 24 h in shallow aluminum trays and stored in a desiccator until tensile strength determination. Tensile strength was determined using a MARK-10 M2-100® tensiometer. Initial tensile strength was determined using a set of nine reference strips that were wetted with tap water before being processed identically to the treatment strips. Tensile strength loss (TSL) for each strip was calculated as a percentage of the initial tensile strength according to the following equation (1) (after Colas et al., 2019):

$$TSL = \left[1 - \left(\frac{Tensile\ Strength_{treatment\ strips}}{Tensile\ Strength_{reference\ strips}}\right)\right] \times \frac{100}{incubation\ time\ (days)} \quad (1)$$

Where "*Tensile Strength* $_{treatment\ strips}$" refers to the maximum tensile strength (lbF) of strips incubated in the field, "*Tensile Strength* $_{reference\ strips}$" refers to the average maximum tensile strength (lbF) of reference strips, and "*incubation time*" refers to the number of days in the deployment period. Tensile strength loss per day (TSL_d) is based on the number of days in the deployment period as the incubation time. Tensile strength loss per degree day (TSL_{dd}) uses a sum of the mean temperature for all deployment days (e,g., degree days) for incubation time. Temperature data from Environment and Climate Change Canada (climate.weather.gc.ca) were obtained from the closest monitoring station to each stream: Windsor A (FCD, GMD, MCC, MMD), Amherstburg (GIB, LMD), and Harrow CDA Auto (BRD). Mean daily temperatures below zero were adjusted to zero, and missing data was estimated using an average of the previous and following days. Vandalization resulting in missing data for site MMD in October and January, site LMD for October, November, and December, site FCD in April, and site BRD in June.

2.5. Benthic invertebrate samples

Benthic macroinvertebrate samples were collected using the standard provincial protocol: the Ontario Benthic Biomonitoring Network (OBBN; Jones et al., 2007). Following OBBN, one pool and two riffle habitats were sampled using a 30 cm wide, 500 µm mesh sized D-net. Samplers travelled in a zig-zag pattern across the stream using the jab-and-sweep method for areas that had little to no-flow, and the kick-and-sweep method for faster flowing streams. Benthic materials were placed in a polyethylene bag with a 1:1 combination of stream water to a formal ethanol preservative solution and sealed using a twist tie. The preservative solution was 15 parts ethanol (95%), 6 parts formaldehyde (37%), and 7 g of Borax per 1L of solution. In the lab the samples were washed and sorted in a randomized order according to Frazao, 2019. Stainless steel U.S.A Standard Test Sieve were used at sizes 4 mm, 1 mm, 0.5 mm, and 0.25 mm, (No. 5, 18, 35, and 60, respectively). Macroinvertebrates were identified to at least the Family level of taxonomic resolution but were identified further if key features were present and undamaged. Macroinvertebrates were then stored in 70% ethanol in labelled 20 mL scintillation vials.

Multiple indices were calculated from macroinvertebrate family and abundance data: the Hilsenhoff Biotic Index (HBI; Hilsenhoff, 1987) which assigns values based on tolerance of organic pollution, Shannon's H index (Shannon, 1948) as a measure of alpha diversity, species richness, the relative abundance of Oligochaete individuals (%Oligochaetes), and the relative abundance of Ephemeroptera, Plecoptera, and Trichoptera individuals (%EPT).

2.6. Statistical analyses

All statistical analyses were carried out using statistical software R 4.0.3. (R Core Team, 2020). Summary plots of environmental variables, DOM optical indicators, and decomposition rates were made using the package *ggplot2* (Wickham, 2016). A correlation matrix of all optical, invertebrate, and decomposition indicators was made using Spearman's rho with the package *corrplot* (Wei and Simko, 2021).

Principal component analysis (PCA), computed in base R and plotted using the package *ggfortify* (Horikoshi and Tang, 2018), was used to examine site spread in multivariate space and identify potential variables driving site groupings. PCAs used only complete observations ($n = 77$). Variables were centered and scaled and collinear variables were removed.

Linear mixed effects models (LMMs) using the *lme4* package (Bates et al., 2015) were used to evaluate the effect of land use and season on environmental variables, decomposition rates, invertebrate metrics, and DOM optical indicators. Variables were transformed to achieve normality, checked visually with histograms. HIX, BIX, A_{254}, PropC3, PropC4, conductivity, discharge, turbidity, TN, DOC, and TSL_{dd} were log transformed. PropC1 and HBI were squared. TSL_d was square-root transformed. Nitrate-nitrite, Ammonia, TDP, and %EPT were cube-root transformed. $SUVA_{254}$, PropC2, FI, species richness, Shannon diversity, and %Oligochaetes were normal without transformation.

Stream type (agricultural, urban), season (spring, summer, fall, winter) and their interaction were treated as fixed effects. Random effects were site and month to account for repeated measures. For models using invertebrate data, month was not included as a random effect as there were only two sampling events. Significance of fixed effects and interactions were determined using type III analysis of variance (ANOVA) tables with Satterthwaite's method. The *emmeans* package (Lenth, 2021) was used to find estimated marginal means (EMMs, least squares) using the Kenward-Roger degrees of freedom (DF) method at a 95% confidence level. Pairwise comparisons and contrasts of EMMs were used to investigate significant fixed effects and interactions using Tukey's p-value adjustment method. Models were checked for homoscedasticity visually and for normality of residuals using Shapiro-Wilks tests.

Variance partitioning of LMMs with the *variancePartition* package (Hoffman and Schadt, 2016) was used to compare indicator variance between study factors (stream type, month, season, site, and residuals). The models were the same as the previous but excluded the interaction between stream type and season.

3. Results

3.1. Stream physiochemical parameters

The concentration of dissolved macronutrients showed distinct seasonal patterns and were typically enriched in AG streams (Fig. 2). TN and nitrate-nitrite had significant type*season interactions ($F(3,69) = 9.96$, $p < 0.0001$; $F(3,69) = 7.75$, $p = 0.00016$) where AG streams had significantly higher concentrations than UR streams for all seasons except fall. In AG streams, TN was highly correlated with nitrate-nitrite ($R = 0.964$, $p < 0.0001$), but not ammonia. In UR streams, TN was highly correlated with nitrate-nitrite ($R = 0.82$, $p < 0.0001$), but also showed moderate correlations with ammonia ($R = 0.48$, $p = 0.0018$). Ammonia concentrations were lower than nitrate-nitrite overall and not affected by land use, but were significantly higher in the winter ($F(3,9.01) = 8.47$, $p = 0.0055$). TDP had a significant type by season interaction ($F(3,69) = 3.05$, $p = 0.034$), where concentrations were significantly lower in the winter, and in UR streams in the fall. DOC had a significant interaction ($F(3,69) = 5.38$, $p = 0.0022$), where only AG streams had a seasonal pattern. There were no significant differences in DOC between AG and UR streams. There were no significant land use or seasonal patterns in turbidity.

The gradient of human-impacted waterways exhibited seasonal changes in physicochemical parameters with mean values conferring strong patterns by stream type (AG, UR; Fig. 2). There was a significant type*season interaction effect for conductivity ($F(3,78) = 14.82$, $p < 0.0001$), where UR streams had significantly higher conductivity than AG streams in every season, and a significant UR seasonal pattern with winter peaks that were not seen in AG streams. Discharge also had a significant interaction ($F(3,69) = 5.01$, $p = 0.0034$), where a significant seasonal pattern was only seen in AG streams, highest in spring. Stream water temperature ($F(3,9.02) = 10.61$, $p = 0.0026$), DO ($F(3,9.36) = 17.17$, $p = 0.0039$), and pH ($F(3,9.29) = 5.17$, $p = 0.023$) had significant seasonal patterns, but were not significantly different between stream types.

3.2. DOM quality

AG streams had significantly higher proportions of terrestrial humic-like components C1 (Fig. 3; $F(1,5.04) = 23.07$, $p = 0.0048$) and C2 ($F(1,5.06) = 119.87$, $p = 0.0001$) than UR streams. C2 was the dominant humic component with anthropogenically linked C1 being less abundant. In UR streams, C3 (microbial humic-like) was the dominant humic-like component. Proportions of C3 and C4 were significantly higher in UR streams (Fig. 3; $F(1,5.01) = 60.55$, $p = 0.00056$; $F(1,5.18) = 56.31$, $p = 0.00057$). $SUVA_{254}$ was the only optical indicator affected by season ($F(3,8.34) = 5.20$, $p = 0.0052$), with significantly higher values in fall than winter or spring. FI was not correlated with land use or season.

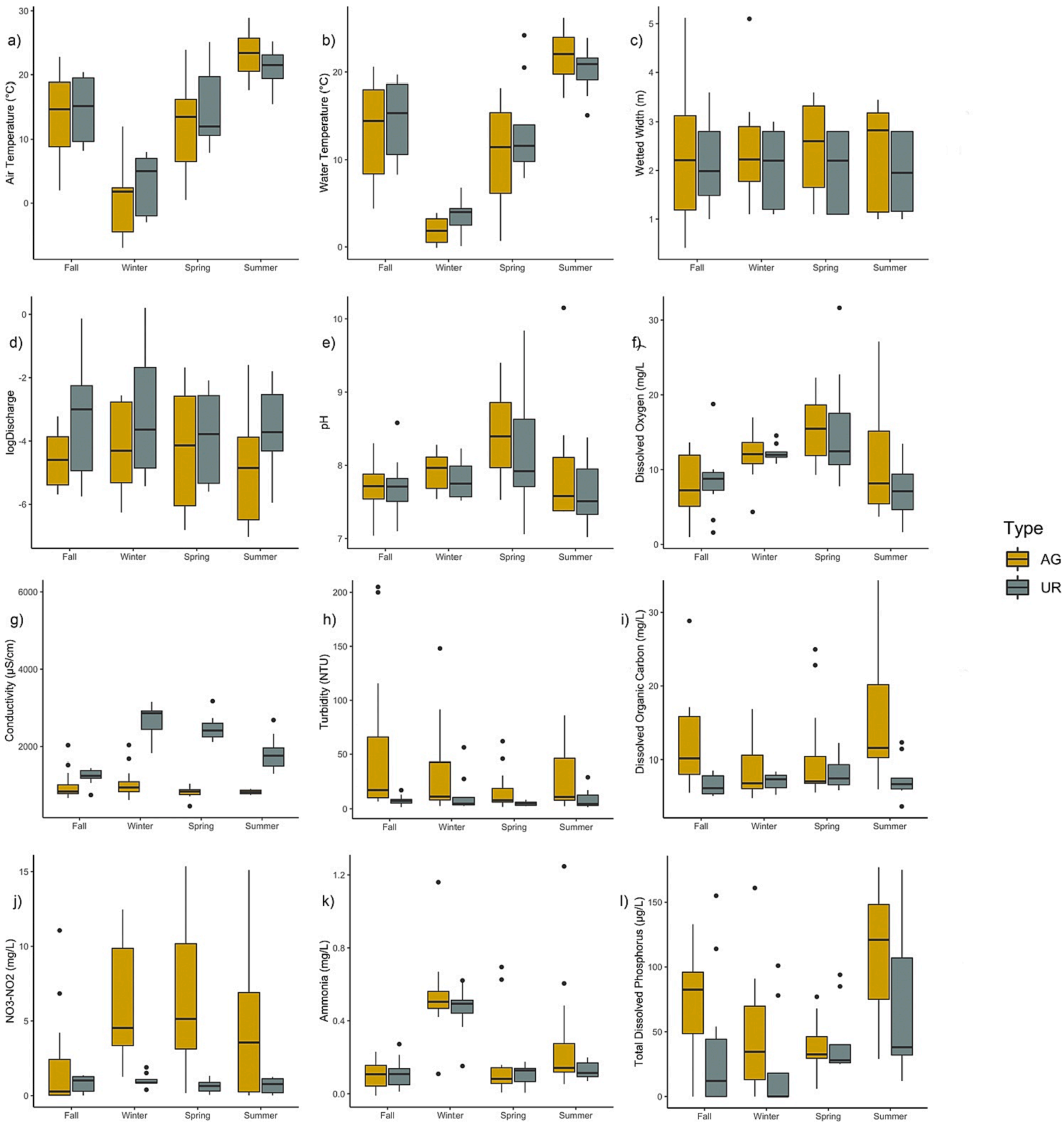

Fig. 2. Temporal summary of environmental variables. Box-whisker plots of physio-chemical variables included in the stream assessment protocol: a Air Temperature (°C), b) Water Temperature (°C), c) Wetted Width (m), d) logDischarge, e) pH, f) Dissolved Oxygen (mg/L), g) Conductivity (μS/cm), h) Turbidity (NTU), i) Dissolved Organic Carbon (mg/L), j) NO_3-NO_2 (nitrate-nitrite, mg/L), k) Ammonia (NH_3, mg/L), l) Total Dissolved Phosphorus (TDP, mg/L). Streams sampled monthly from October 2020 –October 2021.

DOM quality varied with stream physiochemistry and macronutrient concentrations (Fig. S2). Humic-like components C1 and C2, as well as HIX, showed similar relationships with environmental variables. The proportions of component C1 and C2, found in significantly higher quantities in AG streams, were positively correlated with total nitrogen ($R = 0.34$, $p = 0.0014$; $R = 0.57$, $p < 0.0001$) and nitrate-nitrate ($R = 0.23$, $p = 0.033$; $R = 0.45$, $p < 0.0001$), as well as turbidity ($R = 0.34$, $p = 0.0017$; $R = 0.47$, $p < 0.0001$). Proportions of C1 and C2 were negatively correlated with conductivity ($R = -0.54$, $p < 0.0001$; $R = -0.73$, $p < 0.0001$), however only the proportion of C1 negatively correlated with discharge ($R = -0.29$, $p = 0.0067$). C1 and C2 were not correlated with TDP, DO or pH.

Microbial and protein-like components C3 and C4 along with BIX had opposite patterns to the humic components (Fig. S2). The proportion of C3 was strongly negatively correlated to total nitrogen ($R = -0.61$, $p < 0.0001$) and nitrate-nitrite ($R = -0.57$, $p < 0.0001$), while C4 was only moderately negatively correlated to total nitrogen ($R = -0.32$, $p = 0.0028$), and showed no correlation with nitrate-nitrate. The proportion of C4 was the only component negatively correlated with TDP ($R = -0.25$, $p = 0.019$). Both the proportions of C3 and C4 had strong positive correlations to conductivity ($R = 0.58$, $p < 0.0001$; $R = 0.59$, $p < 0.0001$) and negative correlations to turbidity ($R = -0.35$, $p = 0.0012$; $R = -0.34$, $p = 0.0019$). Only the proportion of C3 correlated negatively with DO ($R = -0.25$, $p = 0.024$) and pH ($R = -0.23$, $p = 0.036$). C3 and C4 were not correlated with discharge.

Other optical metrics of DOM quality were also informative. FI was positively correlated with nitrate-nitrite ($R = 0.35$, $p = 0.00098$) and total nitrogen ($R = 0.26$, $p = 0.017$), and negatively correlated with

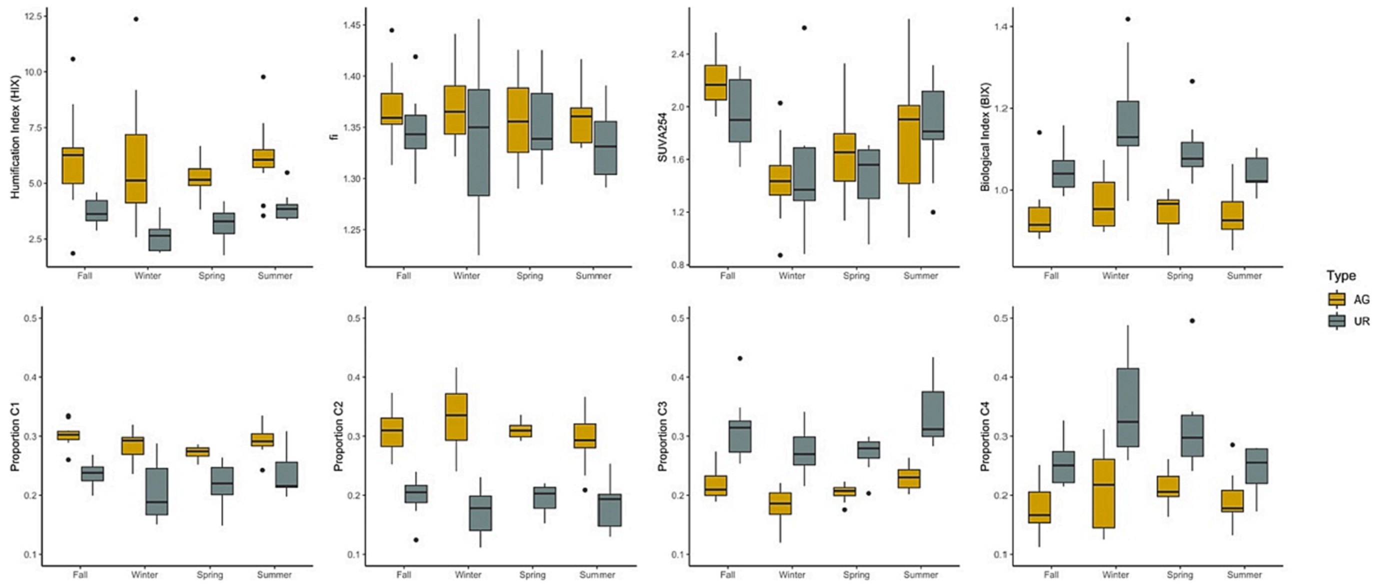

Fig. 3. Temporal summary of DOM quality metrics. Box-whisker plots of fluorescence indices and proportion of PARAFAC components derived from EEM scans. Streams sampled monthly from late October 2020 – early October 2021.

discharge (R = -0.47, $p < 0.0001$), canopy cover (R = -0.23, p = 0.036), and TDP (R = -0.24, p = 0.027). $SUVA_{254}$ was positively correlated with wetted width (R = 0.31, p = 0.0042) and TDP (R = 0.26, p = 0.020), and negatively correlated with nitrate-nitrite (R = -0.43, $p < 0.0001$), total nitrogen (R = -0.31, p = 0.0040), DO (R = -0.41, $p < 0.0001$), and conductivity (R = -0.28, p = 0.0099).

3.3. Cotton strip assay and decomposition rates

Decomposition rates, measured as tensile strength loss per day (TSL_d) or degree-day (TSL_{dd}), were not significantly different between UR and AG streams but varied seasonally (Fig. 4). Decomposition rates (TSL_d) were higher in the warmer seasons (summer and fall), but when temperature corrected (TSL_{dd}), showed the opposite pattern where TSL_{dd} was highest in the winter instead. Seasonal effects were significant on TSL_d ($F(3,8.94) = 10.91$, p = 0.0024) and TSL_{dd} ($F(3, 8.96) = 6.67$, p = 0.012). TSL_d was strongly correlated with TDP (R = 0.57, $p < 0.0001$), and weakly with discharge (R = 0.34, p = 0.0014) and conductivity (R = -0.24, p = 0.031). TSLdd was correlated with ammonia (R = 0.35, p = 0.0010), nitrate-nitrite (R = 0.34, p = 0.0017), and total nitrogen (R = 0.33, p = 0.0025); as well as discharge (R = 0.25, p = 0.023). There were no correlations between decomposition and turbidity.

3.4. Benthic macroinvertebrate biotic indices

Benthic macroinvertebrate indicators varied more by season than stream type (Table 3). Species richness was the only metric significantly different between stream types ($F(1,9) = 9.31$, p = 0.014), higher in AG (23 ± 2.33) than UR streams (15.2 ± 3.22). Species richness was also significantly higher in fall than spring ($F(1,9) = 10.98$, p = 0.0090), but was not correlated with any physiochemical variables. A similar seasonal effect was found in HBI scores ($F(1,4.24) = 9.14$, p = 0.036), with better biotic index scores in the fall (6.75 ± 0.33) than the spring (7.23 ± 0.24). HBI scores for the streams ranged from 5.29 (good water quality; some organic pollution) to 7.92 (poor water quality; very significant organic pollution). HBI was positively correlated with ammonia (R = 0.57, p = 0.047) and %Oligochaetes (R = 0.80, p = 0.0018), and negatively correlated with percent canopy cover (R = -0.56, p = 0.046). Shannon diversity was not linked to stream type or season, but had strong negative correlations with ammonia (R = -0.68, p = 0.010) and % Oligochaetes (R = -0.92, $p < 0.0001$), and positive correlations with percent canopy cover (R = 0.65, p = 0.017). EPT taxa were not very abundant in study streams (2.89 % ±3.83 %) and were not correlated with by stream type or season, but correlated positively with wetted width (R = 0.62, p = 0.024). Oligochaetes were highly abundant (55.1 % ±12.4 %) and had strong positive correlations with DO (R = 0.61, p = 0.030) and ammonia (R = 0.63, p = 0.024).

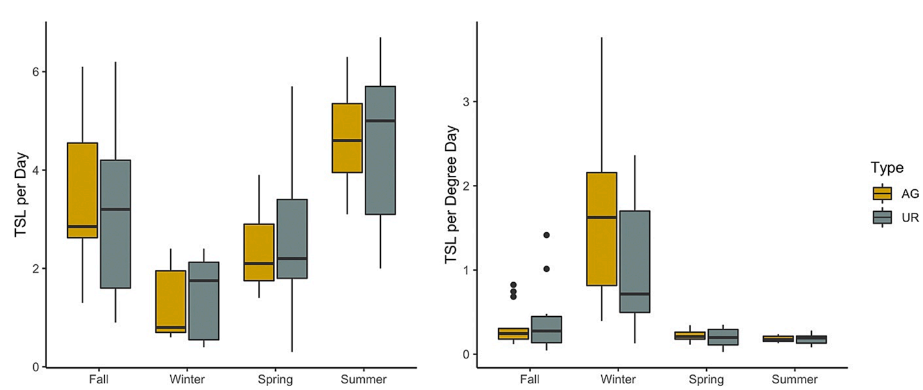

Fig. 4. Seasonal Tensile Strength Loss (TSL). Box-whisker plots of TSL_d and TSL_{dd} by type and season. Streams sampled monthly from late October 2020 – early October 2021. Two outliers removed for clarity in TSL_{dd} graph: 16.21 and 12.70 (AG – winter).

Table 3
Summary statistics for macroinvertebrate indicators (AG n = 8; UR n = 5).

Indicator	Description	Land use	Minimum	Median	Maximum	Mean ± SE
HBI	Hilsenhoff biotic index score. Lower values indicate better water quality	AG	5.73	7.08	7.80	6.98 ± 0.27
		UR	5.29	7.01	7.92	6.95 ± 0.45
Species Richness	Total number of species	AG	14	24	31	23 ± 2.33
		UR	7	13	26	15.2 ± 3.22
Shannon	Shannon's diversity index (H)	AG	0.97	1.25	1.93	1.37 ± 0.14
		UR	0.18	1.09	1.24	0.88 ± 0.21
%EPT	Percentage of EPT individuals out of total individuals	AG	0	0.20	31.08	4.62 ± 3.82
		UR	0	0	0.39	0.11 ± 0.077
%Oligochaeta	Percentage of Oligochaeta individuals out of total individuals	AG	5.04	52.94	75.98	46.20 ± 10.41
		UR	51.37	59.95	95.93	69.27 ± 8.84

SE = standard error of the mean, EPT = Ephemeroptera, Plecoptera, Trichoptera.

3.5. Indicator sensitivity to land use and season

Principal component analysis (PCA) was used to explore relationships between measured parameters and the land use and seasonal gradients (Fig. 5; Fig. S3). Decomposition rates were not included in the final PCA due to high collinearity with water temperature, which had higher loading scores. Principal components (PC) 1 and 2 explained 41.12 % and 29.83 % of the variation respectively. PC1 had an eigenvalue of 62.56, PC2 had an eigenvalue of 27.32, and PC3 had an eigenvalue of 11.54. All other PCs had eigenvalues below 0.15. The land use gradient was best represented by PC1 (Fig. 5a), and the highest loading variables were the proportion of each PARAFAC component and nitrate-nitrite concentrations (loadings $> |0.29|$). Agricultural sites spread along components 1, 2, and nitrate-nitrite concentrations, while urban sites were spread along components 3 and 4. The seasonal gradient was represented by PC2 (Fig. 5b). The highest loading variables on PC2 were water temperature, DO concentration, nitrate-nitrite concentration, $SUVA_{254}$, and the proportion of component 3 and 4 (loadings $> |0.28|$). Spring and winter had high overlap and were characterized by high nitrate-nitrite and DO concentrations, while summer and fall were highly overlapped and characterized by high water temperature and $SUVA_{254}$ values.

Variance partitioning was used to explore indicator sensitivity to environmental and land use gradients (Fig. 6). The proportion of PARAFAC components were most sensitive to the land use gradient, with stream type explaining 67.5–82.9 % of the variance in C1-3 and 54.5 % of the variance in C4. Variance in PARAFAC components was very minimally explained by season (0–7.7 %). Of the invertebrate metrics used, stream type only explained a moderate amount of variance in species richness (35.0 %), Shannon diversity (32.2 %), and a small amount of variance in %Oligochaetes (5.8 %). Species richness was best explained by season (42.1 %), and all other invertebrate metrics had small amounts of variance explained by season (<21.8 %). Most invertebrate metrics were best explained by site-specific factors not accounted for in the land use or seasonal gradients. Decomposition metrics were best explained by season, which accounted for around half of the variance. Variance explained by season was higher than month for all metrics except the proportion of C4 (4.1, 7.7 %), and roughly equal for C3 (7 %, 7.5 %). The proportion of C4 had the most variance explained by residuals (33.0 %) out of all indicators.

Variance of water quality indicators was also explored (Fig. 7). The most sensitive indicators to land use were conductivity (70.5 %), TN (43.7 %), turbidity (28.2 %), nitrate-nitrite (22.3 %), and DOC (16.5 %). Land use only accounted for 2.3 % of the variance in TDP, while season accounted for more (19.2 %). Season explained the majority of the variance in ammonia (47.2 %), and the second-most in $SUVA_{254}$ (27.8 %). Discharge varied the most with site-specific factors (67.8 %), and FI had the most variance explained by residuals (71.1 %).

3.6. Relationships between indicators

Many DOM, decomposition, and invertebrate indicators were significantly correlated to one another (Fig. 8). Decomposition rate TSL_d was negatively correlated to FI ($R = -0.26$, $p = 0.020$), and positively correlated to $SUVA_{254}$ ($R = 0.31$, $p = 0.0054$) and the proportion of C3 ($R = 0.28$, $p = 0.012$). Temperature corrected TSL_{dd} showed the opposite pattern, with no correlation to FI, and a negative correlation to SUVA254 ($R = -0.25$, $p = 0.025$), the proportion of C3 ($R = -0.32$, $p = 0.0037$), and the C:N ratio ($R = -0.35$, $p = 0.0011$). TSL_d was positively correlated to invertebrate metrics %Oligochaetes ($R = 0.81$, $p = 0.0082$) and HBI ($R = 0.79$, $p = 0.0098$), and negatively correlated to Shannon diversity ($R = -0.67$, $p = 0.032$). TSL_{dd} was also positively correlated to %Oligochaetes ($R = 0.72$, $p = 0.024$) and HBI ($R = 0.75$, $p = 0.018$). The only correlation relating DOM and invertebrate indicators was a strong negative correlation between species richness and the proportion of C3 ($R = -0.77$, $p = 0.041$).

4. Discussion

The purpose of this study was to evaluate human-impacted streams and common methods for evaluating the ecosystem health of waterways through a novel combination of structural and functional indicators. The study region, like many around the world, represents one that is almost completely impacted by human activities both urban and agricultural. Understanding these systems is essential for management, and we sought to fill a critical knowledge gap for southwestern Ontario in the Laurentian Great Lakes (*Nayaano-nibiimaang Gichigamin*) basin in North America (Turtle Island). To inform and advance restoration efforts in these all-too-common landscapes, this study provided a more nuanced view of human impacts and confirmed the extent to which seasonality can be accounted for in anthropogenic landscapes, particularly in temperate latitudes.

4.1. Seasonal regime of urbanized and agricultural streams

The seasonal regime of urban and agricultural watersheds results in distinct hydrological and biogeochemical cycles which are likely to shape ecosystem structure and function. Our results were consistent with physiochemical trends previously reported in urbanized and agricultural stream ecosystems (Allan, 2004; Walsh, 2005), however understanding the regimes of these impacts in relation to one another remains underexplored. Although both urbanization and agriculture impact hydrology, urban streams appeared to be more decoupled from natural seasonal water cycles. Urbanized streams had higher, more consistent flows throughout the year while agricultural streams had lower flows and a seasonal pattern that peaked in the spring and was lowest in the summer. Moving from spring to summer, high TDP levels were seen in both stream types and lasted until fall for urbanized streams and winter for agricultural streams. The low summer flows of agricultural streams to farm fields may allow TDP to accumulate and become

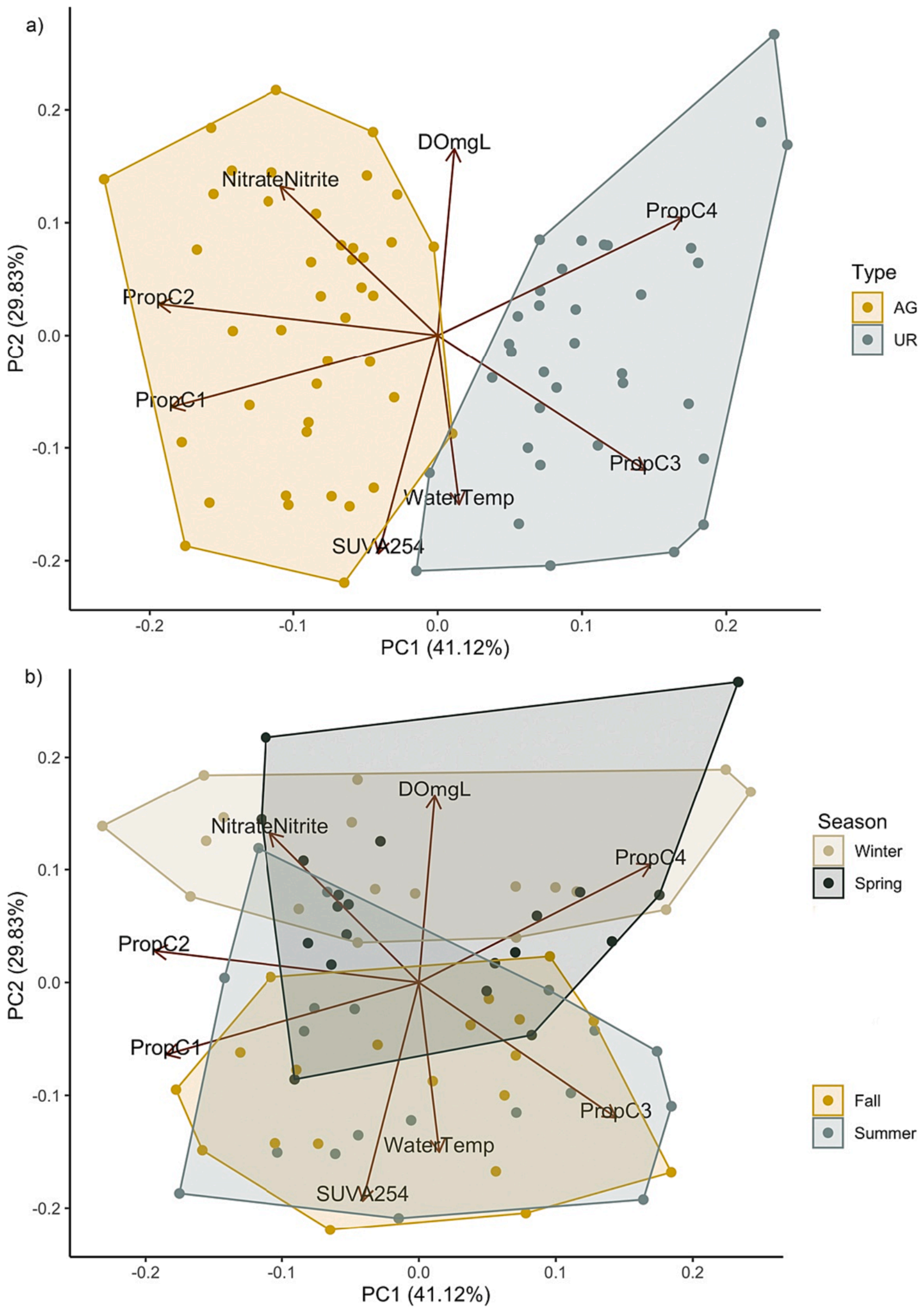

Fig. 5. Principle component analysis (PCA) reveals (a) separation between urban and agricultural land use is driven by changes in DOM components, and, (b) little separation by season based on 77 observations. Collinear variables removed to reduce dimensionality. Each dot represents an observation at a single site and timepoint. Environmental variables, DOM optical parameters, and decomposition rates included in analysis. PC1 and PC2 explain 42.45% and 30.54% of the variation, respectively. Observations grouped by land use type (a) and season (b).

more concentrated prior to being flushed out with elevated flows in the cold seasons. The growing season of agricultural lands also appeared to be reflected in DOC concentrations, where the warm seasons of summer and fall marked a distinct seasonal increase that was not seen in urban streams.

Our data also showed water quality changes during the winter, a season where data are typically limited and many questions remain (Sutton et al., 2021). Conductivity levels increased in all streams in the winter, but peaks were significantly higher in urbanized streams likely due to road salt application and the weathering of concrete infrastructure (Kaushal et al., 2018; Lawson and Jackson, 2021). This is problematic because high levels of dissolved salt pollution and rising alkalinity can increase the amount of ammonium (NH_4^+) converted to ammonia (NH_3), which we observed, and is highly toxic for fish and benthic macroinvertebrates especially in less complex habitats (Liu et al., 2022). Other nitrogen species such as nitrate-nitrite were elevated in the winter and also spring for all streams but levels were significantly higher in agricultural streams. The application of nitrogen-rich fertilizers to cropland in the fall is a common practice in Canada, and multi-region assessments show 47–94 % overwinter losses from the soil to the surrounding environment which may contribute to the high concentrations we observed (Chantigny et al., 2019).

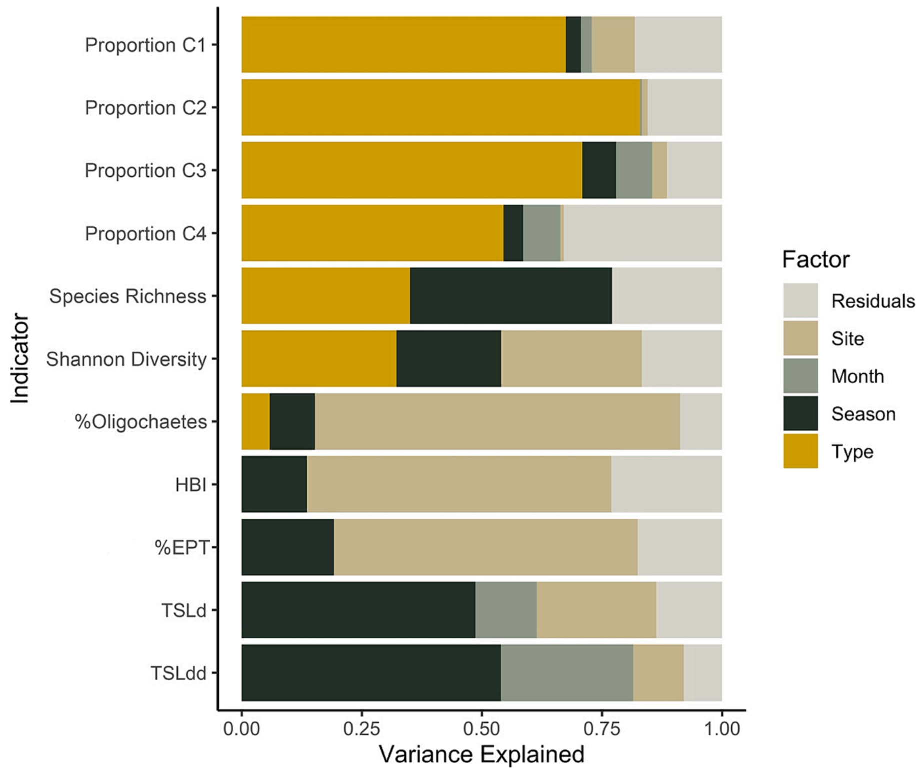

Fig. 6. Indicator variance explained by land use and seasonal gradients confirms sensitivity of PARAFAC components to land use. Variance explained by land use and season based on linear mixed effects models. Variables transformed to improve normality. TSLd, TSLdd = tensile strength loss per day (d) and degree-day (dd); HBI = Hilsenhoff biotic index; EPT = Ephemeroptera, Plecoptera, Trichoptera.

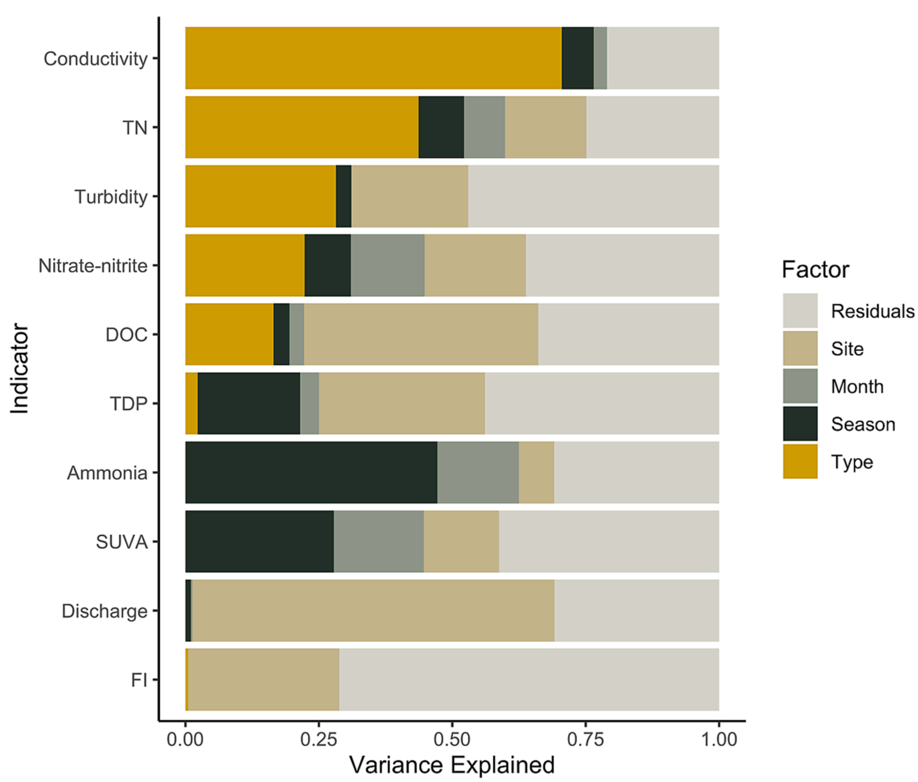

Fig. 7. Water quality indicator variance explained by land use and seasonal gradients highlights sensitivity of conductivity to land use. Variance explained by land use and season based on linear mixed effects models. Variables transformed to improve normality.

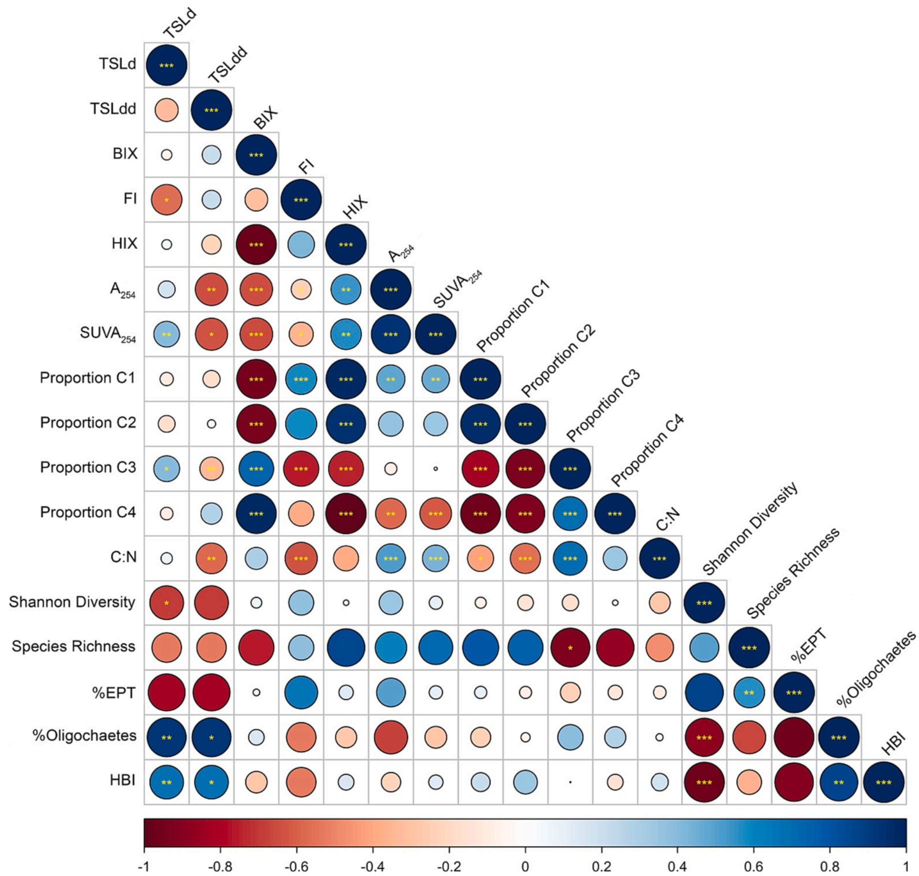

Fig. 8. Spearman correlation plot of carbon indicators. Spearman correlations between optical DOM parameters, decomposition rates, and invertebrate metrics. Correlations involving invertebrate metrics use a reduced dataset (Oct 2020 and Apr 2021), all other correlations use complete observations from Oct 2020-Oct 2021). Circle colour and size corresponds to the spearman correlation coefficient. Asterisks denote p values – ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.

4.2. Cotton strips and macroinvertebrates as indicators of climate and biotic integrity

Although decomposition was strongly affected by season, we did not find that it was affected by land use. Both urbanized and agricultural streams had high TSL_d rates in summer and low rates in the winter. It is not surprising that decomposition rates were higher in the warmer months when temperature and biological activity peak (Duarte et al., 2016). TSL_{dd} rates, which are considered temperature-normalized, were significantly higher in winter suggesting that even though decomposition as a biological process may slow down in cold weather, abiotic processes are still contributing to the breakdown of organic matter. The positive correlations between both TSL_d and TSL_{dd} to discharge highlight the importance of hydrology as one such factor contributing to decomposition rates independent of seasonality. The role of nutrient concentrations on decomposition rates was less clear; although TSL_d correlated with dissolved phosphorus, and TSL_{dd} with dissolved nitrogen species, this may simply be because they share the same seasonal pattern. Decomposition in temperate regions is stimulated by nutrient enrichment, but in impacted streams with chronic eutrophication, rates are likely increased compared to reference conditions but ultimately reach a plateau, unable to be increased any further due to the lack of N and P limitation (Ferreira et al., 2014).

Our data suggest that the cotton strip assay may not be able to discriminate between urbanization and agriculture but may instead be a better indicator of overall ecological integrity and shifts away from reference conditions. While a recent systematic review described the overall relationship between cotton strip decomposition and anthropogenic disturbance as weak (Ferreira et al., 2020), the sensitivity of cotton strips to human impacts and modification of waterways is likely dependent on the scale and type of impact. Reference streams generally have lower decomposition rates than impacted streams (Clapcott et al., 2012; Imberger et al., 2010, and comparing our results to pristine conditions in our region of southern Ontario (Webb et al., 2019) shows that decomposition rates were over three times higher in our study. In this example where the scale of impact is large (ie. pristine to over 80 % disturbance), the cotton strip assay may be useful in defining such a gradient. However, in comparing highly disturbed catchments that have similar ecological quality the cotton strip assay is less useful. We hypothesize that urbanization and agriculture exert different combinations of stressors: in urban streams the generally higher flows and lower nutrient concentrations might result in similar decomposition as agricultural streams where flows are lower but nutrient concentrations are higher. The integration of multiple stressors is part of the appeal of functional indicators like cotton strip decomposition (Palmer and Febria, 2012), however it also makes changes in decomposition rates more difficult to predict and explain without further research into the combinations of synergistic and antagonistic effects responsible (Ferreira et al., 2020).

When assessing ecological integrity, the sampling of benthic macroinvertebrate communities has been one of the most widely implemented biomonitoring techniques due to their variable tolerance to disturbance and organic pollution (Hilsenhoff, 1982). In our study, invertebrate indicators were most responsive to climate and biotic integrity, and only weakly linked to land use. Species richness and diversity were higher in the fall than in spring, possibly because of the habitat instability that comes with spring melts and floods (Death and Winterbourn, 1995) as well as the high levels of ammonia we observed. Land use only had significant effects on species richness but contributed to over a quarter of the variance in both richness and diversity, which were both higher in agricultural streams. The differences in species richness and diversity could reflect higher levels of habitat heterogeneity in agricultural streams, however the absence of land use differences in biotic index scores and percentage of Oligochaetes supports our hypothesis that urban and agricultural streams are similarly degraded. Correlations between high cotton strip decomposition rates and invertebrate indicators of decreased biotic integrity such as low biodiversity, poor biotic index scores, and increased proportion of Oligochaetes supports our conclusion that cotton strips are also effective in measuring overall ecological condition. The trend towards simpler invertebrate communities and more tolerant taxa in human impacted streams is well documented and consistent with our observations (Barrett et al., 2021; Walsh et al., 2005).

4.3. DOM quality is sensitive to land use changes

The incorporation of DOM into management strategies in human impacted landscapes has been suggested in previous studies (Stanley et al. 2012; O'Brien et al. 2017) but has had limited exploration in the Laurentian Great Lakes (*Nayaano-nibiimaang Gichigamin*) basin and in particular where our study was situated in the Lake Erie basin. Whereas cotton strip and benthic macroinvertebrate indicators revealed comparable impacts from urbanized and agricultural land use changes, our data show that DOM signatures were uniquely sensitive to differences between agricultural and urbanized streams and were consistent over month and season. Only $SUVA_{254}$ had a significant seasonal pattern, peaking in the fall and likely reflecting the large inputs of plant litter that occur during that time. Agricultural streams had higher terrestrial humic-like DOM content than urban streams, reflected in high HIX values and proportions of C1 and C2. The urbanized streams in this study were all lined with concrete for a portion of their reaches, limiting hydrologic connectivity to the riparian and hyporheic zones, possibly explaining their low humic content. Humic DOM is exported to agricultural streams from plant and soil organic matter in the surrounding farm fields, driven mostly by climate and hydrology including tile drainage (Dalzell et al., 2007). In contrast, urbanized streams had higher autochthonous DOM than agricultural streams. Both urbanized (Walsh et al., 2005) and agricultural streams (Allan, 2004; Wilson and Xenopoulos, 2009) are known to have high algal biomass. Yet, in our study levels of microbial DOM—components C3 and C4 and BIX—indicative of algal productivity were highest in urbanized streams. Turbidity and light-absorbing aromatic DOM levels (e.g., Components C1 and C2 and $SUVA_{254}$) were higher in agricultural streams, likely limiting the levels of algal productivity possible in agricultural compared to urbanized streams. Previous investigations have shown that stream DOM pools shift from more humic, terrestrially derived carbon in least impacted reference streams to more labile, microbially produced carbon in urbanized (Hosen et al., 2014) and agricultural streams (Williams et al., 2010; Wilson and Xenopoulos, 2009). Our data are consistent with these observations and add that when considering both land uses, DOM composition in urbanized streams appears to be shifted furthest away from reference conditions towards a microbially dominant composition. The temporal stability of land use influences on DOM quality highlights the chronic stress of catchment-scale urban and agricultural impacts on stream ecosystems with implications for microbial carbon cycling and stream greenhouse gas emissions (Hosen et al., 2014; Williams et al., 2010).

4.4. Assessment of highly modified streams and implications for restoration

Intensely farmed and urbanized areas have historically resulted in highly modified headwater streams with drainage prioritized at the expense of ecological condition (Elmore and Kaushal, 2008). There were no suitable reference streams in our study region (*Waawiyaatanong*), where 92 % of the extensive historical prairie, forest, and wetland cover has been cleared and drained to date (ERCA, 2015). There is a historical legacy of the region having rich freshwater resources and supporting high levels of biodiversity (H. Belden & Co., 1881), thus in a restoration context, the streams in this study might be considered examples of a negative reference to "move away from" (Palmer et al., 2005). Although there were no positive reference streams included in this study, given the comparisons to less impacted streams explored in the previous sections we can hypothesize that effective restoration might lower decomposition rates, improve macroinvertebrate richness, diversity, and biotic index scores, as well as decrease the proportion of microbially derived DOM. Evidence of agreement between these measures in the form of significant correlations supports this hypothesis.

One key reason for biomonitoring and the selection of indicators is to assess the health of freshwater ecosystems in terms of decline but also recovery. While the application of indicators used vary widely across streams and rivers, there remains a lack of consensus on what suite of metrics best capture ecosystem recovery (Palmer and Febria, 2012). Our study offers promising insight into how DOM quality may offer a cost-effective approach that is sensitive to watershed-scale changes including not just human impacts but also restoration and stewardship activities. DOM quality was the only indicator able to differentiate between urban and agricultural land uses, any time of the year, and there is potential for the proportion of microbial humic-like DOM to indicate biotic integrity. Some of the most common stream restoration activities include riparian planting and floodplain reconnection (Bernhardt et al., 2005; Craig et al., 2008), which we hypothesize would increase the proportion of terrestrial, non-anthropogenic, humic-like DOM upon restoration success.

In contrast to the sampling of benthic macroinvertebrates or cotton strip deployment, field sampling for EEM-PARAFAC analysis is more practical and simply requires a grab water sample that is filtered and stored until lab processing. Benthic macroinvertebrate sampling requires a large time investment as well as expertise needed to sort and identify animals to a taxanomic resolution suitable for analysis (typically family), which translates to a large cost per sample when outsourcing. Cotton strip assays, while seeming to be the simplest, also requires a large time investment to create all the strips to standardized specifications. Other challenges with the cotton strip assay were the risk of vandalization, needing to adjust deployment length (days) to achieve around 50 % decomposition for each individual stream, and the additional field days needed to retrieve the strips that were deployed. Tensiometers are not standard analytical lab equipment, thus processing likely cannot be outsourced easily which may pose a challenge to moniotoring and bioassessment done in non-academic settings (community groups, citizen science), where the equipment may be more costly to purchase outright.

4.5. Conclusions

Interrogating ecosystems responses across a human-impact gradient is critical in the Anthropocene, and overall, this study furthered our goals to collect baseline integrative ecosystem assessment data for three carbon-based indicators: decomposition, DOM composition, and benthic macroinvertebrates. The combination of structural and functional indicators used gave us a dynamic look at how these ecosystems behave and change over time. Our results also supported our hypotheses and confirmed that the urban and agricultural watersheds in our study region were typical of those seen around the world. The indicator best suited to further investigating anthropogenic impacts is DOM composition, which was highly sensitive to land use stressors and showed distinct urban and agricultural signatures. The four component EEM-PARAFAC model was able to integrate insights from the entire suite of indicators used. In particular, the proportion of microbial humic-like component C3 was correlated to changes in biotic integrity indicated by decomposition rates and macroinvertebrates, and this study provides the first evidence of its kind to link all three. Ease of sampling, seasonal stability, and a more immediate response to shifts in land use compared to invertebrate communities (which may lag; Barrett et al., 2021), makes DOM composition well suited to monitoring impacts as well as restoration successes. Future research could expand on this study to include a broader study area, to confirm patterns across regional geographic and land use differences. Given the lack of consensus or evidence base for ecosystem indicators in support of ecosystem health or recovery (Palmer and Febria, 2012), the relationship between stream DOM quality, ecosystem health, and restoration actions should be interrogated further across different scales and contexts.

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CRediT authorship contribution statement

S. Nolan: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition, Project administration. **A.A. Frazao:** Conceptualization, Methodology, Investigation, Writing – review & editing. **J.D. Hosen:** Methodology, Software, Writing – review & editing. **C.M. Febria:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110991.

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