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Hierarchical Variation in Cellulose Decomposition Across Southern Ontario Reference Streams

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Graduate Program in Geography
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Abstract

The cotton strip assay (CSA) has been promoted as a method of incorporating functional indicators into standardized river assessments by study of organic-matter decomposition. However, operationalization of the CSA as an indicator requires testing the spatial and temporal controls of variance associated with decomposition of cotton strips at regional reference sites. I conducted a hierarchically structured study of decomposition rates in Ontario, Canada. Cotton strips were deployed during the spring, summer and fall in pool and riffle habitats of 22 streams located in three distinct physiographic regions. Partitioning of variation among hierarchical scales associated with rates of decomposition were examined using nested ANOVAs, and comparisons of regional, habitat and seasonal differences were studied using a linear mixed effects model (LMEM). A partial least squares (PLS) regression analysis was performed to identify environmental variables associated with decomposition patterns. I found that variance associated with each successive spatial scale was seasonally dependent, and that temperature was the source of the majority of seasonal distribution of variance. I also found that temperature was be the primary environmental controls of decomposition. By quantifying the natural heterogeneity in decomposition rates, this study will inform biomonitoring practices, enabling progress towards inclusion of the CSA in regional monitoring programs.

Keywords

Decomposition, Cotton Strip Assay, Functional Indicators, Stream Biomonitoring, Reference Condition Approach

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Chapter 1

1 Introduction

1.1 Stream Biomonitoring

Aquatic ecosystem monitoring is an essential component in the assessment of ecosystem health and overall ecological integrity. Biological monitoring is the process of assessing both ecosystem and environmental condition through the use of biological indicators (Jones et al. 2007). The primary concerns regarding the use of bioindicators is the need for acute sensitivity to anthropogenic sources of stress, but to also remain stable in the presence of natural variation over time (Gessner and Chauvet 2002). In addition, to obtain relevant information to track indicator response and improve management decisions, the selection of appropriate indicators for region-specific stressors of interest is required (Young and Collier 2009, Reece and Richardson 1999).

Monitoring programs have typically relied upon structural metrics, which include measures of biological community (e.g. fish, phytoplankton, and invertebrate communities), as well as physiochemical attributes (e.g. nutrients, water chemistry, and channel morphology) (Casotti et al. 2015, Gray et al. 2014). For example, benthic invertebrates are frequently used in biomonitoring practices as a bioindicator because they are relatively stationary and complete their lifecycles within a localized area (Reece and Richardson 1999, Jones et al. 2007). The single point-in-time measurements of benthic invertebrates can then be studied through the use of biotic indices and predictive models to identify compositional changes in the biotic community (Reece and Richardson 1999). As such, their taxonomic community composition can provide information on how changes in the catchment are

impacting the aquatic ecosystem.

The limitations associated with structural indicators can be addressed by incorporating metrics of ecological function into routine stream biomonitoring practices. The definition of ‘function’ used here refers to the rates of biophysical processes taking place within the stream ecosystem (e.g. biogeochemical nutrient cycling, whole-stream metabolism, and organic matter decomposition) (Woodward et al. 2012). The advantage of utilizing ecosystem function is that their measurements are not static, like that of structural metrics, but instead incorporate environmental conditions over time (Feio et al., 2010). In addition to their inherent integration of time, functional indicator methodologies are hypothesized to be more broadly applicable in their implementation as a biomonitoring tool, because they are not expected to be constrained by community taxonomy and biogeography (Friberg et al., 2011, Woodward and Hildrew, 2002). The broad applicability of functional measures could thus allow for various stressor effects and interactions on fundamental patterns and processes to be examined.

Functional indicators are useful in detecting responses in lower trophic organisms, like bacteria and fungi, which are not normally monitored (Sandin et al., 2009). In effect, functional indicators can serve as an early warning to a wide range of disturbances within the catchment (Young et al., 2009). Functional indicators act as an early warning by means of drawing attention to changes in stream condition prior to any distinct shift in primary consumers (i.e. benthic invertebrates) and/or higher trophic level organisms (Sandin and Solimini, 2009). Although attributes of stream function may provide information on the change of stream state earlier than that of structural measures, the relationship between the two is not entirely clear (Bunn et al. 1999). By implementing functional metrics as a

complement to structural metrics in biomonitoring practices, we will be better equipped to recognize how these attributes are related and intertwined.

1.2 Decomposition

In aquatic environments, the process of decomposition is an essential ecosystem service because of the integral role it plays in nutrient cycling (an ecosystem supporting service). The detritivorous community in stream ecosystems is supported by a variety of dead organic material, particularly seasonal inputs of terrestrial leaf litter (Graca et al. 2015). The cycling of nutrient through decomposition is dependent upon abundance, diversity, and feeding activity of heterotrophic consumers (Woodward et al. 2012). The process of decomposition is therefore susceptible to a range of environmental factors through the indirect effects novel stressors can have on the detritivore community.

Organic matter (OM) decomposition has been proposed as a functional indicator for providing an integrated measure of stream ecological integrity (Lecerf et al. 2006, Gessner and Chauvet 2002, Jackson et al. 2016). Decomposition (i.e. the mineralization of organic compounds) in stream ecosystems is initiated with the breakdown of OM, through the combination of solute leaching, microbial conditioning, shredder detritivores feeding and activity, as well as physical abrasion of material (Graça et al. 2015, Abelho 2001). Energy subsidies in the form of allochthonous OM play an integral role in the functioning of shaded headwater streams, where primary production is limited (Pringle et al., 1988, Gregory et al. 1991). Moreover, previous studies have found the process of OM breakdown to be sensitive to a range of stressors associated with anthropogenic activities, including nutrients, temperature and riparian modification (Feio et al. 2010, Palmer and Febria 2012,

Young and Collier 2009, Hagen, Webster and Benfield 2006). It is therefore evident that an array of factors serve to influence the rate at which material is broken down, thus highlighting its potential sensitivity for detecting change in environmental conditions (Ferreira et al. 2015, Jackson et al. 2016).

Decomposition has traditionally been studied using the leaf-litter assay, whereby desiccated leaves of local species are gathered into mesh bags and placed in the stream (Boulton and Boon 1991). However, adopting leaf packs for use in decomposition biomonitoring is hampered by a lack of standardization in leaf litter composition and quality (Tiegs et al. 2013, Fritz et al. 2011). In place of leaf packs, the cotton strip assay (CSA) has been proposed as a solution for monitoring OM decomposition in streams (Slocum, Roberts and Mendelssohn 2009, Tiegs et al. 2007). Artist's canvas has been recommended as the preferred cotton substrate for CSA as it is composed of primarily cellulose (>95%); a carbon-based compound in natural detrital OM (Latter and Walton 1988, Slocum et al. 2009). The decomposition rate of the cotton fabric, studied through the loss of tensile strength, tends to be faster than that of leaf litter thereby increasing its effectiveness by reducing the deployment time necessary to achieve results (Tiegs et al. 2013). The CSA also captures the same environmental controls as that of the leaf litter assay but does so in a more effective manner (Jackson et al. 2016, Tiegs et al. 2007). The next steps towards taking this potential standardized field metric, and operationalizing the CSA as a biomonitoring tool, requires field-testing to determine spatial and temporal parameters surrounding this biomonitoring tool.

1.3 Reference Condition Approach

Initially described by Hughes et al. (1986), the reference condition approach (RCA) to biomonitoring aims to encapsulate the range of natural variability inherent to a given bioindicator at least disturbed or minimally disturbed reference sites (Bailey et al., 2004). The RCA requires the quantification of biological attributes from minimally disturbed stream sites spanning a range of climatic, geological and hydrological properties (Reynoldson et al. 1997). In conjunction with grouping of reference sites based upon the properties of the indicator, environmental predictor variables are used to separate sites based upon their physiochemical properties to create reference condition groups (Reece and Richardson, 1999). By defining the properties of a given region, the RCA can be utilized as an objective benchmark to distinguish biological conditions at test sites that have been impacted by various stressors from comparable regional reference sites (Bunn and Davies 2000, Friberg et al. 2009). However, for the RCA to be implemented effectively, the natural variability of a given indicator must be small enough as to pinpoint deviation in biological status at test sites from reference conditions.

1.4 Hierarchical and Seasonal controls of Decomposition

A critical constraint on the development of decomposition as a biomonitoring tool is the limited knowledge on the natural variability of decomposition, thus hindering the development of decomposition reference conditions (Tiegs et al., 2009). In order to determine the sources of natural variability on decomposition, and better describe their influence, the drivers of decomposition can be viewed as a hierarchy of spatial subsystems; region, watershed, reach, and habitat (Fig. 1) (sensu Hawkins et al. 1993). The nested hierarchical view of

streams is based upon the premise that the overarching larger scale governs the biophysical factors influencing the smaller scales (Frissell et al., 1986).

At the largest spatial extent, decomposition is governed by the underlying geology and regional climatic patterns (Graça et al. 2015). Together, these region-scaled parameters interact to influence broad geomorphic channel properties, water quality variables, and thermal regimes (Young et al. 2005, Irons et al. 1994, Wiley et al., 1997). Geology and physiography shape the channel and determine the stability of the stream banks and floodplain (Minshall, 1984; Lyons et al. 2000). Streams with high loads of fine sediment can limit decomposition rates if the decomposition substrate becomes covered by sediment (Benfield et al., 2001). The relationship between geology and stream processes are also linked by the effects it has on water chemistry properties like conductivity, alkalinity, and hardness (Wiley et al. 1997). Climatic temperature patterns, and stream thermal regimes are two closely coupled factors that underpin decomposition (Graça et al., 2015). Microbial decomposition rates are strongly influenced by temperature, whereby microbial activity increases with temperature, and thus they are likely to vary markedly across latitudinal and climatic gradients (Irons et al 1994; Graça et al 2015).

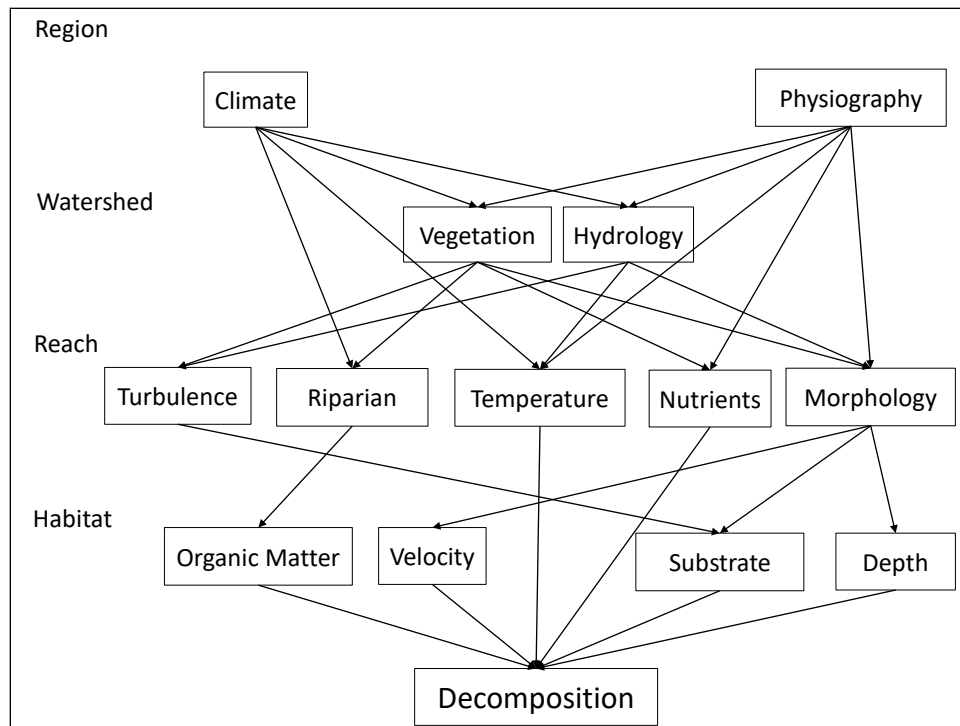


Figure 1. Conceptual model of the environmental controls on decomposition at each hierarchical spatial scale.

At the regional scale, differences in temperature reflect differences found in decomposition rates, however, within regions, temperature differences among streams are often minimal (Chauvet et al 2016). As such, there is less variability among streams within one region, than among streams in several combined regions. For example, Tieg et al. (2009) examined decomposition rates in a geologically uniform region of the Black Forest (Germany) with minimal human impacts. They found that within regions of relatively homogenous geology, decomposition rates were highly consistent among watersheds (Tieg et al., 2009). Habitats within watersheds represent localized conditions of alternating pool-riffle patterns formed by small-scale variation in substrate, depth, slope,

and velocity (Rabeni & Minshall 1977; Minshall 1984; Hawkins et al. 1993). The distinct ecological habitats provided by pools and riffles have been found to be the source of heterogeneity in decomposition within small headwater streams due the effects of variability in localized microbial activity (Clapcott and Barmuta, 2010). For decomposition to be utilized as a functional indicator, these distal and proximate sources of natural variation need to be established (Sandin and Solimini 2009).

The advantage of quantifying spatial heterogeneity with a nested hierarchical design is that controls of decomposition at each scale can be recognized, and thus linkages between ecosystem processes and the scale of influence can be established (Graça et al., 2015). In turn, scale-specific patterns and processes can be meaningfully ascribed to the drivers of both function and structure (Tiegs et al., 2009). However, in addition to taking account of the hierarchical spatial aspect to biomonitoring tools, the temporal heterogeneity of stream ecosystem processes requires explicit consideration when defining reference conditions (Economou et al. 2002; Reece and Richardson, 1999).

Temperate regions have distinct seasonal patterns, thereby making the choice of appropriate temporal scale necessary for the development of any biomonitoring tool, but temporal variability can be often neglected (Boulton 1999, Bunn and Davies 2000). Inter-annually, temperate regions are governed by seasonal hydrologic and temperature regimes, resulting in predictable timing and nature of OM subsidies to streams and the associated breakdown rates (Graça et al. 2015, Abelho 2001). The shifting patterns of stream discharge and temperature create seasonal shifts in resource availability for stream biota (Power et al. 1988). In these areas, broad seasonal patterns in temperate climates differ slightly still across latitudinal gradients (Chapman 1966). Flow variation between seasons

also results in the creation of new microhabitats (Frissell et al. 1986). Reference conditions of decomposition would therefore be expected to vary among seasons in accordance with changes in environmental conditions (Allan and Johnson 1997). This introduces the need to define the season of sampling to be able to ascribe trends in reference conditions (Hawkins et al., 2010).

1.5 Applications to Biomonitoring

Determining the natural variability associated with temporal and spatial heterogeneity is necessary for a mechanistic understanding of the underlying abiotic and biotic processes governing both structure and function of stream ecosystems (Schneider, 2001). By examining the hierarchy of spatial relationships further knowledge can be generated regarding the interpretation of ecosystem patterns and processes (Frissell et al. 1986, Wiley et al., 1997). Furthermore, the use of the reference condition approach provides clear advantages in the implementation of a process-based biomonitoring tool, as it aims to establish benchmarks across spatial scales (Tiegs, Akinwole and Gessner 2009; Gessner and Chauvet, 2002). Establishing a set of decomposition regional reference conditions would further the formation of a comprehensive picture of the scale-dependent processes involved and can thereby aid in the movement towards inclusion of functional indicators into routine stream biomonitoring practices.

2 Research Objectives

The goal of my research study was to understand the role of spatial and temporal variation in rates of cellulose decomposition, as a means of progression towards incorporating functional indicators into current biomonitoring toolsets. To accomplish this goal, three questions were addressed by my study:

- 1) What is the distribution of variation in rates of cellulose decomposition among hierarchically organized spatial scales (region, catchment and habitat) of minimally disturbed reference streams? How does the distribution of variation change between seasons (i.e. spring, summer, fall)?
- 2) Are rates of cellulose decomposition in southern Ontario reference streams different

between riffle and pools, and among physiographic regions, and are these differences dependent upon season?

3) What are the environmental drivers associated with variation in the rate of cellulose decomposition in southern Ontario streams?

2.1 Hypotheses

It is expected that as spatial extent increases with each hierarchical scale, there will be subsequent increases in the sources of variability. As such, region will encapsulate the majority of variance associated with rates of cellulose decomposition, followed by the watershed and habitat scales. The pattern of increasing spatial extent explaining a larger portion of variance will be conserved across seasons, whereby the relative proportions will vary slightly across seasons, but general patterns will be maintained.

With respect to cellulose decomposition in southern Ontario streams, discrete differences in region-scale climatic and physiographic properties will result in differences in decomposition rates. The bedrock streams in the Algonquin Highlands will be the most different from the other two regions because of the interaction the streams have with bedrock geology. Decomposition will be slower in this region because of lower nutrient concentrations in the shallow acidic soils, in addition to the higher proportion of natural land cover present. The two southern regions will have more similar decomposition rates because of the interaction they both have with groundwater. The Norfolk Sand Plains (NSP) regions will have faster decomposition rates because of warmer temperatures in these southernmost streams. The sand-based stream substrate also promotes habitat from stream microbial communities in the lack of interstitial spaces, and thus colonization on

the cellulose substrate. The Dundalk Till Plains (DTP) region will have decomposition rates in between the range of the other two regions. This region has slightly colder water, than that of the sand plains regions, because of groundwater, thus inhibiting rates of decomposition, but still has a nutrient source from the soils in the area. At the habitat scale, pools will have slower decomposition rate than riffle due to increased velocity and turbulence in these habitats.

I predict there will be differences in decomposition among seasons, although these patterns will be consistent across regions. Summer will be the peak in decomposition rates, followed by spring and then fall. Spring will be faster than fall due to the effect of temperature in the fall inhibiting the decomposition processes. The presence of the previous season's leaf litter in the stream during the spring season further promotes decomposition due to the abundant nutrient sources within the warming spring season.

The two primary environmental variables involved in decomposition are stream nutrient concentrations and temperature. The role of the microbial community in decomposition is expected to respond to increases in nutrients and temperature, and as such increase the rate of decomposition.

3 Methods

3.1 Study Design and Site Selection

My study sought to identify the spatial and temporal controls of cellulose decomposition. To do so, a hierarchical study design was used to assess decomposition across four spatial scales in southern Ontario streams. The scales used in this study follow the hierarchical river sub-systems described by Frissel et al. (1986), to partition the variance associated with decomposition between landscapes (i.e. regions), between watersheds within those regions, amongst reaches, and finally between habitats (i.e. pools and riffles) within reaches (Fig 2). The additional component of seasonal variation in rates of decomposition was added to encapsulate the variability associated with temporal shifts in environmental conditions.

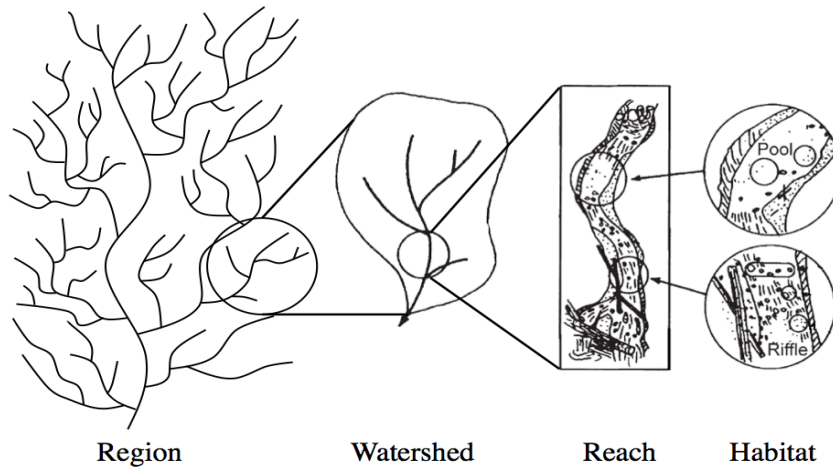


Figure 2. Schematic diagram of the hierarchical scales in this study, adapted from Frissells et al. (1986).

At the largest spatial extent, three physiographic regions were selected to represent landscape-scale differences (Table 1). The three physiographic regions were selected based upon their distinct surficial geology properties, over a discrete latitudinal gradient (Fig 3). The Norfolk Sand Plains (NSP) ($\sim 42^\circ$ latitude) region consists of low gradients streams with sand as the dominant substrate. These alkaline streams are underlain by limestone bedrock and are characterized by rapid soil infiltration rates, which maintain a high groundwater table throughout the year (Chapman 1966). The streams in the Dundalk Till Plains (DTP) ($\sim 44^\circ$ latitude) region have substrates with a mixture of sand, gravel and small pebbles. These streams are fed by groundwater recharge whereby the interaction of underlying sandstone and limestone geology maintains alkaline stream waters. The streams in the Algonquin Highland (AH) region ($\sim 45^\circ$ latitude) flow over felsic-igneous, granite and other Precambrian rock formations. Streams in the Algonquin Highland region differ from the other two, in that they are slightly acidic because of precipitation feeding the bedrock streams and the lack of carbonates in the soils (Chapman, 1966). Each of the three regions represents the selection of distinct stream ecosystem environments across Ontario,

and thereby serves to encompass a range of stream conditions representative of streams in southern Ontario.

Within each physiographic region, six to seven headwater (2nd to 3rd order) streams were selected. Catchments of the selected streams represented the least exposed conditions in terms of the amount of anthropogenic land cover in each region. This selection criteria reduced confounding effects of human activity and increased the relevance of our results for generation of reference condition based bioassessment protocols. As such, site selection focused on identifying catchments with the largest percentage of natural land cover (i.e. forest, scrubland and wetland) possible within the extensively developed regions of southern Ontario. Watersheds were initially selected based on visual assessment of satellite imagery to contain the highest percentage of natural land cover in the catchment and riparian corridor (30 m width) areas as possible. Once potential study sites were selected, ArcGIS 10.0 (ESRI 2010a) and Arc Hydro 2.0 package (ESRI, 2010b) was used to delineate their watersheds. Delineation was based on NASA's Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Digital Elevation Model (DEM) imagery (30 m resolution) and the National Hydro Network stream layer for Ontario (NASA, 2009; Natural Resources Canada, 2016). Selected watersheds were intersected with raster land cover data from Agriculture Canada inventory maps (30 m resolution, AAFC 2012) to determine the proportion of natural land cover using the Geospatial Modeling Environment (Beyeler 2013). The same process was used to obtain land cover data for the 30 m stream buffer area of each headwater watershed, and the riparian corridor extending 100 m upstream of the study site location. The aim of this process was to have a collection of streams with the greatest possible proportion of natural land cover in the

watershed and riparian areas, as well as having 100% natural land cover in the riparian corridor. The final study site selection depended upon accessibility, including distance to roadways, and landowner cooperation. The Dundalk Till Plains (DTP) and Norfolk Sand Plains (NSP) regions each had a total of six streams, and the Algonquin highlands (AH) regions had seven streams (Table 1).

Within the previously determined riparian corridor, the sampling reach was defined by containing distinct riffle and pool habitats in which the decomposition strips could be placed. The selection of the two locations was based upon the expectation that both pool and riffle habitats would have consistent flow throughout the duration of the study.

Table 1. Location (lat/long), elevation (m), area (km²), and land cover descriptions for each study sites and their associated catchments.

Physiography	Stream ID	Latitude	Longitude	Elevation (m)	Area (km ²)	Land Cover (%)		Land Cover in 30m Buffer (%)	
						Agriculture	Natural	Agriculture	Natural
Norfolk Sand Plains	NSP02	42 49 5.16	-80 22 47.07	231	2.54	34	65	3	97
	NSP03	42 44 42.07	-80 28 28.44	210	6.38	66	33	45	55
	NSP17	42 49 50.98	-80 23 24.90	232	4.91	44	55	52	47
	NSP20	42 43 23.94	-80 27 6.00	217	13.57	46	53	32	67
	NSP30	43 3 12.27	-80 22 16.89	247	8.76	43	55	7	92
	NSP12	42 39 56.05	-81 1 24.55	184	4.54	58	42	24	76
	Mean (sd)				220.2 (21.9)	6.78 (3.92)	48.5 (11.5)	50.5 (11.3)	27.2 (19.8)
Dundalk Till Plains	DTP02	44 1 15.43	-79 59 20.64	295	3.4	46	52	26	73
	DTP03	43 59 45.50	-79 59 36.44	334	1.67	38	59	25	74
	DTP08	44 10 15.58	-80 8 39.50	379	2.01	41	59	4	96
	DTP16	44 13 58.97	-80 0 1.88	250	6.72	14	86	8	92
	DTP19	44 12 0.54	-80 5 7.63	323	4.83	20	79	14	86
	DTP40	43 59 24.26	-80 1 27.74	316	1.68	38	62	13	87
	Mean (sd)				316.2 (42.8)	3.39 (2.05)	32.8 (12.8)	66.2 (13.3)	15.0 (8.9)
Algonquin Highlands	AH13	45 13 20.80	-78 54 54.59	326	4.25	0	93	0	81
	AH01	45 22 45.46	-79 8 33.67	340	0.75	0	99	0	100
	AH61	45 26 30.46	-79 7 8.87	335	3.31	0	92	0	100
	AH302	45 12 56.44	-79 1 45.77	331	2.72	0	99	0	100
	AH02	45 15 43.96	-79 5 21.22	330	6.76	0	99	0	99
	AH03	45 20 3.02	-79 6 41.91	308	4.63	2	96	2	98
	AH04	45 22 27.20	-79 8 38.09	332	1.04	1	97	0	100
	Mean (sd)				328.9 (10.2)	3.35 (2.10)	0.4 (0.8)	96.4 (2.9)	0.3 (0.8)

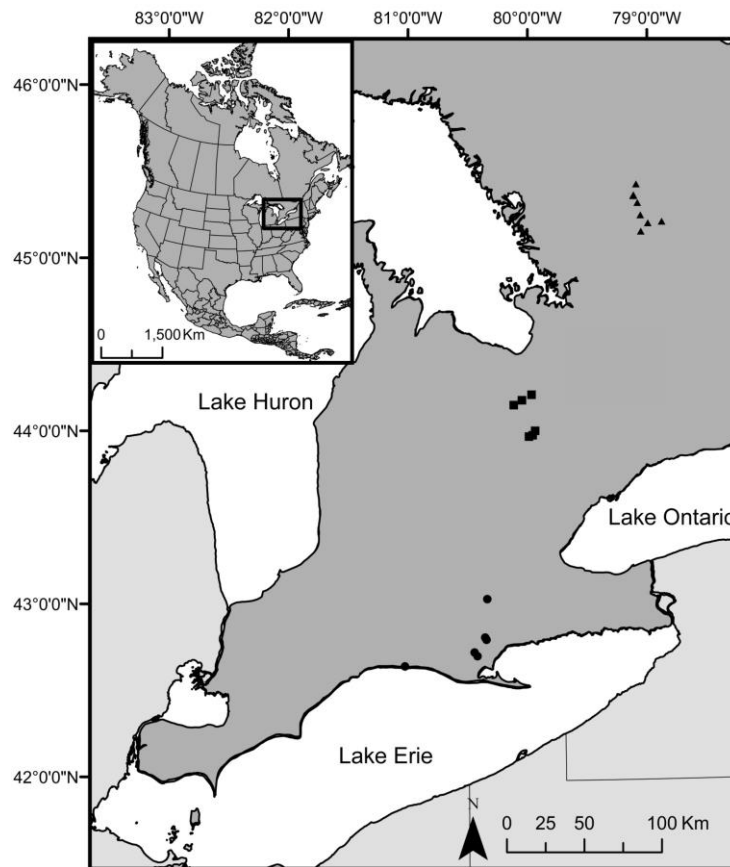


Figure 3. Map showing the location of study sites in the Norfolk Sand Plains (circles), Dundalk Till Plains (squares), and Algonquin Highlands (triangles)

3.2 Cotton Strip Assay

The preparation, deployment, retrieval and processing of the cotton strips followed Tiegs et al. (2013). Fredrix-brand unprimed 12-oz. heavyweight cotton fabric, Style #548 (Fredrix, Lawrenceville, GA, USA) was used as the cotton strip substrate (Tiegs et al. 2013, Slocum, Roberts and Mendelsohn 2009). The fabric was prepared by cutting the material to approximately 2.5 cm x 8 cm strips, with 3 mm frayed ‘fuzz’ along the length of the fabric strip.

In the field, five strips were attached with cable binders to a 1.5 m long chain. Chains were fastened to rebar that was driven into the streambed of pool and riffle habitats. Cotton strips were incubated in the study streams for 28 days in May (spring), August (summer) and November (fall) of 2017 for all regions but the NSP where strips were incubated for 21 days in the summer. The timeline of 28 days was the expected duration to produce an average tensile loss of 50%, which is the preferable percentage loss to provide sufficient information to differentiate the selected study sites, while still maintaining the integrity of the strips themselves (Tiegs et al. 2013).

Upon retrieval, strips were soaked in a tray containing a solution of at least 70% ethanol for 5-10 minutes, after which they were gently brushed off to remove built-up sediment and debris. Cleaned strips were laid flat and covered with folded aluminum foil and put on ice until returned to the lab. In the lab, the cotton strips were dried at 40°C for a minimum of 24 hours. Dried strips were stored in a desiccator until analysis of tensile strength.

Tensile strength (i.e. the force required to break the strip) of the strips was measured using a tensiometer and motorized test stand (Force Gauge, Model M3-100). The ends of each strip were placed in the grips (Mark-10 brand, Model #MG100) and were pulled at a fixed rate of 2 cm/min, until peak tension (lbF) was reached. To assess the overall percent tensile loss, the tensile loss of the treated strip was compared to that of a group of 50 reference strips. Reference strips underwent a mock field deployment by saturating the strip in distilled water, cleaning with 70% ethanol and drying for a minimum of 24 hours at 40°C. The sample tensile strength (measured as peak tension) for each strip was recorded and used to calculate tensile strength loss (%) per day (Eq. 1). To correct for among sample variations in temperature, degree-day was substituted for incubation time. Degree-day was

calculated as the sum of the mean daily temperatures for each incubation period (Benfield 2007).

Equation 1. Tensile Loss

$$\text{Tensile Loss (\%)} = \frac{\left(\frac{\text{Tensile Strength}_{\text{REF}} - \text{Tensile Strength}_{\text{TRT}}}{\text{Tensile Strength}_{\text{REF}}} \right) \times 100}{\text{Incubation Time}}$$

3.3 Site Characterization

Physical properties of each study site were characterized within a 50 m long reach upstream of the deployment location of the cotton strips. Within this reach five transects were established at 10 m intervals (Fig. 4). At each transect, the wetted width of the channel was measured perpendicular to the flow, and depth was measured at five equally spaced intervals. In addition, average velocity was measured by taking instantaneous velocity measurements at the three middle depth measurements of each transect using a stream velocity-meter (Swoffer Instruments, E-230-Model 2100). Riparian canopy cover was estimated at the first, third and fifth transects using a densiometer. Canopy cover measurements were taken facing upstream, downstream, left and right bank at each location, for a total of twelve measurements. The mean of the twelve measurements was calculated to represent overall reach conditions.

At each study site, temperature loggers (TidbiT v2) were placed near the streambed in erosional habitats by attaching the logger to a piece of rebar. Temperature loggers measured and recorded water temperature at 15 minutes intervals for the duration of each deployment. In addition, substrate characterization was completed for each reach using a pebble count of 100 substrate particles by walking the 50 m long reach area to collect

substrate. Substrates that could not be measured in the field (e.g. silt, sand and boulder) were given the median of the range as defined by Wolman (1954). Gravel, pebbles and cobbles were measured based upon the intermediate axis of each particle. The length of the intermediate axis was used to create a particle size distribution, from which the median particle size (D_{50}) for each stream was determined.

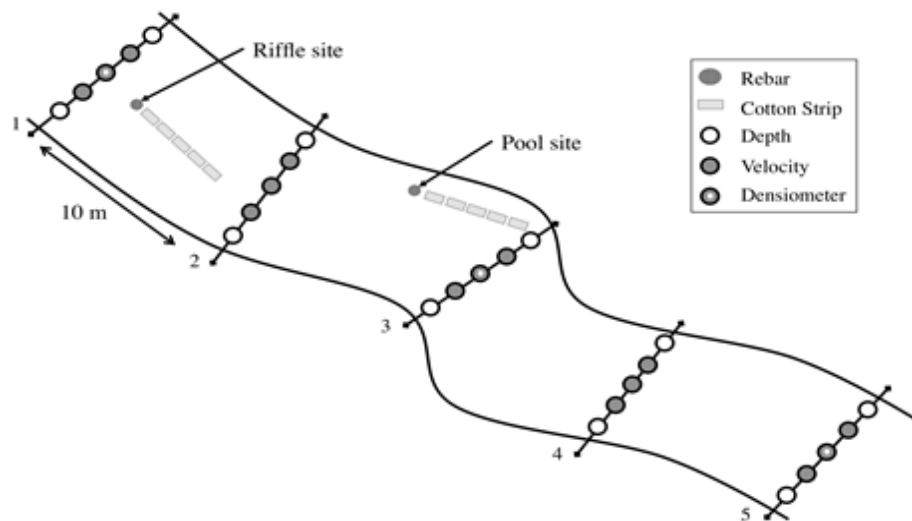


Figure 4. Example site diagram of study reach showing the placement of cotton strips within habitats, in relation to the distribution of transect throughout the reach.

Measurements of water physicochemical properties were collected to characterize water chemistry for each study reach. Specific conductivity ($\mu\text{S}/\text{cm}$) and pH were measured using a multi-meter (YSI, Professional Plus). Grab water samples were taken from a well-mixed, flowing area of the stream reach at 60% depth. A 1 L grab water sample was collected for analysis of total suspended solids. A 250 mL grab sample was also collected for analysis of nitrate-nitrite ($\text{NO}_3^- - \text{NO}_2^-$) and soluble reactive phosphorous (SRP); the dominant bioavailable nutrient forms. Water samples were stored at approximately 4°C in a cooler during field collection. Nutrient samples were kept frozen until sent to an external lab for analysis. At the habitat scale, measurements of instantaneous velocity (Swoffer

Instruments, E-230-Model 2100) over each individual cotton strip were taken to assess the effects of physical fragmentation within each habitat type.

Water samples were analyzed for nitrate-nitrite and soluble reactive phosphorous (NO_3^- - NO_2^- , SRP; ~ 0.25 L) using Flow Injection Analysis automated ion analyzer, whereby the detection limits were $2\mu\text{g/L}$ for NO_3^- - NO_2^- and $1\mu\text{g/L}$ for SRP (Lachat QuikChem, QC8500 FIA Automated Ion Analyzer)(AWWA, 2004).

In the lab, TSS was analyzed by filtering 1 L of distilled water through glass fiber filter paper (Whatman, 934-AH) using a vacuum filtration apparatus, and dried in the oven at 105°C over night. The blank filter papers were then ignited in a muffle furnace for 20 minutes to get the filter dry weight. After measuring for dry filter weight, 750 mL of sample water was filtered with the vacuum apparatus. The oven dried mass provided the value of total suspended solid (TSS; mg/L).

3.4 Data Analyses

Prior to analyses, tensile data were tested for normality, performed using MINITAB version 13.32, and were found to be suitable for applying parametric tests (MINITAB, 2000). A fully nested hierarchical model was used to partition the variance associated with tensile loss per day and tensile loss per degree-day into each successive hierarchical spatial scale. Habitats were nested within streams, and streams within regions. In the nested model, all spatial scales were set as random variables. Seasonal datasets were run separately to highlight the difference in variance distributions among and within seasons.

To assess for differences in daily percentage tensile loss and percentage tensile loss per degree-day, separate linear mixed effects models (LMEM) were used ($p < 0.05$). Habitats

were nested within streams, and streams within regions, with season being tested as an interaction with each nested scale. Season, region and habitat were set as fixed variables, and stream as a random variable.

General linear models were used on untransformed environmental data to summarize physiochemical properties, and to test for differences among regions by season ($p < 0.05$). When the region by season interaction was significant, GLM analyses were followed by Tukey's pairwise post-hoc tests ($p < 0.05$).

Partial least squares (PLS) regression was used to assess the importance of the environmental physiochemical parameters (e.g., pH, specific conductivity, SRP) on decomposition. PLS regression is a multivariate tool used to establish the structure of the relationships between predictor variables and their effect on response variables (Carrascal, Galván and Gordo 2009, Eriksson et al. 1999). The environmental predictor variables (X; \log_{10} and arcsine transformed) were used to produce a set of latent variables (i.e. PLS loadings) that best explain the variance in the daily tensile loss (Y; not transformed) through the simultaneous decomposition of X and Y matrices or vectors (Eriksson et al. 2006). This function works well where (i) the predictors are highly correlated (i.e. there is strong collinear relationship) and; (ii) the dataset has many predictor variables relative to observations (Carrascal et al. 2009).

Separate PLS analyses were conducted for tensile loss per day, and for the temperature corrected tensile loss per degree-day data. The cross-validated goodness of prediction (Q^2) defined as the difference between the predicted and observed values of each individual pass ($Q^2 < 0.097$) was calculated. The total explanatory capacity of the PLS models is given by

the sum of the explanatory capacity (R^2Y) of each component. Only those components that explained more than 10% of the variation of the dependent variable were included. The influence of each X-variable was evaluated by using variable importance on the projection (VIP) scores. Predictors with a VIP of more than one were considered the most relevant for explaining the dependent variable. For important scores the direction of association was determined by examining the loadings on the biplot. All statistical analyses were completed using Statistica 13.3.1 (TIBCO, 2017).

4 Results

Mean tensile loss among all 565 cotton strips was $1.70\% \text{ day}^{-1}$ ($\pm 1.11\% \text{ day}^{-1}$). The greatest tensile loss occurred during summer in a DTP stream ($4.67\% \text{ day}^{-1}$) and smallest occurred in fall, in an AH stream where no loss in tensile strength was detectable. The largest average percent tensile loss for a given season was in the NSP region during the summer ($3.11\% \text{ day}^{-1} \pm 0.82\% \text{ day}^{-1}$). Conversely, the lowest seasonal average was in the AH in the fall ($0.23\% \text{ day}^{-1} \pm 0.16\% \text{ day}^{-1}$). Overall, riffles had larger percent tensile loss ($1.93\% \text{ day}^{-1} \pm 1.09\% \text{ day}^{-1}$), than pools ($1.47\% \text{ day}^{-1} \pm 1.08\% \text{ day}^{-1}$).

4.1 Hierarchical variance partitioning

Results of the nested ANOVA variance components analysis showed that region, stream and habitat explained greater than 80% of the variance in tensile strength loss in all seasons for loss per day and loss per degree-day models (Fig. 5a, b). The distributions of relative variance among spatial scales were conserved between the tensile loss per day, and per degree-day models. However, the relative importance of the region and habitat scales varied among seasons. In the spring, habitat accounted for the largest portion of variance ($\sim 43\%$). Conversely, in the fall, habitat accounted for $\sim 2\%$ and region controlled over 60% of the variance. Variance was more evenly distributed (region $\approx 45\%$; habitat $\approx 25\%$) in summer. The stream scale maintained a consistent allocation of variance between 17 and 20%.

Absolute variance values of the nested ANOVA showed the same pattern of decreasing and increasing variability from spring through fall associated with the habitat and regional

scales, respectively (Fig. 5c). Moreover, assessment of absolute variance showed among season shifts in total variability in tensile loss, whereby the summer had the greatest variability (1.35), followed by the spring (1.04) and fall (1.02). In addition, absolute values emphasized the difference in degree of variability between the two response variables (i.e., tensile loss per day, and tensile loss per degree-day). The degree-day model had less total variance by two orders of magnitude, indicating that most of the variability associated with tensile loss was due to temperature (Fig 5d.).

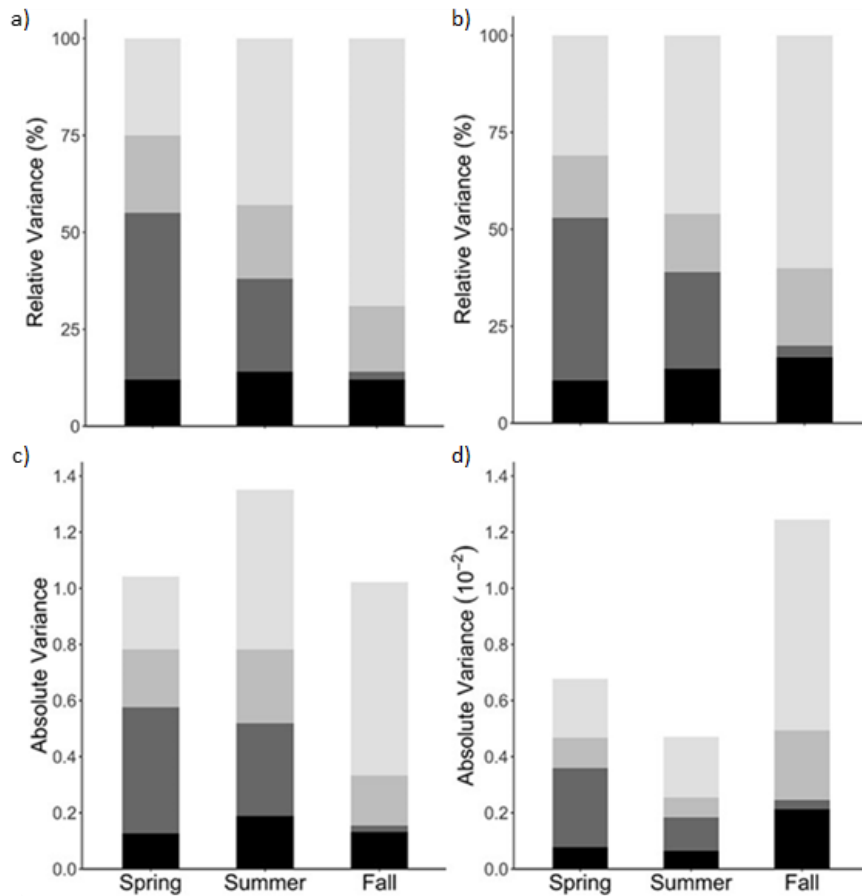


Figure 5. Distribution of variance among habitats (dark grey), nested within streams (medium grey), nested within regions (light grey) among seasons for percent tensile loss per day (a) and percent tensile loss per degree-day (b).

4.2 Seasonal, regional and habitat effects on decomposition

A general linear mixed effects model assessing spatio-temporal patterns of tensile loss per day found no interaction between regions and season ($F_{(4, 48)} = 0.41, p = 0.80$; Table 2). Percent tensile loss per day differed among regions ($F_{(2, 48)} = 29.59, p < 0.0001$), whereby the NSP region had the largest average percent tensile loss per day, followed by the DTP region, and the AH region (Fig 6a). Likewise, tensile loss differed among the three seasons with rates fastest in summer and slowest in fall ($F_{(2, 48)} = 20.45, p < 0.0001$). There was a significant interaction between habitat and region ($F_{(2, 51)} = 5.51, p = 0.007$; Table 2), in which only the NSP exhibited no difference between the two habitat types ($p = 0.483$). There was also a significant interaction between habitat and season ($F_{(2, 51)} = 3.56, p = 0.036$), with fall being the only season where no difference between habitat types was observed ($p = 0.259$).

The same general linear mixed effects model on tensile loss corrected for degree-days also found no interaction between season and region ($F_{(2, 48)} = 0.12, p = 0.88$; Table 3). However, there was also no differences between the three seasons ($F_{(2, 48)} = 0.12, p = 0.88$). A significant difference between regions ($F_{(2, 48)} = 34.25, p < 0.0001$; Fig. 6b) was still detected, with the same pattern of NSP having the largest percent tensile loss, followed by DTP and AH. Likewise, the interaction of region and habitat was significant ($F_{(2, 51)} = 6.92, p = 0.002$), where only the NSP resulted in no differences between the two habitat types ($p = 0.410$). However, an interaction between season and habitat was no longer detected. Habitat differences were significant in the degree-day model ($F_{(1, 51)} = 34.44, p < 0.0001$), indicating that riffles had greater rates of tensile loss than pools.

Table 2. Linear mixed effects model ANOVA table on percent tensile loss per day comparing seasonal differences in decomposition among regions, and seasonal differences between habitats

Source of Variation	Sum of squares	Numerator d.f.	Mean Squares	Denominator d.f.	F	<i>P</i>
Region	213.5	2	106.7	48	29.59	<0.001
Season	147.5	2	73.8	48	20.45	<0.001
Region x Season	5.8	4	1.5	48	0.41	0.80
Stream (Region x Season)	173.4	48	3.6	51	4.45	<0.001
Habitat	27.1	1	27.1	51	33.38	<0.001
Habitat x Region	8.9	2	4.5	51	5.51	0.007
Habitat x Season	5.8	2	2.9	51	3.56	0.036
Habitat x Stream (Region x Season)	41.4	51	0.8	452	5.45	<0.001

Table 3. Linear mixed effects model ANOVA table on percent tensile loss per degree-day among seasons with habitats nested within streams, and streams within regions

Source of Variation	Sum of squares	Numerator d.f.	Mean Squares	Denominator d.f.	F	<i>P</i>
Region	1.5	2	0.8	48	34.25	<0.001
Season	0.01	2	0.003	48	0.12	0.88
Region x Season	0.15	4	0.04	48	1.64	0.18
Stream (Region x Season)	1.1	48	0.23	51	4.96	<0.001
Habitat	0.16	1	0.16	51	35.44	<0.001
Habitat x Region	0.06	2	0.03	51	6.92	<0.001
Habitat x Season	0.02	2	0.01	51	2.18	0.12
Habitat x Stream (Region x Season)	0.23	51	0.005	452	3.86	<0.001

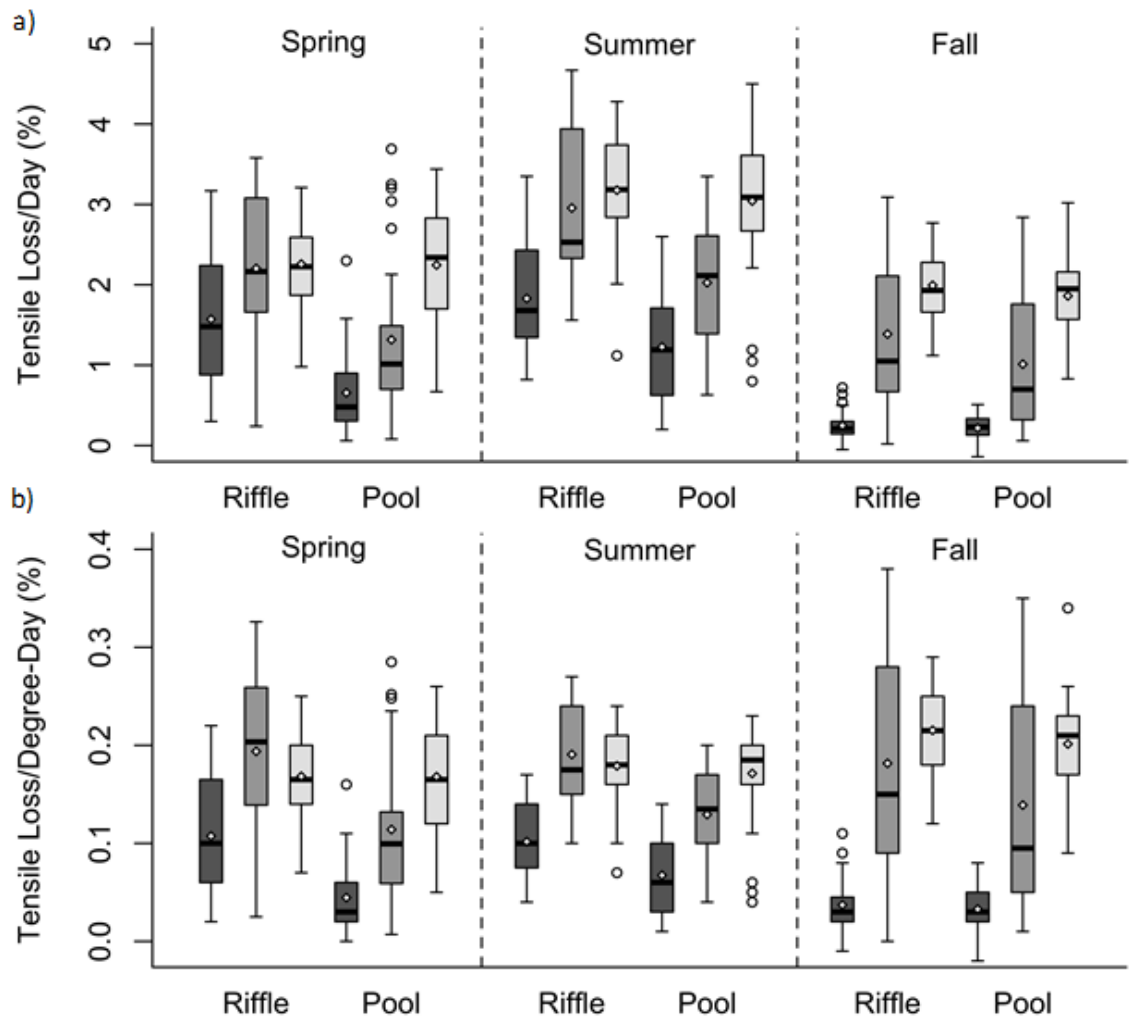


Figure 6. Boxplots summarizing tensile loss for pools and riffles among seasons for the Algonquin Highland region (dark gray), Dundalk Till Plains (medium gray), and Norfolk Sand Plains (light gray). Box plots show the median, interquartile range, and the 5th and 95th percentiles for a) percent tensile loss per day and b) percent tensile loss per degree-day. Mean denoted by the white diamond shapes

4.3 Stream environmental descriptors

A general linear model (GLM) showed that mean percentage natural land cover was significantly greater in the AH ($96.43\% \pm 2.79\%$) compared to the NSP ($50.50\% \pm 10.59\%$; $p < 0.0001$) and DTP ($66.17\% \pm 12.45\%$; $p < 0.0001$). Within the riparian buffer area, the

AH ($96.86\% \pm 6.67\%$) had higher percentage natural land cover than DTP ($84.67\% \pm 8.81\%$; $p < 0.0001$) and NSP ($72.33\% \pm 18.69\%$; $p < 0.0001$), though NSP and DTP did not differ ($p = 0.712$) (Fig 7a). The NSP and DTP did not differ in mean percentage of natural land cover in the catchment ($p = 0.14$) (Fig 7b). Similarly, AH had the largest D_{50} value (3.94 ± 3.71 cm), followed successively by DTP (1.74 ± 0.92 cm), although these two regions were not significantly different ($p = 0.139$). D_{50} values of AH and DTP were significantly larger ($p = 0.002$) than NSP (0.10 ± 0.0 cm) (Fig 8).

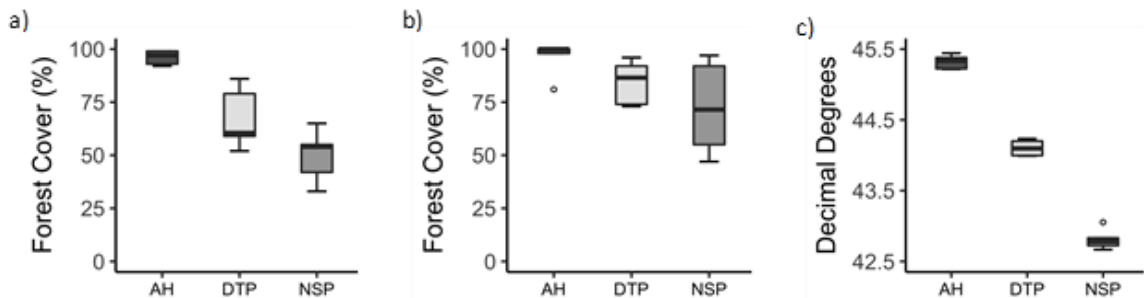


Figure 7. Regional scale variables for streams sampled in the Algonquin Highlands (AH), Dundalk Till Plains (DTP), and Norfolk Sand Plains (NSP) showing the land cover at the watershed (a) the 30m riparian buffer (b) scales, and distribution of sites across latitudes (c). Box plots show the median, interquartile range, and the 5th and 95th percentiles of the sampling event

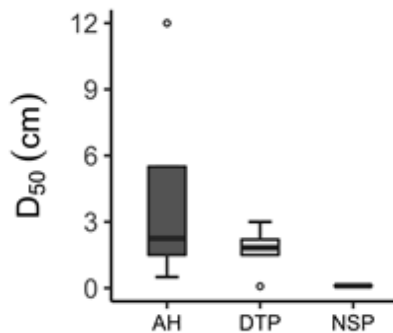


Figure 8. Distribution of D_{50} (cm) substrate characterization for streams sampled in the Algonquin Highlands (AH), Dundalk Till Plains (DTP), and Norfolk Sand Plains (NSP). Box plots show the median, interquartile range, and the 5th and 95th percentiles.

GLMs indicated there was no interaction between region and season for stream width ($p=0.67$) (Fig 9a), depth ($p=0.064$) (Fig 9b) or velocity ($p=0.315$) (Fig 9c). There were significant differences in width ($p=0.002$), depth ($p<0.0001$) and velocity ($p<0.0001$) among regions. Depth was the only property to differ seasonally ($p=0.013$). In contrast, there was a region by season interaction for stream water temperature ($p<0.0001$). In the spring, the DTP streams ($11.66 \pm 1.26^\circ\text{C}$) were colder than the AH streams ($15.25 \pm 1.59^\circ\text{C}$; $p<0.0001$). In the summer, DTP streams ($14.70 \pm 1.80^\circ\text{C}$) were colder than NSP ($17.10 \pm 1.70^\circ\text{C}$; $p=0.038$) and AH ($17.62 \pm 1.42^\circ\text{C}$; $p=0.003$) streams. However, in the fall the AH streams ($6.30 \pm 0.32^\circ\text{C}$) were colder than the NSP streams ($8.97 \pm 0.40^\circ\text{C}$; $p=0.009$) (Fig 9d).

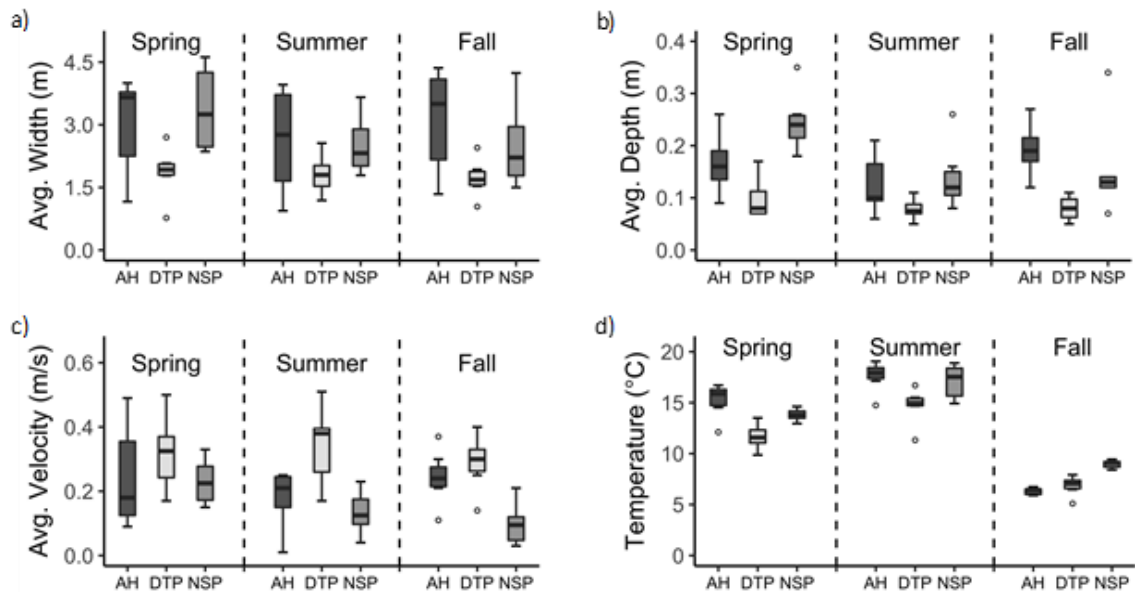


Figure 9. Stream physical properties measured during seasonal (i.e. spring, summer and fall) sampling events in the Algonquin Highlands (AH), Norfolk Sand Plains (NSP), and Dundalk Till Plains (DTP), including average channel width (a), average channel depth (b), average mid-channel velocity (c), and average temperature over deployment period (d). Box plots show the median, interquartile range, and the 5th and 95th percentiles of the sampling event.

A GLM revealed that there was no interaction of region and season among stream conductivities, but streams did differ by region ($p < 0.0001$). The AH streams had lower conductivities ($38.04 \pm 33.17 \mu\text{S/cm}$) compared to the NSP ($514.56 \pm 109.01 \mu\text{S/cm}$) and DTP ($508.28 \pm 83.72 \mu\text{S/cm}$) streams (Fig 10a). There was a region and season interaction for pH ($p < 0.0001$). In the spring, the AH streams (6.13 ± 0.21) had lower average pH than that of the NSP (7.88 ± 0.06 ; $p < 0.0001$) and DTP (8.22 ± 0.05 ; $p < 0.0001$). Similarly, in the summer the AH streams (6.71 ± 0.60) had a lower pH than the NSP (7.86 ± 0.27 ; $p < 0.0001$) and DTP and (7.94 ± 0.24 ; $p < 0.0001$). In the fall, the DTP streams (7.98 ± 0.10) pH differed from the AH (7.01 ± 0.53 ; $p < 0.0001$) and NSP (7.34 ± 0.26 ; $p = 0.029$) (Fig 10b). There was no interaction between region and season for TSS. In addition, there was no regional ($p = 0.25$) or seasonal ($p = 0.07$) differences in TSS (Fig 10).

Streams in the AH region had nutrient concentrations an order of magnitude smaller than the NSP and DTP streams. There was no region and season interaction for nitrate-nitrite concentration ($p < 0.615$). Regional differences were found, whereby average nitrate-nitrite concentrations in the AH ($67.48 \pm 42.36 \mu\text{g/L}$) were significantly lower than the NSP ($1843.67 \pm 1236.26 \mu\text{g/L}$; $p < 0.0001$) and DTP ($1096.94 \pm 543.92 \mu\text{g/L}$; $p < 0.0001$) (Fig 10c). SRP concentrations produced a region by season interaction ($p = 0.026$). Across all seasons, the NSP had higher SRP concentration than the AH region (Sp: $p < 0.0001$, Su: $p = 0.002$, F: $p < 0.0001$). However, DTP streams differed from the NSP in the spring ($p = 0.009$), and fall ($p < 0.0001$), while only differing from AH in the spring ($p = 0.002$) (Fig. 10d).

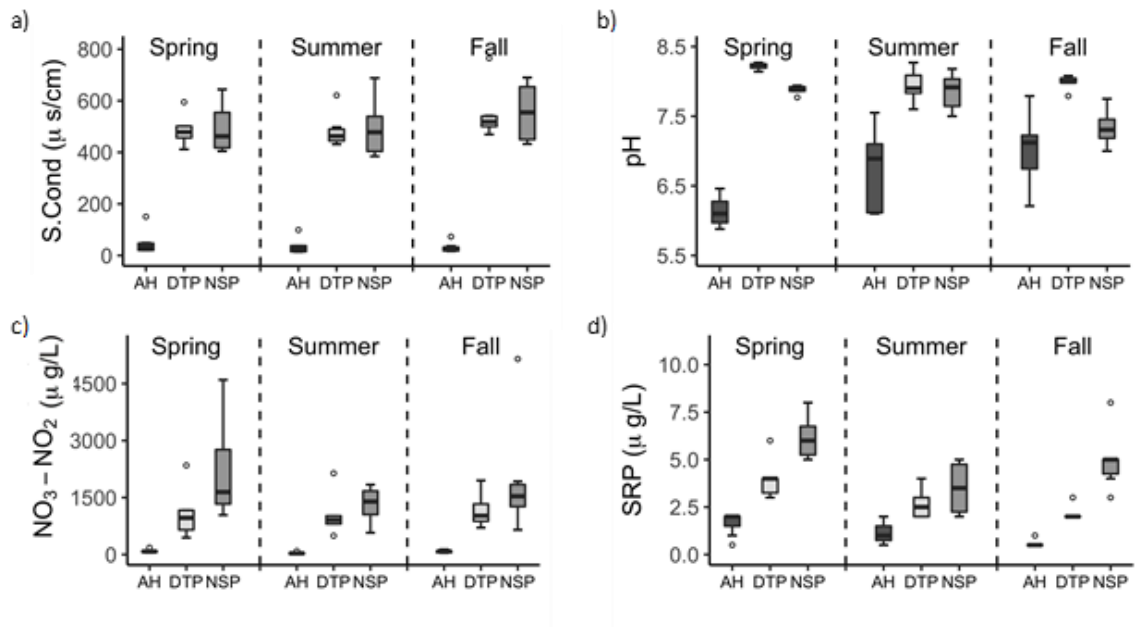


Figure 10. Summary stream chemistry properties measured during seasonal sampling events in the AH, NSP and DTP regions. Box plots show the median, interquartile range, and the 5th and 95th percentiles of the sampling event

4.4 Environmental predictors of tensile loss

PLS analysis on percent tensile loss per day resulted in a significant model ($Q^2 = 0.50$) that contained two components (Fig. 11). The first two components explained 58% of the variance of the independent variables (R^2X) and 68% of the variance of the dependent variable (R^2Y). Six variables (temperature, SRP, latitude, percent natural land cover, conductivity, and $\text{NO}_3\text{-NO}_2$) were found to influence the variance in tensile loss ($\text{VIP} > 1.0$). The first latent vector organized the sites based upon region, whereby the AH region streams separated from the NSP and DTP streams. The environmental descriptors most associated with the first latent variable were natural land cover, latitude, SRP, $\text{NO}_3\text{-NO}_2$, and conductivity. There was a positive relationship between rates of decomposition and the water quality parameters SRP, $\text{NO}_3\text{-NO}_2$, and conductivity. Percent natural land cover and

latitude were negatively associated with rates of decomposition. The second latent vector was positively related to temperature. The warmer and less seasonally variable sites in the AH were separated from the cooler and more variable sites in the NSP and DTP. Sites also separated by season with the cooler fall samples clustering apart from the more similar summer and spring samples.

PLS analysis on tensile loss per degree-day resulted in one significant model component ($Q^2 = 0.49$). The degree-day model explained 51% of the variance of the independent variables (R^2X) and 54% of the variance of the dependent variable (R^2Y). In the degree-day model, SRP, latitude, conductivity, percent natural land cover, NO_3-NO_2 and pH were found to be highly influential variables ($VIP > 1.0$). The direction of relationships in the per day model were maintained in the degree-day model, with negative associations between decomposition rates and latitude, and land cover, as well as positive associations with SRP, NO_3-NO_2 , pH, and conductivity.

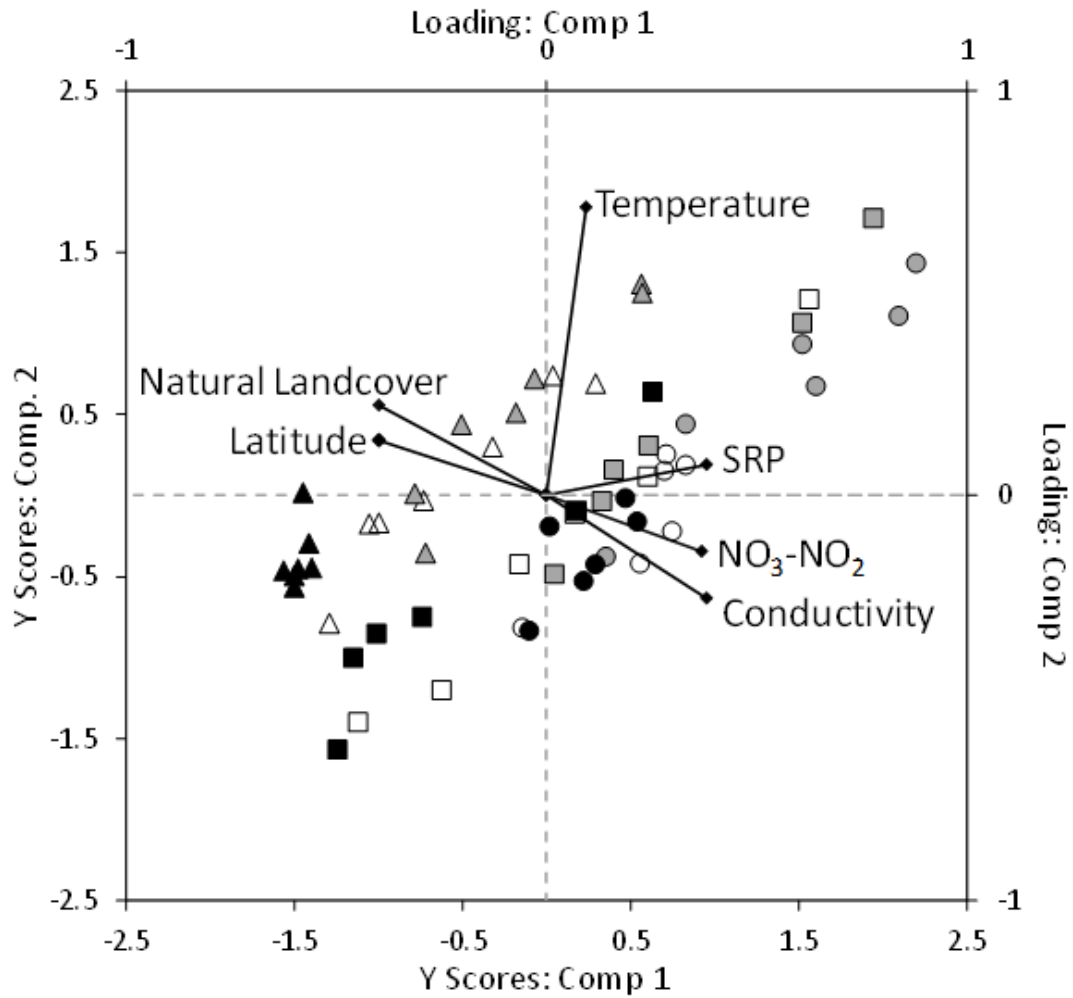


Figure 11. Scores and loadings biplot for the PLS regression analysis of percent tensile loss as influenced by environmental descriptor variables considered to be important in the model ($VIP > 1.0$). Response variable scores are represented on the primary axes, and loadings on the secondary axis. Variable loadings show the association between predictor environmental variables and the response variables (tensile loss) via proximity to the origin. Algonquin Highlands represented by the triangles, Dundalk Till Plains represented by the squares, and Norfolk Sand Plains by the circles. Hollow markers represent spring, grey represent summer, and black represent fall.

5 Discussion

5.1 Hierarchical and Seasonal Variance in Decomposition

Fundamental theories of spatial ecology predict that ecological variation should increase with spatial extent (Wiens, 1989; Cooper et al. 1998; Tiegs et al., 2009). However, my assessment of seasonal distributions of variance in decomposition among the three hierarchically nested spatial scales showed that agreement with this hypothesis was seasonally dependent. Indeed, a reallocation of variability among the hierarchical spatial scales in association with the change in the seasons was observed. Specifically, the largest portion of the variance progressively changed from the habitat scale to the regional scale from spring through the fall. This finding suggests that the relative importance of drivers of decomposition are changing throughout the year.

The decrease in variation at the habitat scale, from spring to fall suggests a homogenization of the environmental attributes of pool and riffle habitats through the summer and fall months. The distinct boundaries of these two habitats are not always marked, but they do represent different ecological habitats, with unique biota and physical properties (Hawkins et al, 1993, Wallace et al 1997). The two most important factors differentiating between riffles and pools are stream water velocity and the associated effects on organic matter storage. A decline in the amount of variation in velocity from spring to fall was observed in my study, which may explain the reduced amount of variation at the habitat scale. Variability in stream velocity has been linked to rates of decomposition by Tiegs et al (2009), who argued that physical fragmentation of leaf material was a driver of habitat related variability in decomposition. With similar flow rates between the habitats, the

amount of physical abrasion and fragmentation on the fabric strips would become more comparable.

In addition to the consistency of flow rates between habitats, the observation of decreased variance associated with the habitat scale in the fall may also be a result of increased similarity between habitat streambed detrital matter storage and transport. Although I did not measure these variables I did observe a general increase in abundance of litter across habitat types in the fall, therefore both habitats would be exposed to more similar microhabitat resources and conditions. The quantity of leaf litter and other streambed detrital matter has been shown to influence rates of decomposition stems from field studies and small-scale experiments (e.g., Richardson 1991, Rowe and Richardson 2001, Tieggs et al. 2008). For example, Tieggs et al (2008) tested the hypothesis of litter quantity promoting microbial leaf decomposition through higher concentrations of fungal spores in stream water. However, contrary to expectations, higher quantities of litter did not lead to faster microbial decomposition and the effects caused by alteration of litter quantities on leaf decomposition were relatively weak (Tieggs et al 2008). Though accumulated detrital matter might not have a known effect on microbial decomposition, it does have an effect on the microhabitats available for other components of the heterotrophic community. The relationship between higher volumes of accumulated organic matter and associated debris has primarily been linked to the abundance of benthic invertebrates, and their role in the breakdown process. The relationship is more strongly shown by the effect of benthic invertebrates with increasing organic matter in streams (Richardson, 1991; Rowe and Richardson, 2001). The relationship with cotton strip decomposition and invertebrates is

still not entirely clear. Further studies are needed to test the role that accumulated leaf litter may have on microbial decomposers at the habitat scale.

In addition to the decrease in habitat variability, I observed an increase in the amount of variability associated with the region scale, as the seasons progressed from spring through fall. Several past studies of decomposition in streams have found regional scale to be the dominant source of variation (e.g., Tiegs et al 2009, Young et al 2008). However, these studies have not observed the seasonal effect that was observed in my study. Moreover, the observed increase in regional scale variability was disproportionately large due to the decline in variability at the habitat scale, suggesting that regional scale variability was due to a change in the relative influence of regionally scaled drivers.

Observed regional differences in water temperature were found to be a large source of variation in decomposition. However, it does not appear that the seasonal shift in variance allocation is due to temperature, as the pattern of variance allocation was conserved when the analysis was run using degree-day corrected decomposition rates. An alternative driver of the increase in regionally scaled variance in the fall could be a seasonal divergence of regional flow regimes, and its subsequent influence on the source of stream water. The balance of hydrologic exchange between surface and subsurface plays an integral role in determining numerous water quality parameters at the regional scale (e.g. conductivity, pH, alkalinity, and temperature). The importance of source water can also be traced to changes in the degree of hyporheic exchange on thermal regimes (Johnson, 2004). Hyporheic flow paths may influence breakdown rates, whereby decomposition would be higher where surface water down-welled into the sediments than at sites where

groundwater upwellings predominated (Boulton and Quinn, 2000). However, it was beyond the scope of my study to test these hypotheses and thus future studies are needed to understand the mechanisms behind the observed seasonal pattern of regional variance.

5.2 Habitat, Regional, and Seasonal Differences in Decomposition

Assessment of uncorrected decomposition data showed seasonal differences in decomposition, including an interaction between season and habitat. However, these differences could be attributed to differences in water temperature associated with seasonal climatic conditions as these differences were not observed when degree-day corrected decomposition data was analyzed. This finding was expected as temperature is a fundamental driver of microbial activity and thus decomposition (Boyero et al., 2011, Ferreira & Chauvet, 2011) and illustrates the importance of accounting for temperature differences when assessing decomposition measurements across seasons and among different thermal regimes.

My study observed differences in decomposition rates between pools and riffles following my expectation of faster breakdown rates in higher flow, riffle patches. Habitat related differences in decomposition have been frequently noted in the literature (e.g., Tiegs et al 2008, Boyero et al., 2011). However, past studies are mixed on whether decomposition is greater in riffles or pools. Studies observing greater decomposition in riffles have cited hydraulic factors leading to increased physical abrasion as the cause of increased decomposition (Tiegs et al 2009). Moreover, studies have pointed to the depositional sediments in pools increasing burial of organic substrates reducing microbial activity

(Suberkropp & Chauvet, 1995; Benfield et al., 2001). In contrast, studies finding greater decomposition in pools have suggested that increased retention of fine particulate organic matter in pools and increased nutrient availability in depositional sediments may enhance microbial activity (Boulton & Quinn, 2000; Claret et al., 2001; Calpcott and Barmuta 2010).

Contrasting effects of habitat on decomposition may be the result of regionally-scaled differences in habitat structure. Indeed, I observed inter-regional differences in the effect of habitat on decomposition rate in my study. For example, the finer sand and silt sediments of the Norfolk Sand Plains (NSP) did not produce the same distinction between habitat types as seen in the other two regions. Both riffle and pool habitats in the NSP consisted of similar substrates (i.e., sand), and similar amounts of substrate burial were observed between habitats on retrieval. In contrast, the Algonquin Highlands (AH) streams had distinct differences in substrate size and hydraulic condition with larger cobble and gravel in riffle habitats compared to predominantly fine sediments and organic debris in pools.

Comparison of decomposition among the three physiographic regions revealed that all regions were different; differences that were independent of seasonally driven temperature effects. This finding is consistent with my prediction of differences in regional rates of decomposition resulting from variation in physicochemical water quality parameters associated with regionally scaled physiography. For example, glacial outwash deposits, found in the Dundalk Till Plains and the Norfolk Sand Plains, produce high hydraulic connectivity and thus high rates of groundwater input, which directly affects water

temperature and water chemistry descriptors, such as conductivity and alkalinity (Wiley et al. 1997; Hladyz et al 2010). Indeed, the importance of physiographic-related water quality parameters was supported by the results of our PLS analysis, which indicated that conductivity and pH were predictors of variation in decomposition among the regions and seasons.

Past studies have found conductivity, and pH to be controls of decomposition (e.g. Jenkins & Suberkropp, 1995, Clivot et al., 2013). Although conductivity was found to be a highly influential variable through the PLS analyses, the relationship between conductivity and decomposition is not clear. In hardwater streams (i.e. greater conductivity and greater pH) decomposition has been found to proceed at a faster rate (Rosset et al 1982, and Suberkropp, 1991). The relationship with strictly pH is clearer as stream water pH has been found to affect the fungi decomposer communities (Suberkropp, 1992, Chamier, 1992), resulting in slower breakdown rates in acidic streams (e.g., McGeorge et al. 1991). For example, Hildrew et al (1984) pointed to seasonal differences in pH as a primary factor driving differences in cotton substrate decomposition rates. They found that among season differences pH had a positive relationship with decomposition, whereby there was a reduction in microbial activity in some streams of low pH (Thompson and Bärlocher, 1989, Griffith and Perry 1994). The Norfolk Sand Plains was the only region to follow the pattern proposed by Hildrew et al. (1984), with a negative relationship being observed. In particular, during the fall season, the relationship between decomposition and pH has been found to be strongest, when pH reaches a seasonal minimum (Jenkins et al., 2013).

Past studies have also observed that land use within a stream's catchment area is an important predictor of decomposition (Encalda et al 2010). These studies have generally observed increased decomposition with increased anthropogenic land use (Benfield et al 2000, Lecerf et al., 2006, Imberger et al., 2008). I also observed increased decomposition with reduced amounts of catchment scale forest cover, although disentangling the effect of land use from that of physiography and latitude is difficult because these landscape variables were inherently confounded in my study area. My results do indicate that regional land cover patterns are influencing decomposition through control of stream nutrient concentrations. As the microbial community obtains a proportion of their nutrient requirements from the water column (Suberkropp, 1998), the relationship between breakdown rates and bioavailable nutrients, such as SRP and nitrate-nitrite, have been regularly noted in the literature (Suberkropp & Chauvet, 1995; Ferreira and Chauvet, 2011; Griffiths and Tiegs, 2016). Though SRP and nitrate-nitrite were found to be highly influential variables through our PLS analyses, relationship between the decomposition and nutrients are not always monotonic (Bergfur et al 2007, Pozo et al 2014; Chauvet et al 2016). For example, a large-scale pan-European study by Woodward et al. (2012) compared decomposition rates of leaf litter at impacted and corresponding reference sites but found no clear or consistent response to nutrient concentrations by the microbial decomposers. Our study followed the theoretical response pattern of higher nutrients being influential in determining decomposition rates, but the lack of direct relationships in other studies elucidates the need for further research into this dynamic interaction.

Riparian land use influences key variables, such as nutrient, temperature, canopy cover and suspended sediments (e.g. Young et al. 2005, Casotti et al., 2015, Silva-Junior et al., 2014, Graça et al 2015, Sponseller and Benfield, 2001), as such I expected that greater percentage natural land use within this area would be more important than catchment-wide landuse. Contrary to expectation, natural landuse at the catchment scale was found to be a more important predictor of decomposition. Although catchment landuse was found to be more important in this study, riparian areas are frequently highlighted for their importance in nutrient-temperature interactions in streams, and their subsequent effects on microbial communities (Sridhar and Bärlocher, 1997). In addition, others have pointed to the effect that riparian vegetation can have on the water chemistry and therefore the activity of aquatic hyphomycetes (Bärlocher and Graça, 2002; Graça et al 2015). Riparian areas have been highlighted as a crucial component of the landscape for their role in influencing stream ecosystem processes (Correll 2000). In particular, small, forested headwater streams are often densely shaded by riparian vegetation, thus derive most of their energy and carbon from decomposition processes (Wallace et al., 1997). By working to establish how riparian and catchment-wide landuse interrelate, the impacts of landscape alteration on decomposition can be more clearly presented, and thus strengthen decomposition as a bioindicator.

6 Applications for Biomonitoring

My study of spatial and temporal heterogeneity in decomposition across spatial scales provides critical information to further promote decomposition as a biomonitoring practice. By studying reference streams, with the greatest possible natural land cover in the catchment and riparian area, I have provided evidence that applications of the CSA method to observe changes in rates of decomposition likely needs to incorporate scale and season specific effects.

The seasonal reallocation of hierarchical variance among scales established that habitat-scale variance is reduced in the fall, but not in other seasons. This finding suggests that monitoring data from different habitat would not be comparable if sampling was undertaken during the summer or spring seasons. Riffle habitats have been previously recommended as the standard habitat type to be used for studying decomposition, as sediment deposition and burial are less likely in riffles (Young et al 2008). We have established that selection of riffles as the standard habitat type would only be important in spring and summer, but not in fall. Moreover, fall monitoring would avoid need for habitat specificity and the requirement to identify comparable habitats would be eliminated, simplifying biomonitoring protocols.

The decrease in overall magnitude of variance in decomposition across all seasons, established that seasonality of monitoring is less important of a consideration if high quality temperature data can be generated. However, given this is more resource intensive

variable, our study also shows that sampling within a season can generate quality reference data.

My finding of regional differences in rates of decomposition points to the need for development of a stream classification system for regional RCA models. This finding is contrary to hypotheses that adoption of a functional indicator would increase regional applicability of a biomonitoring program (e.g. Gessner and Chauvet 2002; Bunn and Davies 2000; Young et al 2008; Tiegs et al 2009). Indeed, our study suggests that even the relatively modest physiographic differences between the NSP and DTP regions necessitate individual reference stream groups. The importance of finer-scale physiographic attributes for CSA is comparable to what has been observed for monitoring with benthic macroinvertebrates in southern Ontario (Yates and Bailey 2010). Moreover, it suggests that the complex mosaic of glacial deposits in southern Ontario could require a substantial number of reference groups to enable accurate assessment of biological conditions at test sites.

7 Future Research

Future research is needed to establish the potential of stream decomposition, and more specifically the CSA, by testing aspects of variation that were beyond the scope of this study to address. First, the present study sought to purposefully minimize variation at the riparian scale, which hinders the ability to detect whether this parameter does indeed contribute to variation in decomposition. To further dissect the role of land use on decomposition rates, reference condition rates established from the present study could be compared to rates produced from test sites that incorporate a range of natural and disturbed areas in the watersheds and riparian areas. Second, studies are needed that test the importance of regional variation in decomposition at reference sites to the establishment of biological status at test sites. These studies should select test sites within and across physiographic regions to establish if deviation of test sites from reference conditions requires highly resolved reference groups. Such a study could be conducted in an area similar to that of the Norfolk Sand Plains that is heavily dominated by row-crop agricultural.

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Curriculum Vitae

Education

Master of Science, Geography

October 2018

Dissertation Title: Hierarchical variation in cellulose decomposition across southern Ontario reference streams. Supervisor: Dr. Adam G. Yates
Western University, London, Ontario

Bachelor of Science, Honors Specialization in Environmental Science **October 2015**

York University, Toronto, Ontario

Industry and Research Experience

MSc Research Project

September 2016-Present

- Developed a hierarchical study design to partition the variation associated with decomposition among spatial scales, and thus creating comprehensive picture of scale-dependent processes
- Used ArcGIS along with land cover data to locate study sites with high proportions of natural land cover
- Communicated with landowners regarding visiting their property and to obtain permission
- Conducted field characterization of streams over three seasons using methods from both CABIN and OSAP manuals
- Organized shipping of field water samples for laboratory nutrient testing
- Analyzed data using RStudio, Systat statistics software and associated figures made using RStudio and Sigma plot
 - General linear models, linear mixed effects model, partial least squares regression analysis, variance partitioning using nested ANOVAs
- Involved property landowners by communicating results to interested landowners in plain language

NSERC's (CREATE) Internship

July-August 2018

Dept. of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences

- Involved in fisheries research studies while participating in internship placement with the DFO
 - Assessing the fish friendly nature of low velocity head turbines on the Severn River dam
 - Entrapped fish using angling and boat electrofishing, passed fish through a low velocity hydroelectric turbine, and following assessed injury and mortality rates following
 - Tagged all species captured with PIT (passive integrates transponder) tags, and a subset of target species with telemetry tags for assessment of delayed mortality and/or long-term fitness

Research Assistant**January 2018-April 2018***Western University, Department of Geography*

- Under contract to the Fresh Water Quality Monitoring and Surveillance (FWQMS) Division of Environment and Climate Change Canada (ECCC), completed watershed delineation of all possible watersheds (7000+) in the Athabasca River and Peace River basins
- Ran overlapping intersections of geophysical (i.e. geology, physiography, topography, hydrology) and climate data for all watersheds in concordance with the CABIN database for future use in reference condition bioassessment studies

Asset Management Specialist**August 2015- July 2016***GM Blueplan Engineering*

- Halton Region's Downspout Disconnection Program
 - Communicated with members of the public on the importance of disconnecting and on the details of Halton Region's disconnection program
 - Utilized and maintained MS Access relational database and Manifold GIS database
 - Collaborated with project management on troubleshooting database query malfunctions to better field staff efficiency and data quality
 - Co-managed a team of four summer students, which included training, daily data QA/QC, and weekly one-on-one revision of field data collected
- Peterborough street light assessment
 - Assessed the structural integrity of streetlight poles
 - Used QGIS and Microsoft Access to record streetlight assessment data
- Thorold Flow Monitoring
 - Assisted in the sewer smoking process, whereby fractures in storm and wastewater sewer lines were located to ensure proper management, maintenance, and repair of municipal sewage systems
- Saskatchewan Parks Asset Management
 - Digitized and geo-referenced park data into Manifold

Assistant Aquatic Resource Technician**July – August 2015***Conservation Halton*

- Worked alongside CH's aquatic monitoring team in the collection, entry and analysis of water resources data specific to: groundwater, surface water, water quality (e.g. benthics and chemistry) and fish habitat assessments
- Conducting electrofishing surveys in streams across Halton region, and identified specimens to the species level
- Kept accurate and organized records of site visits and data collected for data entry in CH's long-term monitoring database

Research Practicum Student**May 2015- July 2015***York University, Department of Biology*

- Responsible for the sorting, pinning, labeling, and data logging of bee specimens collected in Chile by a graduate student, under the supervision of Dr. Laurence Packer

Volunteer Research Assistant**September - December 2014***York University Department of Geography*

- Under the supervision of Dr. Andrew Medeiros, worked in a palaeolimnology laboratory enumerating midge flies from sediment samples collected in Inuvik, NWT
- Gained skills in using a dissecting microscope, slide preparation, and identification of midge fly larvae using dichotomous keys

Publications

Bower, S.D., Kooner, H., Ludwig, H., Lumb, S., Raina, **J.**, **Webb**, J., Zrini, Z., O'Connor, C.M., and S.J. Cooke. (In Review). Diel patterns of hooking depth for active and passive angling methods in bluegill and pumpkinseed. *Bulletin of Marine Science*.

Presentations

Webb, J.R.*, Painter, K.J., Pearce, N., Bailey, R.J., Tiegs, S.D., Yates, A.G., Hierarchical variation in cellulose decomposition across southern Ontario reference streams. Society for Freshwater Science Conference, Detroit, MI, USA. (* denotes presenter)

Bower, S.D., Kooner, H., Ludwig, H., Lumb, S., Raina, **J.**, **Webb**, J., Zrini, Z., O'Connor, C.M., and S.J. Cooke*. Diel patterns of hooking depth for active and passive angling methods in bluegill and pumpkinseed. Fish at Night Conference, Miami, FL, USA.

Professional Development and Training

The Science and Management of Stressors in the Great Lakes Basin **September 2017**
University of Guelph, School of Environmental Sciences

- Introduced to Scenario analysis (SA) process to analyze possible future events by considering alternative possible outcomes relative to current and historical situations.
- Prepared a policy brief designed to communicate science to the public for the purpose of recommending changes to environmental policies
- Studied the Ecological Risk Assessment (ERA) framework using bow-tie analysis

CABIN Project Manager Level Training **June 2018**
Canadian Rivers Institute

- Participated in CABIN Training Program to gain necessary skill and knowledge to conduct freshwater benthic invertebrate monitoring and assessment to a nationally acceptable standard, using online CABIN resources (database, analytical and reporting tools)
- Online course consisting of up to 6 theoretical modules about the program and use of the online tools, and practical field course providing certification in CABIN sampling methods.

Practical Field Research Experience

York University, Department of Geography

- Various field courses throughout undergraduate degree, including:
 - Urban Watershed Management and Biodiversity field course, focused on applied conservation and analytical techniques in watershed management, restoration ecology and biodiversity
 - Fish and Fisheries: The Science Behind Conservation and Management Field course on the foundations of fisheries science, contemporary management, and conservation strategies, in addition to learning the practical skills needed for fisheries assessment and research
 - Environmental Change in Lake Ecosystems Field Course on general limnology of lakes, in the Frontenac Arch, with emphasis will be on examining factors (physical, chemical, and biological) that determine the abundance and distribution of zooplankton and phytoplankton within and among lakes

Teaching Experience

Teaching Assistant

September 2016-December 2017

Western University, Department of Geography

- GEOG2122A – Spatial Techniques
- GEOG2152G- Geography of Hazards
- GEOG2310A- Weather and Climate

Volunteer Experience

Conservation Volunteer

May 2014-Present

Nature Conservancy of Canada

- Volunteered alongside the Nottawasaga Valley Conservation Authority in completing restoration projects in the Minnesing Wetlands, including;
 - Installing Christmas tree sediment traps along various rivers to restore stream meander properties
 - Tree planting in previous cattle rangeland areas to restore natural canopy coverage

Volunteer Terrestrial Monitor

June 2014- May 2016

Toronto & Region Conservation Authority

- Responsibilities include visiting the assigned site up to ten times per year to survey for the presence of a set of indicator species (flora, fauna; lichens)
- Attended seasonal training sessions to learn the correct identification and recording methods

Community Stewardship Volunteer

May 2014- August 2015

City of Toronto Parks & Forestry

- Stewardship activities include weeding invasive non-native plant species, planting and maintaining native vegetation, and monitoring specific site conditions
- Monitoring activities include water chemistry, birds, vegetation and aquatic invertebrates

Certifications and Training

Comprehensive WHIMIS Certification

Standard First Aid & CPR/AED

Ontario G Class Drivers License

Canadian Safety Council ATV Training Certification

Class II Backpack Electrofishing Certification