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Comparison of Heterotrophic Activity in Forested Streams Originating from Wetland and Agricultural Tile Drainage Sources

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Abstract

Agricultural development of lands in southern Ontario, Canada, have resulted in many headwater streams being sourced by agricultural tile drains instead of wetlands. Tile drainage inputs can influence stream conditions (i.e., temperature, hydrology, and water chemistry) that are important drivers of ecological function. To assess the influence of agricultural tile drainage inputs on stream ecosystem function, I compared heterotrophic activity (i.e., organic matter breakdown and benthic respiration) in forested streams originating from wetland and agricultural tile drainage sources over four seasons. I found a reduction in heterotrophic activity in the tile-sourced stream, particularly in the summer, that appeared to be due to reduced stream temperatures from influxes of groundwater. Reduced heterotrophic activity was also evident in downstream network segments. My findings suggest there may be a widespread reduction in heterotrophic activity in streams across agricultural regions where tile drainage is prevalent.

Keywords

Summary for Lay Audience

In southern Ontario, Canada, many headwater streams were historically sourced by wetlands. However, with agricultural developments, numerous wetlands were drained and consequently many streams are now sourced by agricultural tile drains. Agricultural tile drainage is a subsurface drainage system that removes excess water from soils, through the use of underground pipes, for improved crop production. Although tile drainage has agronomic benefits, tile drainage inputs can influence stream conditions (i.e., temperature, hydrology, and water chemistry) that are important in maintaining ecosystem function; the natural processes that control the movement of energy and matter through an environment. To assess the influence of agricultural tile drainage inputs on stream ecosystem function, I used a stream network within the headwaters of the Thames River Basin, where adjoining streams had different sources (i.e., wetland versus tile drainage). I compared consumption rates of carbon and oxygen by microbes (heterotrophic activity), largely bacteria, fungi, and archaea, between the wetland-sourced and tile-sourced streams over four seasons. Heterotrophic activity was assessed through rates of microbial respiration and organic matter (OM) breakdown. Respiration is a metabolic process that breaks down organic carbon, while consuming oxygen, to produce carbon dioxide and energy. OM breakdown is the process of breaking down complex organic matter (e.g., leaves) into simpler inorganic matter (e.g., carbon dioxide, inorganic forms of nutrients) to be cycled back into the environment. OM breakdown was measured using the cotton strip assay, which is a method to compare streams' capacities to process organic matter by assessing cellulose breakdown. I found a reduction in heterotrophic activity in the tile-sourced stream, particularly in the summer. This reduction in heterotrophic activity appeared to be due to colder stream temperatures from increased groundwater inputs, as tile drainage pipes intersected the water table and extracted more groundwater. Reduced heterotrophic activity was also evident in downstream network segments. Additionally, I found less variation in heterotrophic activity along the tile-sourced stream. Therefore, if my findings are representative of how tile drainage has affected headwater streams more broadly, there may be a widespread reduction in heterotrophic activity in headwater streams across agricultural regions where tile drainage is prevalent.

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1 Introduction

Stream ecosystems are vital natural resources that support life on Earth by contributing to the hydrologic cycle and providing habitats, drinking water, and food (McKinney, 1963). However, streams are vulnerable to human activities as they are influenced by the landscapes they flow through (Hynes, 1975; Vannote et al., 1980). In order to inform management actions that protect stream ecosystems from human activities, stream health assessments are required to provide information about degradation and its causes (Vörösmarty et al., 2010). One stream health assessment method that has been increasingly promoted is measuring heterotrophic activity (i.e. the consumption of carbon and oxygen from benthic microbes, largely bacteria, fungi, and archaea), as it is sensitive to changes in environmental variables (e.g., temperature, velocity, and nutrients) caused by human activities.

One human activity that that has expanded over the last 200 years and poses a major threat to stream ecosystems is agricultural land use (Allan & Castillo, 2007). Agricultural activities, such as fertilizer application, tillage, and land clearing, can degrade stream ecosystems by altering environmental variables that control essential ecosystem processes associated with biological activity. For example, agricultural activities can lead to changes in stream velocity, temperature, and nutrients, and thereby change the rate of carbon processing by altering the capacity of heterotrophs to respire and breakdown organic matter (OM). One agricultural activity that has pronounced impacts on the hydrologic network is agricultural tile drainage; a method to drain wet soils for improved crop production. Agricultural tile drainage has been shown to alter environmental variables (Gedlinske, 2014). However, there is a lack of study assessing the effects of tile drainage inputs on stream heterotrophic activity. Therefore, the goal of my thesis is to study the effects of tile drainage inputs on stream heterotrophic activity.

1.1 Heterotrophic Activity

Heterotrophs are organisms that gain energy via external food sources, such as living or dead organic matter (Allan & Castillo, 2007). Many microorganisms (i.e., fungi,

bacteria, and archaea) are heterotrophic as they consume dead organic matter to acquire energy (Allan & Castillo, 2007). Through consumption, microorganisms break down and release carbon, making their activity a driving factor in OM breakdown and respiration processes. Therefore, heterotrophic activity is defined as the consumption of carbon and oxygen by organisms that rely on external food sources. However, in most streams, the vast majority of heterotrophic activity is associated with microbial organisms, such as fungi, bacteria, and archaea. Indeed, compared to microbes, contributions to heterotrophic activity from macro-organisms are typically negligible and most assessments of heterotrophic activity focus on microbial organisms alone. Thus, the amount of heterotrophic activity is frequently determined by microbial metabolism and abundance, which are controlled by environmental variables, such as temperature, water quality, and hydrology (Moat et al., 2002).

OM breakdown is the process of breaking down complex organic matter (e.g., leaves, wood) into simpler inorganic matter (e.g., carbon dioxide, inorganic forms of nutrients) to be cycled back into the environment. OM breakdown consists of many sub-processes including: physical fragmentation, microbial activity, invertebrate feeding, and their joint effects (Hauer & Lamberti, 2017). Physical fragmentation is a controlling factor of OM breakdown as abrasion and fragmentation breaks larger particles of organic matter into smaller pieces exposing more surface area to microbial action and facilitating consumption for macroinvertebrates (Benfield et al., 2001). Microbial activity, performed primarily by bacteria and fungi, softens organic tissue making it more palatable to invertebrates (Allan & Castillo, 2007). Macroinvertebrates, such as insect larvae, feed on organic matter causing increased fragmentation leading to increased microbial activity (Graça, 2001). The rate of OM breakdown is controlled by these sub-processes (microbes, macroinvertebrates, and fragmentation), which are, in turn, controlled by environmental variables that influence heterotrophic activity.

Linked to OM breakdown is the rate of heterotrophic respiration, which can be used as an indicator of biomass and activity of heterotrophs on organic substrate (Hauer & Lamberti, 2017). Respiration is a metabolic process that breaks down organic carbon,

while consuming oxygen, to produce carbon dioxide and energy (Urry et al., 2016). As organic carbon is being broken down for respiration, it is also driving OM breakdown. The level of heterotrophic respiration is indirectly controlled by environmental variables that influence the rate of heterotrophic activity.

1.2   Environmental Controls on Heterotrophic Activity

Hydrological changes in stream environments can greatly alter the level of heterotrophic activity. Increases in water flow, turbidity, and velocity result in more abrasion and fragmentation of organic matter, exposing more surface area to microbial action, and subsequently increasing OM breakdown (Benfield et al., 2001). For example, Ferreira and Graça (2006) demonstrated that increased stream velocities promoted OM breakdown through stimulating microbial activity via increased oxygen and nutrient levels. However, extreme flow events may temporarily decrease heterotrophic activity from benthic scouring and loss of biofilms (Allan & Castillo, 2007).

Stream hydrology naturally varies over time due to changing amounts of precipitation associated with storm events and seasonality. Increased precipitation from storm events is directed to stream systems, causing an increase in water flow. Available water supply varies between seasons. In temperate environments, the greatest amount of flow is in the spring after winter snowmelt, and lowest in the summer from increased evaporation rates via warmer air temperatures and longer days (Brown et al., 2013). For example, dos Santos Fonseca et al. (2013) found that increased flow velocities, as seen during high precipitation events/seasons, caused leaf litter to be more labile, resulting in greater breakdown rates. Although heterotrophic activity is enhanced by moderate increases in stream flow, it may not increase from greater seasonal flow, as seasonal changes in flow, temperature, and nutrients are linked (Griffiths & Tiegs, 2016). For example, cold spring temperatures may hinder heterotrophic activity although there is an increase in stream flow from snowmelt.

The level of nutrients, especially nitrogen and phosphorus, in streams greatly influences heterotrophic activity, as nutrients can be a limiting factor, particularly when in short supply. Heterotrophic bacteria and fungi require nutrients in order to survive and perform biological processes optimally; therefore, an increase in nutrients can be beneficial to their survival and activity (Allan & Castillo, 2007; Gulis et al., 2004). However, increased nutrient loadings in nutrient-rich environments can have negative ecological impacts, such as eutrophication, on streams (Evans-White et al., 2009; Lecerf et al., 2006). Eutrophication leads to an increase in algal and macrophyte growth and therefore an increase in respiration, which can create anoxic and acidic stream conditions (Yang et al., 2008). Low oxygen and pH levels can impair heterotrophic survival, thereby decreasing heterotrophic activity (Dodds & Welch, 2000). Indeed, a study analyzing the relationship between OM breakdown and nutrient concentrations, performed by Woodward et al. (2012), found a hump-shaped relationship over large nutrient gradients suggesting a subsidy-stress response of heterotrophic activity to nutrient addition. Nitrogen and phosphorus inputs also vary seasonally due to the effects of the growing season and hydrology (Allan & Castillo, 2007). Naturally, there would be reduced nutrient concentrations in streams during the growing season due to nutrient uptake in plants, however, in agricultural landscapes, extensive fertilizer applications during this period can conceal this effect and continue to enhance nutrient concentrations (Allan & Castillo, 2007).

Contaminants (e.g., pesticides) can harm aquatic systems as they decrease heterotrophic survival rates and activity (Dangles et al., 2004). High concentrations of contaminants can lead to increased deformities and mortality rates in macroinvertebrates as well as impact invertebrate abundance, drift, and emergence, resulting in a decrease in heterotrophic activity (Jeffries et al., 2010; Rolland, 2000; Woodward et al., 1997). Moreover, Artigas et al. (2012) found that fungal communities with exposure to the fungicide, tebuconazole, had a decrease in biomass and enzymatic activities, leading to a decrease in OM breakdown. Furthermore, some contaminants, such as heavy metals, can alter the pH of aquatic systems, slowing heterotrophic activity where pH ranges exceed

survivable conditions (i.e. 6.5 to 8.5 on the pH scale) (Dangles et al., 2004; Ministry of Environment and Energy, 1994).

Temperature strongly influences the level of heterotrophic activity, as microbes and invertebrates cannot regulate their internal temperature making their metabolism temperature dependent (Griffin, 1981). Stream temperatures are influenced by many factors including climatic differences between regions, groundwater inputs, and seasonality (Allan & Castillo, 2007). Warmer temperatures favour biological processes and increase the rate of heterotrophic activity. Thus, summer and winter seasons have the fastest and slowest rates, respectively. However, temperatures tending towards the extremes can have detrimental impacts on heterotrophs, as organisms have optimal temperature ranges where growth and fertility rates are highest. For example, Sridhar and Bärlocher (1993) found that aquatic hypohmycetes, an important fungal decomposer, had lower growth rates below 15°C and above 25°C, signifying that intermediate temperatures were best. Temperature ranges that favour heterotroph health will consequently favour heterotrophic activity.

Another factor that strongly influences stream temperatures is the amount of groundwater input. Groundwater inputs regulate stream temperatures, as groundwater lacks contact with surface temperatures, making streams cooler in the summer and warmer in the winter in comparison to streams with mainly surface inputs. For example, Kaandorp et al. (2019) found that groundwater inputs to streams buffered stream temperatures, providing an area of stable thermal conditions for organisms during winter. Consequently, the amount of groundwater inputs to streams may also influence the level of variation in heterotrophic activity within and among seasons.

1.3 Effects of Agricultural Land Use on Heterotrophic Activity

In North America, agricultural regions have been developed for over two hundred years (Sharitz et al., 1992). Landscapes with fertile soils were used for intensive row crop

cultivation while shallow soils were used for lower intensity pasture agriculture (Yates & Bailey, 2010). Agricultural development modifies the landscape, consequently altering nearby streams, as they are influenced by the landscapes they flow through (Hynes, 1975; Vannote et al., 1980). Agricultural activities, such as pesticide application, result in stressors entering stream ecosystems. These activities effect stream hydrology, water chemistry, and temperature, and consequently alter fundamental stream ecosystem functions, including heterotrophic activity.

Agricultural land use can impact stream hydrology, although the amount of impact is dependent on crop evapotranspiration rates, soil infiltration capacity, and scope of drainage and irrigation systems (Allan & Castillo, 2007). Cropping practices that compact soil and reduce soil infiltration, along with drainage systems and removal of natural vegetation, can increase the volume and velocity of runoff during precipitation events (Paul & Meyer, 2001; Wang et al., 2001). For example, Schottler et al. (2014) found that runoff levels were highly correlated with the proportion of agriculture (i.e. soybeans) in the watershed, mainly driven by changes in crop evapotranspiration rates and loss of wetlands. An increase in runoff consequently results in an increase in stream flow and velocity, potentially increasing physical OM breakdown.

Increasing nutrient (e.g., fertilizers rich in phosphorus and nitrogen) and contaminant (e.g., pesticides) inputs, attributed to agricultural land use, can also impact stream ecosystems. Pesticides (e.g., insecticides, herbicides, fungicides) and fertilizers, used for crop protection and growth, respectively, enter stream systems through runoff, groundwater, and drainage systems (Skinner et al., 1997). Many studies have found that streams with increased proportions of agricultural land use in their catchment area have increased concentrations of phosphorus and nitrogen (Allan, 2004; Carpenter et al., 1998; Omnerik, 1977). For example, Goolsby and Battaglin (2001) demonstrated that fertilizer application was the leading factor for increased nutrients in streams draining agricultural areas. Fertilizer and manure are typically applied to agricultural soils in the spring and fall seasons, whereas pesticides may be applied to crops throughout the growing season (Skinner et al., 1997). In low-nutrient streams, nutrient loadings can increase

heterotrophic activity, whereas streams with excess nutrients and contaminants can decrease heterotrophic activity by reducing heterotroph survival (Allan & Castillo, 2007).

Agricultural activities can increase stream temperatures by removing riparian vegetation and the associated shading it provides. For example, Moore et al. (2005) found that stream temperatures significantly increased, upwards of 5°C, where riparian forest was removed. Heterotrophic activity can increase with warmer stream temperatures, as long as the temperature remains within optimal range (Allan & Castillo, 2007).

1.4   Effects of Agricultural Tile Drainage on Heterotrophic Activity

One of the ways agricultural land use can alter stream environments is through the implementation of agricultural tile drainage. Agricultural tile drainage is a subsurface drainage system that removes excess water from soils, through the use of underground pipes, for improved crop production (Dierickx, 1990; Gilliam et al., 2015). The underground pipes collect water, lower the water table, and export water to nearby streams. Agricultural tile drainage enhances crop production by lengthening the growing seasons and increasing the area of suitable cropland (Du et al., 2005; Fausey, 2005; Kornecki & Fouss, 2001; Moore, 2016). Although tile drainage has agronomic benefits, it also has potential negative impacts to the natural stream system by altering the hydrologic, thermal and nutrient regimes (Figure 1).
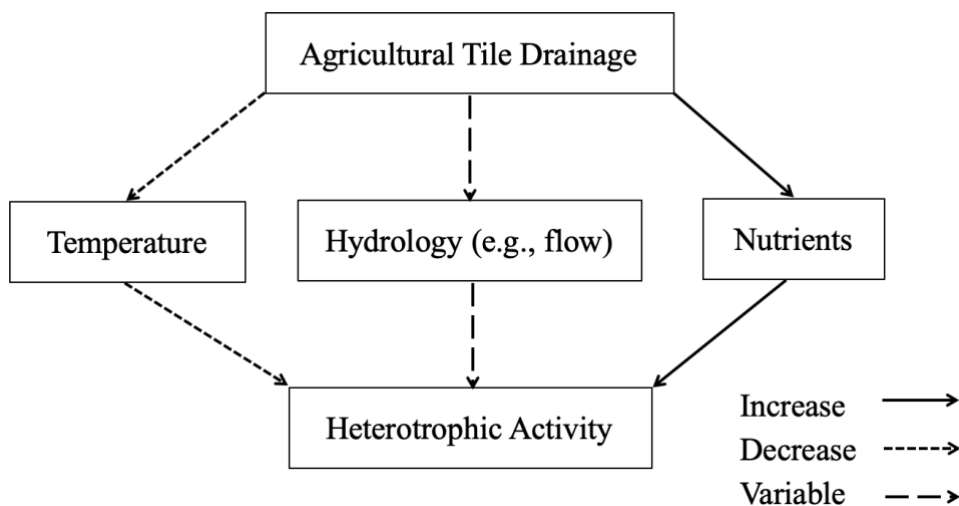
Figure 1. The effects of agricultural tile drainage on heterotrophic activity in the summer, fall, and spring seasons. Increase, decrease, or variable refers to predicted change in heterotrophic activity associated with the stressor.

In areas with significant wetland loss and/or the implementation of tile drainage, stream flows tend to increase in magnitude and frequency during precipitation events (Allan, 2004; Kulhavý et al., 2007). Tile drains serve as a conduit that speeds the movement of water through soils to streams. Moreover, tiles amplify the effect of wetland loss by further reducing the land's capacity to store excess water, resulting in water that is quickly directed downstream, leaving the stream susceptible to heavy precipitation events and lower base flows from inconsistent water supply and larger channels (Poff et al., 1997). Agricultural tile drainage also may lead to alterations of stream channel form, as channels are straightened and entrenched (i.e., deepened) to accommodate drain connection and to deal with greater stream flows during precipitation events (Allan & Castillo, 2007). However, in areas where agricultural tiles are set near or below the water table, drainage to streams may enhance baseflow and reduce seasonal variation in stream flow through increased groundwater inputs, although this may not mitigate the flashy regime associated with increased tile inputs during storm events. Indeed, the direct conduit from agricultural tile drainage results in greater subsurface runoff (via tile) and reduced surface runoff. For example, Klaiber et al. (2020) found that mean total runoff was 396% (95% via subsurface flow and 5% via surface flow) greater for tile drainage whereas surface runoff was 85% lower, compared to undrained fields.

During precipitation events when stream flow has increased, OM breakdown can accelerate via increased physical fragmentation and stimulated microbial activity. However, during extreme flow events, heterotrophic activity may decrease from microbial community disturbance (Allan & Castillo, 2007).

Agricultural tile drainage provides a direct conduit for nutrients and contaminants from agricultural fields to streams, as opposed to wetland systems that remove excess nutrients through various filtration mechanisms (i.e., physical, chemical and biological processes; Allan & Castillo, 2007; Herrera, 2009; Vymazal, 2016). Because water inputs from tile drainage may be flashy in association with precipitation events, nutrient loadings from tile drainage may also be pulsed and sporadic in timing. Although there is often a decrease in surface runoff associated with tile drainage, the subsurface pathway for nutrients may be more impactful, as drainage water can enter the stream faster and have less contact with soils (Gentry et al., 2000; Reid et al., 2012; Zhang et al., 2009). Many studies have found that agricultural tile drainage can transport substantial amounts of phosphorus and nitrogen (e.g. Arenas Amado et al., 2017; Baker et al., 1975; David et al., 2010; King et al., 2015). For example, Smith et al. (2015) found that 25-80 % of phosphorus applied to agricultural fields was lost through tile drainage. In regards to contaminants, a study conducted by Kronvang et al. (2004) found that pesticides were transported in drainage water, impacting invertebrate species through significant mortality. Furthermore, nutrient loadings can be beneficial to heterotrophic activity in low-nutrient streams while they can be detrimental in high-nutrient streams.

Agricultural tile drainage water has limited time and ability to interact with external warming/cooling factors, such as sunlight and air temperature, as underground pipes rapidly drain transporting water (Vought et al., 1998). Therefore, during warmer seasons, surface sourced inputs, such as from wetlands, may be warmer in comparison to tile drainage inputs, as there is increased exposure to external warming factors (Vought et al., 1998). In contrast, during colder seasons, surface inputs have more exposure to cooling factors, possibly making them colder than tile drainage inputs. Streams draining agricultural tiles may also have significant groundwater inputs, if the tile drainage system

is installed below or near the water table, resulting in colder/warmer stream temperatures during the summer/winter, respectively, in comparison to wetland-fed streams. As heterotrophic activity can decrease with cooler stream temperatures and increase with warmer stream temperatures (within optimal temperature range), tile drainage inputs may have variable impacts on ecosystem functions driven by heterotrophic activity.

The effects of agricultural tile drainage on stream parameters (i.e., hydrology, water chemistry, and temperature) are well studied in literature. To summarize, agricultural tile inputs can potentially impact stream conditions by altering thermal and hydrological regimes, and increasing nutrient loads. Although there are many studies analyzing the effects of agricultural tile drainage on streams by measuring structural metrics, there is a lack of study measuring functional metrics (i.e., heterotrophic activity). The use of heterotrophic activity as a functional metric is important to further the understanding of agricultural tile drainage impacts on stream ecosystem function.

2   Research Objectives

The goal of my study was to increase understanding of how agricultural tile drainage impacts stream ecosystem function, and heterotrophic activity in particular, by assessing OM breakdown and benthic respiration rates in streams originating from wetland and agricultural tile drainage sources. Another goal of my study was to identify if the impact from agricultural tile drainage on stream heterotrophic activity varies by season (i.e., summer, fall, winter, and spring seasons) and scale (i.e., segment and reach scale). My thesis addressed these knowledge gaps by completing two related studies. First, a **Segment Assessment Study** to address the following research objective:

1. Examine the temporal patterns in heterotrophic activity among stream segments over a year.

Second, a **Reach Comparison Study** to address the following research objectives:

1. Assess differences in heterotrophic activity among stream segments, and determine if those differences are related to stream position and season.

2. Determine what environmental factors are associated with heterotrophic activity across the stream network.

2.1 Predictions

Segment Assessment Study

1. I predict that stream segments will follow the same temporal patterns in heterotrophic activity, with greatest rates in the summer months when warm stream temperatures are optimal for heterotrophic activity, and smallest rates in the winter months when cold stream temperatures inhibit heterotrophic activity. However, I predict that the tile segment will have lower heterotrophic activity, in comparison to the marsh segment, in all seasons except for the winter. This is because stream temperatures in the tile segment will be colder in the summer and warmer in the winter, in comparison to the marsh segment, from influxes of groundwater regulating stream temperatures.

Reach Comparison Study

1. I predict there will be a difference in heterotrophic activity between the marsh and tile segments. I predict that stream position will be related to those differences with differences being greater at the source of the segments, rather than the end of the segments. I predict that season will also be related to differences in heterotrophic activity between the marsh and tile segments, with differences being greatest in the summer, and smallest in the winter.

2. I predict that stream temperature and nutrient concentrations will be most strongly associated with heterotrophic activity, as they are the primary environmental variables controlling microbial metabolism.

3   Methods

3.1   Study Area

My study assessed stream ecosystem functioning in a headwater stream network in the agricultural region of southwestern Ontario, Canada (Figure 2). Southwestern Ontario experiences a humid continental climate, due to proximity to Laurentian Great Lakes, with temperatures averaging 27 °C in July and -10 °C in January (Goverment of Canada, 2021). The average annual precipitation of this region is approximately 1025 mm (Goverment of Canada, 2021). The geology of this region is dominated by calcareous Paleozoic age bedrock. Prior to the 1800s, wetlands and forests dominated Southwestern Ontario's landscape (Butt et al., 2005). Wetlands were drained and forests were removed for agriculture, resulting in the agriculturally dominated land use seen today (Butt et al., 2005). Many streams in southern Ontario historically drained groundwater fed wetlands (Butt et al., 2005). However, with the drainage of wetlands and expansion of tile drainage over the last 100 years, many streams in this region are now sourced by tile drains collecting water beneath agricultural fields (Kokulan, 2019).

My study took place within the headwaters of Nissouri Creek, located within the Thames River Basin. Nissouri Creek's drainage area is 30.9 km^2 and is primarily comprised of agricultural fields (86%), with some forested (12%) and few wetland (1%) areas (Ministry of Environment, 2012). Agricultural activities in this area consist of a mixture of crop cultivation and livestock (Ministry of Environment, 2012). Crop cultivation in Nissouri Creek's watershed consists primarily of corn (40%) with some forage and fodder crops (12%), soybean (10%), and grains (5%) while livestock consists primarily of poultry with some cattle and pigs (Ministry of Environment, 2012).

The headwater network I studied was contained within a 55-acre woodlot primarily composed of cedar/yellow birch. My study streams were composed of one first-order stream, one second-order stream, and the adjoining second-order trunk stream (Figure 2). The first-order stream (hereafter tile segment) drains a 50-acre tiled, agricultural field. The second-order stream (hereafter marsh segment) continuously drains

a 3-acre marsh and intermittently drains a 15-acre marsh. The third-order trunk stream (hereafter combined segment) drains the marsh and tile segments.

To examine the temporal patterns of heterotrophic activity among stream segments (Segment Assessment Study), 3 sampling sites along the stream network with 1 site along each stream segment were used (marsh segment: MARS01; tile segment: TILS01; combined segment: COMS02; Figure 3). Sites were continuously sampled from May of 2020 through May of 2021. Sites were comparable in bank full and wetted widths, depth, velocity, canopy cover and substrate (Table 1).

To see if there is a difference in heterotrophic activity among stream segments and positions (Reach Comparison Study), 9 sampling sites were established along the stream network, with 3 sites along each stream segment (marsh segment: MARS00, MARS01, MARS02; tile segment: TILS00, TILS01, TILS02; combined segment: COMS01, COMS02, COMS03). MARS00, TILS00, COMS01 were located at the initiation of their respective branches. MARS01, TILS01, and COMS02 were located in the middle of their respective branches (approx. 180 m, 125 m, and 165 m from source, respectively). MARS02, TILS02 and COMS03 were located at the end of their respective branches (approx. 325 m, 195 m, and 365 m from source, respectively); with MARS02 and TILS02 located just before the branches adjoin. Substrate was dominated by sand at five of the sites (MARS01, TILS01, TILS02, COMS02, COMS03). In contrast, MARS00 and MARS02 were silt-dominated and gravel dominated the substrate at COMS01 and TILS00. All sites had full-forested canopy cover. Sites were sampled over a 3 to 5-week period in each of the four seasons. The summer sampling period took place between July 23, 2020 to August 19, 2020; the fall sampling period took place between October 13, 2020 to November 9, 2020; the winter sampling period took place between January 27, 2021 to March 9, 2021; the spring sampling period took place between April 14, 2021 to May 20, 2021. Due to vandalization, data for site MARS00 during the fall are missing.
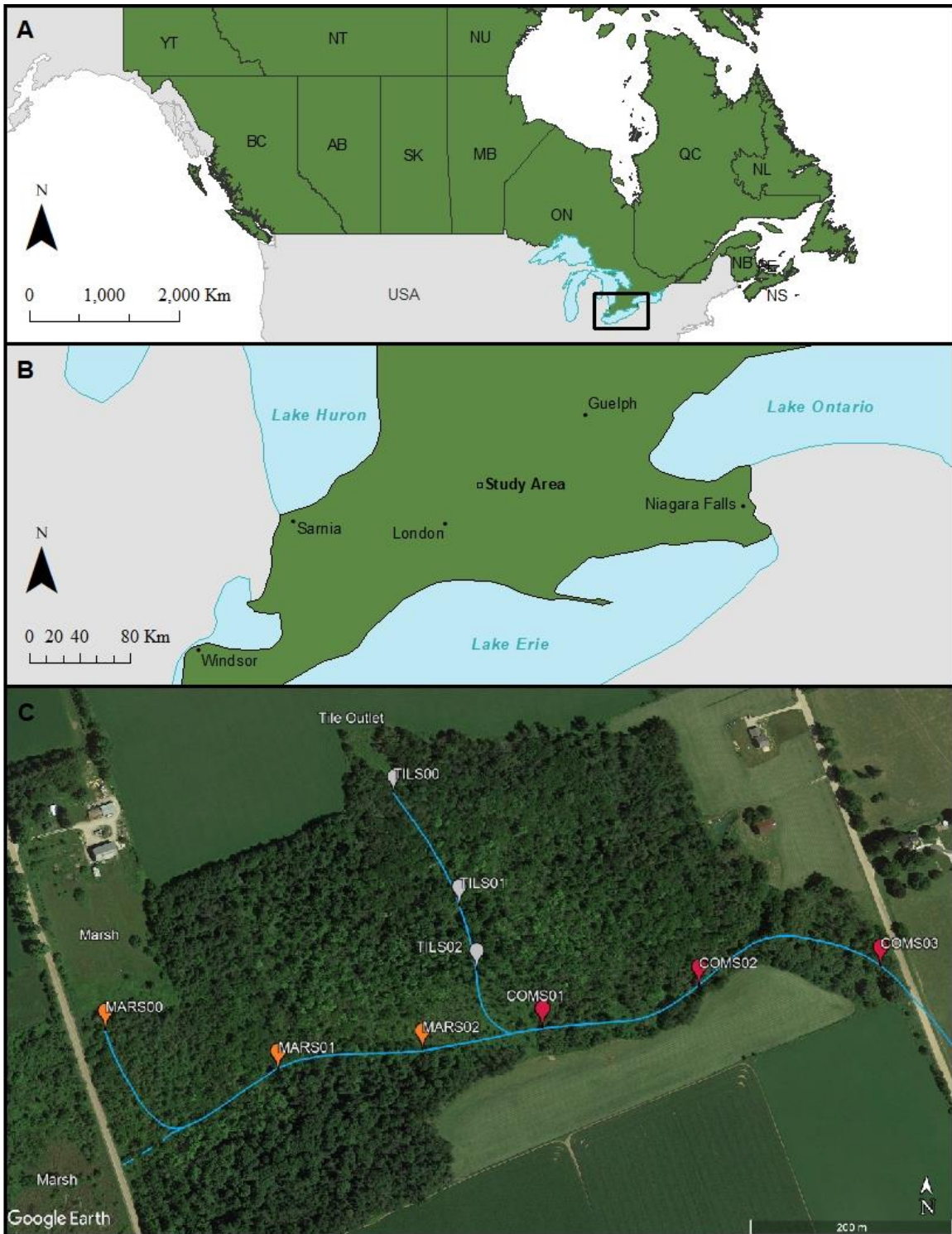
Figure 2. Maps displaying (A) the location of study region within Canada, (B) the location of study area within the study region of southern Ontario, and (C) the location of sampling sites (marsh, tile and combined sites denoted in orange, grey, and magenta, respectively) on headwater branches of Nissouri Creek draining a marsh (Western Branch) and a tile outlet (Northern Branch).

Table 1. Physical characteristics of stream reaches used for the Segment Assessment Study during the summer from a forested network located in southern Ontario, Canada.

Site	Bank Full (m)	Wetted Width (m)	Depth (cm)	Max Velocity (m/s)	Canopy Cover	Substrate
MARS01	1.8	0.9	5.0	0.011	Full	Sand
TILS01	2.8	1.1	3.2	0.073	Full	Sand
COMS02	2.4	1.1	4.5	0.066	Full	Sand
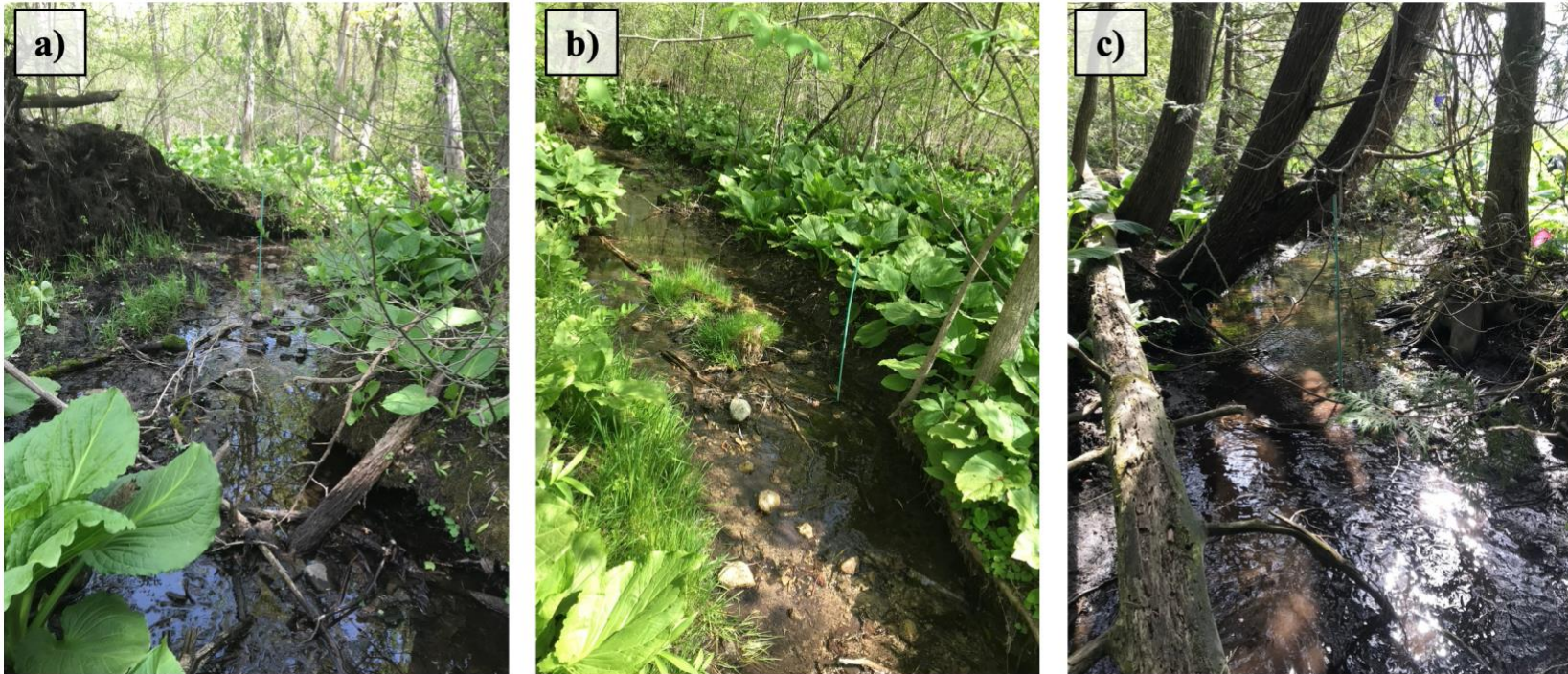
Figure 3. Spring photos of the three stream sites; MARS01 (a), TILS01 (b), COMS02 (c), used for the Segment Assessment Study from a forested network located in southern Ontario, Canada.

3.2   Data Collection

OM breakdown was measured using the cotton strip assay (CSA). Preparation, deployment, retrieval and processing of the cotton strips followed procedures in Tiegs et al. (2013). In brief, six cotton strips (2.5cm by 8cm with 3mm length frayed edges) cut from Fredix-brand unprimed 12-oz. heavyweight cotton fabric, Style #548 (Fredrix, Lawrenceville, GA, USA), were anchored to the streambed in riffle habitats of each stream using rebar. Strips were incubated for approximately three to five weeks, depending on the season, to achieve an average tensile loss of 50%. Following incubation, the strips were retrieved and sterilized in 70% ethanol to inhibit further decomposition, unless they were to be processed for respiration determination (see below).

In the lab, cotton strips were dried at 40°C for 24 hours before being used for analysis of tensile strength. Tensile strength was measured using a tensiometer and test stand with a pull rate of 2 cm/min. Tensile strength in treated strips was compared to reference strips that underwent the same processes, but were incubated in distilled water. Loss of tensile strength, used to assess the rate of OM breakdown, was calculated using equation (1).

$$(1) \qquad \text{Tensile Loss (\%)} = \frac{\left(1 - \frac{\text{Tensile Strength}_{\text{TRT}}}{\text{Tensile Strength}_{\text{REF}}}\right) \text{x } 100}{\text{Incubation Time}}$$

Measurements of respiration were collected following the procedure from Tiegs et al. (2013) in all seasons, except for the winter due to limitations with the oxygen sensors in sub-zero temperatures. At each site, six 200 mL chambers (3 control chambers and 3 chambers containing 2 strips each) were filled with stream water, capped, and placed on the streambed for 2 hours. Dissolved oxygen (DO) was measured using an Ultrapen (Model PT5, Myron L Company) before and after the 2-hour incubation. Upon removal from the chambers, strips were sterilized for 30 seconds in ethanol and then taken to the lab to be dried at 40°C for 24 hours. Strip respiration was calculated using equation (2) modified from Hauer and Lamberti (2017).

$$(2) \quad R_{OM} = \left[\frac{(DO_{OM\ start} - DO_{OM\ end})}{t_{OM}} \right.$$

$$\left. - \frac{(DO_{Control\ start} - DO_{Control\ end})}{t_{Control}} \right] \times Volume_{H_2O}Chamber$$

Water temperature was measured every hour using HOBO loggers (UA-002-64, Onset) at the same locations and intervals of the cotton strips. Average daily temperatures, as well as average daily minimum and maximum temperatures were calculated for each day.

3.3 Stream Environment Sampling

Channel characteristics (width and depth of channel, velocity, substrate, and canopy cover) were measured for the Segment Assessment Study. Stream stage (depth at a single point; indicator of stream flow) and water temperature was recorded every 30 minutes over the duration of the study using level loggers (U20-001-04, Onset). Channel form measurements were completed once during the summer to measure channel width and depth at 5 evenly spaced transects spanning 10 times bank full width of channel. Riparian canopy cover was measured using a densiometer at 3 (lower, center and upper) of the 5 afore-mentioned transects. In addition, average velocity was obtained by measuring velocity, using a velocity-meter, once at the deepest point across the channel at each transect. Velocity measurements were also taken for each strip of the Reach Comparison Study.

Water chemistry and dominant bioavailable nutrient forms (i.e., dissolved organic carbon (DOC), nitrate-nitrite (NO_2^- + NO_3^-), soluble reactive phosphorus (SRP), specific conductivity (SPC), and pH) were measured at each site. Water chemistry was sampled using a handheld YSI sonde to collect instantaneous measures of SPC and pH. Dominant bioavailable nutrient forms were measured by collecting grab water samples in a turbulent region of the stream. Samples were shipped to the Biogeochemical Analytical Service Laboratory in Edmonton, Alberta, Canada, and analyzed for DOC, using a Total Organic

Carbon Analyzer (detection limit of 0.1 mg/L as C) and nitrate-nitrite and SRP, using an Automated Ion Analyzer (detection limit for of 1 ug/L as N, and 2 ug/L as P).

3.4   Data Analysis

<u>Segment Assessment Study</u>

To examine the temporal patterns of heterotrophic activity among stream segments, timeseries plots were generated depending upon the type of data that was available. For data types that were measured as a snapshot of current conditions (i.e., water chemistry, heterotrophic activity), values were assigned to the sampling event. For data types that were measured continuously (i.e., water level, stream temperature), values were averaged by day over the sampling period. Timeseries plots were visually analyzed to detect and compare trends through time.

<u>Reach Comparison Study</u>

A general linear model (GLM) was used to assess spatio-temporal differences in stream ecosystem functioning ($\alpha = 0.05$; obj. 1). A fully nested hierarchical model was used where positions were nested within stream segments. Fixed effects were season and segment as well as their interaction (season x segment), and position (nested in segment and season). GLM analyses were followed by Tukey's pairwise post-hoc tests ($\alpha = 0.05$). The GLM analysis and post-hoc tests were performed in TIBCO Statistica (version 13.5). All means are presented with plus/minus standard deviation.

Partial least squares (PLS) regression was used to weigh the importance of physical variables (i.e., velocity, depth), stream temperature (i.e., degree day/day, average daily stream temperature range), and water chemistry (i.e., DOC, nitrate-nitrite, SRP, pH, SPC) variables on tensile loss/day (obj. 2). For data types with snapshot measurements (i.e., physical variables, water chemistry, tensile loss), values were assigned to the sampling period. For data types with continuous measurements (i.e., stream temperature), values were summarized over the sampling period. Specifically, degree day/day was calculated by totaling the average daily temperatures for each incubation period and dividing it by the incubation time, in days. Moreover, average daily stream temperature

range was calculated by averaging mean daily stream temperatures over the incubation period. All variables were normalized prior to analysis. The goodness of prediction fit (Q^2), which compares the observed values to the predicted values, was used to evaluate model performance ($Q^2 > 0.097$). To evaluate the total explanatory capacity of the model, the sum of each component's explanatory capacity (R^2Y) was calculated and only components that explained more than 10% of the variation of tensile loss were retained. The influence of each factor was assessed using variable importance on the projective (VIP) scores and only factors with significant (VIP > 1.0) scores were considered important for explaining tensile loss. X scores of the significant variables were examined to determine the direction of association. The PLS regression was performed in TIBCO Statistica (version 13.5).

4   Results

4.1   Segment Assessment Study

Averaged over each sampling event throughout the year, the marsh segment had the greatest annual mean tensile loss (2.83 ± 1.11 %/day), followed by the combined segment (2.49 ± 1.01 %/day), and the tile segment (1.99 ± 1.01 %/day; Figure 4). Tensile loss for all stream segments steadily increased to a maximum from June to September (marsh: 4.28 %/day; tile: 3.89 %/day; combined: 4.28 %/day) before gradually decreasing to a minimum (marsh: 0.95 %/day; tile: 0.59 %/day; combined: 0.99 %/day) in early March, then increased in April before declining in May. The greatest observed tensile loss occurred in the combined segment (4.28 %/day) during the August 11 to 31 sampling period. In contrast, the smallest tensile loss was observed in the tile segment (0.58 %/day) during the sampling period from January 27 to March 9. The range of tensile loss over the study year was similar for all stream segments at around 3.3 %/day.

The combined segment had the greatest annual mean respiration (0.138 ± 0.015 mg O_2 hr^{-1}), followed by the marsh segment (0.136 ± 0.015 mg O_2 hr^{-1}), and the tile segment (0.125 ± 0.031 mg O_2 hr^{-1}; Figure 4). Respiration for all stream segments increased from July to early August. However, respiration for the tile and marsh segments

continued to increase into late August before decreasing in September, whereas respiration for the combined segment decreased in late August before increasing in September. Respiration for the tile stream continued to steadily decrease into November. In contrast, respiration for the marsh stream increased in October before decreasing in November and respiration for the combined stream decreased in October before increasing in November. From April to May, respiration for the combined and tile segment increased, but decreased in the marsh segment. Maximum respiration occurred in the marsh segment (0.168 mg O_2 hr^{-1}) during the August 11 to August 31 sampling period, whereas minimum respiration occurred in the tile segment (0.082 mg O_2 hr^{-1}) during the March 9 to April 15 sampling period. The range of respiration values over the study year was greatest in the tile segment (0.077 mg O_2 hr^{-1}), followed by the marsh segment (0.050 mg O_2 hr^{-1}), and the combined segment (0.042 mg O_2 hr^{-1}).

Mean daily average stream water temperatures differed by less than half a degree between the marsh (8.3 ± 6.6 °C), tile (8.8 ± 4.3 °C), and combined (8.5 ± 6.3 °C) segments throughout the study year (Figure 4). Stream temperature for all segments steadily increased from June through September before gradually decreasing in February, then increased in April to May. However, the timing of maximum and minimum daily average temperatures varied among the segments. On average, the tile segment was around 3 °C colder from July to August and about 3 °C warmer from December to February, in comparison to the marsh and combined segments. Mean, maximum, and minimum daily average stream temperatures were coldest from December to February in the marsh segment (mean: 0.9 ± 1.0 °C, max = 4.0 °C, min = -2.2 °C), followed by the combined segment (mean: 1.4 ± 1.0 °C, max = 4.9 °C, min = -1.3 °C), and the tile segment (mean: 4.0 ± 1.4 °C, max = 8.0 °C, min = -3.3 °C). On the other hand, mean, maximum, and minimum daily average stream temperatures were warmest from July to August in the marsh segment (mean: 17.5 ± 1.1 °C, max = 26.6 °C, min = 12.3 °C), followed by the combined segment (mean: 16.9 ± 0.9 °C, max = 22.4 °C, min = 12.1 °C), and the tile segment (mean: 14.4 ± 0.5 °C, max = 18.0 °C, min = 12.0 °C). Average daily temperature ranges were greatest in the combined segment (4.2 °C), followed by the marsh segment (4.0 °C), and the tile segment (2.8 °C).

Differences in mean daily water level was less than 0.01 m between the marsh (0.11 ± 0.02 m) and tile segment (0.12 ± 0.03 m), but was, on average, twice as high in the combined segment (0.20 ± 0.04 m; Figure 4). Moreover, the maximum water level occurred in the combined segment (0.54 m), whereas the minimum water level was measured in the tile segment (0.06 m). Over the period of study, water level range was also greatest in the combined segment (0.4 m), followed by both the marsh and tile segment (0.2 m). An increase in mean water level was apparent in all stream segments from the summer (i.e., June 20 to September 21) (marsh: 0.10 ± 0.01 m; tile: 0.08 ± 0.01 m; combined: 0.19 ± 0.02 m) to fall season (i.e. September 22 to December 20) (marsh: 0.013 ± 0.02 m; tile: 0.13 ± 0.02; combined: 0.22 ± 0.03 m). Afterwards, mean water levels decreased from the fall to the winter (i.e., December 21 to March 19) (marsh: 0.11 ± 0.02; tile: 0.12 ± 0.02m; combined: 0.19 ± 0.04 m) season and remained similar into the spring season (i.e., March 20 to June 19) (marsh: 0.12 ± 0.02 m; tile: 0.13 ± 0.01 m; combined: 0.19 ± 0.03 m).

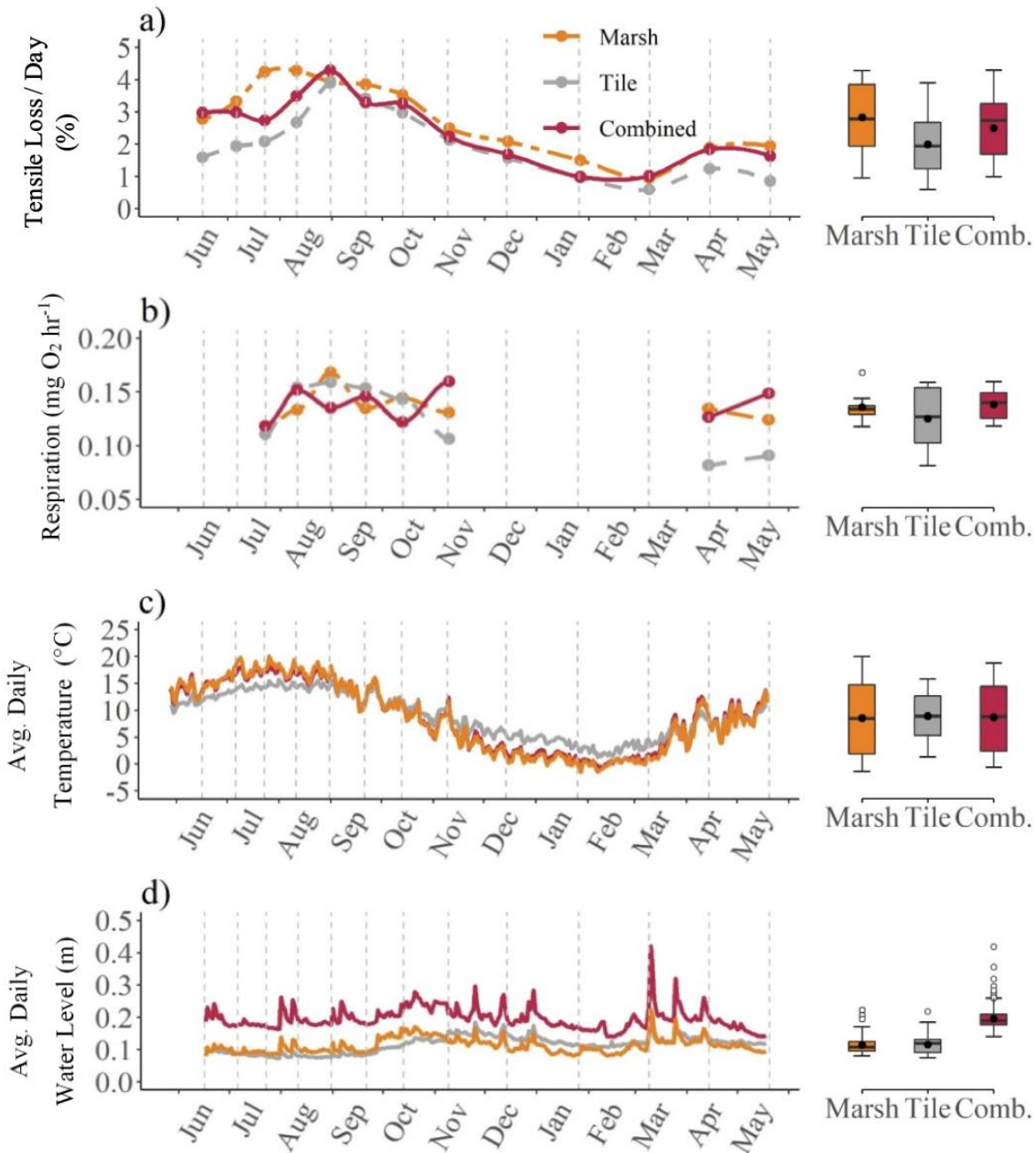**Figure 4.** Time series of tensile loss (a), respiration (b), average daily stream temperature (c), and average daily water level (d) with boxplots (25th, 50th, and 75th percentiles; whiskers denote ±1.5 x interquartile range; black dots denote mean) for the marsh (two dash, orange), tile (dash, grey), and combined (solid, magenta) segments from June 2020 to May 2021. Grey dashed lines denote sampling events.

SPC was, on average, greatest in the tile segment (704.6 ± 18.9 uS/cm), followed by the marsh (680.6 ± 48.7 uS/cm), and combined segment (664.2 ± 32.7 uS/cm; Figure 5). For all stream segments, SPC increased in July then steadily decreased into September, where SPC increased before decreasing in October. All conductivities increased in November. However, in December, SPC at the marsh and combined segments decreased while SPC at the tile segment increased. In January, SPC for all stream segments continued on the same trend of increasing in January before decreasing into April, and increasing in May. Maximum SPC occurred in the tile segment (724.0 uS/cm) during the May 27 to June 16 sampling period, while minimum SPC occurred in the marsh segment (584.0 uS/cm) during the March 9 to April 14 sampling period. SPC range was greatest in the marsh segment (158.0 uS/cm), followed by the combined segment (109.1 uS/cm), and the tile segment (59.0 uS/cm).

On average, pH was greatest in the combined segment (8.09 ± 0.20), followed by the tile segment (8.04 ± 0.22), and marsh segment (7.88 ± 0.26; Figure 5). For all stream segments, pH increased to a maximum in early July (marsh: 8.25; tile: 8.35; combined: 8.43) before slightly decreasing into September, then rapidly decreasing into October before increasing in November. Furthermore, pH for all stream segments decreased to a minimum in December (marsh: 7.28; tile: 7.53; combined: 7.67) before increasing in March and slightly decreasing in May. The marsh segment exhibited the greatest range (0.97), followed by the tile segment (0.82) and combined segment (0.76).

Mean SRP was greatest in the marsh segment (11.2 ± 5.4 ug/L as P), followed by the combined segment (8.5 ± 5.2 ug/L as P), and the tile segment (6.7 ± 5.4 ug/L as P; Figure 5). For all stream segments, SRP was steady from June to early August, when it began decreasing to a minimum in October (marsh: <1 ug/L as P; tile: <1 ug/L as P; combined: 1.0 ug/L as P) before steadily increasing to a maximum in December (marsh: 22.0 ug/L as P; tile: 16.0 ug/L as P; combined: 16.0 ug/L as P) and plateauing into May. SRP was at least 16 times greater from October to December among all stream segments. SRP range was 140 % greater at the marsh segment (21.5 ug/L as P) than the tile segment (15.5 ug/L as P) and combined segment (15.0 ug/L as P).

Average nitrate-nitrite concentrations were at least 160 % and as much as 330 % greater (10544 ± 3263 ug/L as N) at the tile segment than the combined segment (6511 ± 2188 ug/L as N) and marsh segment (3214 ± 1485 ug/L as N; Figure 5). For all stream segments, average nitrate-nitrite concentrations were similar from June to early August before decreasing in late August, where the tile and combined segments reached a minimum (tile: 5640 ug/L as N; combined: 1960 ug/L as N). All stream segments then increased in September prior to decreasing in November, where the marsh segment reached a minimum (1280 ug/L as N). Average nitrate-nitrite concentrations for all stream segments then increased in December and plateaued into May. Mean nitrate-nitrite concentrations at the tile segment were more than 210 % greater than the marsh and combined segment from December to May, as opposed to 132 % greater from June to November. Maximum nitrate-nitrite concentrations for the marsh (6340 ug/L as N) and combined (9160 ug/L as N) segment occurred in late July, whereas maximum nitrate-nitrite concentrations occurred in late May for the tile segment (15000 ug/L as N). Nitrate-nitrite concentration range for the tile segment (9360 ug/L as N) was as least 2160 ug/L as N and as much as 4300 ug/L as N greater than the combined (7200 ug/L as N) and marsh segment (5060 ug/L as N).

On average, DOC was greatest in the marsh segment (15.6 ± 9.7 mg/L as C), followed by the combined segment (13.1 ± 8.4 mg/L as C), and the tile segment (7.9 ± 6.8 mg/L as C; Figure 5). For all stream segments, DOC varied through time; however, it was unrelated to season. Maximum DOC occurred in the marsh segment (29.0 mg/L as C), followed by the combined segment (25.6 mg/L as C), and the tile segment (18.9 mg/L as C), whereas minimum DOC occurred in the tile segment (2.0 mg/L as C), followed by the combined segment (2.6 mg/L as C), and the marsh segment (3.5 mg/L as C). DOC range was greatest in the marsh segment (25.5 mg/L as C), followed by the combined segment (23.0 mg/L as C) and the tile segment (16.9 mg/L as C).
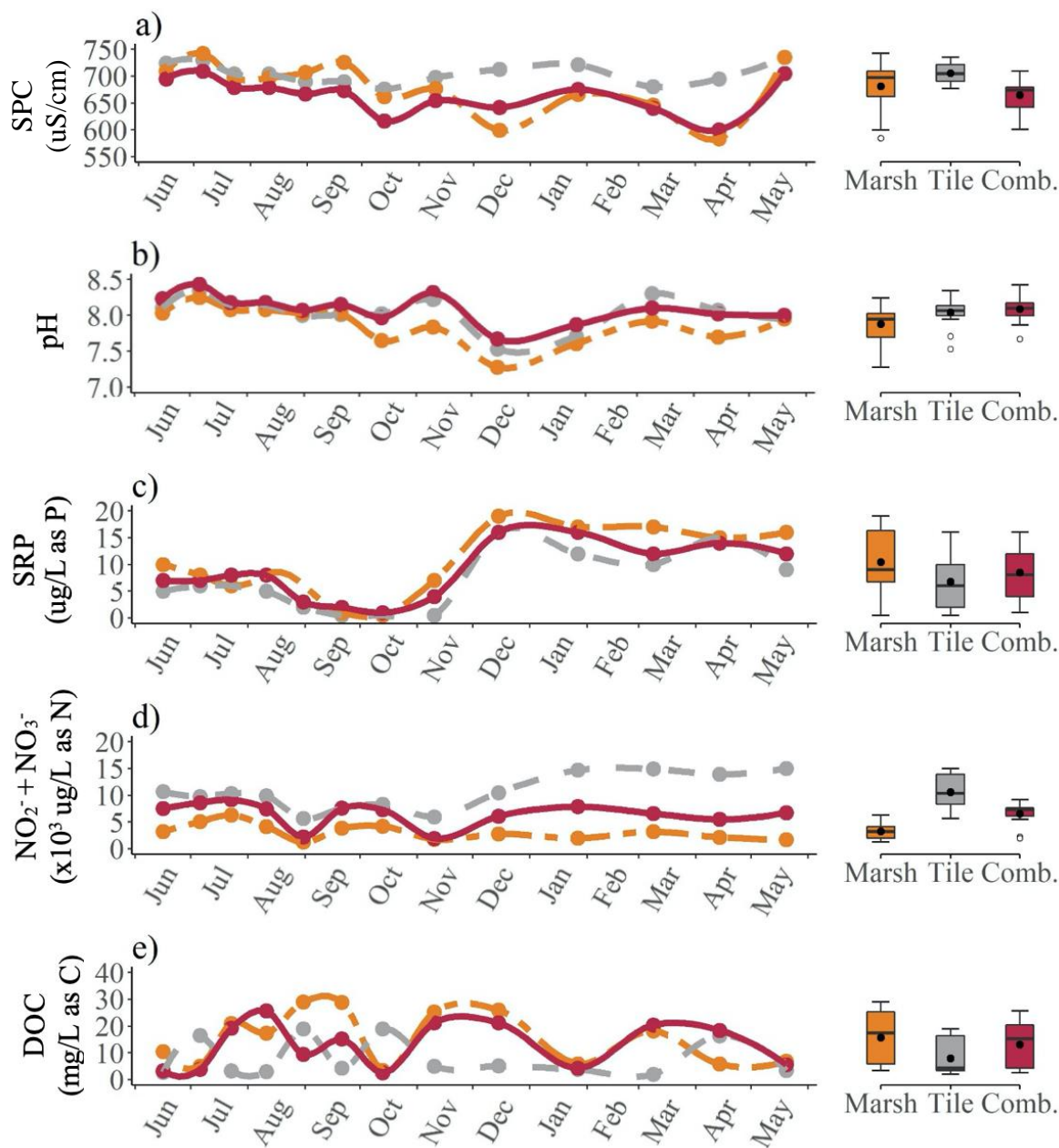
Figure 5. Time series of SPC (a), pH (b), SRP (c), nitrate-nitrite (d), and DOC (e) with boxplots (25th, 50th, and 75th percentiles; whiskers denote ±1.5 x interquartile range; black dots denote mean) for the marsh (two dash, orange), tile (dash, grey), and combined (solid, magenta) segments over 13 sampling events from June 2020 to May 2021. Closed circles denote each sampling event.

4.2   Reach Comparison Study

Mean tensile loss among all cotton strips was 1.64 ± 1.0 % day (Figure 6). For all stream segments, tensile loss was greatest in the winter, and smallest in the summer. Maximum tensile loss occurred at the middle of the marsh segment in the summer (4.61 %/day), whereas minimum tensile loss occurred at the top of the tile segment in the winter (0.33 %/day). Among all seasons, average tensile loss was lowest in the tile segment ($< 2.26 \pm 0.72$ %/day). In contrast, average tensile loss was greatest in the combined segment for the fall (2.00 ± 0.49 %/day) and summer season (3.42 ± 0.47 %/day), and greatest in the marsh segment for the spring season (1.54 ± 0.40 %/day). For all seasons, the relative order of stream position varied with segment, with larger differences occurring at the marsh and tile segments in certain seasons than the combined segment. At the marsh segment, variation in tensile loss among positions occurred in the summer, fall, and spring seasons, while there was little variation in the winter season. At the tile segment, variation in tensile loss among positions occurred in the summer, and fall, while there was little variation in the winter and spring season. At the combined segment, variation in tensile loss among positions only occurred in the fall season, where positions were stratified with decreasing tensile loss from the top to bottom positions.

The general linear model assessing spatio-temporal differences in tensile loss indicated that season, stream segment and position factors, as well as the season by location interactions were all significant ($p < 0.05$; Table 2). Tensile loss was greater in the marsh and combined segments than the tile segment for all seasons (Figure 6). However, the greatest difference between the tile and marsh/combined segments was seen in the summer while the smallest difference was seen in the fall. Furthermore, tensile loss was lowest at the upper tile and marsh sites in all but the winter season, when average tensile loss at the upper marsh position was higher than the other positions, and average tensile loss at the upper tile position was similar to the other positions. Tensile loss at the middle position of the marsh segment was highest in all seasons except for the winter season, where it was lower than the top position. Tensile loss was also greatest in the middle position of the tile segment for the summer and fall seasons, while it was similar to the other positions in the winter and spring seasons. Indeed, average tensile loss at the

middle position of the tile segment was at least 164 % greater than the top position in the summer and fall season.

Table 2. General linear model results for percent tensile loss per day.

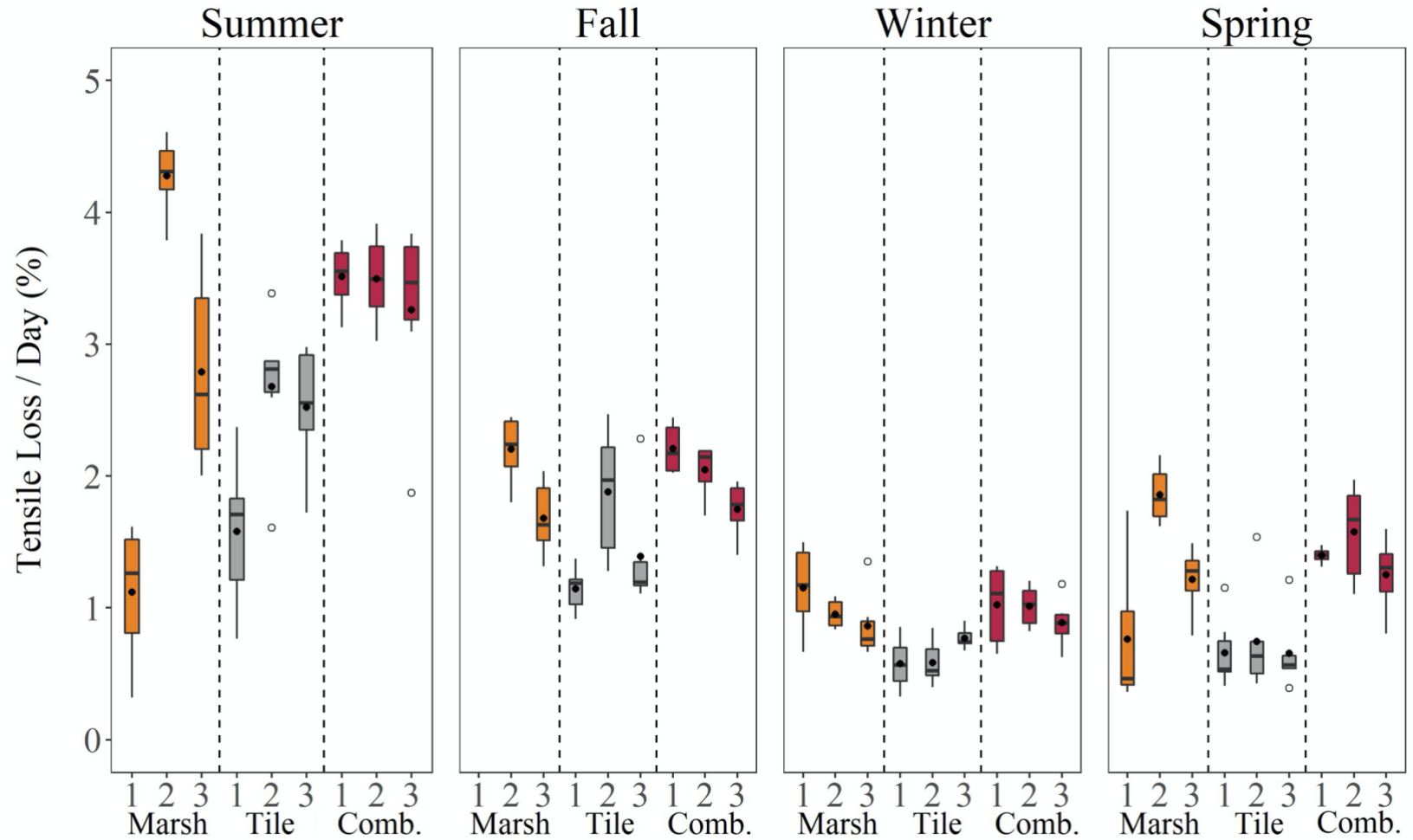| Source of Variation	Sum of Squares	Mean Squares	F	p
Season	119.60	39.87	289.74	< 0.001
Segment	17.16	8.58	62.37	< 0.001
Position(Segment)	20.85	3.48	25.26	< 0.001
Segment x Season	4.56	0.76	5.52	< 0.001
Position(Segment) x Season	20.41	1.20	8.72	< 0.001
Error	24.08	0.14		

Figure 6. Boxplots summarizing tensile loss along each position (1-top, 2-middle, 3-bottom) among summer, spring, fall, and winter seasons for the marsh segment (orange), tile segment (grey), and combined segment (magenta). Box plots show the mean, median, interquartile range, and the 5th and 95th percentiles for percent tensile loss per day.

Percent contribution calculations revealed that season explained the majority of variation (58 %) in tensile loss in the studied stream network (Figure 7). In contrast, location related factors of segment and position cumulatively explained just under 20 % of the variation with position explaining just under 2 % more variation than segment. Interaction terms cumulatively explained just over 12 % of the variation with the position and season interaction explaining just over 7 % more variation than segment and season interaction. 11 % of the total variation was statistically unexplained.
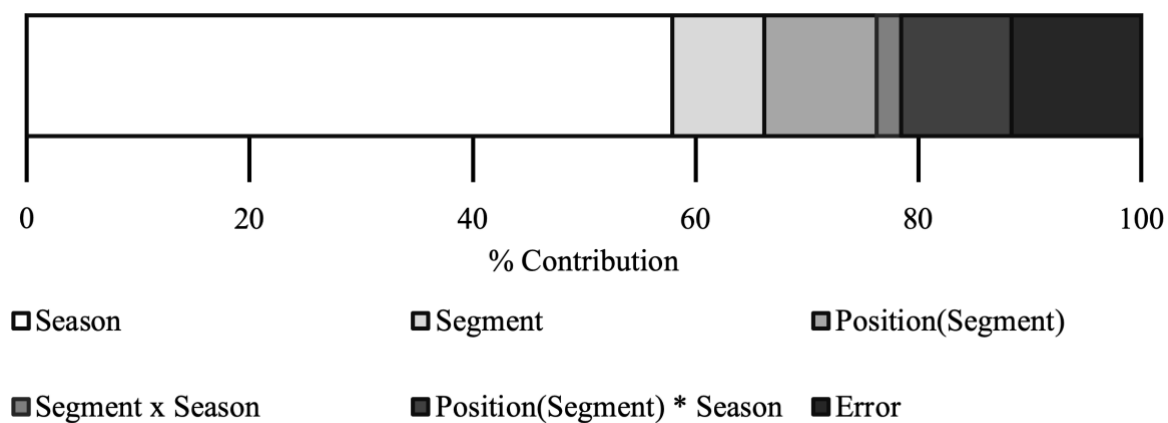
Figure 7. Percent contribution for general linear model of tensile loss/day. Grey scale from light to dark represents contribution of season, segment, position (segment), segment x season, position (segment) x season, and error.

Degree day/day were greatest in the summer (> 12.5 °C/day) and lowest in the winter (< 6.2 °C/day) regardless of segment or position (Figure 8). Degree day/day range was greatest in the marsh segment (18.6 °C/day), followed by the combined segment (17.2 °C/day), and the tile segment (12.8 °C/day), regardless of season or position. Maximum and minimum degree day/day were observed at the bottom of the marsh segment (max: 18.9 °C/day, min: 0.3 °C/day) in the summer and winter, respectively. Degree day/day were consistently between 5.6 °C/day and 13.0 °C/day at the top of the marsh and tile segments, whereas other segment positions had ranges of at least 11.6 °C/day among seasons.

Average daily stream temperature range was greatest in the spring season (5.8 ± 2.4 °C) and smallest in the winter season (1.5 ± 1.3 °C), regardless of segment or position (Figure 8). The maximum average daily temperature range was observed at the middle position of the marsh segment (8.1 °C) in the spring, whereas the minimum temperature range was observed at bottom of the marsh segment in the winter, and the top of the tile segment in the fall (0.7 °C). In the middle of the marsh segment, the maximum average daily temperature range was 8.1 °C, while the minimum was 1.0 °C, making it the position with the greatest range (7.1 °C). At the top of the tile segment, the maximum average daily temperature range was 1.5 °C, while the minimum was 0.7 °C, making it the position with the smallest range (0.8 °C). Furthermore, the top of the marsh and tile segments had ranges of average daily stream temperature range at least 2 times smaller than the other segment positions.

Average pH was greatest in the summer (8.04 ± 0.23), followed by the spring (7.85 ± 0.28), fall (7.81 ± 0.16), and winter season (7.72 ± 0.13), regardless of segment or position (Figure 8). Maximum pH occurred at the bottom of the tile segment (8.20) in the summer, whereas minimum pH occurred at the top of the marsh segment (7.30) in the winter. The greatest pH range occurred at the top of the marsh segment (0.62), whereas the smallest pH range occurred at the top of the tile segment (0.24), regardless of season. In the summer and spring, pH at the top of the marsh and tile segments was at least 0.40 units smaller and as much as 0.53 units greater, respectively, than the other two positions in those segments. In contrast, pH range in the winter was less than 0.36, regardless of segment or position.

Average SPC was greatest in the winter (705.5 ± 43.9 uS/cm), followed by the summer (703.0 ± 34.4 uS/cm), fall (668.1 ± 53.0 uS/cm), and spring season (654.4 ± 76.7 uS/cm), regardless of segment or position (Figure 8). Maximum and minimum SPC both occurred in the spring. However, maximum SPC occurred at the top of the marsh segment (799 uS/cm) whereas minimum SPC occurred at the bottom of the marsh segment (577.2 uS/cm). Regardless of season and position, the marsh segment had the greatest range in SPC (221.8 uS/cm) and was at least 2.5 times greater than the range in SPC at the combined segment (90.5 uS/cm) and tile segment (81.3 uS/cm). SPC at the top

of the marsh segment was always at least 82.0 uS/cm greater than at the other two positions, regardless of season. Additionally, SPC ranges at the top of the marsh (22.0 uS/cm) and tile (18.9 uS/cm) segments were, at minimum, 2 times smaller than other segment positions (> 45.4 uS/cm), regardless of season.

Average SRP was greatest in the winter (11.89 ± 3.14 ug/L as P) and spring (11.89 ± 2.37 ug/L as P) season, whereas it was smallest in the fall season (3.00 ± 2.12 ug/L as P), regardless of segment or position (Figure 8). Regardless of season or position, the marsh segment had the greatest average SRP (9.67 ug/L as P), followed by the combined segment (9.67 ug/L as P), and the tile segment (6.54 ug/L as P), which was at least 137 % smaller. Maximum SRP occurred at the middle of the marsh segment (17.00 ug/L as P) in the winter, whereas minimum SRP occurred at the middle of the tile segment (< 1.00 ug/L as P) in the fall. Furthermore, SRP range was greatest at the bottom position of the marsh segment (12.00 ug/L as P), whereas SRP range was smallest at the top of the tile segment, as well as the top and middle of the combined segment (8.00 ug/L as P), regardless of season

Average nitrate-nitrite concentrations across all locations were greatest in the winter season (9037 ± 4394 ug/L as N) and smallest in the fall season (3541 ± 2612 ug/L as N; Figure 8). Moreover, average nitrate-nitrite concentrations were at least 2 times greater at the tile segment (11298 ± 3600 ug/L as N) than the combined (5583 ± 2430 ug/L as N) and marsh (4867 ± 4058 ug/L as N) segment, regardless of season and position. Maximum nitrate-nitrite concentrations occurred at the top of the tile segment in the spring (16300 ug/L as N), whereas minimum nitrate-nitrite concentrations occurred at the bottom of the marsh segment in the fall (408 ug/L as N). Regardless of position, nitrate-nitrite concentration range was greatest at the marsh segment (11892 ug/L as N), followed by the tile segment (10370 ug/L as N) and the combined segment (6740 ug/L as N), which was at least 150% smaller. Furthermore, nitrate-nitrite concentration range was 2 times greater at the top of the marsh segment than the other two positions, and had an average that was 4 times greater.

Average DOC was greatest in the summer season (22.04 ± 7.81 mg/L as C), and smallest in the spring season (9.96 ± 7.79 mg/L as C), regardless of segment or position (Figure 8). Average DOC was greatest in the combined segment (18.44 ± 8.63 mg/L as C), followed by the marsh segment (15.01 ± 8.67 mg/L as C), and the tile segment (11.72 ± 9.65 mg/L as C), regardless of position. Maximum DOC occurred at the top of the tile segment in the summer (27.70 mg/L as C), while minimum DOC occurred at the middle of the tile segment in the winter (2.00 mg/L as C). DOC range was greatest at the top of the tile segment (22.90 mg/L as C), regardless of season. On the other hand, DOC range was smallest at the middle of the tile segment (3.00 mg/L as C), which was at least 7.4 times smaller than the other two positions in the tile segment.

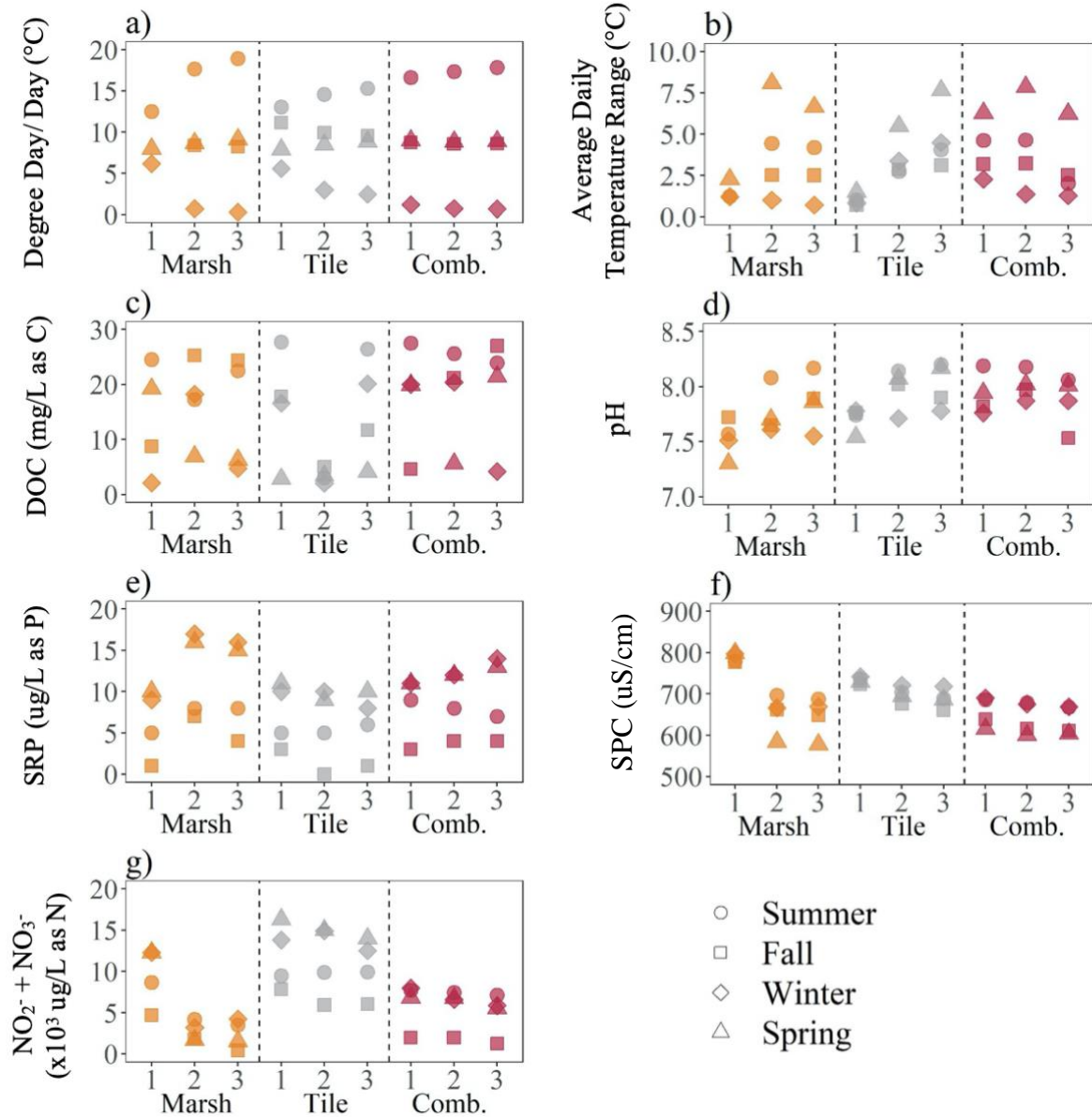**Figure 8.** Dot plots summarizing environmental variables – degree day/day (a), average daily stream temperature range (b), DOC (c), pH (d), SRP (e), SPC (f), and NO_2^-+NO_3^- (g) – along each position (1-top, 2-middle, 3-bottom) among summer (circle), fall (square), winter (diamond), and spring (triangle) seasons for the marsh segment (orange), tile segment (grey), and combined segment (magenta).

PLS analysis on percent tensile loss per day resulted in a significant model ($Q^2 =$ 0.590) that contained one component. The component explained 24.5 % of the variance of the independent variables (R^2X) and 67.7 % of the dependent variable (R^2Y). Degree day/day (VIP = 1.98), and pH (VIP = 1.42) were found to influence the variance in tensile loss (VIP > 1.0) with degree day/ day having the strongest association. Furthermore, degree day/day and pH were positively associated with tensile loss. Response variable scores showed that sites were clustered by season, where winter sites typically had the smallest rates of tensile loss and summer sites had the largest rates (Figure 9). Sites in the fall and spring were grouped together between the winter and summer season, although fall observations of tensile loss were skewed more to the positive end of the axis than were those from spring.
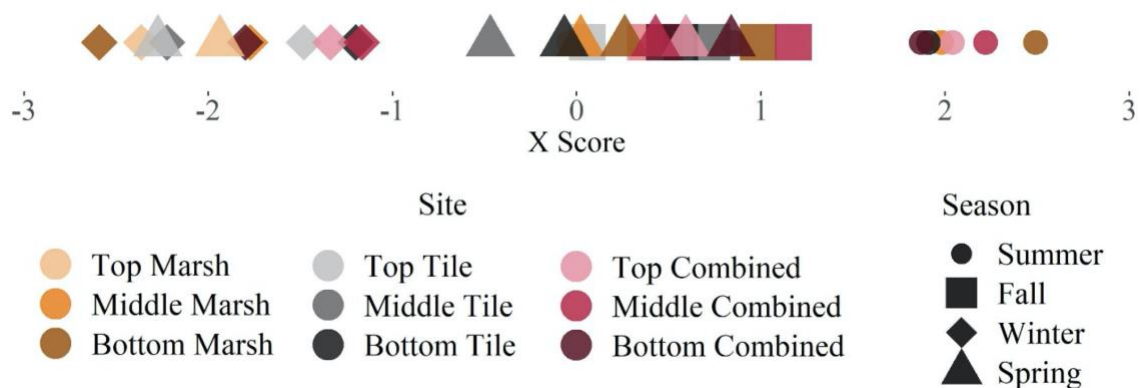

Figure 9. Response variable scores (x scores) for the PLS regression analysis of percent tensile loss per day along each position (colour scale from light to dark represents top to bottom position) among summer (circle), fall (square), winter (diamond), and spring (triangle) seasons for the marsh segment (orange), tile segment (grey), and combined segment (magenta).

5   Discussion

5.1   Comparison of heterotrophic activity to other studies

Rates of tensile loss observed in my study were within the range of variation of tensile loss observed in past studies of temperate forested streams for the spring, summer and fall seasons. For example, a study performed by Webb et al. (2019) examining forested streams in southern Ontario, Canada, with significant amounts of agricultural in the catchments observed an average tensile loss of 1.64 ± 1.01 %/day across the spring, summer, and fall seasons while my study had a slightly higher but comparable average tensile loss of 1.91 ± 0.96 %/day. Furthermore, Webb et al. (2019) had a range in tensile loss of 0.09 – 4.03 %/day which, except for having a slightly lower maximum, encapsulates my tensile loss range of 0.65 - 4.28 %/day. Additionally, a study of 20 forested streams in northern Michigan, USA, with little human activity in the catchments established mean tensile loss rates of 1.8 ± 0.7 %/day, which was comparable to my average tensile loss rates of 1.8 ± 0.4 %/day measured during the fall (Tiegs et al., 2013). Moreover, my fall tensile loss rates were also comparable to rates found at the lower-most range of tensile loss observed in least-disturbed temperate forest streams across the globe (Tiegs et al., 2019). Finally, a study performed by Kielstra et al. (2019) in southern Ontario, Canada, during the spring season observed a median tensile loss of 2.43 %/day, which was about twice as large as the median tensile loss observed in my study (1.22 %/day); however, my rates were at the lower-end of their range which may be a reflection of the urban nature of many of the streams used in their study.

As far as I could tell from the literature, there are no other studies that have looked at heterotrophic activity in the winter season in temperate regions. As a starting point for comparisons, my study observed a much lower tensile loss in the winter season, with an average of 0.87 ± 0.20 %/day, than the spring, summer, and fall seasons. Therefore, my study provides initial insights into the rate of heterotrophic activity in cold regions (i.e., air temperatures below 0°C). Future studies are needed to define typical winter rates of heterotrophic activity in temperate forested streams.

I found temperature to be the primary driver controlling differences in tensile loss among seasons. Indeed, I observed tensile loss to be fastest in the summer when stream temperatures were warmest, and slowest tensile loss in the winter when stream temperatures were coldest. My finding is consistent with several other studies who also observed greater tensile loss in warmer seasons (e.g. Fernandes et al., 2012; Ferreira & Chauvet, 2011; Griffiths & Tiegs, 2016; Webb et al., 2019). In contrast to other studies, I found greater tensile loss in the fall than the spring. However, my streams were typically warmer in the fall than spring season, whereas streams in past studies were typically warmer in the spring (Griffiths & Tiegs, 2016; Webb et al., 2019), further indicating that temperature is a key driver of seasonal differences of tensile loss.

Rates of respiration observed in my study followed similar trends across the summer, fall, and spring seasons when compared to another study in a temperate region (e.g., Bott et al., 1985). I observed greatest rates in the summer, followed by the fall, and spring; however, average rates of respiration across seasons were still very similar to each other (< 0.02 mg O_2 hr^{-1} apart). Likewise, a study performed by Bott et al. (1985) on forested streams in Michigan, USA, observed the same trend across seasons, with rates of respiration being greatest in the summer, followed by the fall, and spring.

5.2 Effect of agricultural tile drainage on heterotrophic activity

I consistently observed lower rates of tensile loss in the tile segment than the marsh segment. Thus, if these streams were comparable prior to tile drainage, my findings suggest that the construction of the tile system led to reduced rates of heterotrophic activity. Impaired heterotrophic activity in the tile segment appears to be due to the influx of additional groundwater year-round and associated reduction in stream temperatures, as temperature was significantly associated with tensile loss. The effect of lowered temperatures was strongest in the summer, when stream temperatures and heterotrophic activity differed the most between segments, and almost neglible in the fall, when stream temperatures and heterotrophic activity were most similar between

segments. Consistent with my findings, a past study found neglible differences in decomposition between tile drained and natural woodlots streams during the fall (Vought et al., 1998). However, the year-long time span of my study provides additional evidence of the seasonal effects of agricultural tile drainage on stream heterotrophic activity.

I observed similar yet fluctuating rates of respiration between the tile and marsh segments in the summer and fall seasons. However, in the springtime, I observed lower rates of respiration in the tile segment. This suggests that the difference in stream sources was not reflected in the rate of respiration, except for in the spring. My observation of similar rates of respiration across the summer and fall season suggest that seasonal changes were not reflected in the rate of respiration.

During the winter, I observed lower rates of tensile loss at all positions in the tile segment despite this segment having the warmest stream temperatures. Possible explanations for the lower rates despite the presence of warmer water are 1) decreased diversity and density of the heterotrophic community, and 2) water temperatures below the threshold for heterotrophic activity. Firstly, the composition of heterotrophic communities may have been altered by other factors that I did not measure, such as contaminants (e.g., pesticides) originating from the agricultural field. Indeed, previous studies have found that microbial litter decomposition was significantly reduced in the presence of agricultural pesticides as a result of declines in microbe abundance and diversity (Rasmussen et al., 2012; Schäfer et al., 2007). Secondly, the difference in water temperatures between the tile and marsh segments may not have mattered because temperatures in both segments were below that needed to stimulate microbial activity. Indeed, average stream temperatures for both segments in the winter season were consistently below 5 °C, which is the same temperature range other studies have found heterotrophic activity to be greatly suppressed (Koske & Duncan, 1974; Sridhar & Bärlocher, 1993). Further studies are needed to pinpoint the cause(s) of lower rates of tensile loss despite warmer stream temperatures.

I observed less variation in tensile loss among positions in the tile segment than the marsh segment suggesting that the implementation of tile drainage may have homogenized the rate of heterotrophic activity along the stream segment. The contrast in longitudinal warming was strongest in the summer, when the marsh segment exhibited the most substantive longitudinal warming trend (1.7 °C increase from top to bottom position), in contrast to the tile segment (0.9 °C increase from top to bottom position), even though both segments had similar stream temperatures at the upper-most positions. The differences in longitudinal warming between the two segments may be the combined effect of channel shortening and increased capture of groundwater associated with the installation of tile drainage. Indeed, the tile segment is 130 m shorter than the marsh segment, likely in part because the tile segment was truncated by drainage pipes that replaced the upper-most portions of the stream network during the implementation of tile drainage. With less aboveground distance to travel, water from the tile would not have as much time to warm before reaching the lower reaches of the tile segment, compared to water in the marsh segment. Additionally, the tile segment had at least 5 times more discharge at the upper-most position than the marsh segment, suggesting that the tile segment may have greater influxes of groundwater than it did prior to the implementation of tile drainage. Thus, a greater volume of cool groundwater being redirected to the stream through tile drainage pipes may also explain the slower longitudinal warming pattern of the tile segment. Overall, the combined effects of stream network truncation and influxes of groundwater may explain the reduced amount of heterotrophic activity throughout the tile segment relative to the marsh segment.

My examination of drivers of tensile loss showed that pH was positively associated with tensile loss. This association was seen in all segment positions and was strongest in the summer, when pH levels were greatest. pH has been found to be an important control in other studies of tensile loss (Clivot et al., 2013; Griffith & Perry, 1994; Suberkropp, 1995; Thompson & Bärlocher, 1989; Webb et al., 2019). However, other studies finding pH to be a driver of tensile loss had pH ranges greater than 2.0 (Suberkropp, 1995; Webb et al., 2019), whereas the greatest pH range in any given season in my study was 0.6 and that range was only found at one site, whereas all other

sites had pH ranges within 0.2. As daily fluctuations in pH can be as much as 0.5 units in streams (Allan & Castillo, 2007), it seems unlikely that a pH range of 0.6 would make a biological difference and thus, it is unlikely that pH in my study had a strong influence on tensile loss. Rather, it is more likely that the pH association is an artifact of pH covarying with temperature, as sites with warmer stream temperatures typically also had higher pH.

5.3 Effect of agricultural tile drainage downstream

I observed influences of the agricultural tile drain downstream of the tile segment, especially in the summer season. Tensile loss in the combined segment more closely resembled the marsh segment, which is likely due to having similar temperature patterns. However, when the tile and marsh segments converged, the addition of colder water from the tile segment caused the warming trend of the marsh segment to reset to a temperature closer to that observed midway down the marsh segment. The reset suggests that the combined segment would have been warmer without inputs from the tile segment, suggesting that tile drainage decreased stream temperatures downstream. As temperature was found to be a driver of tensile loss in my study, it is probable that colder stream temperatures in the combined segment lowered tensile loss beyond what would be observed in the absence of tile drainage. Furthermore, stream temperatures at the lower-most position of the combined segment were colder than the lower-most position of the marsh segment, despite being 40 m longer. This suggests that inputs from the tile segment had thermal influences greater than 365 m downstream. However, as the combined segment continued to warm downstream, those influences gradually weakened and thus likely did not persist substantially further down the studied stream network.

6 Applications

My study has identified possible impacts of agricultural tile drainage on heterotrophic activity in one stream network. However, agricultural tile drainage is widespread throughout the world. Indeed, around 11 % of the world's agricultural fields are tile drained (ICID, 2018). In North America, 27 % of cropland is tile drained with 14 % of that tile drainage occurring in Canada (ICID, 2018). In southern Ontario, Canada,

45 % of agricultural fields are tile drained, with some regions as high as 85 % (OMAFRA, 2011). Thus, if my findings are representative of how tile drainage has affected headwater streams more broadly, there may be a widespread reduction in heterotrophic activity in streams across agricultural regions where tile drainage is prevalent. There may also be less variability in heterotrophic activity along stream networks. Furthermore, in areas where headwater streams are predominately sourced by agricultural tile drains that intersect the water table, streams may be colder at both the source and downstream network.

My study comparing heterotrophic activity in streams sourced by wetlands and agricultural tile drains is useful to inform managers of the possible impacts agricultural tile drainage can have on stream heterotrophic activity. Stream ecosystem function, measured by heterotrophic activity, provides essential ecosystem services such as maintaining water quality and food webs to provide human societies with drinking water and food, wastewater disposal areas, aesthetic/recreational opportunities, and water for municipal/industrial/agricultural uses (Meyer et al., 2005). In particular, my findings of lowered heterotrophic activity, reduced variation of heterotrophic activity downstream, and colder stream temperatures from agricultural tile drainage provides managers with critical information that can assist with setting land use management targets that protect stream ecosystem function.

Many studies have been proposing the CSA as an effective tool for monitoring stream health, as tensile loss is sensitive to changes in environmental conditions (e.g. Griffiths & Tiegs, 2016; Tiegs et al., 2013; Webb et al., 2019). By demonstrating that tensile loss measured using the CSA is sensitive to thermal changes, my study provides further support for the use of the CSA as a biomonitoring tool, particularly for the assessment of thermal effects resulting from human activities. Thermal effects are one of the main types of impacts that human activities can have, particularly in urban and agricultural land use where there are reductions of riparian shading, and alterations in groundwater influxes and channel morphology (Sullivan & Adams, 1991).

7   Future Research

The findings of my research suggest three future lines of research:

1. My study comparing trends in stream heterotrophic activity between tile drain and wetland sourced streams over a year has enabled me to identify the seasonal effects of tile drainage on heterotrophic activity. However, interannual variability in those trends remains unknown. To determine how much the effects of agricultural tile drainage on heterotrophic activity differs over years, and see how trends change in response to different conditions (e.g., warmer winters, wetter summers, etc.), my study could be repeated in the future. Answering these questions would further knowledge of the effects of agricultural tile drainage on stream heterotrophic activity under various annual conditions.

2. Future research is needed to dissect my finding of lower rates of heterotrophic activity despite the presence of warmer water in the tile drained segment during the winter season, and determine if this finding was caused by lowered diversity and density of the heterotrophic community. Genomics techniques could be used to assess the communities and see whether there is a difference in diversity and density of bacteria/fungi between the marsh and tile drained segments, and whether the degree of tile drainage influences that difference.

3. Replicate studies need to be performed in other streams and temperate regions to determine if trends in heterotrophic activity between tile drain and wetland sourced streams in my study are consistent across all tile drained streams. Particularly, replicate studies would be useful in determining whether trends are consistent across regions with different uses for tile drainage, such as comparing tiles that drain the water table to tiles that drain pooling water on the surface. These studies would determine whether the effects of agricultural tile drainage can be generalized regionally and/or by usage.

8   Conclusion

Agricultural tile drainage in southern Ontario is common and likely to increase with continued agricultural intensification. Tile drainage may impact ecosystem function as it influences stream environmental conditions, such as stream temperatures, water chemistry, and hydrology, that drive ecosystem function. My study, comparing ecosystem function (via heterotrophic activity) between marsh-sourced and tile-sourced streams, provided evidence that agricultural tile drainage may lower heterotrophic activity along the stream network. Colder stream temperatures, via the influx of groundwater, were associated with this reduction in heterotrophic activity. Reduced stream ecosystem function can negatively impact the essential services stream ecosystems provide, such as maintaining water quality and food webs for human and wildlife survival, making my findings critical to helping managers set land use management targets that protect stream ecosystem function. Further studies are needed to determine if reduced heterotrophic activity from agricultural tile drainage, found in my study, is consistent across all tile-sourced streams.

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