Assessing Stream Function Across an Agricultural Gradient in Three Ecoregions of Alberta

by

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Abstract

Streams provide essential ecosystem services including nutrient cycling and uptake, organic matter processing and ecosystem production and respiration. Stream ecosystem functioning provides an integrated metric of biological structures and processes that can respond to anthropogenic land use activities including removal of riparian areas and nutrient enrichment. Agricultural land use is common in the Boreal Transition, Parkland and Grassland ecoregions of Alberta, where streams are exposed to a range of land use intensities and levels of nutrient loading. Two key indicators of ecosystem function are stream metabolism and organic matter processing, which incorporate carbon cycling and community structure. This thesis aims to describe the direct and indirect effects of anthropogenic land use on stream metabolism and decomposition in streams within agriculturally-impacted areas of Alberta. I studied 34 streams along a gradient of nutrients over three years in spring, summer and fall. Metabolism was measured with the single station open-channel method over 3–5-day deployments in each season. Variation in stream physicochemical properties was analyzed across regions and seasons, and direct impacts on metabolism were studied through generalized linear mixed modelling and indirect impacts through structural equation modelling. Stream metabolism was driven primarily by water temperature and dissolved organic carbon concentrations and does not appear to be strongly influenced by increased concentrations of nutrients. Land cover has indirect impacts on metabolism by controlling changes in light and dissolved organic carbon availability. Decomposition was measured in-stream using litterbags deployed from June to September. Decomposition was assessed through decay rates and stoichiometry. Contrary to metabolism, decomposition was directly correlated with increasing total nitrogen concentrations, which increased microbial decay rates and lowered litter carbon:nutrient ratios. This research

showcases the diverse impacts of anthropogenic land use on stream function and the importance of nutrient criteria and riparian management for preserving ecological integrity.

Preface

This thesis is an original work by Emily Barrie and is divided into four distinct chapters. The first chapter is a general introduction to the thesis that provides background information on the objectives and project design. Chapters two and three are manuscripts intended for publication. The fourth chapter is a general conclusion that provides a summary and recommendations for future work. Chapters 2 and 3 are written in plural to reflect the collaborative work. Supervisory authors were Drs. S.E. Tank and R.D. Vinebrooke with further support from Dr. G.S. Piorkowski and M. Kobryn.

Chapter 2

Barrie, E., Kobryn, M., Tank, S. E., Vinebrooke, R. D., and Piorkowski, G. S. Environmental drivers of stream metabolism across land use gradients in agricultural ecoregions of Alberta, Canada

Chapter 3

Barrie, E., Kobryn, M., Tank, S. E., Vinebrooke, R. D., and Piorkowski, G. S. Microbial activity and nutrients impact organic matter decomposition in agricultural streams of Alberta, Canada

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Chapter 1. General Introduction

1.1 Project Background

1.1.1 Stream Ecosystems in Agricultural Regions

Stream ecosystems are important for biogeochemical cycling, agricultural water use, habitat, and recreation. The strong link between stream ecosystems and human activity has led to significant environmental stressors (Allan 2004, Sabater et al. 2018). Anthropogenic uses of streams can alter ecosystem health and function through changes to carbon cycling (Cole et al. 2007), eutrophication (Carpenter et al. 1998, Dodds and Smith 2016), and climate change (Whitehead et al. 2009, Piggott et al. 2015). Agricultural land use in particular can have many impacts on stream health through non-point source pollution of nutrients and pesticides, and changes to riparian vegetation (Vitousek et al. 2009, Beketov et al. 2013). The variety of impacts agriculture can have on steam ecosystems requires examination of which drivers alter ecological integrity.

Agriculture is often associated with increases in non-point source nutrient pollution and removal of riparian areas. The riparian areas of streams are important for providing a transition zone between the stream and uplands that controls the movement of materials between the two areas. Increasing nutrients can lead to eutrophication, increases in toxic blooms of cyanobacteria, shifts in species composition and changing biogeochemical functioning such as carbon cycling and decomposition (Burrell et al. 2014). Agricultural impacts on stream ecosystems can be mitigated through watershed management plans and the prescription of beneficial management practices. Appropriate management plans include the examination of biological indicators to assess preventative or restorative designs to preserve stream health.

1.1.2 Functional Assessments of Stream Ecosystem Health

Baseline structural metrics such as water chemistry and algal or invertebrate species composition are often used to characterize stream health. The inclusion of functional metrics that incorporate ecosystem processes in water quality monitoring can allow for more sensitive assessments that more directly quantify change in ecosystem services (Young and Collier 2009, Feio et al. 2010, Verdonschot and Lee 2020). Functional metrics allow for the assessment of the interacting biotic and abiotic components of stream ecosystems (Palmer and Febria 2012). Functional metrics can also indicate changes to ecological integrity where structural metrics may not due to decoupling between structural traits and function (Feckler and Bundschuh 2020). Therefore, functional metrics are complementary to structural assessment and necessary for a holistic approach to assessing stream health.

Stream metabolism and organic matter decomposition are becoming more common methods of assessing stream function due to their ease of measurement and the lowering costs of equipment (Young et al. 2008). Stream metabolism incorporates an integrated measure of gross primary production and ecosystem respiration within streams, while decomposition relates to local food web dynamics and carbon availability. Both of these metrics have the potential to respond to agricultural land use change and associated impacts (Piscart et al. 2009, Griffiths et al. 2013, Clapcott et al. 2016, Wagenhoff et al. 2017). Stream metabolism has been shown to vary across land use types and anthropogenic intensity (Bernot et al. 2010, Pearce et al. 2020). Decomposition, often measured through leaf litter breakdown, can be affected through changes to temperature and flow regimes (Ferreira et al. 2006, Griffiths and Tiegs 2016, Manning et al. 2018, Yeung et al. 2018, Palmer and Ruhi 2019) as well as nutrient concentrations in streams (Ferreira et al. 2015, Manning et al. 2015). By assessing links between the stream environment and agriculture though stream metabolism and organic matter decomposition, a comprehensive assessment of ecosystems' responses to agricultural intensity can be completed.

The effects of agriculture on stream function may also vary depending on specific geographic and climatic conditions (Woodward et al. 2012). In Alberta, streams in the Parkland, Grassland and Boreal ecoregions vary in their climate, dominant soil types, and land use but are all extensively affected by agriculture (Palliser Environmental Services, 2008). The variability of Alberta's ecoregions thus provides an ideal opportunity for assessing the impacts of anthropogenic land use on stream function. Stream function can be affected through direct changes in local characteristics such as nutrient concentrations, canopy cover, water temperature, and channel characteristics, but also more indirectly by regional characteristics including climate and watershed morphology (Bernot et al. 2010, Handa et al. 2014, Farrell et al. 2018). Therefore, it is critical to understand how variation across ecoregions relates to the impacts of agriculture on streams. By understanding the environmental conditions within streams and watersheds of each ecoregion and across nutrient gradients, this project will allow for the determination of steam functional indicators' responses for individual ecoregions.

1.2 Research Objectives

The purpose of this thesis is to examine how stream function shifts along a gradient of nutrients and agricultural land use. Through describing ecoregion and land use effects on stream health, the impacts of agricultural land cover can be determined, which will be essential when developing watershed management plans. This thesis consists of two data chapters each with their own objectives. The first chapter assesses direct and indirect drivers of stream metabolism along a gradient of agricultural intensity where our primary objectives were to: (i) determine the influence of local environmental variables affected by land use change on stream metabolism, and (ii) describe how variation in watershed-scale characteristics indirectly impact stream metabolism. We hypothesized that watershed land cover and morphology would have indirect impacts on metabolism by controlling local conditions such as light, temperature and discharge. We also predicted that removal of riparian vegetation and increased nutrients would result in higher metabolic rates across all seasons. The second chapter assesses organic matter decomposition in response to nutrient enrichment in agricultural ecoregions with the objectives of: (i) examining microbial and invertebrate decay rates and their drivers, and (ii) assessing changes in detrital stoichiometry along a gradient of increasing nutrient concentrations. We hypothesized that increasing concentrations of essential nutrients of nitrogen and phosphorus would lead to faster decay rates and decreasing carbon: nutrient ratios of litter due to enhanced decomposer activity.

1.3 Study Design

The study was conducted in agricultural areas of the Boreal Transition Zone, Parkland and Grassland ecoregions of Alberta. Major river basins containing our study sites include the Athabasca, Red Deer, Oldman, North Saskatchewan, South Saskatchewan, and Battle rivers, with headwaters in the Rocky Mountains and Western Alberta. The Grassland and Parkland regions experience high levels of agriculture, while agricultural development in the Boreal is confined to the Dry and Central Mixedwood subregions. We assessed 34 streams ranging from 3rd-5th Strahler order that were chosen to be wadable with similar physical characteristics in width and accessibility. Streams were chosen to span a gradient of increasing concentrations of nitrogen and phosphorus. Sampling was conducted in the spring, summer and fall seasons to account for temporal variation. The study spanned three years between 2017-19 with repetition between some sites to account for long-term trends. We measured stream metabolism for 3-5 day

periods in each season and organic matter decomposition between June and September. To assess the impacts of agriculture on stream function, we compared our metrics to gradients of nutrient enrichment, physical and chemical conditions, and land cover within the watershed.

1.4 Significance

The use of integrated measures of stream health will provide more comprehensive knowledge of the effects of agriculture across ecoregions in Alberta. The knowledge generated from this research will contribute to academic literature on how stream ecosystem function is impacted by nutrient loading and land use change in varying ecoregions. Understanding the impacts of nutrients and agriculture at a functional level will be important for developing watershed management plans for streams in agricultural areas. Current numerical water quality guidelines for Alberta surface waters only relate to major lakes and rivers. Therefore, there is a need for more explicit water quality standards for lower order stream reaches in these agricultural regions (Alberta Department of Health 1970, Government of Alberta 2018). This project is also a component of Alberta Agriculture and Forestry's "Nutrient Objectives for Small Streams in Agricultural Watersheds of Alberta" which aims to recommend nutrient criteria for streams in these regions. Water quality management plans can be based on specific ecoregion responses determined from their impacts on functional response within the provincial project. Furthermore, changes to drivers of stream function altered by land use change such as temperature, canopy cover and light can also be directly targeted for mitigation by management plans. This project will evaluate the importance of riparian areas and land cover within the watershed.

Chapter 2. Environmental drivers of stream metabolism across land use gradients in agricultural ecoregions of Alberta, Canada

2.1 Introduction

Stream metabolism is an integrated measure of gross primary production (GPP) and ecosystem respiration (ER), where the difference reflects net ecosystem productivity (NEP), and acts as a sensitive indicator of stream function (Young et al. 2008). Stream metabolism was first quantified by Odum (1956) with the description of daily oxygen curves to calculate production and respiration. In recent years, methods of measuring diurnal oxygen have become more refined and accessible allowing for more robust metabolism measurements (Hoellein et al. 2013). Measurements of diel oxygen to find primary production and respiration allows for the integration of all organisms and processes influencing organic matter processing in streams, making metabolism a sensitive environmental metric (Hall and Hotchkiss 2017). Stream metabolism has been used as an indicator to discern the effects of nutrients (Frankforter et al. 2010, Pearce et al. 2020), anthropogenic activities (Beaulieu et al. 2013, Griffiths et al. 2013, Yates et al. 2013, Clapcott et al. 2016, Arroita et al. 2018), temperature (Demars et al. 2011), light (Nebgen and Herrman 2019a, Reisinger et al. 2019), discharge (Blaszczak et al. 2019, O'Donnell and Hotchkiss 2019) and watershed morphology (Jankowski and Schindler 2019) on overall stream function.

Many studies have used stream metabolism as an indicator of ecosystem health in agricultural areas (Griffiths et al. 2013, Alberts et al. 2017), but responses to land use can vary among ecoregions (Frankforter et al. 2010, Gurung et al. 2019). Due to the variety of stressors on metabolism related to agriculture, it is necessary to assess how regional trends relate to local drivers of metabolism. Previous studies have been conducted across wide spatial scales (Bernot et al. 2010, Appling et al. 2018b) but the focus on agricultural gradients in these studies is limited. Regional variation in factors such as climate, landscape morphology and land cover can influence and interact with local environmental variables to control metabolism within watersheds (Bernot et al. 2010, Bogard et al. 2020). In Alberta, streams in the Parkland, Grassland and Boreal ecoregions vary owing to differences in the associated climate, dominant soil types and land use of their catchments, but extensive modifications of the landscape for agriculture are common to all ecoregions (Palliser Environmental Services 2008). The broad ecoregion variability as well as strong agricultural gradient presents Alberta as an ideal area to study metabolic responses to environmental variables across a differing land-use intensity within varying ecoregions.

In addition to the direct effects of agricultural land use and nutrient rich soils on the functioning of Albertan streams (Prepas and Trew 1983), several broad drivers that vary seasonally can be expected. For example, precipitation and discharge regimes can affect seasonal metabolism through increases in dissolved organic carbon (DOC) and scouring of primary producers. Seasonal increases in chlorophyll *a* can be associated with changes in GPP due to greater numbers of primary producers and alterations in the number of grazers (Riley and Dodds 2012, Alberts et al. 2017). Regional temperature regimes impact metabolism through temperature dependent enzymes and shifting community structures (Yvon-Durocher et al. 2012). Increased land use pressure has also been associated with increased seasonal variation as a result of associated temporal variation in plant biomass, flow, nutrient availability and reduced riparian shading (Clapcott et al. 2016).

Agricultural land use is often associated with increases in non-point source pollution to receiving waterbodies, with nutrients being of particular concern for the eutrophication of aquatic ecosystems (Carpenter et al. 1998, Dodds and Smith 2016). Eutrophication of stream ecosystems can have detrimental impacts on stream functioning but impacts on metabolism have varied across studies depending on location, the type of agricultural land use, urbanization and latitude. Bernot et al. (2010) found ammonium and nitrate to be important in influencing GPP and ER, but found no relationships with soluble reactive phosphorus. Another large regional study of agricultural areas found no relationship between metabolism and nutrients, but found relationships between GPP and phosphorus when light was corrected for within agricultural areas of the United States (Frankforter et al. 2010). Pearce et al. (2020) also reported that even when total phosphorus (TP) concentrations increased downstream of a sewage treatment facility, there was no difference in GPP within mid-order streams. Taken together, these studies suggest that variation in the response of metabolism to nutrients may more often be due to other environmental factors that need to be examined.

Agricultural land use practices can often lead to reduced or full removal of the riparian vegetation that acts as an important buffer between streams and adjacent lands. The reduction of the riparian buffer can reduce the attenuation of nutrients in runoff from adjacent fields and

inputs of allochthonous carbon from vegetation that will impact stream metabolism (Alberts et al. 2017). Increased dissolved organic carbon in streams associated with loss of riparian areas can limit GPP through reductions in light and increase ER by stimulating heterotrophic processes (O'Donnell and Hotchkiss 2019). Intact riparian cover can also contribute allochthonous organic material to the stream, which increases microbial respiration (Riley and Dodds 2012).

The main goal of this research was to understand how stream metabolism varies across a wide range of environmental conditions and to infer potential influences of agricultural land use across contrasting ecoregions. Research objectives were to (i) characterize regional variation in drivers of stream metabolism, (ii) determine the influence of proximal environmental variables impacted by land use change on stream metabolism, and (iii) describe how regional variation in watershed morphology and land cover affect stream metabolism. We measured GPP, ER, and NEP across agricultural gradients in three ecoregions, with measurements across three seasons to determine the responses to variables altered by land use change. We hypothesized that watershed morphology and land cover, including a gradient of anthropogenic intensity will have indirect impacts on metabolism by altering local conditions such as temperature, light, and discharge. We also expected higher nutrient concentrations and increasing temperature regimes from riparian alterations to increase GPP and ER. This paper will provide insights into how stream metabolism responds to direct (stream level) and indirect (watershed level) impacts of agricultural land use across regions and offer information into watershed management practices necessary to preserve ecological integrity.

2.2 Methods

2.2.1 Study Area

A total of 34 third-to-fifth Strahler-order perennial streams in the Parkland, Grassland and Boreal ecoregions were selected for this study (Figure 2.1). All streams within the Boreal ecoregion were contained within the Dry Mixedwood subregion, as agricultural activity is most prevalent in this subregion. There were no high-quality reference sites available in these regions as all watersheds have been impacted to some extent by agricultural land use. Sites were selected from a suite of candidate sites that span a gradient of agricultural intensity, and finalized based on site accessibility and measured concentrations of nitrogen and phosphorous to confirm the existence of a nutrient gradient. In 2017, 10 Grassland sites and three Parkland sites were sampled. A total of 10 Parkland sites were sampled in 2018 with an additional two sites in each of the Boreal and Grassland. The final field season in 2019 involved 12 Boreal sites. All sites were accessed in spring (May), summer (July or August), and fall (September or October) for a total of 123 site visits.

The three ecoregions vary in their physical and climatic characteristics with the Grassland being warmer and drier and the Boreal being cooler and wetter (Natural Regions Comittee 2006). Major watersheds containing our study sites include the Athabasca, Red Deer, Oldman, North Saskatchewan, South Saskatchewan and Battle rivers. Annual average precipitation (from 1981-2010) for our study watersheds ranged from an average of 492.5 mm in the Boreal (Moose Wallow Station), to 492.4 mm in the Parkland (Olds Climate Station) and 380.2 mm in the Grassland (Lethbridge A Climate Station) (see Figure 2.1 and Table S1.1 for climate station locations). Mean annual temperature within the watersheds ranges from 2.2°C in the Boreal (Moose Wallow Climate Station) to 5.9 °C in the Grassland (Lethbridge A Climate Station) (Environment and Climate Change Canada, 2020) The Boreal is characterized by Luvisolic and the Grassland by Chernozemic soils while the Parkland includes both soil types. Surficial geology across sites varied but was dominant in Glaciolacustrine Deposits and Stagnant Ice Moraine. The Grassland and Parkland are characterized by more glacial till than the Boreal. Riparian vegetation is variable due to specific landowner practices, but also varies regionally with the Grassland being characterized by more grasses and shrubs than the Parkland and Boreal ecoregions, which are naturally treed (AAFC 2019, Natural Regions Committee 2020). Beaver activity occurs within the study region but was not studied so their influence on flow and stream metabolism could not be evaluated.

2.2.2. Data Collection

Streams were visited twice during each of the spring, summer and fall seasons, with 4-5 days between site visits. Stream width, mean velocity, mean depth, and discharge were measured during each site visit using an Acoustic Doppler Velocimeter (ADV) (SonTek FlowTracker 2, San Diego, USA) when navigable, and an Acoustic Doppler Current Profiler (ADCP) (Teledyne StreamPro, Poway, CA, USA) when water levels were high. All flow measurements were taken at a run section of stream reach with near-laminar flow, and discharge was calculated using the mid-section method (Turnipseed & Sauer, 2010). Samples for total nitrogen (TN), total phosphorous (TP), chlorophyll *a*, and dissolved organic carbon (DOC) were also collected once

during every deployment period. Water samples were taken mid-stream and mid-depth and placed on ice for transport to ALS Environmental Laboratories (Edmonton and Calgary, Alberta) for analyses (Table S1.2). An EXO2 multi-parameter sonde (YSI, Yellow Springs, Ohio) was deployed mid-stream for 4-5 day periods to measure dissolved oxygen (DO), temperature, pH, conductivity and turbidity at 10-minute intervals. The sonde was calibrated before each deployment for conductivity, pH, turbidity and local oxygen saturation following manufacturer's protocols. Sonde probes were thoroughly cleaned prior to each deployment and were deployed with an added wiper in 2019 to prevent biofouling.

In addition to measured *in situ* variables, we acquired growing degree-days and precipitation data from Alberta Climate Information Service (ACIS) weather stations (Table S1.2) (ACIS, 2020). Antecedent cumulative precipitation occurring 3, 8 and 14 days prior to each sonde deployment was compiled. Varying lengths of antecedent condition were chosen to represent the direct impacts of different precipitation regimes on stream metabolism. Watersheds were delineated using the ArcGIS Spatial Analyst extension in ArcMap software (ArcGIS v. 10.4; Esri Canada, Toronto, ON). Government of Alberta ArcHydro Phase 2 Data (AEP 2018) was used to support watershed delineation. Land cover data based upon optical satellite and radar imagery with a spatial resolution of 30 m (AAFC 2019) was aggregated within each watershed boundary through summation. Coarse land cover categories were created for each watershed by adding the areas of crops (cropland), tame pasture and forage (pasture), treed area types (forest); also included were the aggregate areas of open water, wetlands, developments (residential, industrial) and native grassland (Table S1.3, Figure S1.1). Total land cover was converted to a proportion by dividing the total area of the class by the watershed area for comparative analyses across watersheds. Watershed morphological characteristics were also calculated and included watershed area (km²), mean slope of the land area in watershed (%), overall watershed slope from start to outlet of main channel (%), drainage density (total length of streams in watershed basin divided by area), and shape factor (ratio of basin length to width).

2.2.3. Metabolism Models

Metabolism was calculated using the one-station, open channel method (Odum 1956, Hall and Hotchkiss 2017). We calculated daily GPP and ER for each 4-5 day deployment in spring, summer and fall for 109 site-season combinations (Table S1.4). A total of 14 deployments were not modelled due to stagnant water or equipment failures. Metabolism metrics were calculated using the 'streamMetabolizer' package in R (Appling et al. 2017, 2018a). The model 'b_np_oipi_tr_plrckm.stan' was chosen, which used inverse Bayesian modelling with no pooling of K_{600} (the gas exchange coefficient normalized to a Schmidt number of 600) to account for observation and process errors. Not pooling K_{600} was also better suited for shorter deployments (Appling et al. 2018b). The model fitted daily parameters by fitting observed and modelled DO for each separate 24 h period using a Bayesian Markov chain Monte Carlo (MCMC) fitting procedure. K_{600} priors were selected based on measured values in a similar landscape (Campeau et al. 2014). The model contained a function to convert daily K_{600} into an oxygen- and temperature- specific gas exchange constant (Appling et al. 2018b). GPP and ER hyper-parameters were set based on the default model setting, which uses a range of literature values (Hall 2016).

Data required for the model included diurnal (i.e., 10-minute) DO concentration (mg L⁻¹), diurnal water temperature, diurnal light data, and stream depth, which was calculated as the mean depth from the 10-station discharge transects that were collected on deployment and retrieval. The light data included in the model were obtained using two methods. In 2019, light was measured using an Odyssey photosynthetically active radiation (PAR) logger (Dataflow Systems, Christchurch, New Zealand) deployed on the stream bank, away from the influence of shading from vegetation, to measure incident irradiance. The logger measured PAR at 10-minute intervals for the length of the deployment. In 2017-18, solar radiation data were retrieved from nearby weather stations (ACIS 2020), which measured irradiance hourly in W m⁻². ACIS data were converted to PAR using the "convert SW to PAR" function in streamMetabolizer (Appling et al. 2017), which is based on numerical coefficients from Britton & Dodd (1976). Light data were interpolated to match the 10-minute intervals using the 'calc light merged' function, which merged observed data from the weather stations with modelled data based the solar time at specific geographic co-ordinates at the specified interval. Comparisons of Odyssey recorded light and ACIS weather stations data were completed by comparing the final metabolism metrics of GPP, ER and K₆₀₀ for the spring 2019 deployments and are shown in Figure S1.2. The mean relative percent differences for all sites are presented in Tables S1.5A-C. A Welch t-test was performed for each variable to compare methods and no significant differences were found (GPP, t₅₇=-0.288, p=0.774; ER, t₅₇=0.963, p=0.340; K₆₀₀, t₅₇=-1.264,

p=0.212). Weather station data were therefore used when stream bank collected light was unavailable.

Metabolism models were inspected by viewing the fit of modelled DO relative to observed DO and confirming MCMC convergence with the Gelmin-Rubin statistic (Rhat). Convergence of MCMC indicates that enough samples have been drawn from the posterior distribution to characterize its shape and location (Hobbs and Hooten, 2015). Chains with metabolism parameters that did not converge (i.e., Rhat >1.1) were run with more steps (Appling et al. 2018b). Results that did not fit ecological expectations (GPP<0, ER>0) were discarded, which occurred on approximately 2% of days, possibly due to observation error from sedimentation or inadequate flow across the sensors. In total, 98 valid models of site-season combinations were used for further analyses and comparisons.

2.2.4 Statistical Analyses

Physical and chemical properties of the sites were assessed and compared across ecoregions. Between-site variation in physicochemical parameters was assessed using a principal components analysis (PCA) for individual seasons (Oksanen et al. 2019). All variables except water temperature and PAR were log₁₀- transformed to meet assumptions of linearity and scaled before being input to the PCA. Differences across ecoregions and seasons were assessed with a two-way Analysis of Variance (ANOVA) (Type I) using ecoregion and season as factors, and Tukey HSD post-hoc tests. The residuals for TN, TP, Chlorophyll *a* and DOC did not meet normality assumptions, and thus these water chemistry variables were log₁₀-transformed before being input to the ANOVA. Variation in GPP and ER across ecoregion and season were also assessed using two-way ANOVA and Tukey post-hoc, after being log₁₀-transformed to meet normality requirements.

All metrics of stream metabolism (calculated daily) and flow (measured at sensor deployment and retrieval) were averaged for each deployment. In cases where water quality data were missing, average antecedent data from the two months preceding the assessment period were used if available to fill the missing data record (two instances with all water quality data and six for chlorophyll *a*). Data were thoroughly explored to assess for outliers, normality, collinearity and possible relationships between metabolism and environmental drivers. NEP was calculated as the sum of GPP and ER, and the GPP:ER ratio was used to determine if sites were

net heterotrophic or autotrophic (Hall and Hotchkiss 2017). To assess local (i.e., site-specific) drivers of GPP and ER, generalized linear mixed models (GLMM) were used to accommodate the non-normal distribution of the data and repeated site measurements in the 'lmer4' package (Bolker et al. 2009, Bates et al. 2015). A goodness of fit test in the 'fitdistrplus' package was used to evaluate the distributions of GPP and ER (Delignette-Muller and Dutang 2015), and considered the log normal and Gamma distributions, which are both suitable for continuous data. The Gamma fit was most appropriate for both GPP and ER (Table S1.6). GLMMs were run with a log link function and using the Laplace approximation (Bolker et al. 2009, Bates et al. 2015).

The GLMM predictors were stream physicochemical and environmental variables that could alter metabolism at the scale of a local stream reach. Fixed effect variables included for GPP were PAR, TN, TP, water temperature, DOC, discharge, chlorophyll a, and antecedent precipitation. Possible predictors of ER were identified as TN, TP, water temperature, DOC, discharge, chlorophyll a, and antecedent precipitation. In both cases, antecedent precipitation was included as a local factor to account for seasonality and variation between years in precipitation regimes. Season was also included as a fixed effect. All variations of antecedent precipitation data (i.e., 3, 8, and 14 day) were tested with generalized linear relationships with GPP and ER. The strongest relationship between metabolism and total antecedent precipitation was seen with 8 days, so only this metric was used in further analyses to avoid issues of collinearity. Models were assessed to ensure variance inflation factors (VIF) of less than 3 which led to Season being removed as a fixed effect (Zuur et al. 2009). A random intercept effect term of site was chosen to represent the repeated structure of the data. The complete models were then assessed via multi-model inference to determine the top models and highest weighted variables. Akaike's Information Criterion corrected for small sample sizes (AIC_c) was used to evaluate models due to a low number of observations. Top models were considered as those within $\Delta 2$ AIC_c of the best model. Model relative weights were calculated using the 'MuMIn' package in R (Barton 2019). The Akaike Weight (ω) for each explanatory variable was also calculated as the sum the relative weight of each model within which the variable occurred (Burnham and Anderson 2002). Model residuals were assessed for normality and homogeneity of variance, compared against the null model and convergence was assessed through the 'allFit' function (Bates et al. 2015). All predictor variables were scaled for the analysis using a z-score.

A structural equation model (SEM) was used in addition to the GLMM to better understand regional trends informed by local characteristics. A piecewise SEM was chosen due to repeated measurements at each site in the dataset. Piecewise SEMs (pSEMs) are confirmatory factor analyses based on directed separation tests and can account for random effects and smaller data sets (Shipley 2009, Lefcheck 2016). The pSEM uses partial correlation coefficients to show relationships between variables in the model and produces standardized coefficients, which are important for comparing the magnitude of effects in the model. Data were transformed to meet normality requirements and scaled using a z-score. The hypothesized causal relationships are presented in Figure 2.2, which shows the hierarchical relationship of the regional and streamspecific variables. Within the pSEM, direct influences on GPP and ER were described with linear mixed effect models based on the highest weighted variables in the GLMMs and included "Site" as a random intercept effect. Following the GLMM results (see section 2.3.3), pSEM descriptors of GPP included PAR, water temperature, DOC and discharge, while descriptors of ER included water temperature, DOC and precipitation. Watershed-scale metrics, which included watershed land cover, watershed morphology, and precipitation have site-specific values and were input as linear models. Watershed land cover and morphology were expressed as gradients using a PCA approach to simplify multiple variables and preserve degrees of freedom, after land cover was arcsine-transformed and watershed morphology was log₁₀transformed (See sections 2.3.1 and 2.3.4). All models in the pSEM were evaluated to ensure assumptions were met and then evaluated using the 'piecewiseSEM' package (Lefcheck 2016). All analyses were conducted using R 3.6.2 (R Core Team, 2019).

2.3 Results

2.3.1 Site and Regional Characterization

Selected stream physicochemical characteristics are presented in Table 2.1 and summarized as PCAs by season in Figure 2.3. Physicochemical characteristics explained different levels of variation between sites in the three seasons with between 56.1 and 67.2% variation explained. Streams within each ecoregion tended to cluster together but most so in the summer PCA. In spring, Grassland sites were characterized by greater pH and PAR while the Boreal was more associated with greater DOC. Parkland sites showed the greatest spread in the PCAs. The summer PCA explained the greatest amount of variance and a regional gradient was evident across the first principal component (43.1%). Along the gradient, Grassland sites were more correlated to PAR and pH, and Boreal sites were correlated to discharge and DOC. Parkland sites were spread more throughout the PCA space than the Grassland and Boreal sites. The fall physicochemical variables explained a cumulative 56.5% of variation where Grassland sites were still correlated with higher PAR and pH.

Detailed seasonal and regional differences in physicochemical properties are presented in Figure 2.4. Mean water temperature differed marginally across ecoregions ($F_{2,89}=2.66$, p=0.075) where streams in the Grassland region (Mean=16.24°C ± 0.81°C Standard error) were warmest. Water temperature differed significantly across seasons ($F_{2,89}=78.08$, p<0.001) as the summer season had the highest mean water temperature in the study streams (19.53 ± 0.38 °C). All seasonal temperatures were significantly different from each other based on the Tukey post-hoc test, with no significant interaction effect ($F_{4,89}=1.58$, p=0.186). Mean PAR during deployments also varied significantly across regions ($F_{2,89}=28.88$, p<0.001) and seasons ($F_{2,89}=40.36$, p<0.001). The Boreal ($289.31 \pm 24.27 \mu$ mol m⁻² s⁻¹) had significantly less light than the Grassland ($490.96 \pm 29.75 \mu$ mol m⁻² s⁻¹) and Parkland ($443.91 \pm 28.56 \mu$ mol m⁻² s⁻¹) regions based on post-hoc comparisons. The fall ($236.08 \pm 21.82 \mu$ mol m⁻² s⁻¹) also had the lowest PAR while there was no significant difference detected between spring ($477.03 \pm 22.48 \mu$ mol m⁻² s⁻¹) and summer ($450.35 \pm 30.27 \mu$ mol m⁻² s⁻¹). There was a significant interaction term ($F_{4,89}=2.74$, p=0.033) where the Boreal summer PAR decreased compared to spring and other regions.

TN did not vary seasonally or show an interaction, but did vary significantly between ecoregions ($F_{2,89}$ =7.21, p=0.001). The Grassland (M=0.85±0.09 mg L⁻¹) streams had significantly lower TN concentrations than the Boreal streams (M=0.64 ± 0.15 mg L⁻¹) based on post-hoc comparisons. In contrast, mean TP overall varied seasonally ($F_{2,89}$ =3.62, p=0.031), but not regionally. The concentrations of TP were highest (M=0.22 ± 0.23 mg L⁻¹) in the summer and significantly greater than the fall (M=0.15 ± 0.05 mg L⁻¹). Chlorophyll *a* did not vary significantly across seasons, but showed a small ecoregion effect ($F_{2,89}$ =2.96, p=0.057) where streams in the Parkland had higher concentrations than the Boreal streams. DOC varied regionally ($F_{2,89}$ =12.74, p<0.001) where the Boreal streams (M=27.94 ± 2.90 mg L⁻¹) had significantly greater concentrations than streams in either the Parkland (M=16.88 ± mg L⁻¹) or Grassland (M=15.23 ± 3.82 mg L⁻¹). Site-specific discharge varied significantly by ecoregion ($F_{2,88}$ =3.21, p=0.045) where the streams in the Parkland had the highest discharge overall (1.09 ±0.48 m³/s). There was also a marginal seasonal difference in discharge ($F_{2,88}$ =2.82, p=0.065)

with highest discharge typically occurring in the spring $(1.17 \pm 0.42 \text{ m}^3/\text{s})$. However, it should be noted that sampling over three years could be confounding ecoregion differences.

In addition to the demarcation by ecoregion, study sites also showed clear variation by watershed land cover and morphology as evident in Figure 2.5. The PCA for watershed land cover (Figure 2.5A) showed a clear regional gradient from Grassland to Boreal sites, along a gradient from exposed and cropland sites to more forest-covered and wetland dominated watersheds. The PCA for watershed morphology did not show clear separation among ecoregions, but did separate a gradient of watershed size, slope, and drainage area, with a gradient of large to small watersheds with increasing slope and decreasing drainage density along PC1, which explained 50.7% of the variance. Cropland is more common in the Parkland and Grassland ecoregions while agricultural practices in the Boreal are dominated by anthropogenically-altered pasture. Both TP and TN varied across the proportion of anthropogenic land use in the watershed and the land cover gradient (Land Use PC1). While TN rose more across the land-cover gradient (PC1), with positive values indicating greater watershed coverage of trees and wetlands, TP increased along with increasing proportion of anthropogenic land use and was driven by Parkland sites (Figure 2.6). Riparian areas at all sites are managed by landowners and often partially or completely removed. Upstream of study sites, the riparian areas can remain more treed due to topographic constraints or differing land use objectives.

2.3.2 Patterns in Stream Metabolism

Across all sites and seasons, mean daily GPP and ER ranged from 0.19 to 11.02 gO₂ m² day⁻¹ and -0.31 to -22.05 gO₂ m² day⁻¹ respectively (Figure 2.7). Overall, there were significant differences among ecoregions for GPP ($F_{2,89}$ =6.56, p=0.002), with GPP in the Boreal (M=2.62±0.42 gO₂ m⁻² day⁻¹) significantly lower than the Parkland (M=3.67±0.41 gO₂ m⁻² day⁻¹) and Grassland (M=4.10±0.39 gO₂ m⁻² day⁻¹) based on a Tukey post-hoc test. ER did not vary significantly among ecoregions ($F_{2,89}$ =1.48, p=0.234), but the range of ER values tended to vary more among ecoregions than for GPP. Seasonal differences in ER were significant (F=_{2,89} = 15.00, p<0.001), where the summer had the highest rates of ER, and Fall the lowest. However, the interaction between ecoregion and season was not significant. GPP was significantly different across seasons as well ($F_{2,89}$ =4.98, p=0.009) with fall (2.34±0.36 gO₂ m⁻² day⁻¹) being significantly less productive than the summer (4.58±0.48 gO₂ m⁻² day⁻¹) based on post hoc

comparisons. GPP and ER were generally coupled across seasons where production increased with respiration; however, there were some exceptions, primarily in the summer 2019 Boreal deployments where ER greatly exceeded GPP (Figure 2.8). The range of ER across sites was also highest in summer and – alongside GPP – most constrained in the fall.

NEP also varied significantly among ecoregions ($F_{2,89}=12.77$, p<0.001) with Grassland (M=0.48±0.33 gO₂ m⁻² day⁻¹) sites exhibiting significantly greater NEP than the Boreal (M=-3.18±0.71 gO₂ m⁻² day⁻¹) and Parkland (M=-1.47±0.48 gO₂ m⁻² day⁻¹) (Figure 2.9). While there was no significant variation in NEP across seasons, there was a significant interaction between seasons and ecoregions ($F_{4,89}=3.80$, p=0.007). The Boreal NEP decreased in summer relative to other seasons, while summertime increases in NEP occurred in the Grassland and Parkland. Only 11 of the 31 Grassland deployments were heterotrophic (a total of 35.5%), with the remaining sites having positive NEP. In contrast, both Boreal (82.9% of sites), and Parkland (71.9% of sites) sites were predominantly heterotrophic (Table S1.4).

2.3.3 Local Drivers of Metabolism

Our GLMM analysis to assess local drivers of metabolism returned four models within two Δ AIC_c of the top model for local drivers of GPP (Table 2.2). The best model for GPP included PAR (β =0.239, p=0.001), DOC (β =0.167, p=0.013), water temperature (β =0.279, p<0.001) and discharge (β =-0.187, p<0.001). Across all top models, the best predictors of GPP included discharge, DOC, water temperature, and PAR (each with a weighted importance of 1.00), followed by TP (0.343), chlorophyll *a* (0.156) and antecedent precipitation (0.153). DOC, TP, water temperature and light were all positively related to GPP. Discharge and chlorophyll *a* were negatively correlated with GPP, although the coefficient for chlorophyll *a* was not significant (β =-0.085, p=0.250). The GLMM analysis for ER resulted in six models within Δ 2 AIC_c of the top model. Discharge (β =0.092, p=0.108), DOC (β =0.233, p<0.001), precipitation (β =0.209, p<0.001), and water temperature (β =0.399, p<0.001) represented the best model for ER. Across all top models, the highest weighted variables (weighted importance of 1.00) were DOC, precipitation, and water temperature. Discharge was also present in three models (0.54), while TP (0.27) and TN (0.22) were each present in two models. All model-included variables were positively correlated with ER.

2.3.4 Regional Drivers of Metabolism

Multiple pSEMs were similarly assessed through an AIC approach to describe the hierarchal relationships between regional and local variables (Shipley 2013). Watershed morphology and land cover were included in the pSEMs via two separate PCAs, using the first axis of individual PCAs to describe variation across individual watersheds (Figure 2.5). The initial model (Figure 2.2) represented how GPP and ER were expected to be impacted directly by local environmental conditions (discharge, PAR, TN, TP, DOC, and water temperature) and indirectly by watershedscale variables (watershed morphology, land cover and antecedent precipitation capturing temporal changes). Outputs from the initial hypothesized model showed a poor fit (Fisher's C=89.47, p<0.001, df=42). Therefore, a second alternative pSEM was run with the local predictors defined by the results of the GLMMs and the PCA outputs of regional land cover and watershed morphology (Table S1.7). This model was statistically valid (C=22.20, p=0.677, df=26) with an AIC of 94.21. A final alternative model was run to include fraction of human land use (calculated as the cumulative proportion of cropland, pasture and developed land in watershed) in place of general land cover (i.e., Land Cover PC1). While this model was also statistically valid (Fisher's C=37.187, p=0.072, df=26), it had a higher AIC at 111.18. Therefore, the second model was chosen as the optimal model.

The optimal model showed a hierarchal relationship between regional and local variables (Figure 2.10). While watershed morphology was not significantly correlated with any direct predictors of metabolism (DOC, discharge, water temperature), it was a significant predictor of land cover where smaller, steeper watersheds were correlated with more forest cover (standard path coefficient= 0.190, p=0.047). Land cover PC1 was related to metabolism by being positively correlated to DOC (0.552, p<0.001), indicating a positive relationship between DOC and increasing proportions of forested and wetland cover classes. Land cover also had a negative relationship with PAR (-0.460, p<0.001), representing a decrease in light in more forested watersheds. Water temperature had a strong residual correlation with increased watershed forest cover (0.230, p=0.013). Residual correlations were used to represent the relationship between water temperature and land cover as they are both influenced by other unspecified variables.

Antecedent precipitation (as defined in 2.2.2) was assessed as a direct predictor of GPP and ER (see below), but also land cover, DOC and discharge. Precipitation was significantly positively related to Land Cover PC1, as greater precipitation was correlated with more trees and

wetlands within the watersheds (0.3772, p<0.001). However, precipitation was not a significant predictor of DOC, water temperature or discharge. Furthermore, the relationship between water temperature and PAR indicated that water temperature was positively correlated with PAR (0.634, p<0.001). There was no evidence for a relationship between DOC and discharge.

In addition to precipitation, water temperature, DOC, PAR and discharge were assessed as direct predictors of either GPP or ER. Direct predictors of GPP within the pSEM (marginal $r^2=0.30$, conditional $r^2=0.48$) differed somewhat when compared to the GLMM results. The only significant driver of GPP was water temperature (0.438, p<0.001). PAR was positively, but not significantly, correlated with GPP (0.164, p=0.162). Discharge and DOC had no correlation with GPP. Conversely, ER (marginal $r^2=0.42$, conditional $r^2=0.45$) was significantly correlated with all tested local variables. Precipitation (0.297, p<0.001), DOC (0.333, p<0.001) and water temperature (0.428, p<0.001) were all positively correlated with ER, with water temperature having the greatest influence amongst all local variables. GPP and ER had a significant positive residual correlation (0.530, p<0.001).

2.4 Discussion

This research showcases the ecological variation across geographically proximate watersheds and ecoregions and how this variation drives stream metabolism. Our study comprises a large geographical region with a gradient of natural and land use practices as well as local environmental factors that influence metabolism. We found that GPP and ER were coupled and varied among ecoregions where the Grassland had the highest rates of autotrophy likely due to greater incidence of light. Land cover, which described regional gradient, was a better indirect predictor of metabolism than ecoregion as it represented riparian conditions. Local factors such as DOC, PAR and precipitation were important in predicting GPP and ER with temperature exhibiting the greatest effect. Unlike originally hypothesized, nutrients were not a direct regulator of metabolism. All local variables that were significant predictors of metabolism are heavily influenced by riparian vegetation, which is further represented by the land cover gradient used in this study. This paper aims to identify the importance of riparian conservation for preserving ecological integrity.

2.4.1 Regional and seasonal trends of stream physicochemical characteristics

This study enables an assessment of stream metabolism across a gradient of nutrient concentrations, within multiple ecoregions. Concentrations for both TN and TP were often in excess of previously published numeric guidelines for Alberta surface waters (Alberta Department of Health 1970, Government of Alberta 2018). DOC and TN in our study were highly correlated and have been previously shown to increase in forested watersheds with agricultural activity due to litter decomposition and fertilizer application (Jiang et al. 2014). However, high concentrations of TN, TP and DOC in the Boreal are also likely driven by wetland cover within the watersheds. Other studies in Alberta also show an increase in TN and TP with agriculture. Specifically, Bayley et al. (2013) found that TN and TP in wetlands in the Boreal Transition Zone increase with agriculture. We also saw seasonally variation where TP was higher spring and summer most likely due to high phosphorus loads during spring runoff, (Jedrych 2008).

In addition to nutrient concentrations, there was further regional variation seen in chlorophyll a, water temperature and PAR, where chlorophyll a was lower in Boreal streams and water temperature and PAR were highest in the Grassland streams. Although chlorophyll *a* is expected to increase with agriculture due to greater light and nutrient availability, previous studies have shown that chlorophyll a concentrations in Alberta have not been related to agricultural intensity and tend to be more related to ecoregion (Carr et al. 2005, Bayley et al. 2013). Water temperature and PAR reflect both the expected climatic and seasonal variation (Strong and Leggat 1992), generally increasing in the summer with the exception of PAR in the Boreal. The variation in PAR reaching the stream in the Boreal region may be due to higher canopy cover but could also be due to the above-average amount of precipitation seen in summer 2019 (Environment Canada, 2020). Discharge in our study also varied where the Parkland sites typically had higher rates of discharge than the Grassland and Boreal sites with the exception of the summer 2019 season. This is consistent with higher precipitation during the sampling period. While runoff associated with precipitation in the Boreal can be low due to surficial geology and wetland cover (Devito et al. 2017), precipitation is still indicative of temporal changes between sampling years.

2.4.2 Stream metabolism in watersheds along a regional gradient of land cover

The range of GPP and ER were within the range of expected ecological values (Bernot et al. 2010), which have been shown to range from 0.1 to $16.2g O_2 m^{-2} d^{-1}$ for GPP and -0.4 to-23.1 g $O_2 m^{-2} d^{-1}$ for ER. GPP and ER were highly coupled at most sites, though ER sometimes did exceed GPP leading to more net heterotrophy at our sites. High rates of ER paired with GPP can indicate a large portion of ER is autotrophic respiration by algal biomass or that there are large inputs of organic material upstream leading to greater respiration (Hall and Beaulieu 2013). The high prevalence of net autotrophy in the Grassland ecoregion is consistent with greater light availability driving GPP, and ER being less connected to allochthonous inputs as a result of lower levels of precipitation.

While Grassland sites had the highest GPP in each season, ER showed no regional variation. Higher GPP rates in the Grassland region are likely driven by lower proportions of forested areas and higher proportions of cropland within the watershed as streams in row-crop agricultural areas tend to be more productive than streams in forested areas (Griffiths et al. 2013). While DOC was a significant predictor of ER, and varied significantly between ecoregions, ER itself did not show this same ecoregion variation. This may be explained by varying amounts of organic matter subsidies due to different riparian vegetation along the forest to grassland gradient.

2.4.3 Temperature regulates metabolism in agricultural streams

Both GPP and ER increased with water temperature, indicative of the temperature dependence of metabolic processes (Demars & Manson, 2013). Additionally, GPP and ER showed significant seasonal differences, with seasonal variation being greatest for ER. The seasonality of stream metabolism in our study was consistent with streams in areas of high anthropogenic activity that show greater temporal variation in GPP and ER (Clapcott et al. 2016). Seasonal trends across all regions are likely being primarily driven by water temperature and PAR as evident by their associated high variance inflation, where higher values correspond to high GPP and ER. Temperature in our study has stronger influence on ER, often leading to net heterotrophy, as seen in past studies due to increased organism metabolic activity (Demars et al. 2011, Yvon-Durocher et al. 2012). Furthermore, greater PAR associated with less riparian shading has been suggested to lead to increased water temperature in low flows (Nebgen and

Herrman 2019b). As a result, NEP will be negative more frequently with both warmer seasons and greater reductions to riparian areas. We also saw ER decrease in the fall, most likely due to lower water temperatures but also possibly due to limited woody debris trapping leaf litter which is consistent with more land use alterations (Houser et al. 2005). Overall land use changes resulting in increased water temperatures over summer seasons can have important implications for increased carbon emissions from these streams (Song et al. 2018).

The effect of PAR on GPP was also found to be highly weighted in our models. We measured PAR using two different methods that captured incident light at the stream surface influenced by riparian cover, but no attenuation of light in the stream due to DOC or turbidity. Frankforter et al. (2010) found that light influences GPP, where GPP decreases as canopy cover increases. While our study sites often saw the removal of riparian vegetation leading to open canopy cover, the use of the open-station method allows for the inclusion of upstream riparian areas that may be more natural (Hall and Hotchkiss 2017). Due to the relationship between GPP and PAR, we can infer that more closed canopy cover will result lower GPP. However, the appropriate riparian conditions needed to maintain healthy ecosystem metabolism requires further study.

Although season was not included in the models due to high variance inflation, the impacts of seasonality are visible in PAR and water temperature. Our sites were only monitored for short-term deployments, which makes it difficult to infer seasonal trends. While we did detect some seasonal differences in GPP and ER, our deployments could also have been impacted by short-term disturbances. Clapcott et al. (2016) suggested that deployments should exceed 5 days to accurately measure stream health, while Munn et al. (2020) recommends 14 days. The resolution of our study could be improved by comparing longer-term data in our sites to short term deployments and assessing seasonal trends over longer time periods.

2.4.4 Watershed land cover has indirect impacts on stream metabolism

The study region represents a gradient of land cover and agricultural intensity in the Boreal, Parkland and Grassland ecoregions. The geographic gradient in our study enables examination of regional variation in natural land cover as well as agricultural practices (PC1 in Figure 2.5B). We also examined watershed morphology as it has been shown to influence metabolism where wider, flatter streams tend to have greater GPP due to increased light availability, and stream metabolism in steeper watersheds is less sensitive to temperature (Jankowski and Schindler 2019). However, watersheds were not selected to account for gradients in size and shape; thus, impacts on stream function could not be adequately evaluated. Linkages between local and regional characteristics were assessed with a piecewise structural equation model. The model (presented in Figure 2.10) suggests that total land cover within the watershed is a better explanatory variable for local variables and stream metabolism than just the influence of human activities. Our findings contrast with past research studying local and regional effects on metabolism. Bernot et al. (2010) also looked at regional and proximal environmental variables impacts on stream metabolism. This study looked at a wider regional gradient and scale that this study and compared urban and reference streams. Unlike our study, they found nutrients impacted GPP and ER and that geographic controls on metabolism were less important than land use change. We expected to see the influence of anthropogenic land use on stream metabolism, but the regional gradient provided the better model. This is likely because the regional gradient incorporates upstream riparian conditions. Riparian areas at all sites are managed by landowners and often partially or completely removed. Upstream of study sites, the riparian areas can remain more natural due to differing land uses, thus other natural regional conditions such as wetland cover can influence local stream environments.

Both TN and TP increased with anthropogenic land use in our study, but we found low variable weights for TP and TN in our models for GPP and ER. Furthermore, there seemed to be little evidence of the land cover gradient of increasing treed areas and pasture impacting TP concentrations. While our data consisted of a wide range of TN and TP including high concentrations, the effects of nutrients were not evident on GPP and ER. The impact of nutrients on metabolism is often masked by other variables such as temperature and flow regimes (Frankforter et al. 2010). It is likely that any impacts of nutrients were not evident due to strong effects of temperature and PAR. This is similar to past studies examining nutrients and temperature (Pearce et al. 2020). Alternatively, the effects of nutrients may be limited due to high background concentrations and limited inorganic nutrient concentrations being detectable. However, TN did increase along the land cover gradient and was correlated with DOC, which was found be highly important for driving both GPP and ER as it was present in all models. The correlation between DOC and TN suggests that N in our study is more organic and could be related to runoff of organic material. DOC was positively related to GPP indicating that

autotrophic respiration may be an important component of stream metabolism in these regions (Cole et al. 1982, Hall and Beaulieu 2013). There is also a positive relationship between ER and DOC which could be driven by increases in bacterial respiration with increases in DOC during high flows (Demars, 2019). The increases in DOC across a land cover gradient also point to greater control of regional factors on stream metabolism than nutrients.

Watershed land cover including natural vegetation and agriculture was a significant driving factor for DOC, PAR and water temperature, which all impacted metabolism. Ecoregion was strongly correlated with DOC, which was much higher in the Boreal likely due to the higher proportion of wetlands within the watershed and high carbon flux in the region (Gergel et al. 1999, Lapierre et al. 2013) (Figure S2). Land Cover PC1 is also an indicator of the climatic differences across regions, which is indicative of a gradient in light and temperature across our streams. Latitudinal gradients are often expected to drive changes in GPP and ER due to the sensitivity of metabolism to temperature, where higher latitude sites tend to have lower NEP. However, temperature trends can be impacted by watershed land use and cover (Gurung et al. 2019). Water temperature was the only variable that was significantly correlated with GPP as evidenced by the GLMM analysis to determine local drivers. This was unexpected due to the evidence for higher ER sensitivity to temperature and the influence on PAR on GPP (Demars et al. 2011). The open station method did allow for the inclusion of upstream riparian impacts that were not directly measured; thus, the lack of significance by PAR could be because in our study PAR was not directly limited by riparian vegetation at all sites. Furthermore, PAR does have direct influences on water temperature which is highly correlated with GPP and ER. PAR was related to the regional gradient represented in the negative correlation with land cover indicating that regional trends a stronger driving force than local light limitation.

Discharge was found to have divergent impacts on metabolism by increasing ER and decreasing GPP (Table 2.2). Precipitation, which ultimately drives increases in discharge, was only present in models describing ER. The varying response of discharge and precipitation may be related to the decoupling of GPP and ER seen especially during the summer measurements collected in the Boreal region. High discharge can impact GPP through scouring of autotrophs attached to surfaces, and/or lower light availability due to increased turbidity and greater water depths. ER tends to be more resilient to high flows as there is greater fragmentation of organic

material needed for decomposition (O'Donnell and Hotchkiss 2019, 2020). With increased precipitation, there can also be greater DOC runoff from soils which enters waterways and leads to increased respiration (Demars 2019). Discharge was the only proximal variable present in the GLMMs that was not significantly correlated with any variable in the pSEM. This is likely because discharge controls on GPP are primarily related to short-term disturbances and not related to the regional scale. While precipitation did not significantly influence discharge, there was a positive correlation (Table S7), which could explain the increase in ER with discharge in the GLMMs.

2.5 Conclusion

This project conveys the influence of regional variation and agricultural land modifications on stream metabolism. Metabolic variables are strongly influenced by the latitudinal changes (ie. PAR and water temperature) with ecoregion. The agricultural practices within the Boreal, Parkland and Grassland ecoregions all vary, which can influence metabolism through alterations to DOC, PAR and water temperature. Water temperature was the most important regulator of metabolism for agricultural streams and can be influenced by seasonal changes and removal of riparian areas. While the impacts of TN and TP were not evident in this study, DOC was an important driver for both GPP and ER and was correlated with land cover, likely due to variation in forest and wetland cover. Land cover had important indirect effects on metabolism by correlating to PAR and DOC. All important proximal variables are highly connected to the landscape and riparian vegetation. Riparian areas near agricultural streams can act as buffers for nutrient runoff, provide allochthonous leaf litter used in heterotrophic respiration and control light availability and temperature regimes in the water. Riparian management is key mitigation technique for restoring and maintaining natural metabolic processes (Alberts et al. 2017). Therefore, maintenance of natural riparian buffer zones is recommended to help preserve the ecological integrity of streams in areas of high agricultural intensity.

2.6 Tables

Table 2.1. Select stream physicochemical variables, presented as a mean (± standard deviation) across all deployments.

Site	Latitude	Longitude	Width (m)	Depth (m)	Discharge (m ³ /s)	Chl a (µg/L)	TN (mg/L)	TP (mg/L)	DOC (mg/L)
Buffalo Creek	53.01	-110.87	4.8±0.2	0.46±0.21	0.20 ±0.07	6.06±0.82	1.23±0.23	0.16±0.05	18.8±4.3
Bullshead Creek	49.96	-110.61	6.48±0.66	0.45±0.05	1.13 ±0.68	13.46±6.95	0.77±0.12	0.28±0.41	10.1±5.4
Beaverhill Creek	53.75	-112.68	6.11±0.22	0.85±0.07	0.03 ±0.03	29.90±13.27	2.02±0.22	0.33±0.11	23.5±2.1
Connor Creek	54.02	-114.92	7.20 ±0.41	0.89±0.27	1.13 ±1.44	4.81±2.25	2.36±0.44	0.33±0.18	39.0±11.5
Dogpound Creek	51.79	-114.36	7.76±0.78	0.43±0.19	0.88 ±0.91	1.70±0.95	0.30±0.15	0.02±0.02	6.2±1.4
Eagle Creek	51.94	-114.43	3.94±0.76	0.43±0.17	0.03 ±0.02	5.83±1.11	0.41±0.26	0.02±0.01	7.2±5.2
Grizzlybear Creek	53.11	-110.64	2.92 ±0.28	0.50±0.16	0.15 ±0.17	25.50±17.39	3.60±0.90	0.76±0.66	43.5±15.1
Goose Creek	54.34	-114.93	6.04±0.36	0.72±0.28	1.48 ±0.84	3.94±1.28	1.32±0.30	0.09±0.01	25.8±4.5
Horse Creek	54.33	-114.69	3.12±0.61	0.79±0.20	0.30 ± 0.27	1.56±0.52	1.17±0.25	0.02±0.01	29.7±6.4
Kneehills Creek	51.48	-113.11	9.80±0.33	0.48±0.09	0.22 ±0.20	14.28±12.43	1.35±0.59	0.14±0.11	29.3±34.3
Little Paddle River	53.95	-115.02	5.96±0.65	1.09±0.72	3.63 ±3.49	8.15±9.13	2.30±0.10	0.19±0.09	38.9±10.2
Lasthill Creek	52.36	-114.46	12.11±0.63	0.58±0.12	0.66 ± 0.64	6.65±4.40	1.38±1.39	0.05±0.02	12.7±6.0
Lloyd Creek	52.74	-114.14	8.36±0.39	0.42±0.15	0.24 ±0.23	13.62±20.32	1.24±0.37	0.14±0.06	17.3±6.9
Mosquito Creek	50.25	-113.55	9.62±0.55	0.53±0.04	0.63 ±0.05	7.30±8.83	0.48±0.21	0.04±0.02	5.9±3.7
Matzhiwin Creek	50.84	-111.93	4.78±1.77	0.38±0.22	0.42 ±0.32	4.00±1.09	0.57±0.19	0.09±0.04	7.8±4.3
Namepi Creek	54.08	-112.98	9.30±0.0	1.30±0.00	1.46 ±0.00	3.45±0.00	3.82±0.00	0.43±0.00	56.8±0.0
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Onetree Creek	50.73	-111.69	6.51 ±0.76	0.65±0.08	0.97 ±0.45	9.81±7.18	0.71±0.12	0.21±0.12	7.5±0.9
Seven Persons Creek	49.9	-110.85	3.90±0.35	0.50±0.04	0.30 ±0.22	3.08±1.82	0.56±0.12	0.06±0.02	8.8±4.5
Pipestone Creek	53.03	-113.27	6.75±2.37	0.61±0.13	1.02 ± 1.41	12.85±4.89	1.65±0.49	0.26±0.11	18.5±4.3
Pine Creek	54.7	-112.97	5.81±1.20	0.45±0.40	0.27 ± 0.34	12.06±12.65	2.73±0.05	0.22±0.05	43.8±10.1
Pothole Creek	49.52	-112.8	5.24±0.20	0.32±0.03	0.26 ±0.11	7.58±6.61	0.47±0.26	0.09±0.06	5.5±3.9
Ray Creek	52.00	-113.6	1.25 ± 0.05	0.27 ± 0.00	0.14 ± 0.00	2.82±0.00	0.97±0.00	0.18±0.00	16.3±0.0
Rosebud Creek	51.32	-113.33	5.09±0.48	0.31±0.02	0.56 ±0.23	19.63±15.54	1.17±0.32	0.15±0.09	26.4±29.5
Sturgeon River	53.83	-113.28	17.74±1.80	0.80±0.24	4.31 ±5.35	12.58±10.38	1.43±0.34	0.16±0.06	17.7±5.3
Strawberry Creek	53.31	-114.05	13.48±0.65	0.40±0.27	0.48 ±0.30	4.50±3.41	1.07±0.20	0.05±0.03	17.1±3.5
Threehills Creek	52.00	-113.57	1.99±0.62	0.62±0.09	0.06 ±0.09	10.01±3.38	1.41±0.24	0.31±0.08	21.8±3.9
Tomahawk Creek	53.35	-114.66	5.80±1.78	0.68±0.40	0.59 ±0.85	7.53±4.95	2.38±0.12	0.17±0.01	63.8±14.8
Unknown Creek	50.02	-112.75	3.01±0.51	0.56±0.14	1.02 ±0.47	6.35±0.81	0.56±0.09	0.15±0.10	3.9±1.0
Upper Sturgeon River	53.58	-114.89	10.60±0.11	1.10±0.00	0.48 ±0.00	3.66±0.00	1.43±0.00	0.10±0.00	39.7±0.0
Weed Creek	53.3	-113.98	8.54±1.04	0.78±0.09	0.25 ±0.27	4.65±1.89	1.23±0.20	0.08 ± 0.08	16.5±0.9
Weiller Creek	52.99	-113.22	3.05±0.42	0.70±0.07	0.16 ±0.06	46.60±25.80	1.65±0.50	0.84±0.31	13.3±2.7

Table 2.2. Top GLMM models of GPP and ER based on multi-model inference of all possible combinations of environmental variables. Marginal (R^2_m) describes the variance explained by the fixed effects and conditional (R^2_c) includes variance explained by fixed and random effects (site). Models are written to include intercept and coefficients.

	AICc	Δ AIC _e	ω	R ² m	R ² c
GPP ~			1		1
0.832 - (0.187×Discharge) + (0.167×DOC) + (0.239×PAR) + (0.279× Water Temperature)	348.3	0	0.412	0.327	0.663
0.820- (0.192×Discharge) + (0.153×DOC) + (0.231×PAR) + (0.092×TP) + (0.269×Water Temperature)	349.1	0.79	0.278	0.342	0.669
0.810 - (0.08×Chlorophylla a) - (0.182×Discharge) + (0.135×DOC) + (0.237×PAR) + (0.142×TP) + (0.260×Water Temperature)	350.2	1.95	0.156	0.337	0.672
0.829 - (0.190×Discharge) + (0.166×DOC) + (0.242×PAR) + (0.03×Precipitation) + (0.275×Water Temperature)	350.3	1.98	0.153	0.329	0.665
ER ~					
$1.392 + (0.092 \times \text{Discharge}) + (0.233 \times \text{DOC}) + (0.209 \times \text{Precipitation}) + (0.399 \times \text{Water Temperature})$	412.2	0	0.278	0.449	0.601
1.392 + (0.238×DOC) + (0.221×Precipitation) + (0.399×Water Temperature)	412.6	0.43	0.225	0.443	0.591
1.386 + (0.090×Discharge) + (0.221×DOC) + (0.216×Precipitation) + (0.388×Water Temperature) + (0.071×TP)	413.4	1.29	0.146	0.457	0.606
1.385 + (0.224×DOC) + (0.216×Precipitation) + (0.386×Water Temperature) + (0.075×TP)	413.7	1.58	0.126	0.452	0.598
1.386 + (0.088×Discharge) + (0.215×DOC) + (0.207×Precipitation) + (0.398×Water Temperature) + (0.057×TN)	413.9	1.74	0.117	0.459	0.611
1.384 + (0.215×DOC) + (0.217×Precipitation) + (0.385×Water Temperature) + (0.067×TN)	414.0	1.89	0.108	0.455	0.604

2.7 Figures



Figure 2.1. Map of study region showing the Boreal, Parkland and Grassland ecoregions, individual study sites, and weather stations used to determine light regimes and precipitation.



Figure 2.2. Hypothesized relationships among regional and local variables affecting GPP and ER tested using a piecewise SEM (see also Figure 2.10).



Figure 2.3. PCAs for (A.) Spring, (B.) Summer, and (C.) Fall showing variation in select physicochemical characteristics across sites and coloured according to ecoregion.



Figure 2.4. Boxplots of select average physicochemical variables across ecoregions and seasons, showing the distribution of site means within individual seasons. Within plots, the box demarcates the 25th and 75th percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outlier (Quartile±1.5*Inter-Quartile Range). Outlier data points are shown using dots. Significance testing was done using a two-way ANOVA where (.) represents a p-value of less than 0.1, * less than 0.05, **less than 0.01, and *** less than 0.001, and n.s. indicates not significant.



Figure 2.5. A) Principal Components Analysis (PCA) of watershed morphology based on transformed and scaled data. Sites are colour coded by ecoregion. Area indicates watershed area in hectares, watershed slope indicates mean watershed slope from start of main channel, shape factor is the ratio of basin length to width, and drainage density is the summed length of all channels in the watershed divided by the watershed area. B) A PCA of land cover where sites are coded by region. Land cover variables were input as the arcsine of the proportion in the watershed from Agriculture and Agri-Food Canada's Annual Crop Inventory. Both "Morphology PC1" and "Land Cover PC1" were used in the pSEM.



Figure 2.6 Relationships between TN, TP and proportion of anthropogenic land use in the watershed (sum of developed, cropland and pasture), and the land cover gradient (Land Cover PC1, where positive values indicate more trees and pasture).



Figure 2.7. Box-plots to show ranges in GPP and ER across seasons and ecoregions. Within plots, the box demarcates the 25th and 75th percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outlier (Quartile±1.5*Inter-Quartile Range). Outlier data points are shown using dots. Significance testing was done using a two-way ANOVA where (.) represents a p-value of less than 0.1, * less than 0.05, **less than 0.01, and *** less than 0.001.



Figure 2.8. Mean daily GPP and ER within each season of deployment (spring, summer, and fall). The black line is a 1:1 line where points below are heterotrophic and points above are autotrophic.



Figure 2.9. Box plots of within-season mean daily NEP across sites for each ecoregion and season. NEP>0 is autotrophic and NEP<0 is heterotrophic. Significance testing was conducted using a two-way ANOVA where (.) represents a p-value of less than 0.1, * less than 0.05, **less than 0.01, and *** less than 0.001. Within plots, the box demarcates the 25^{th} and 75^{th} percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outlier (Quartile±1.5*Inter-Quartile Range).



Figure 2.10. Best fitting pSEM based on multi-model inference. Solid lines depict positive paths while dashed represents negative paths. Grey represents non-significant relationships and variables. Diagram includes standardized path coefficients and marginal R² values. Full model statistics are as follows: C=22.20, p=0.677, df=26, and AIC=94.21. Residual correlations are indicated by double-sided arrows. Only local variables with a weight of 1.00 in the GLMMs were assessed.

Chapter 3. Microbial activity and nutrients impact organic matter decomposition in agricultural streams of Alberta, Canada

3.1 Introduction

Organic matter decomposition is a key stream function that provides insights into carbon cycling and food webs (Hall et al. 2000, Gessner and Chauvet 2002, Tank et al. 2010). Decomposition integrates the effects of abiotic factors, such as temperature and physical abrasion with biological processes, including microbial activity and detritivory (Webster and Benfield 1986, Ferreira et al. 2015, Griffiths and Tiegs 2016). These processes can all be affected practices relating to increasing agricultural land use due, including increases in nonpoint source nutrient pollution, shifts in available allochthonous material, and changes to temperature and flow regimes. Thus, the sensitivity of decomposition to the conversion of natural land cover to agricultural uses makes it an important metric for assessing stream functional health. Decomposition can also vary across ecoregions through shifts in invertebrate and microbial community structure and stream physicochemical properties (Hladyz et al. 2010, Pozo et al. 2011). Therefore, it is important to assess how decomposition responds to agriculture across a broad diversity of ecoregion types. Studying decomposition in three ecoregions with varying degrees of land modification to agricultural uses can therefore provide insight into decomposition changes along a gradient of agricultural intensity in a geographic area of natural variation.

There are several key parameters known to affect decomposition that can vary across ecoregions or gradients of disturbance. Agricultural land use changes have been shown to impact decomposition through increased nutrients (Ferreira et al. 2015), which stimulate microbial activity and the colonization of leaf litter, and in turn the microbial priming increases shredder decay rates (Manning et al. 2015). However, increasing nutrient concentrations can also limit shredder decomposition when concentrations are elevated enough to have detrimental effects on invertebrate communities creating a "u-shaped" response to nutrients by invertebrates (Woodward et al. 2012). Water temperature, which is often higher in anthropogenically altered streams due to removal of riparian canopies, can also increase decomposition rates (Fernandes et al. 2009, Griffiths and Tiegs 2016). Finally, velocity can impact decomposition through increased physical fragmentation and stimulation of microbial conditioning of litter (Ferreira and Graça 2006, Ferreira et al. 2006). Increased decomposition rates due to disturbance have important ecological implications for stream ecosystems. Increased rates of decomposition are indicative of increasing shredder and microbial species abundance and diversity (Gessner et al. 2010), but can lead to lower carbon resources later on and thus lower shredder abundance (Cross et al. 2006). Furthermore, increased microbial activity can result in increased CO₂ production which has positive feedback implications for climate warming (Boyero et al. 2011).

Decomposition rates are assessed through the use of decay rates to describe the litter breakdown processes (Petersen and Cummins 1974, Benfield et al. 2017). Decay rates of specific litter allow for the assessment of how litter quality can impact decay, but a standardized substrate allows for assessments across different streams (Young et al. 2008). Decay rates are often assessed within mesh litter bags. Coarse mesh bash bags include litter exposed to invertebrates while fine mesh bags exclude invertebrate decomposers. Coarse mesh bags are more common as they can mimic natural decomposition, but fine bags and the comparison of the ratio of coarse to fine bags allows for more comprehensive understanding of processes regulating decomposition (Ferreira et al. 2020). Cotton-strip assays are becoming a more common substrate for assessing decomposition, with some proponents arguing for this substrate type as a global standard (Colas et al. 2019). However, a naturally occurring litter will provide more ecologically realistic insights into stream functioning as a response to local changes and reflect natural processes.

Changing litter stoichiometry during decomposition provides insight into microbial and invertebrate community functions through analyzing changes in nutrient demands across food webs (Sterner and Elser 2002). The C:N and C:P ratios of organic matter change during decomposition depending on the nutritional needs of the decomposer communities and stream water concentrations (Manning et al. 2016). Detrital consumers can be limited by both N and P, as N is needed for the N-rich enzymes required to acquire C from complex polymers, while there is also a high P requirement for RNA (Elser et al. 2003, Sinsabaugh et al. 2009). Microorganisms can obtain these nutrients from the litter, or from stream water when litter quality is poor. Thus, nutrients can be released in the decomposition process when the litter is more nutrient rich and retained when litter quality is poor due to retention of the nutrients within the microbial-litter complex as carbon is mineralized (Suberkropp 1998, Findlay 2010). Microbial conditioning of litter also increases the proportion of decomposition that is attributable to detritivores by increasing the nutritional quality of their food source (Kaushik and Hynes 1968, Gulis et al.

2006). Understanding how C:N:P ratios change as decomposition progresses provides insight into the biogeochemical functioning of decomposer communities and how the quality of detrital food resources change with progressive litter decomposition.

The purpose of this chapter is to assess how agricultural land use associated with nutrient enrichment affects organic matter decomposition and processing, and how this effect may vary across a series of three diverse ecoregions. We assess how nutrient enrichment influences microbial and invertebrate activity by assessing decay rates and stoichiometry in coarse and fine litterbags across a gradient of nitrogen and phosphorus concentrations in three agricultural ecoregions of Alberta. Our main objectives were to: (i) determine the response of microbial and invertebrate decay to nutrient enrichment; and (ii) evaluate detrital quality via an assessment of changing litter stoichiometry with decomposition. We hypothesized that increasing nutrients would lead to faster decay rates and that nutrient poor litter will have decreasing carbon: nutrient ratios of litter due to enhanced microbial activity.

3.2 Methods

3.2.1 Study Region and Project Overview

Organic matter decomposition was assessed at 34 sites across the Boreal, Parkland and Grassland ecoregions of Alberta (Figure 3.1). Sites were chosen to span a gradient of anthropogenic land uses, which were intended to reflect a corresponding gradient in concentrations of total nitrogen and phosphorus. The project was completed over three years: In 2017, decomposition was measured at 12 Grassland and 3 Parkland sites; in 2018, decomposition was measured at 10 Parkland (including the 3 Parkland sites from 2017) and 2 Boreal sites; and in 2019 decomposition was measured at 12 Boreal sites including the 2 sites measured the year prior. In total, 39 litterbag deployments were completed across 34 sites. However, the final statistical analysis was constrained to 32 deployments across 27 sites due to damage to some bags resulting in inability to complete analyses and two streams drying out or becoming stagnant (Figure S2.1).

Different climatic and geographical features characterize the three ecoregions. The Grassland is typically the warmest region and has higher proportions of native grass and shrubland land covers than the other ecoregions. The Boreal is the coolest ecoregion and characterized by more forest land cover; this study focused on the Dry Mixedwood subregion of the Boreal, where conversion of aspen and spruce forest to cropping and pasture systems is more prevalent than more northern subregions. Soils also differ regionally, with the Boreal dominated by Luvisolic soils and the Grassland by Chernozemic soils. The Parkland is a transition between the two soil orders and vegetation types, with a combination of aspen parkland and fescue grasslands (Natural Regions Comitteee, 2006). Surface waters in the Boreal region are characterized by higher concentrations of phosphorus, often leading them to be naturally mesotrophic or eutrophic (Prepas and Trew 1983). Additionally, 28.5% of all land in Alberta is commercially fertilized and 21.6% of farms apply manure, which can lead to increases stream water nutrients and thus in eutrophication (Carpenter et al. 1998, Dorff and Beaulieu 2014, Maheaux et al. 2016). Elevated concentrations of nutrients in stream waters across a range of climatic and geographic variables provide unique opportunities to study organic matter decomposition in this region.

3.2.2 Litterbag Deployment and Retrieval

Organic matter decomposition was assessed with litterbags, which were chosen over standard cotton substrates to better understand the natural decomposition processes in the streams (Chauvet et al. 2016). Reed canary grass (*Phalaris arundinacea*) was chosen as the decomposition substrate because it is found in agricultural riparian areas throughout our study region, enabling a common substrate to standardize decay rates among sites. A common source of reed canary grass was collected at a stream within the Parkland region of Alberta in the preceding fall for the 2017 and 2019 field seasons, and in the early spring for the 2018 field season.

Twelve fine (0.2 mm) and twelve coarse (2 mm) mesh-sized litterbags, constructed from Nitex nylon mesh, were filled with approximately 7.0 g of dried reed canary grass and strung along a vinyl-coated nylon wire alternating between fine- and coarse-bags. Separate sized meshes were chosen to account for microbial and invertebrate decomposition. The 24 bags were deployed at each stream site in late spring via attachment to the bottom of the deepest part of the stream while wire ends were anchored to the bank to simulate decomposition of riparian vegetation occurring at the banks. Following deployment, two coarse- and two fine-mesh bags were removed from the stream immediately (representing time-zero), with subsequent bags being removed every two to three weeks until early fall (a total of 6 retrievals, including time zero).

3.2.3. Physicochemical and Land Use Data Collection

Water quality samples and discharge measurements were taken upon each deployment and during every other bag retrieval (i.e., three measurements total, at deployment, at the third and at the sixth retrievals). Water samples were collected at mid-stream and mid-depth and analyzed for total nitrogen (TN), total phosphorus (TP), and dissolved organic carbon (DOC). Water chemistry analyses were completed at ALS Environmental Laboratories (Calgary and Edmonton, Alberta) following standard protocols (Table S2.1). Stream flow and associated measurements were taken with an Acoustic Doppler Velocimeter (ADV) (FlowTracker2, SonTek Inc., San Diego, CA) to calculate stream velocity, discharge, total stream width and mean depth.

Daily water temperature was measured over the course of the 2017 and 2019 deployments using a HOBO Pendent Data Logger (UA-002-64; Onset Computer Co., Bourne, MA). In 2018, and at some 2017 sites where the logger failed, mean water temperature was calculated from point measurements taken during the deployment using a multi-parameter water quality sonde (EXO2, YSI, Yellow Springs, OH) that was deployed at select sites for 4-day periods and intermittently during the litterbag deployment with a handheld multi-parameter sonde (SmarTroll, In Situ, Pittsburgh, PA). In addition to water temperature, pH was also collected intermittently using the deployed multi-parameter water quality sonde and a handheld multi-parameter sonde. Point measurements of water temperature and pH were collected on 9 to 17 days over the course of the deployments. However, to standardize measurements across sites and parameters only those measurements coinciding with bag retrieval were used to find mean pH and temperature.

For each sampling site, watersheds were delineated using the ArcGIS Spatial Analyst extension in ArcMap software (ArcGIS v. 10.4; Esri Canada, Toronto, ON) and Alberta ArcHydro Phase 2 Data (AEP 2018). Land cover was assessed using optical satellite and radar imagery with a spatial resolution of 30 m (AAFC 2019), and was aggregated within each watershed through summation as categories of cropland, tame pasture and forage (pasture), and treed area types (forest); also included were the aggregate areas of open water, wetlands, developments (residential, industrial) and native grassland. The proportion of land use in each

watershed was found as the quotient of total area of land use type and watershed area. Anthropogenic land use was defined as the sum of developed land, cropland and forage cover.

3.2.4 Litter Decomposition and Stoichiometry

After litter bag retrieval, bags were stored in a fridge at 4°C until they could be processed (up to one week). Bags were rinsed gently with distilled water and then dried in an oven at 60 $^{\circ}$ C for a minimum of three days to determine dry weight. The samples were then ground to 0.5 mm using a general purpose sample mill (FOSS CT 293 Cyclotec). After milling, samples were oven dried for 12 hours at 50°C and placed in a desiccator to cool. The ash-free dry mass (AFDM) for each litterbag was then determined by combusting 250 mg of ground sample in a muffle furnace at 550°C for 40 minutes with a ramp speed of 5°C min⁻¹ (Benfield et al. 2017). Samples were cooled in a desiccator and weighed to find the mass of the ash remaining. The percentage of organic matter (%OM) in each sample was found by subtracting the weight of the combusted sample from the dry mass of the subsample and dividing by the original subsample weight. The %OM of the sample was then multiplied by the dry weight to find the AFDM of the litter bag. For samples where AFDM could not be measured due to limited sample size, we calculated the AFDM based on the relationship between AFDM and total carbon (g; see below) ($r^2=0.9977$). Decay rates (k) were calculated using the slope of the regression between the natural log of percent AFDM remaining and days since deployment. We used the decay rate from fine bags to represent microbial decomposition (k_{microbial}), and from coarse bags to represent total decomposition (k_{total}), which represents the combined decomposition by microbial and invertebrate communities. Invertebrate decomposition rates were calculated as $k_{total} - k_{microbial} =$ k_{shredder} (Manning et al. 2016). Ratios of k_{shredder} to k_{microbial} were calculated to assess relative contributions of shredder and microbial activity to decomposition, with k_{shredder}/k_{microbial}>1 indicating a greater relative shredder contribution.

The percentage of carbon and nitrogen contained within the ground samples were measured at the Alberta Agriculture and Forestry Soil and Water Laboratory (Lethbridge, Alberta) using a FlashSmart CHNS/O Element Analyzer with Multivalve Control (ThermoFisher Scientific Inc.). Percent phosphorus was measured at the Natural Resources Analytical Laboratory at the University of Alberta (Edmonton, Alberta) using the PO₄-P colourimetric method after sulfuric acid digestion (US EPA, 1993). Percentages of C, N and P were converted to grams by multiplying by the litter bag dry mass, and molar ratios of C:N and C:P were calculated following conversion to molar units.

3.2.5 Data Treatment and Statistical Analyses

Physiochemical characteristics (TN, TP, DOC, pH, water temperature, velocity) were averaged for the entire deployment for each site. For measurements where TN or TP were below the detection limit (4% of TN samples and 5% of TP samples), half of the detection limit (0.10 and 0.01 mg L⁻¹ respectively) was used (USEPA, 2000). Physicochemical differences across ecoregions were tested with a 1-way ANOVA and assumptions verified. Velocity, discharge and N:P were log-transformed for analysis. Molar ratios of water column N:P were calculated for each site using TN and TP.

Variation in decay across ecoregions for the classes of decay rates ($k_{shredder}$, $k_{microbial}$, and k_{total}) was assessed using 1-way ANOVAs. Linear models were used to assess environmental drivers of decay. Input variables to these models included water temperature, TN, TP, DOC, the fraction of anthropogenic land use, velocity, ecoregion and stream pH. Variables were scaled and the variance inflation factor (VIF) checked. Due to DOC and TN being highly correlated (r=0.71), DOC was removed from the analysis to ensure VIF<3 (Zuur et al. 2010). Global models were assessed with multi-model inference to find the best model, and models within $\Delta 2$ AICc were retained (Burnham and Anderson 2002, Barton 2019). All model residuals were assessed to validate assumptions and outliers were checked. Models were compared against the intercept only model.

Although a common substrate was used across all litterbags, litter was collected for each deployment during the preceding year, and in some cases during different seasons. As a result, 1-way ANOVAs were conducted to see if the initial (day zero) C:N and C:P ratios were consistent across all deployments. There was variation in the nutrient composition of the bags tested at day zero between years ($F_{2,65}$ =48.5, p<0.0001) where the starting C:N ratio of 2018 bags (mean= 54.4± 1.13 S.E.) was significantly lower than both 2017 (88.0 ± 2.53) and 2019 (91.6±4.46) (Tukey post-hoc comparison, p<0.05). Initial C:P also varied significantly ($F_{2,58}$ =7.65, p=0.001) where 2017 had the highest ratio (1534.4 ± 120.41) compared to 2018 (1184.2 ±52.22) and 2019 (1192.4±28.37). Therefore, we calculated a standardized Δ C:N and Δ C:P over the length of our deployments as the mean of the final bags within each site subtracted

from the mean of the starting bags within each site (Manning et al. 2016). To assess influences on changes in litterbag stoichiometry, we looked at the effect of decay rates, water column nutrients, ecoregion and stream nutrient limitation using ANOVAs to compare regional differences and linear regressions to assess drivers of C:N and C:P. All predictor variables were scaled and assumptions validated through residuals. All analyses were conducted using R version 3.6.2 (R Core Team, 2019).

3.3 Results

3.3.1 Ecoregion Characteristics and Nutrient Limitation

Physicochemical characteristics differed among sites and ecoregions (Figure 3.2). Mean water temperature ranged from 15.0-20.2 °C across all sites. Water temperature varied significantly between ecoregions with deployment-specific temperatures in the Grassland (mean=18.8 °C \pm 0.40 S.E.) significantly greater than those in the Parkland (17.1 \pm 0.43 °C) (1way ANOVA and Tukey HSD; F_{2.29}=4.716, p=0.017). Total nitrogen varied by more than an order of magnitude across sites, with a range of 0.28-4.35 mg L^{-1} (median of 1.38 mg L^{-1}), indicating that streams spanned a range from oligotrophic to eutrophic (Dodds et al. 1998). Concentrations of TN did not vary significantly between ecoregions, but the Grassland streams did have the lowest concentrations with mean values in the oligotrophic range $(0.77 \pm 0.22 \text{ mg L}^{-1})$ ¹) (F_{2.29}=2.861, p=0.073). Similar to TN, TP also ranged by nearly an order of magnitude from 0.015 to 1.20 mg L^{-1} (median of 0.16 mg L^{-1}). This range corresponded with a classification of three streams as oligotrophic, with the rest falling in the mesotrophic to eutrophic range (Dodds et al. 1998). Concentrations of TP also showed no significant difference between ecoregions. Unlike TN and TP, DOC did vary significantly across ecoregions (F_{2.29}=8.173, p=0.002) where streams in the Boreal had greater concentrations of DOC $(34.0 \pm 5.04 \text{ mg L}^{-1})$ than streams in either the Parkland $(18.3 \pm 3.17 \text{ mg L}^{-1})$ or Grassland $(9.5 \pm 2.25 \text{ mg L}^{-1})$, which did not differ from each other. Site-specific velocity also varied significantly between ecoregions, where Grassland sites had a slightly higher mean velocity $(0.22 \pm 0.06 \text{ m s}^{-1})$ (F_{2.29}=3.408p=0.047). Similarly, study-site discharge showed a small but significant difference between ecoregions (F_{2.29}=3.533, p=0.042), with sites in the Boreal region $(0.73 \pm 0.20 \text{ m}^3 \text{ s}^{-1})$ having significantly higher discharge than those in the Parkland $(0.38 \pm 0.15 \text{ m}^3 \text{ s}^{-1})$. Finally, pH was also found to differ between ecoregions ($F_{2,29}=18.66$, p<0.001), with sites in the Grassland having the highest pH (8.59 \pm 0.006), and sites in the Boreal the lowest (7.74 \pm 0.08). Across all sites, increasing

proportions of human land use at the watershed scale had a positive effect on mean concentrations of TP (β =0.467, p=0.023). There was no measurable effect of anthropogenic land-use on TN concentrations (β =1.004, p=0.269) (Figure 3.3).However, a nutrient gradient was present across all streams as TN increased with TP (p<0.001; r²=0.716).

Stream water nutrient ratios (as TN and TP) were determined across all sites and years, and used to assess possible limitation of ecological processes (e.g., decomposition) based on comparisons to Redfield ratios, and the limitation endpoints for microalgal growth laid out in Hillebrand and Sommer (1999) (Figure 3.4). There was a wide range of N:P ratios across sites, ranging from 8.0-198.5 (median = 19.2) but most streams were within the range of co-limitation (Hillebrand and Sommer 1999). An ANOVA comparing N:P across ecoregions found a marginal difference between ecoregions ($F_{2,29}$ =2.656, p=0.087), although this was likely driven by the substantial apparent P-limitation at Horse Creek in the Boreal (N:P=198.5). Upon removing Horse Creek as an outlier, no significant difference in N:P was found among ecoregions ($F_{2,28}$ =1.847, p=0.176). However, the Boreal ecoregion did tend to have the highest incidence of apparent P-limitation.

3.3.2 Variation in Decay Rates and their Drivers

There was significant AFDM loss across all sites, with a mean final mass that was 25.86±1.54% of the deployed mass. Variation in decay rates was assessed across decomposition type and ecoregion. Comparisons of k_{shredder}, k_{microbial} and k_{total} across ecoregions are presented in Figure 3.5A. The two-way ANOVA indicated significant variation in decomposition rates among ecoregions, with Boreal sites having faster overall decomposition rates than the Grassland and Parkland sites ($F_{2,83}$ =5.299, p=0.007). k_{microbial} (0.012 ± 4.5x10⁻⁴ d⁻¹) was greater than k_{shredder} (0.006 ± 9.1x10⁻⁴ d⁻¹), and all three types of decomposition (microbial, invertebrate and total) varied significantly where k_{total} was highest (0.018 ± 1.2x10⁻³) ($F_{2,83}$ =46.848, p<0.001). There was no interaction effect between ecoregion and the type of decomposition ($F_{4,83}$ =0.453, p=0.770). Comparisons of the ratio of k_{shredder}/k_{microbial} across ecoregions indicated no cross-ecoregion variation in this metric ($F_{2,27}$ =1.025, p=0.372) (Figure 3.5B), with microbial decomposition exceeding invertebrate decomposition in all but five deployments.

Environmental drivers of microbial, shredder and total decay were assessed with linear regression and multi-model inference (Table 3.1A). Four models were returned within $2.0 \Delta AIC_c$

of the top model for microbial decay rates. The best model for predictors of $k_{microbial}$ included a positive relationship with TN (β =0.0015, p<0.001) and a negative relationship with the proportion of anthropogenic land use in the watershed (β =-0.0010, p=0.019). Variable weights were determined for all top models, with TN present in all models (variable weighted, ω =1.0), anthropogenic land use present in three models (0.80), mean velocity in two models where it had a positive effect on $k_{microbial}(\omega$ =0.42), and pH in one model only where there was a negative relationship (ω =0.17) (Table 3.1B).

There were four models within 2.0 ΔAIC_c of the top model for shredder decay rates. The most highly weighted variable was TN, which was present in all models (Table 3.1A). The best model for k_{shredder} included mean velocity (β =0.018, p=0.036) and TN (β =0.002, p=0.007). The percent of anthropogenic land use was also present in two models as a negative predictor (ω =0.34). Velocity was also highly weighted (ω =0.8) and increased k_{shredder}. The variables pH (ω =0.14) and TP (ω =0.21) were also each present in one model each and had a negative relationship with k_{shredder}.

Five models were found for describing k_{total} . The best model for total decay included mean velocity (β =0.003, p=0.008), TN (β =0.007, p<0.001) and TP (β =-0.003, p=0.091). The highest weighted variables in total decay models were mean velocity and TN, which were present in all models (ω =1). Anthropogenic land use (ω =0.31) and pH (ω =0.29) were present in two models and had negative effects on k_{total} , while TP (ω =0.28) was only present in the top model.

A similar analysis was conducted for the $k_{shredder}/k_{micorbial}$ ratio but the top models were found to be not significantly different from the intercept only model. A comparison of the $k_{shredder}/k_{micorbial}$ ratio to TN and TP saw a general increase in invertebrate decay with increasing nutrients (Figure 3.6), but the relationships were not significant.

3.3.3 Litterbag Stoichiometry

Initial litter stoichiometry varied due to differences in substrate retrieval times but tended to be carbon rich with high C:N and C:P ratios. Over the course of the deployment, the ratios of C:P and C:N decreased in both types of bags (Figure 3.7) (p<0.001 for all models). The initial C:N of the litter bags was 77.29 ± 2.68 and decreased to 57.07 ± 2.49 , while C:P decreased from

1284.8±43.87 to 1043.46±60.00. At all time points, the litter was above Redfield proportions (i.e., 106:16:1).

The change in litter stoichiometry was evaluated as the mean C:X_{final}-C:X_{start} such that a negative Δ C:X signifies a carbon:nutrient decrease and relative N or P increase. Overall, there was a general trend of decreasing (i.e., negative Δ) C:N and C:P in all streams with a median of - 22.24 and -254.33, respectively. The change in litter nutrient content was assessed across bag types and ecoregions using 2-way ANOVA (Figure 3.8). The change in C:P (i.e., Δ C:P) did not vary between bag types or ecoregions. In contrast, there was a significant difference between ecoregions in Δ C:N (F_{1,54}=9.82, p<0.001), with the Boreal C:N ratio decreasing (greater N retention) more than the Parkland and Grassland ecoregions. While Δ C:N did not vary significantly among bag types, there was a marginally significant interaction effect (F_{2,54}=2.95, p= 0.061).

To understand the effect of within-stream nutrient concentrations on this change in stoichiometry, we compared nutrient ratios of the final bags to mean stream water concentrations of TN and TP for the Boreal and Parkland ecoregions, which had a sufficient number of final retrievals to enable this assessment (Figure 3.9). In the coarse-mesh bags deployed in the Boreal region, there was a decrease in C:P with increasing TP (β =-0.465, p=0.013), but no significant relationship between final C:N and TN (β =-0.05, p=0.119). In the fine-mesh bags of the Boreal, there was no significant relationship between either TN and final C:N (β =-0.041, p=0.134) or TP and final C:P (β =-0.314, p=0.103). In contrast, there were marginal, or significant relationships between C:N and TN in the coarse- (β =-0.129, p=0.002) and fine-bags (β =-0.059, p=0.053), and between C:P and TP in the coarse (β =-0.701, p<0.001) and fine bags (β =-0.297, p=0.020) in the Parkland. Across all sites, the relationship between final C:N and TN was significant in both the coarse-mesh (β =-0.094, p=0.002) and fine-mesh (β =-0.071, p=0.008) bags. The relationship between final C:P and TP was also significant for both coarse- (β =-0.562, p<0.001) and fine-mesh (β =-0.239, p=0.012) bag types (Figure 3.9).

The influence of stream water nutrient concentrations on litter stoichiometry was additionally assessed for both fine- and coarse-mesh bags using multiple linear regressions (Table 3.2). Here, models testing the influence of TN or TP, the corresponding decay rate parameter (k_{total} or k_{microbial}), and their interaction were built for each bag type, and for each of

 Δ C:N and Δ C:P (Table 2). The Δ C:N in the fine-mesh bags was not impacted by TN in this model, but did decrease significantly with increasing k_{microbial} (β =-15.026, p=0.002), indicating that the relative concentration of N at the end of the deployment was higher with increasing microbial activity. As both microbial activity and TN increased, the Δ C:N increased, meaning more litter N was remineralized. However, there was also a significant interaction between k_{microbial} and mean concentrations of TN in the stream water (β =8.149, p=0.044) suggesting the effects of k_{microbial} and TN have varying effects on Δ C:N in the fine bags. The Δ C:N in the coarse bags was only affected by k_{total} (β =-10.243, p=0.015), where the proportion of N retained in the litter increased with increasing decomposition rates.

In contrast to the result for controls on Δ C:N, Δ C:P ratios were most strongly controlled by water column concentrations of TP. In the fine bags, there was a significant decrease in Δ C:P in streams with higher TP concentrations, indicating more P was retained in litter with increasing stream water nutrient concentrations (β =283.23, p=0.0205). However, the model itself was not significant (F_{3,25}=2.147, p=0.1195) suggesting there could be other variables controlling the change in litterbag P composition that were not assessed. The effect of TP was also significant in the model for coarse bags (β = -417.7, p=0.002), where there was higher retention of P in the litter as TP increased. Linear relationships between Δ C:N and Δ C:P and microbial and total decay rates are shown in Figure 3.10, reinforcing that decay rates significantly affected Δ C:N (p<0.05), but not Δ C:P.

3.4 Discussion

Here, we describe organic matter decomposition across a nutrient gradient within three agricultural regions of Alberta. Despite clear differences between our ecoregion sites in temperature, velocity and DOC, there was little variation in decomposition responses across the three diverse ecoregions that we studied. Instead, we found that increasing nutrients were a primary control on decomposition of organic matter, and that stream water concentrations of TN and TP increasing with agriculture across all regions. We propose that increasing nutrients enhanced decomposition of organic material through reducing nutrient limitation to microbial communities and possibly also due to reductions or limited natural effects of detritivory. Multivariate assessments indicate that microbial activity increased by TN is most important for driving decomposition, and that invertebrate-mediated processes are less important. Finally,

through assessing changes in litter stoichiometry we found broad-scale increases in litter nutrient content with stream water nutrient concentrations, most likely as a result of microbial activity. Overall, these findings suggest bottom-up rather than top-down forces regulate decomposition of organic matter in our study sites.

3.4.1 Decay rates and microbial contribution do not vary greatly between ecoregions

Across the three diverse ecoregions that we studied, decomposition rates were similar to those found for leaf litters of similar quality in nutrient enriched streams from other ecoregions, despite slight variation between studies in terms of litter material and litterbag mesh size (Hladyz et al. 2010, Manning et al. 2015). We assessed ecoregion differences due to broad variation in climate, vegetation, and soil, which can lead to variation in stream physicochemical characteristics that are important to consider when assessing decomposition responding to anthropogenic perturbations such as agriculture (Hladyz et al. 2010, Pozo et al. 2011). While streams from the Boreal, Parkland, and Grassland ecoregions varied in some key parameters including water temperature, DOC, pH and velocity, the response of decomposition to expected regional gradients was muted. In particular, while there was some difference of region on overall rates of decomposition, we found no difference in the relative contribution of microbial decomposers to the total decomposition rate.

Decomposition rates overall varied by region where the Boreal region typically had greater rates of decomposition than those observed in the Grassland and Parkland ecoregions. However, ecoregion was not an important driver for any type of decomposition in the linear models. Our ecoregions are largely differentiated by climate, which led to significantly different water temperatures between the regions. Previous studies have shown that temperature increases organic matter decomposition rates (Griffiths and Tiegs 2016). However, other studies have supported that temperature is not an over-riding determinant of decomposition rates across ecoregions and that local factors can be more important (Pozo et al. 2011). The Boreal in our study had lower temperatures but higher concentrations of DOC and TN, which may exert more control on decomposition rates than water temperature.

The roles of microbial and shredder activity in decomposition of organic matter were similar among the studied ecoregions, which suggests that the difference in decomposition rates between ecoregions is driven by physicochemical variation rather than shifts in community structure (Hladyz et al. 2010). Overall, the rates of microbial decomposition were highest and made up the largest proportion of decomposition in our litterbags. While we saw a slight increasing proportion of invertebrate decomposition with increasing TN, there was no significant relationship between the proportion of invertebrate-mediated decomposition and concentrations of nutrients. This is contrary to a similar study by Manning et al. (2016) that saw an increase in the proportion of shredder-mediated breakdown in high nutrient streams. Increased nutrient ratios of litter due to microbial conditioning typically increase invertebrate activity (Kominoski et al. 2015), but increases in agricultural activity and thus nutrient concentrations may lower carbon resources in streams and thus lower consumer populations (Cross et al. 2006). However, further studies into detritus quantity would be needed to test the impacts of this in our study systems. Additionally, increased N and P can inhibit invertebrate abundance is typically lower in prairie streams so the low invertebrate decay rates could be a result of natural variation in abundance (Tank et al. 2010). An increased proportion of microbial decomposition has important implications in stream function due to increased carbon loss as CO₂ from increased respiration (Rosemond et al. 2015).

3.4.2 Microbial activity and total nitrogen drive decomposition in Alberta's agricultural ecoregions

Anthropogenic land use within our watersheds ranged from 5-93% across all sites and was correlated to increasing concentrations of nutrients suggesting that pollution (i.e., urban uses) or land use alteration (i.e., agricultural uses) increases nutrient delivery to streams. Mean concentrations of nutrients during the litterbag deployments often exceed previously published numeric guidelines for Alberta surface waters (Alberta Department of Health 1970, Government of Alberta 2018). Additionally, TN and DOC were highly correlated indicating nitrogen in the streams is primarily organic. This is further substantiated by the majority of our inorganic nitrogen measurements being below detection limits.

Total nitrogen was an important driver of decay rates in our streams. Increased TN was significantly related to higher rates of $k_{microbial}$ which is consistent with other studies (Ferreira et al. 2015). The positive effect of TN on $k_{microbial}$ suggests N-limitation of microorganisms in our study streams and is consistent with our streams being predominantly N-limited or co-limited (Hillebrand and Sommer 1999). Other studies have also seen increased microbial decay rates

along gradients of increasing concentrations of N and/or P (Kominoski et al. 2015, Manning et al. 2018). While TN was an important driver of k_{microbial}, increasing TP had limited effect. Variation in TP may be less important for regulating decay in our streams due to more fungal activity than bacterial in microbial colonization of the litter. Aquatic fungi, typically comprised of aquatic hyphomycetes, also tends to exceed bacteria in litter decomposition (Gessner et al. 2007). Further, fungi are often have higher N requirements (Manning et al. 2015, Jabiol et al. 2018) and are more likely to colonize lower quality litter such as reed canary grass (Kominoski et al. 2015). Also, algae within our streams are most likely N- or co-limited, and the presence of increased algae in the biofilm can stimulate fungi leading to increased decomposition (Francoeur et al. 2020). However, TN was highly correlated with DOC suggesting that TN is associated with more organic material, and early decomposition is being driven by the carbon limitation of microbial heterotrophs (Soong et al. 2020).

In contrast to the direct effects of TN, anthropogenic land use (in our study, the sum of cropland, pasture and developed land) had a negative relationship with decay rates in our models, suggesting that changes in watershed land cover will influence decomposition rates above and beyond the role of increasing nutrient concentrations alone. Increased anthropogenic land use often leads to the removal of riparian areas, which can increase light into surface waters. While light availability has been shown to increase organic matter production and thus stimulate microbial activity, this tends to occur in lower nutrient streams (Evans-White and Halvorson 2017). Furthermore, exposure of UV-B radiation also damage fungi (Newsham et al. 1997) and microbes. Increased land use can also increase sedimentation in the water column which can negatively affect microbial enzymatic pathways and thus reduce the rate of decomposition (Piggott et al. 2015). The presence of shredder species is often limited in prairies due to lower allochthonous inputs, and agricultural land use conversions can further decrease these inputs (Tank et al. 2010).

In addition to anthropogenic land use and nutrient concentrations, we assessed other likely drivers of decay rates including velocity, temperature and pH. Velocity was positively associated with $k_{microbial}$ in two of our top models. Increasing velocity can be important in driving physical abrasion of the leaf litter, but also decreases boundary layers between microbial mats and the water column, which can limit oxygen depletion (Bruder et al. 2016, Juvigny-Khenafou et al.

2020). Though the positive relationship between velocity and litterbag mass loss can also indicate that physical fragmentation is an important driver of increased decomposition. Velocity was also an important driver of $k_{shredder}$ and k_{total} , likely due to increased fragmentation in the larger mesh bags. However, velocity impacts often vary with year and may not be a primary driver in longer experiments (Yeung et al. 2018). This study limited repeated sites between years thus repeated experiments over many years are needed to properly infer the impact of velocity on decomposition in streams.

Water temperature appeared to not affect microbial, shredder or total decay rates in our study. This was unexpected as temperature is expected to increase microbial decomposition due to increased cellular reaction rates (Yvon-Durocher et al. 2012). Water temperature in our study varied slightly between sites where the Grassland typically had the highest mean temperatures, but there may not have been enough variation between sites to see an effect, particularly given that our deployments ranged through the majority of the open water season (i.e., from late spring to early fall). Also, water temperature can negatively impact the influence of fungi due to decreased sporulation rates (Fernandes et al. 2009). In a similar study, Pozo et al. (2011) also found that temperature was not a driving factor in decay rates.

While pH was not present in all models, the negative correlation between pH and k_{microbial} was unexpected as microbial decay has been shown to increase in more alkaline waters with increased fungal and microbial growth (Suberkropp and Chauvet 1995, Riipinen et al. 2009). Microbial decomposition tends to increase with pH in the 6-8 range though the response beyond this may decrease (Ferreira et al. 2020). In addition to the impact microbial breakdown processes, pH can affect the community composition of shredders and has been shown to increase invertebrate decomposition in the 5-6.6 pH range (Riipinen et al. 2009, Ferreira et al. 2020). The limited pH gradient in our study, where all sites ranged from 7.4-8.8, and lack of information on community composition makes it difficult to distill the impacts of pH on decomposition in these streams.

3.4.3 Increased stream water concentrations of nutrients lower detrital nutrient ratios

We assessed changes in detrital stoichiometry with decomposition and how stream water nutrients and microbial- and invertebrate-mediated decomposition influenced these changes.

Overall, litterbag C:N and C:P decreased at our sites which appeared to be driven by microbial decay and water column nutrients. When considering changes in C:N, microbial priming of litter is stimulated by periphytic algae (Kuehn et al. 2014), which appears to be N or co-limited in these streams (Hillebrand and Sommer 1999). Further, our initial mean litter N:P of 17.8 was well in excess of the culture-based N:P stoichiometry for common aquatic hyphomycetes, which are important fungal decomposers (9.7; Brosed et al. (2017). Taken together, these metrics indicate that water column nitrogen may limit microbial activity in these streams. Microbial immobilization of N from the water column is consistent with the decrease in C:N we saw in our litter. The change in ΔC :N in our study was primarily driven by microbial activity in our models and likely due to fungal activity (Sinsabaugh et al. 2009). Microbes, particularly aquatic hyphomycetes, assimilate nutrients from the water column and immobilize N in the litter through the release of N-rich enzymes needed to acquire C from polymers when there is low quality litter that cannot meet microbial nutrient requirements (Parton et al. 2007, Pastor et al. 2014). Similar results were reported by Manning et al. (2016), who found that increasing nutrient content led to the convergence of stoichiometric ratios within the litter. Increasing TN increases microbial conditioning, which allows for the changes in C:N associated with fungal colonization. Fungal colonization and immobilization of N provide more accessible litter for invertebrate decomposition, and fungi are also an important component of stream food webs.

There was a decrease in C:P ratios with increasing TP in the water column, which is consistent with other studies using alternate litter types (Scott et al. 2013). The decrease of C:P ratios in the litter was not related to decomposition type, but there was an interaction between k_{total} and TP indicating that elevated phosphorus was associated with increased shredder activity. This finding is similar to Manning et al. (2016) who saw a relationship between decreasing C:P ratios of litter, increased nutrient enrichment and shredder induced decay. Shredder stoichiometry is typically lower than the litter they consume and the interaction may be an effect of lowered litter C:P due to increased TP and microbial activity, and thus increased invertebrate decay (Elser et al. 2000). The model for Δ C:P was not significant for the fine (microbial) bags suggesting that TP did not interact with microbial activity. This is unexpected as microbes have high P-demands due to P-rich RNA (Elser et al. 2003). Manning et al. (2015) saw a similar effect of stream water phosphorus affecting C:P more than C:N. They suggested that this is because C:N is mediated by fungal biomass and C:P is decoupled with fungal biomass due to cellular P storage. Fungi are often more N-limited due to flexible P storage in cells which could be the reason that microbial activity is not driving C:P. Alternatively, microbial community shifts can influence P without a difference in decay rates, as shifts in species composition or diversity can lead to different nutritional requirements while biomass remains the same (Gessner et al. 2007). A second possible reason for the change in Δ C:P with increased TP is abiotic sorption. Microbial biofilms can enhance adsorption of inorganic materials, such as inorganic, leading to decreases in Δ C:P (Mehring et al. 2015). Further analysis of the phosphorus content of the litter and stream as well as species identification are needed to interpret the exact mechanisms.

3.5 Conclusion

Overall, decomposition and mass loss increased along gradients of nitrogen and phosphorus concentrations in our sites. We conclude that: (i) microbial activity was more important than shredder activity in driving decomposition; (ii) nutrients, specifically TN, increased microbial activity; and (iii) there was convergence of C:N and C:P in our litterbags as stream water nutrients increased, which is likely due to microbial activity and in-stream TP availability. Increased stream water nutrients were positively correlated with anthropogenic land cover in our study, which could further influence decomposition if there is removal of riparian zones and subsequent reductions in the amount allochthonous material entering the stream. Alternatively, decomposition could vary due to regional changes along the anthropogenic land cover gradient. Even though there was a negative relationship between increased anthropogenic land use and decomposition, the impacts of nutrients and DOC with increased land use have important implications for stream function. Stimulation of decomposition rates can affect community structures and alter food web dynamics, as well as increase microbial respiration. If decay rates then lower with increased nutrients (i.e. a hump shaped relationship of decay rates with nutrients), this can be an indication of nutrient pollution causing decrease in dissolved oxygen and a reduction in aerobic metabolism (Woodward et al. 2012). To minimize the impacts of nutrients on stream function and to preserve ecological integrity, natural buffer zones should be retained in riparian areas within the Grassland and Parkland. Additionally, TN and TP can have impacts on stream function and reductions in nutrient runoff through riparian management and non-point source pollution decreases should be focus of management plans. This research demonstrates the strength of organic matter decomposition as an indicator of stream function in

response to anthropogenic land use change across ecoregions, and provides essential information needed to make management decisions.

3.6 Tables

Table 3.1A. Linear models describing microbial and total decomposition based on multi-model inference of all possible combinations of environmental variables. All variables are mean variables for the watershed and the entire deployment period. Models display standardized coefficients.

	AICc	ΔAIC_{c}	ω	R ² multiple	R ² adjusted
kmicrobial~					
Intercept + (0.0015)TN - (0.001)%Anth	-288.88	0	0.414	0.36	0.32
Intercept + (0.0015)TN - (0.0007)%Anth +				0.40	0.33
(0.0005)Velocity	-287.63	1.25	0.222		
Intercept + (0.0014)TN + (0.0009)Velocity	-287.38	1.50	0.197	0.34	0.28
Intercept + (0.0013)TN - (0.0009)%Anth -				0.26	0.21
(0.0004)pH	-287.08	1.80	0.168		
K _{total} ~					
Intercept + (0.003)Velocity + (0.007)TN -					0.38
(0.003)TP	-231.17	0	0.28	0.44	
Intercept + (0.003) Velocity + (0.004) TN	-230.70	0.47	0.22	0.38	0.34
Intercept + (0.002)Velocity + (0.004)TN -					0.37
(0.001)%Anth	-230.58	0.59	0.21	0.43	
Intercept + (0.003) Velocity + (0.003) TN -					0.37
(0.002)pH	-230.37	0.80	0.19	0.43	
Intercept + (0.002) Velocity + (0.004) TN -					0.38
(0.001)%Anth – (0.001)pH	-229.24	1.93	0.11	0.46	
Kshredder~					
Intercept + (0.018) Velocity + (0.002) TN	-241.29	0.00	0.31	0.28	0.22
Intercept + (0.017) Velocity + (0.004) TN -	-240.51	0.78	0.21		0.25
(0.008)TP				0.32	
Intercept $-(0.008)$ %Anth $+(0.002)$ TN	-240.39	0.90	0.20	0.26	0.20
Intercept + (0.013)Velocity - (0.005)%Anth +	-239.76	1.53	0.14		0.23
(0.002)TN				0.31	
Intercept + (0.018) Velocity - (0.002) pH +	-239.62	1.67	0.14	0.30	0.23
(0.002)TN					

Table 3.1B. Relative variable importance based on Akaike weights for models returned in in top linear models investigating drivers of decay rates. Higher weighted variables indicate higher relative importance.

Variable	Weight (ω)					
	Kmicrobial	Ktotal	Kshredder			
TN	1	1	1			
Mean Velocity	0.42	1	0.80			
% Anth	0.80	0.31	0.34			
pН	0.17	0.29	0.14			
ТР	0	0.28	0.21			
Water Temperature	0	0	0			
Ecoregion	0	0	0			

Table 3.2. Models testing influence of decomposer activity and total nutrients on the change in litter stoichiometry over the deployment. Decomposition in fine-mesh litter bags is modelled relative to microbial decay rates ($k_{microbial}$), while mass loss from coarse-mesh bags is modelled relative to microbial and invertebrate decay rates (k_{total}).

	Intercept	Water Column Nutrient	Decomposition Rate (k)	Interaction	Model Significance	Multiple R ^{2/} Adjusted R ²
$\Delta C:N \sim$						
TN+k _{microbial} +	-21.380	-6.149	-15.026	8.149	F _{3,26} =6.927	0.442/
TN* k _{microbial}	<i>p<0.001</i>	<i>p</i> =0.234	<i>p=0.002</i>	<i>p</i> =0.044	<i>p=0.001</i>	0.3801
TN+k _{total} +	-26.386	-3.204	-10.243	4.730	F _{3,26} =3.773	0.3033/
TN* k _{total}	p<0.001	<i>p</i> =0.461	<i>p</i> =0.015	<i>p</i> =0.233	<i>p=0.023</i>	0.2229
Δ C:P ~						
TP+ k _{microbial} +	-205.29	-283.23	-31.27	186.53	F _{3,25} =2.147	0.2049/
TP* $k_{microbial}$	<i>p</i> =0.0152	<i>p=0.0205</i>	<i>p</i> =0.7198	<i>p</i> =0.1340	<i>p</i> =0.1195	0.1095
TP+ k _{total} +	-455.2	-417.7	-101.5	397.4	F _{3,22} =5.62	0.4338/
TP* k _{total}	p<0.001	<i>p=0.002</i>	<i>p</i> =0.4279	<i>p=0.010</i>	<i>p</i> =0.005	0.3566

3.7 Figures



Figure 3.1. Study region showing in the range of study sites across the Boreal Mixedwood, Parkland and Grassland ecoregions of Alberta, Canada. Major cities and nearby weather stations are also represented.



Figure 3.2. Box-plots to show variation of physiochemical characteristics across sites within the ecoregions. Results of 1-way ANOVA analyses are shown within each panel with lettering showing the results of the Tukey HSD post-hoc test where applicable. Within plots, the box demarcates the 25th and 75th percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outlier (Quartile±1.5*Inter-Quartile Range). Outlier points are shown using dots.



Figure 3.3. Relationship between mean total phosphorus (A) and mean total nitrogen (B) and the proportion of anthropogenic land use in the watershed. Grey bands represent the 95% confidence interval of the regression line. R^2 represents multiple R^2 and R^2_A is adjusted for differences in sample size. (C) The nutrient gradient present across all sites. In panels A and B the shaded area indicates the 95% confidence interval around the regression line.


Figure 3.4. N:P molar ratios (as total nitrogen: total phosphorus) during deployments across ecoregions on a log scaled y-axis. Dashed lines represent known limitations for stream microalgae, where less than 13 indicates N-limitation, between 13 and 22 indicates co-limitation and >22 indicates P-limitation (Hillebrand and Sommer 1999). The solid grey line represents the Redfield Ratio. Within plots, the box demarcates the 25^{th} and 75^{th} percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outliers (Quartile±1.5*Inter-Quartile Range). Outlier points are shown using dots. Statistical results are presented in 3.3.1.



Figure 3.5. A) Decomposition rates (k; day⁻¹) based on microbial, invertebrate and combined activity across ecoregions. B) The ratio of invertebrate (K_s) to microbial (K_m) decomposition rates as $k_{shredder}/k_{microbial}$ across ecoregions. A ratio of <1 signifies that microbial decomposition was dominant. Within plots, the box demarcates the 25th and 75th percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outliers (Quartile±1.5*Inter-Quartile Range). Outlier data points are shown using dots. (n.s. = not significant at α =0.050; *** = p<0.001).



Figure 3.6. Relationships between the ratio of K_s/K_m and mean stream TN and TP. Shaded area indicates the 95% confidence interval around the regression line. Relationships were not significant (p>0.05).



Figure 3.7. Mean within-site C:N and C:P for fine (left panels) and coarse (right panels) bags relative to time (days) since initial deployment. Linear regression outputs are provided in each panel. The shaded area indicates the 95% confidence interval around the regression line.



Figure 3.8. Boxplots showing variation in ΔC :N and ΔC :P across ecoregion and litterbag type (coarse vs. fine). A negative change indicates that the C:nutrient ratio decreased over the deployment, and relative nutrient content increased. Two-way ANOVA results are presented (n.s. = not significant at α =0.050; *** = p<0.001). Within plots, the box demarcates the 25th and 75th percentile, the bar indicates the data median, and the whiskers indicate highest and lowest points excluding outlier (Quartile±1.5*Inter-Quartile Range). Outlier data points are shown using dots.



Figure 3.9. Relationships between mean stream water nutrient concentrations and C:N (A,B) and C:P (C,D) content of final bags in each ecoregion. Statistical outputs are presented in section 3.3.3. Shaded area indicates the 95% confidence interval around the regression line.



Figure 3.10 The linear relationship between ΔC :N and ΔC :P and decay rates. Significance for each linear model is displayed in each panel. The shaded area indicates the 95% confidence interval around the regression line. Microbial represents changes in fine mesh bags while total shows change in coarse mesh bags.

Chapter 4. Summary of stream function in Albertan agricultural streams

4.1 Summary of Findings

This thesis describes the relationships between agricultural land cover and stream function across a regional gradient. By assessing stream metabolism and organic matter decomposition in streams in the agricultural areas of the Boreal, Parkland and Grassland regions this research illustrates that the removal of riparian areas and increase in nutrient runoff from agricultural lands can alter stream ecosystem function. The regional gradient can alternatively provide insight into varying ecosystem function where the Boreal streams would also be influenced by the high wetland cover within their watersheds. Previous studies have used stream metabolism and decomposition as indicators of stream health as they encompass environmental processes over time across many levels of biological organization and can reveal broad changes to ecosystems (Clapcott and Barmuta 2010, Silva-Junior et al. 2014). This research contributes to knowledge that organic matter decomposition and metabolism are sensitive environmental metrics that can be used to infer the effects of land use change.

We assessed direct and indirect impacts of agriculture on stream function. In chapter 2, we found metabolism was indirectly affected by watershed land cover. Land cover within the watershed is often altered with agriculture and can include impacts on riparian vegetation. The effects of land cover on local stream conditions have important implications for management of riparian areas. The natural riparian vegetation along stream banks controls light and water temperature which can result in lower rates of GPP and ER. Water temperature was the most important local variable driving GPP and ER and was further influenced by light and canopy shading. The effects of nutrients and the amount of allochthonous material in an ecosystem will affect productivity with increases in respiration. Respiration is also more sensitive to temperature, which could lead to greater net heterotrophy if riparian areas of forested streams are not as light or temperature limited.

In chapter 3, we assessed organic matter decomposition and found decay rates generally decreased with anthropogenic land use in the watersheds after other variables were accounted for and were more strongly affected by water velocity and nutrient concentrations. Decomposition type did not vary between ecoregions, but the rates were slightly higher in the Boreal where TN

and DOC were also higher. Microbial decomposition was driven by TN, possibly due to fungal activity and the stoichiometric ratios of the litter converged with increasing TN and TP. Riparian vegetation can influence nutrient concentrations in the streams and microbial decay rates. This also has implications for streams acting as carbon sources with climate change. Nutrients have strong impacts on microbial decomposition and can increase decay rates. For invertebrate decomposition, the hump-shaped response to nutrients seen in other studies (Woodward et al. 2012) and our limited contribution of invertebrates possibly due to agricultural activity indicates that excessive concentrations of nutrients could also reduce decomposition rates. However, the naturally lower populations of shredders in muddy-bottom prairie streams could also be a limiting factor in invertebrate decay rates.

The assessment of anthropogenic impacts on stream function in this study will provide important information for watershed management plans (Wagenhoff et al. 2017). Riparian buffer zones that limit vegetation removal and restrict livestock access to stream banks can reduce erosion, maintain natural light availability and temperature regimes, and limit nutrient delivery to streams (Clapcott and Barmuta 2010). This in turn will effect metabolism as both ER and GPP tend to increase with removal of riparian vegetation (Burrell et al. 2014). Riparian areas will also influence the types of vegetation available for decay and decomposition rates. Management plans should include uncultivated or restored riparian buffer zones to mitigate the impacts of agriculture on stream function.

4.2 Considerations and Limitations

Our study compared stream ecosystem function across a gradient of agricultural intensity. One important consideration is that all sites are within anthropogenically-altered watersheds, so it is difficult to distill the complete impacts of agriculture. While the nutrient gradient approach does allow inferences of the impacts of non-point source pollution on decomposition, alternative drivers can be difficult to infer. For example, the regional gradient also varies across the study streams. Variation in land cover, such the influence of wetland cover in the Boreal, could also be important in driving stream ecosystem functioning. The inclusion of reference sites that are similar to agricultural sites in morphology and climate would allow for a more holistic comparison of the effects of land cover alteration on metabolism and decomposition (Young et al. 2008). In addition to limitations through lack of reference sites, this study spanned three

years where not all sites were repeated. Due to variation in discharge and precipitation across years, and the long-term runoff effects in the Boreal ecoregion (Devito et al. 2016), a longer-term study would provide better understanding of these impacts on stream ecosystem functioning,

Metabolism measurements have improved with technological advancements but are still limited by environmental conditions and variation in modelling techniques. Modelling of metabolism had progressed significantly since first described by Odum (1956). A variety of software exists from spreadsheet methods (Holtgrieve et al. 2010, Riley and Dodds 2013), modelling of CO₂ flux (Bortolotti et al. 2016), to multiple varieties of Bayesian modelling of diurnal oxygen (Grace et al. 2015, Appling et al. 2018a). One primary limitation in modelling stream metabolism is estimating the gas exchange coefficient (k). While directly estimating k can provide improved accuracy, the process can be expensive and time-consuming across a large study area. Modelling k, often normalized to a 600 Schmidt number of k₆₀₀, can be done from oxygen time series (Hall and Ulseth 2019, Nifong et al. 2019). The 'streamMetabolizer' package uses state-space models to estimate k and account for process errors, but error would still be lowest with direct measurements (Appling et al. 2018a). Futher, large inputs of groundwater can bias metabolism results by appearng to decrease primary production and increase respiration (Hall and Tank, 2005). Groundwater inflows should be measured and corrected for in any future studies.

An important component in understanding decomposition is knowing species composition and biomass. We cannot infer if increased agriculture will impact species richness within microbial communities due to their high functional redundancy. We recommend further microbial and invertebrate monitoring due to the possible decoupling of species structure and function (Feckler and Bundschuh 2020). Finally, we used the relative concentration of TN and TP to assess nutrient limitation, which represent all forms of N and P, instead of the dissolved and inorganic forms that are more bioavailable to microbes. However, our sites had low levels of dissolved inorganic nitrogen (DIN) with >50% of our sites being below detection limits during all water quality retrievals. However, TN and TP can still be effective especially when inferring nutrient limitation, as DIN and soluble reactive phosphorus proportions can be highly variable (Dodds 2003).

4.3 Future Research

This research illustrates the importance of agricultural land cover and associated changes in environmental conditions such as water temperature, TN and DOC on stream function. Further research should continue to look at the effects of nutrients on invertebrates and microbes through species identification and analysis of food web stoichiometry (Welti et al. 2017). Species identification should also be conducted to assess for possible decoupling of structure and function (Feckler and Bundschuh 2020). Structural metrics are also necessary to complement stream function, so we recommend further assessment of microbial and invertebrate communities in our understanding of these ecosystems. Further, studies using nutrient diffusing substrata would allow for a direct determination of nutrient limitation in these streams. Analysis of DOC composition is also crucial due its importance in driving GPP and ER. Finally, our study was conducted using relatively short deployments and with few sites repeated between years. The use of long-term monitoring of metabolism and repeated decomposition would enable a more complete assessment considering temporal changes.

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Supplementary Data

Appendix 1. Supplementary Data for Chapter 2

Ecoregion	Site	Site Code	Latitude	Longitude	ACIS Station
Boreal	Connor Creek	CNR02	54.02	-114.92	Evansburg 2 AGCM
Boreal	Goose Creek	GSE02	54.34	-114.93	Fort Assiniboine AGCM
Boreal	Horse Creek	HRS01	54.33	-114.69	Fort Assiniboine AGCM
Boreal	Little Paddle	LIT01	53.95	-115.02	Evansburg 2 AGCM
	River				
Boreal	Lasthill Creek	LST01	52.36	-114.46	Hespero AGCM
Boreal	Lloyd Creek	LYD02	52.74	-114.14	Crestomere AGCM
Boreal	Namepi Creek	NAM02	54.08	-112.98	Smoky Lake AGDM
Boreal	Pine Creek	PNE02	54.70	-112.97	Atmore AGDM
Boreal	Strawberry Creek	STW01	53.31	-114.05	St. Francis AGCM
Boreal	Tomahawk Creek	TOM01	53.35	-114.66	St. Francis AGCM
Boreal	Upper Sturgeon River	USR02	53.58	-114.89	Evansburg 2 AGCM
Boreal	Weed Creek	WED01	53.30	-113.98	St. Francis AGCM
Grassland	Bullshead Creek	BUL02	49.96	-110.61	Medicine Hat RCS
Grassland	Kneehills Creek	KNE03	51.48	-113.11	Keoma AGCM
Grassland	Mosquito Creek	MSQ02	50.25	-113.55	Barons AGCM
Grassland	Matzhiwin Creek	MTZ01	50.84	-111.93	Rosemary IMCIN
Grassland	Onetree Creek	ONE01	50.73	-111.69	Rosemary IMCIN
Grassland	Seven Persons Creek	PER01	49.90	-110.85	Seven Persons IMCIN
Grassland	Pothole Creek	POT01	49.52	-112.80	Raymond IMCIN
Grassland	Rosebud Creek	RSB03	51.32	-113.33	Keoma AGCM
Grassland	Unknown Creek	UNK03	50.02	-112.75	Barons AGCM
Parkland	Buffalo Creek	BUF01	53.01	-110.87	Gilt Edge North AGCM
Parkland	Beaverhill Creek	BVH01	53.75	-112.68	Oliver AGDM
Parkland	Dogpound Creek	DOG01	51.79	-114.36	Olds College AGDM
Parkland	Eagle Creek	EGL01	51.94	-114.43	Olds College AGDM
Parkland	Grizzlybear	GRZ01	53.11	-110.64	Gilt Edge North AGCM
	Creek				
Parkland	Pipestone Creek	PIP01	53.03	-113.27	Wetaskiwin AGCM
Parkland	Ray Creek	RAY01	52.00	-113.60	Three Hills AGCM
Parkland	Sturgeon River	STU03	53.83	-113.28	Oliver AGDM
Parkland	Threehills Creek	THR01	52.00	-113.57	Three Hills AGCM
Parkland	Weiller Creek	WEI01	52.99	-113.22	Wetaskiwin AGCM

Table S1.1. ACIS Weather stations used to gather climatic variables for nearby sites.

Environmental Variable	Units	Method Reference
Total Suspended Solids (TSS)	mg/L	APHA 2540 D-Gravimetric
Turbidity	NTU	APHA 2130 B-Nephelometer
Dissolved Kjeldahl nitrogen (DKN)	mg/L	APHA 4500-NORG (DKN)
Total Nitrogen (TN)	mg/L	APHA 4500 N-Calculated
Ammonia (NH ₃)	mg/L	APHA 4500 NH3-NITROGEN
		(AMMONIA)
Nitrate+Nitrite (NO ₃ -+ NO ₂ -)	mg/L	CALCULATION
Nitrite (NO ₂ -)	mg/L	EPA 300.1 (mod)
Nitrate (NO ₃₋)	mg/L	EPA 300.1 (mod)
Total Phosphorus (TP)	mg/L	APHA 4500-P PHOSPHORUS
Total Dissolved Phosphorus (TDP)	mg/L	APHA 4500-P PHOSPHORUS
Dissolved Orthophosphate (SRP)	mg/L	APHA 4500-P PHOSPHORUS
Total Kjeldahl nitrogen (TKN)	mg/L	APHA 4500-NORG (TKN)
Dissolved Organic Carbon (DOC)	mg/L	APHA 5310 B-Instrumental
Phaeophytin <i>a</i>	µg/L	EPA 445.0 ACET
Chlorophyll <i>a</i>	µg/L	EPA 445.0 ACET

Table S1.2. Methods and units of water chemistry analyses completed by ALS Environmental Laboratories

Site			Pe	rcent of L	and Cover in	Watershed			
	Cropland	Developed	Exposed	Forest	Grassland	Pasture	Shrubland	Water	Wetland
Beaverhill Creek	30.3	3.5	0.11	20.8	7.1	11.2	4.4	8.8	13.6
Buffalo Creek	57.4	2.3	0.41	1.8	8.3	14.3	7.1	1.3	7.1
Bullshead Creek	9.8	1.2	0.28	2.6	80.8	0.34	3.7	0.45	0.8
Connor Creek	15.1	1.9	0.06	35.5	0.39	39.0	0.98	0.36	6.8
Dogpound Creek	47.0	2.7	0.36	19.0	21.1	7.9	0.52	0.2	1.3
Eagle Creek	21.3	2.8	0.71	28.4	23.2	21.6	0.44	0.13	1.5
Goose Creek	1.9	1.0	0.03	75.8	0.06	2.9	2.1	1.8	14.3
Grizzlybear Creek	48.4	2.4	0.77	2.7	14.5	11.7	10.5	2.4	6.6
Horse Creek	17.5	2.1	0.37	43.9	0.02	15.6	0.82	0.24	19.5
Kneehills Creek	75.3	2.9	0.57	2.3	9.3	2.6	1.2	0.67	5.1
Lasthill Creek	20.5	2.9	0.45	30.0	18.2	20.0	1.1	0.77	6.0
Little Paddle River	12.9	4.1	0.28	39.3	0.08	29.4	1.1	0.26	12.6
Lloyd Creek	15.6	2.2	0.32	41.1	3.6	27.8	0.64	1.1	7.7
Matzhiwin Creek	40.6	1.7	0.72	0.22	44.7	2.9	0.84	2.6	5.8
Mosquito Creek	35.9	2.3	0.25	1.7	44.1	4.6	8.3	0.45	2.3
Namepi Creek	42.9	2.7	0.03	24.4	0.09	20.4	0.84	0.24	8.4
Onetree Creek	24.2	3.5	1.4	0.2	54.2	5.5	1.2	3.0	6.9
Pine Creek	17.8	1.6	0.02	41.6	0.28	18.4	2.1	2.2	16.0
Pipestone Creek	50.2	3.8	0.19	13.7	3.1	23.4	0.51	1.4	3.8
Pothole Creek	38.1	2.3	0.4	0.05	53.4	2.5	1.3	1.5	0.49
Ray Creek	79.2	2.5	0.21	4.3	2.5	5.1	2.7	0.03	3.5
Rosebud Creek	72.9	3.4	0.82	2.6	8.8	3.4	0.48	1.2	6.6
Seven Persons Creek	39.5	1.5	0.35	0.13	55.0	0.25	1.7	1.0	0.7
Strawberry Creek	31.9	3.1	0.54	22.8	0.99	32.3	0.29	0.26	7.8
Sturgeon River	36.6	10.0	0.62	25.8	0.69	19.0	0.42	5.2	5.0
Threehills Creek	68.2	2.4	0.3	6.3	5.8	5.6	5.0	1.1	5.2
Tomahawk Creek	4.3	2.0	0.11	50.4	1.0	31.6	0.78	0.37	9.3
Unknown Creek	87.7	2.6	0.8	0.06	4.6	2.8	0.24	0.13	1.2
Upper Sturgeon River	2.7	1.7	0.37	50.2	0.47	37.8	0.7	0.36	5.7
Weed Creek	37.0	3.6	0.14	18.4	0.79	30.9	0.33	0.19	8.8
Weiller Creek	69.6	8.6	0.33	6.8	2.5	6.3	0.52	1.1	4.5

 Table S1.3. Percent of land cover in watershed based on AAFC Crop Inventory Data

Site (Creek Name)	Year	Season	Davs Modelled	GPP	ER
Beaverhill Creek	2018	Fall	1	1.05±0.00	-3.83±0.00
Beaverhill Creek	2018	Spring	2	6.66±1.64	-13.79±4.03
Beaverhill Creek	2018	Summer	1	2.98±0.00	-5.18±0.00
Buffalo Creek	2018	Spring	2	3.43±0.01	-9.18±3.32
Buffalo Creek	2018	Summer	2	5.98±0.44	-16.46±4.93
Bullshead Creek	2017	Fall	2	2.79±0.45	-0.95±0.44
Bullshead Creek	2017	Spring	1	3.24±0.00	-5.39±0.00
Bullshead Creek	2017	Summer	2	8.37±0.88	-6.94±0.03
Connor Creek	2019	Spring	2	2.07±0.36	-2.10±0.66
Connor Creek	2019	Summer	2	2.88±1.50	-18.70±2.29
Dogpound Creek	2017	Fall	2	2.15±1.07	-2.49±1.10
Dogpound Creek	2017	Spring	4	5.07±1.32	-4.23±1.22
Dogpound Creek	2017	Summer	2	6.17±3.71	-6.10±0.82
Dogpound Creek	2018	Fall	4	1.10±0.05	-0.98±0.20
Dogpound Creek	2018	Spring	2	1.31±0.18	-2.50±0.18
Dogpound Creek	2018	Summer	2	10.16±0.24	-5.96±0.01
Eagle Creek	2018	Fall	3	0.78±0.06	-1.06±0.06
Eagle Creek	2018	Spring	2	1.62±0.09	-2.37±0.46
Eagle Creek	2018	Summer	2	1.62 ± 0.09	-2.37±0.46
Goose Creek	2019	Fall	2	0.51±0.04	-2.01±0.48
Goose Creek	2019	Spring	4	2.24±0.24	-3.56±0.19
Goose Creek	2019	Summer	3	0.70±0.09	-7.54±2.30
Grizzlybear Creek	2018	Fall	1	4.61±0.00	-5.33±0.00
Grizzlybear Creek	2018	Spring	2	2.73±0.30	-3.48±0.09
Horse Creek	2019	Fall	3	0.73±0.45	-7.56±5.81
Horse Creek	2019	Spring	4	0.88±0.11	-2.68±0.45
Horse Creek	2019	Summer	2	1.35±0.17	-3.24±0.61
Kneehills Creek	2017	Fall	2	2.38±0.71	-2.36±0.22
Kneehills Creek	2017	Spring	2	3.65±0.38	-2.21±0.33
Kneehills Creek	2017	Summer	1	5.03 ± 0.00	-5.34 ± 0.00
Kneehills Creek	2018	Fall	2	5.09±0.18	-5.16±0.96
Kneehills Creek	2018	Spring	3	5.57±0.41	-4.42 ± 0.38
Kneehills Creek	2018	Summer	2	7.92±0.53	-5.72±1.27
Lasthill Creek	2018	Fall	2	0.84±0.20	-1.49±0.15
Lasthill Creek	2018	Spring	3	2.30±0.15	-2.49±0.19
Lasthill Creek	2018	Summer	2	4.61±0.28	-3.84±0.26
Lasthill Creek	2019	Fall	3	2.47±0.13	-1.98 ± 0.04
Lasthill Creek	2019	Spring	3	1.02±0.19	-2.37±0.14
Lasthill Creek	2019	Summer	3	2.87±0.57	-3.85 ± 0.60
Little Paddle River	2019	Spring	2	0.42 ± 0.26	-3.43±0.24
Little Paddle River	2019	Summer	1	0.36 ± 0.00	-9.25±0.00
Lloyd Creek	2018	Fall	2	2.51±0.45	-4.64±1.28
Lloyd Creek	2018	Spring	3	5.20±0.75	-5.06±0.61
Lloyd Creek	2018	Summer	2	4.55±0.40	-3.74 ± 0.06
Lloyd Creek	2019	Fall	3	7.46±2.39	-16.92±1.59
Lloyd Creek	2019	Spring	3	1.65 ± 0.40	-2.57±0.65
Lloyd Creek	2019	Summer	3	8.23±2.87	-13.41±2.47
Matzhiwin Creek	2017	Fall	2	1.68±0.03	-1.92±0.01
Matzhiwin Creek	2017	Spring	2	5.38±0.72	-4.72±0.40
Matzhiwin Creek	2017	Summer	2	4.56±0.40	-4.56±0.73

 Table S1.4. Descriptions for Metabolism Measurements and Deployments

Mosquito Creek	2017	Spring	2	4.11±0.31	-2.67±0.03
Mosquito Creek	2017	Summer	2	8.70±2.05	-5.02±0.91
Namepi	2019	Summer	2	5.08±0.77	-17.25±3.61
Onetree Creek	2017	Fall	2	3.58±0.31	-5.24±0.29
Onetree Creek	2017	Spring	2	3.10±0.40	-3.47±0.45
Onetree Creek	2017	Summer	2	8.50±0.92	-4.92±0.22
Pine Creek	2019	Spring	4	2.36±0.11	-3.27±0.13
Pine Creek	2019	Summer	4	11.02±4.07	-22.05±3.09
Pipestone Creek	2018	Spring	2	1.73±0.25	-3.18±0.06
Pipestone Creek	2018	Summer	2	2.17±0.16	-2.60±0.27
Pothole Creek	2017	Fall	2	5.14±0.26	-0.51±0.07
Pothole Creek	2017	Spring	1	3.77±0.00	-2.86±0.00
Pothole Creek	2017	Summer	2	3.83±0.69	-3.23±0.18
Ray Creek	2018	Spring	4	2.22±0.89	-5.88±1.77
Rosebud Creek	2017	Fall	2	2.32±0.55	-2.54±0.19
Rosebud Creek	2017	Spring	2	2.53±0.20	-1.64±0.25
Rosebud Creek	2017	Summer	2	5.42±0.84	-4.87±0.42
Rosebud Creek	2018	Fall	2	1.93±0.03	-1.29±0.05
Rosebud Creek	2018	Spring	3	4.90±0.50	-3.35±0.45
Rosebud Creek	2018	Summer	2	5.39±0.05	-4.35±0.33
Seven Persons Creek	2017	Fall	1	1.14 ± 0.00	-0.71±0.00
Seven Persons Creek	2017	Spring	2	3.04±0.51	-4.03±0.49
Seven Persons Creek	2017	Summer	2	3.04±0.51	-4.03±0.49
Strawberry Creek	2019	Fall	2	0.33±0.06	-0.31±0.01
Strawberry Creek	2019	Spring	3	0.79±0.29	-0.84 ± 0.30
Strawberry Creek	2019	Summer	3	0.48±0.16	-1.03±0.17
Sturgeon River	2017	Fall	2	5.74±0.09	-6.19±0.11
Sturgeon River	2017	Spring	1	$1.74{\pm}0.00$	-4.97 ± 0.00
Sturgeon River	2017	Summer	2	6.49±0.95	-6.40±0.63
Sturgeon River	2018	Fall	2	2.49±0.56	-2.68±0.24
Sturgeon River	2018	Spring	1	1.73 ± 0.00	-6.35±0.00
Sturgeon River	2018	Summer	2	3.80±0.28	-3.77±0.24
Threehills Creek	2017	Spring	4	7.58±1.36	-8.04 ± 1.57
Threehills Creek	2017	Summer	1	2.76 ± 0.00	-3.45 ± 0.00
Threehills Creek	2018	Spring	3	5.71±0.79	-5.07±0.41
Threehills Creek	2018	Summer	1	4.80 ± 0.00	-9.48 ± 0.00
Tomahawk Creek	2019	Fall	3	1.74±0.37	-3.24±1.57
Tomahawk Creek	2019	Spring	2	3.37±0.67	-5.76±0.30
Tomahawk Creek	2019	Summer	2	0.25±0.06	-9.48±2.98
Unknown Creek	2017	Spring	2	0.19±0.02	-5.33±0.86
Unknown Creek	2017	Summer	1	0.75 ± 0.00	-2.40 ± 0.00
Upper Sturgeon River	2019	Spring	3	2.41±0.77	-5.20±1.52
Weed Creek	2019	Fall	3	0.53±0.25	-0.55±0.46
Weed Creek	2019	Spring	3	2.58±0.70	-2.47±0.69
Weed Creek	2019	Summer	3	4.83±2.52	-9.25±5.51
Weiller Creek	2018	Fall	3	0.75±0.11	-0.62±0.18
Weiller Creek	2018	Spring	2	5.59±0.66	-5.56±1.16
Weiller Creek	2018	Summer	2	4.86±0.39	-5.38 ± 1.10

Table S1.5. Mean metabolism metrics calculated based on ACIS weather station and stream bank Odyssey PAR with relative percent difference where (a) is GPP g $O_2 m^3 day^{-1}$, (b) is ER g $O_2 m^3 d^{-1}$, and (c) is $K_{600} day^{-1}$. Average metabolism metrics were calculated for Spring 2019 for all Boreal sites and compared via different light collection methods and compared to show relative percent difference between sites across all days metabolism was calculated.

A. Gross I	Primary Production			
Site	Weather Station	Stream Bank	Mean Absolute Error	Mean Relative Percent Difference
GSE02	2.01	2.24	0.23	10.98
HRS01	0.83	0.88	0.05	5.58
LIT01	0.35	0.42	0.07	27.23
LST01	1.07	1.02	0.05	4.87
LYD02	1.91	1.65	0.25	14.40
PNE02	2.18	2.36	0.18	7.95
STW01	0.71	0.79	0.09	12.88
TOM01	3.38	3.37	0.01	0.19
USR02	2.38	2.41	0.23	9.86
WED01	2.38	2.58	0.44	18.79
Mean	1.70	1.77	0.17	10.82

B. Ecosy	stem Respiration			
Site	Weather Station	Stream Bank	Mean Absolute Error	Mean Relative Percent Difference
GSE02	-3.00	-3.56	0.56	17.50
HRS01	-2.53	-2.68	0.15	6.27
LIT01	-2.68	-3.60	0.92	29.36
LST01	-1.78	-2.37	0.59	28.66
LYD02	-2.79	-2.57	0.21	8.07
PNE02	-2.88	-3.27	0.39	12.58
STW01	-0.77	-0.84	0.08	10.93
TOM01	-5.77	-5.76	0.01	0.19
USR02	-4.30	-5.20	0.90	17.69
WED01	-2.30	-2.47	0.43	18.54
Mean	-2.79	-3.12	0.40	14.23

C. Gas E	Exchange Constant			
Site	Weather Station	Stream Bank	Mean Absolute Error	Mean Relative Percent Difference
GSE02	3.35	4.63	1.28	33.41
HRS01	4.74	5.08	0.34	7.16
LIT01	2.92	4.29	1.37	38.09
LST01	0.41	1.37	0.96	114.23
LYD02	2.72	2.89	0.36	14.36
PNE02	4.00	5.22	1.22	26.65
STW01	7.27	7.95	0.68	8.99
TOM01	8.48	8.47	0.02	0.20
USR02	1.34	2.25	0.90	50.85
WED01	1.33	.92	0.59	34.29
Mean	3.58	4.34	0.78	32.52

Gross Primary Production Distribution					
	Gamma	Log Normal			
Goodness Of Fit Statistic					
Kolmogorov-Smirnov	0.086	0.102			
Cramer-Von Mises	0.073	0.252			
Anderson-Darling	0.467	1.556			
Goodness of Fit Criteria					
AIC	425.904	438.806			
BIC	431.076	443.976			
Ecosystem Respiration	n Distribution				
	Gamma	Log Normal			
Goodness Of Fit Statist	Goodness Of Fit Statistic				
Kolmogorov-Smirnov	0.104	0.104			
Cramer-Von Mises	0.177	0.194			
Anderson-Darling	1.134	1.176			
Goodness of Fit Criteria					
AIC	492.030	492.327			
DIC	407 200	407 407			

Table S1.6. Distribution Statistics for Gross Primary Production and Ecosystems Respiration

 based on log normal and gamma distributions

Table S1.7. Partial structural equation model (pSEM) results for the most optimal model (C=22.20, p=0.677, df=26 and AIC of 94.21), with p-values and standardized path coefficients indicated for each modelled relationship. Increasing Land Cover PC1 represents increasing forest over and increasing morphology PC1 represents smaller, steeper watersheds.

Response	Predictor	p-value	Standardized path coefficient
GPP	Discharge	0.9224	0.0091
GPP	DOC	0.5273	0.0659
GPP	PAR	0.162	0.1642
GPP	Water Temperature	0.0001	0.4379***
ER	Antecedent Precipitation	0.0006	0.2973***
ER	DOC	0.0003	0.3332***
ER	Water Temperature	0	0.4275***
DOC	Morphology PC1	0.1688	-0.1241
DOC	Land Cover PC1	0	0.5523***
DOC	Discharge	0.4838	-0.0611
DOC	Antecedent Precipitation	0.3086	0.096
Discharge	Land Cover PC1	0.8255	-0.0255
Discharge	Antecedent Precipitation	0.6557	0.0506
Discharge	Morphology PC1	0.1325	-0.1618
Water Temperature	PAR	0	0.6339***
Water Temperature	Antecedent Precipitation	0.1504	0.12
Water Temperature	Morphology PC1	0.2166	-0.1016
PAR	Land Cover PC1	0	-0.4604***
Land Cover PC1	Morphology PC1	0.0473	0.1895*
Land Cover PC1	Antecedent Precipitation	0.0001	0.3772***
~~GPP	~~ER	0	0.534***
~~ Land Cover PC1	~~Water Temperature	0.0131	0.2294*



Figure S1.1. Proportions of land cover within the watershed colored by ecoregion. Categories are aggregates based on AAFC data.



Figure S1.2 Comparisons of metabolic variables calculated based on light from Odyssey logger on stream bank and nearby ACIS weather stations in Spring 2019. R² represents adjusted R²

Appendix 2. Supplementary Data for Chapter 3

Table S2.1. Methods and units of water chemistry analyses completed by ALS Environmental Laboratories

Environmental Variable	Units	Method Reference
Total Nitrogen (TN)	mg/L	APHA 4500 N-Calculated
Total Phosphorus (TP)	mg/L	APHA 4500-P PHOSPHORUS
Dissolved Organic Carbon (DOC)	mg/L	APHA 5310 B-Instrumental



Figure S2.1. All sites where there was complete deployment of coarse or fine bag types (n=62). Point represent average between two bags of the same type collected on the day.



Figure S2.2. Average C:N Ratio of bags retrieved over course of deployment (n=62). Point represent average between two bags of the same type collected on the day.



Figure S2.3. Average C:P Ratio of bags retrieved over course of deployment (n=62). Point represent average between two bags of the same type collected on the day.



Figure S2.4. Relationships between $k_{microbial}$ and $k_{shredder}$ and stream nutrient concentrations. Relationships were tested in and importance in shown in Table 3.1.


Figure S2.5. Relationships between mean concentrations of nutrients in stream water and C:N (A,B) and C:P (C,D) content of final bags including all streams. Statistical outputs are presented in section 3.3.3. Shaded area indicates the 95% confidence interval around the regression line.