UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

LE PÉRIPLE DU MÉTABOLISME BACTÉRIEN À TRAVERS LE PAYSAGE FLUVIAL DU SAINT-LAURENT

A BACTERIAL METABOLIC JOURNEY THROUGH THE ST. LAWRENCE RIVERSCAPE

MÉMOIRE PRÉSENTÉ
COMME EXIGENCE PARTIELLE DE LA
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PAR
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Une illustration originale du « périple du métabolisme bactérien à travers le paysage fluvial du Saint-Laurent », réalisée spécialement pour ce mémoire par l'artiste Jasmyne Flournoy.

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Three years ago, I made the 2,326 km drive from Florida to Quebec, my car filled to the brim with possessions and my spirit bubbling with anticipation. I was eager to be a part of a unique project, to live in a new country, and to possibly pick up a bit of French along the way. While my French has only improved slightly since I arrived, my knowledge, curiosity, and ability to learn have grown more than I could have expected. I have also developed a love for rustic *chalets* and maple syrup. I could not have done any of this without the people who have supported and challenged me along the way.

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Preface

This work has been partly funded by a NSERC-Create scholarship awarded to Elizabeth Grater as part of the EcoLac training program and well as NSERC-Discovery grants awarded to François Guillemette (supervisor) and Paul del Giorgio (co-supervisor) and a Fondation de l'UQTR grant awarded to François Guillemette. The scientific cruises on the St. Lawrence River that were necessary for conducting this study were funded by the Réseau Québec Maritime and the Government of Quebec through the Odyssée Saint-Laurent research program. This thesis is a contribution to the research program of the UQTR Chair on the ecology of the St. Lawrence River awarded to François Guillemette.

Résumé

En tant que mécanisme clé de la dégradation, de la rétention et de la production de matières organiques dissoutes (DOM), les bactéries fournissent des informations utiles sur les processus de traitement de la DOM au sein des systèmes fluviaux. En raison de sa sensibilité en tant que traceur et de son caractère essentiel au sein des écosystèmes, le métabolisme bactérien est largement utilisé pour étudier le fonctionnement des systèmes aquatiques et le devenir de la matière organique dans ces mêmes systèmes. Bien que nos connaissances sur les processus bactériens se soient considérablement développées au cours de la dernière décennie, la complexité du métabolisme bactérien nous laisse encore perplexes et pour cela, elle se doit d'être davantage étudiée. Le couplage et le découplage entre la respiration bactérienne (BR) et la production bactérienne (BP), ainsi que la réponse de l'activité bactérienne aux changements hydrologiques et morphologiques d'un système fluvial, par exemple, sont encore largement sous-étudiés. Cela dit, le but de ce mémoire est de fournir une étude approfondie du métabolisme bactérien et de la consommation du carbone dans un grand écosystème fluvial, plus particulièrement en réponse aux changements dans la composition de la MOD, de son hydrologie et de sa morphologie. Ce faisant, ce mémoire comblera des lacunes dans nos connaissances sur le fonctionnement des systèmes fluviaux.

Dans les deux premiers chapitres de ce mémoire, nous explorons le répertoire des connaissances et les questions irrésolues liées au fonctionnement du métabolisme bactérien, à la dynamique de la DOM et des nutriments dans les systèmes fluviaux. Par la suite, dans le chapitre 3, nous menons une enquête approfondie sur les multiples facettes du métabolisme bactérien (BP, BR, consommation bactérienne de carbone (BCC) et de l'efficacité de croissance bactérienne (BGE)), ainsi que sur les changements dans la composition de la DOM et des nutriments dans le fleuve Saint-Laurent (SLR). Les objectifs de cette étude sont 1) d'évaluer BR, BP, BCC et BGE spatialement et temporellement dans le SLR, 2) d'explorer l'influence des sous-unités fluviales, comme les lacs fluviaux, et de l'hydrologie sur le métabolisme bactérien, 3) de déterminer les

variables environnementales influençant le métabolisme bactérien et 4) de prédire les fonctions globales du SLR, quant au traitement et au transport de la DOM.

En juillet 2017 et 2018, le navire de recherche Lampsilis, propriété de l'Université du Québec à Trois-Rivières (UQTR), a été déployé sur le SLR afin de réaliser une vaste campagne d'échantillonnage sur une cinquantaine de stations distribuées longitudinalement et latéralement. À chaque site, l'eau a été recueillie à l'aide d'un échantillonneur Goflow à 1 m de profondeur. En ce qui a trait aux mesures de DOM et de nutriments, nous avons eu recours à une digestion en milieu humide par persulfate pour évaluer les concentrations de carbone organique dissous (DOC), à une digestion avec persulfate combinée à une analyse colorimétrique pour mesurer les concentrations d'azote et de phosphore, et enfin à une caractérisation optique (fluorescence et absorbance) associée à une modélisation PARAFAC pour déterminer la composition de la DOM. Concernant le métabolisme bactérien, les taux de BR ont été évalués avec des capteurs optiques d'oxygène dans des flacons fermés. Pour leur part, les taux de BP ont été obtenus via une méthode de marquage à la leucine. Enfin, des plaques Biolog Ecoplates^{TM} contenant un colorant tétrazolium et des substrats de carbone furent employées pour déterminer la capacité de dégradation du carbone des communautés bactériennes. Les résultats ont révélé un clivage entre les concentrations stables de DOC et les modèles dynamiques de composition de la DOM et du métabolisme bactérien, mettant ainsi en évidence l'indépendance existante entre les fonctions de transport et de « réacteur » du système fluvial. Les tendances longitudinales de BP, BR, BCC et BGE indiquent toutes une augmentation vers l'aval, un schéma contradictoire à ce qui est normalement observé dans les grands fleuves. Ces résultats sont probablement attribuables à l'augmentation de la DOM d'origine terrestre et du phosphore à mesure que le fleuve devient progressivement plus connecté à l'environnement terrestre vers l'aval. Dans les conditions de haut débit d'eau, les concentrations de carbone et de nutriments se sont avérées plus élevées en moyenne par rapport aux conditions normales, alors que l'activité métabolique bactérienne était plus faible en moyenne, ce qui suggère que la capacité des bactéries à traiter les matériaux est diminuée lors des périodes de haut débit d'eau. Enfin, le point d'admission des eaux usées de la ville de Montréal constituait un point chaud (hotspot) d'activité bactérienne, activité stimulée par les concentrations soudainement élevées de

nutriments. Cependant, dans les lacs fluviaux, l'activité bactérienne a diminué de l'amont à l'aval, suggérant une réduction progressive du carbone labile dans ces zones, probablement due à une hausse du temps de séjour de l'eau.

Le dernier chapitre de ce mémoire résume l'essentiel de nos résultats, ainsi qu'un examen des recommandations de travaux qui devraient être prochainement menés sur le fleuve Saint-Laurent. Sur le plan collectif, ce mémoire présente une analyse approfondie du métabolisme bactérien dans le contexte d'un vaste système fluvial et fournit de l'information contrainte spatialement sur l'impact des activités humaines sur le fonctionnement du Saint-Laurent et sa capacité de réponse qui pourront être profitables aux futures initiatives de gestion du bassin versant du fleuve St-Laurent.

Mots-clés : Métabolisme bactérien, traitement du carbone, fonctionnement des rivières, paysage fluvial, matière organique dissoute, effluents urbains

Summary

As a key mechanism degrading, retaining, and producing dissolved organic materials (DOM), bacteria provide useful information about the processing abilities of DOM within river systems. Due to its sensitivity as a tracer and ubiquity within ecosystems, bacterial metabolism is widely used to investigate the functioning of aquatic systems and the fate of organic materials within these systems. While our knowledge of bacterial processing has expanded greatly in the past decade, the complexities of bacterial metabolism are still perplexing to us and must be studied further. The coupling and uncoupling between bacterial respiration (BR) and bacterial production (BP) and the response of bacterial activity to hydrological and morphological changes within fluvial systems, for instance, are still widely understudied. With that said, the aim of this master's thesis is to provide an investigation of bacterial metabolism and carbon consumption within a large river ecosystem, specifically in response to changes in DOM composition, hydrology, and morphology, in order to fill in knowledge gaps surrounding river functioning.

In the first two chapters of this master's thesis, we explore the repertoire of knowledge and remaining questions related to bacterial metabolic functioning and DOM and nutrient dynamics within rivers. After, in chapter 3, we provide an investigation of the multiple facets of bacterial metabolism (i.e. BP, BR, bacterial carbon consumption (BCC), and bacterial growth efficiency (BGE)) and DOM composition and nutrient changes throughout the St. Lawrence River (SLR). The objectives of this study are to 1) assess the BR, BP, BCC, and BGE spatially and temporally within the SLR, 2) explore the influence of riverine units, such as fluvial lakes, and hydrology on bacterial metabolism, 3) determine possible drivers of bacterial metabolism within the river, and 4) predict the large scale processing and transport functions of the SLR.

We fulfilled these objectives by conducting two field campaigns aboard the Lampsilis research vessel, sampling longitudinally and laterally within the SLR in the summer of 2017 and 2018. At each sample site (44 sites for year 1, 51 sites for year 2), water was collected using a Go-Flo water sampler at 1 m depth. For DOM and nutrients measurements, we used a wet persulfate method to measure the concentrations of dissolved organic carbon, colorimetric analysis with persulfate digestion to measure concentrations of nitrogen and phosphorus, and optical characterization (i.e. fluorescence and absorbance) and PARAFAC modeling to determine the DOM composition. For bacterial metabolism, we utilized oxygen optical sensors in closed flasks to measure rates of BR, the radioactive leucine tagging method to measure rates of BP, and Biolog Ecoplates™ containing a tetrazolium dye and carbon substrates to measure the carbon degradation capacity of the bacterial communities. Results revealed a disconnect between stable concentrations of DOC and the dynamic patterns of DOM composition and bacterial metabolism, highlighting the relative independence of the transport and reactor functioning of the river. Longitudinal trends of BP, BR, BCC, and BGE, showed an increase in each facet downstream, an inverse pattern to what is normally observed in large rivers. This unique trend was likely driven by an increase in terrestrial DOM and phosphorus as the river became gradually more connected to the terrestrial environment downstream. During high waterflow conditions, concentrations of carbon and nutrients were higher on average compared to normal conditions, while bacterial metabolic activity was lower on average, suggesting that high flows reduced the ability of bacteria to process materials within the river. Inputs of sewage from the city of Montreal provided a hotspot of increased bacterial activity, driven by high concentrations of nutrients. Bacterial activity decreased, however, from the start to the end of the fluvial lakes, suggesting a removal of labile carbon within these areas, likely driven by an increase in water residence time.

The final chapter of this master's thesis provides a general conclusion of our findings as well as an examination of recommendations for future work along the St. Lawrence River. Collectively, this thesis yields an extensive analysis of bacterial metabolism throughout a large river and provides observations that I hope can help guide future management initiatives throughout the SLR watershed.

Keywords: Bacterial metabolism, carbon processing, river functioning, riverscape, dissolved organic matter, urban effluent

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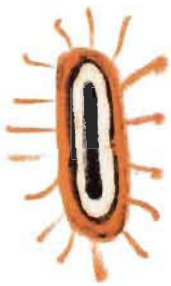
List of Abbreviations and Acronyms

AA	*Amino Acid*
BCC	*Bacterial Carbon Consumption*
BGE	*Bacterial Growth Efficiency*
BP	*Bacterial Production*
BR	*Bacterial Respiration*
CA	*Carboxylic Acids*
CDOM	*Colored-Dissolved Organic Matter*
CH	*Carbohydrate*
CO_2	*Carbon Dioxide*
DOC	*Dissolved Organic Carbon*
DOM	*Dissolved Organic Matter*
EEM	*Excitation Emission Matrix*
O_2	*Oxygen*
LSP	*Lake St. Pierre*
PARAFAC	*Parallel Factor Analysis*
SLR	*St. Lawrence River*
TN	*Total Nitrogen*
TP	*Total Phosphorus*





Chapter 1

Project Description

1. 1 **Holes in Our Knowledge**

Due to their sensitivity to change, their importance at the base of food webs, and their role in carbon processing, bacteria are key tracers of aquatic ecosystem change. Despite this, few studies have conducted a thorough investigation of the multiple facets of bacterial metabolism (i.e. BR, BP, BGE, and BCC) in response to fluctuations in the carbon and nutrient pool. Instead, studies often focus on only one variable of bacterial metabolism, extrapolating the others from it. Because the frequent coupling and uncoupling within bacterial activity is not yet fully understood, using one metric to calculate others can lead to biased results (del Giorgio et al., 2006). By studying DOM alterability and bacterial flexibility in unison, we gain unique insight into how the two variables are connected and how they respond to different environmental conditions. With this strong base, we can then start to predict the effects of anthropogenic land use changes on bacterial metabolism, food web dynamics, and greenhouse gas budgets.

With scattered knowledge of bacterial metabolism and carbon consumption within fluvial systems, a cumulative study is needed to strengthen the links between bacterial

activity and carbon pool dynamics in rivers. **In this study, we will use the St. Lawrence River, a large river along the border of South East Canada and North East USA, to conduct a comprehensive examination of the bacterial processes in response to changes throughout a riverscape.** By studying these mechanisms within a large complex river, we can pinpoint multiple variables that drive bacterial metabolism within an understudied system.

1. 2 **Objectives**

The St. Lawrence River (SLR), the second largest river in North America, is a central resource for riverine communities in eastern Canada. Unlike many large rivers that originate with headwater streams, the SLR receives much of its water from Lake Ontario, a large lake known for its low concentrations of DOM and nutrients (Frenette et al., 2012; Hudon et al., 2017). From its headwaters at Lake Ontario, the SLR flows for 450 km before reaching the saline waters of the estuarine transition zone and the Gulf of the St. Lawrence, east of Québec City (Fig. 1.1) (Frenette et al., 2012).

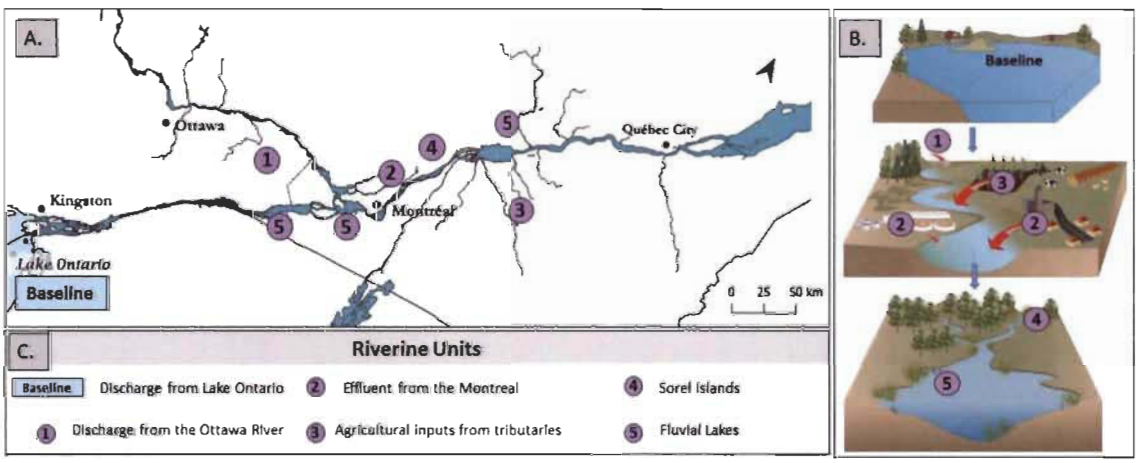


Figure 1.1: The St. Lawrence River with the 5 studied riverine units identified in A. a map of the SLR and B. a conceptual model of the SLR. Panel C. explains each of the riverine units (i.e. discharge of Lake Ontario, discharge from the Ottawa River, the effluent from Montreal, the Sorel Islands, and the fluvial lakes).

Within the center of the SLR, a deep, fast moving channel, created by historical and current dredging, transports highly processed water from the Great Lakes to the estuary. This section of the river, known as the main water mass, stays laterally isolated from the rest of the river (Frenette et al., 2012). In the northern section of the river, inputs from the Ottawa River, the biggest tributary draining into the SLR, deposit dark, humic water from a relatively pristine, forested catchment into the river, creating the northern water mass. Downstream of the Ottawa River, smaller tributaries, draining forested and agricultural watersheds, input additional subsidies of organic matter and nutrients into the northern water mass (Frenette et al., 2012). The lack of lateral mixing, due to the SLR's unique bathymetry, creates two water masses, each containing distinct physical and chemical properties, that run parallel to each other (Fig. 1.2) (Frenette et al., 2012; Poirier-Larabie et al., 2020; Rice et al., 2006). Because of their distinct sources, the main and northern water mass also contain distinct conductivities, around 300 µS/cm and 100 µS/cm for the main water mass and northern water mass, respectively (Kestrup and Ricciardi, 2010; Yang et al., 1996).

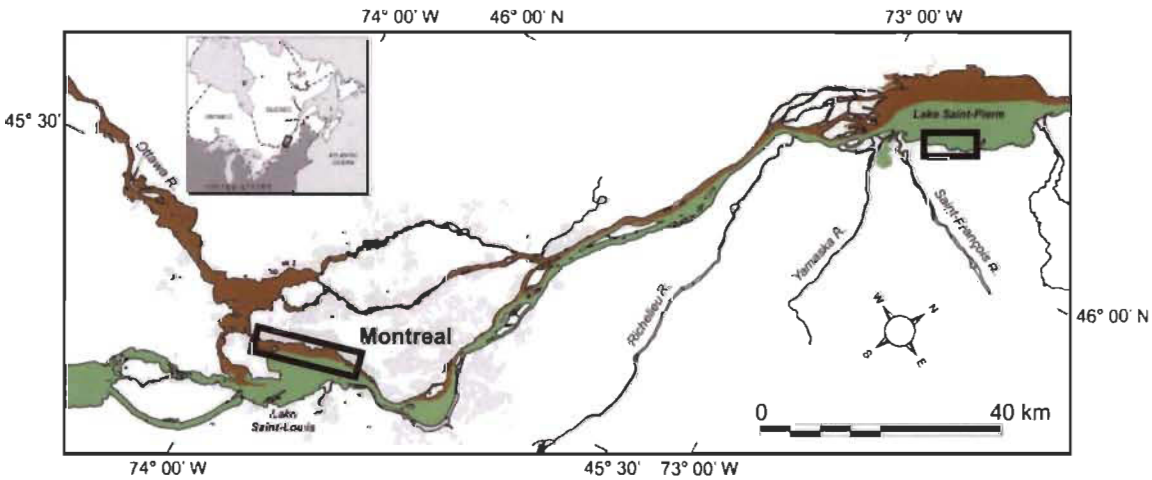


Figure 1.2: The St. Lawrence River, split into the main (green) water mass, originating at Lake Ontario, and northern (brown) water mass, originating at the Ottawa River (adapted from Poirier-Larabie et al., 2020).

Besides lateral separation, the SLR contains a variety of discontinuities that have the potential to add or remove DOM, significantly influencing the composition of materials within the SLR (Fig. 1.1). For instance, wastewater from urban regions is

discharged into the SLR, with varying levels of treatment occurring in advance (Hudon et al., 2017). From the greater area of Montreal alone, primary-treated effluent from over 3 million people is dumped into the SLR, contributing one of the highest inputs of nutrients along the river (Frenette et al., 2012; Hudon et al., 2017). In addition, fluvial lakes and archipelagoes throughout the river create areas of reduces water residence time, potentially affecting the processing and transportation of DOM (Frenette et al. 2012). Each of these discontinuities have the potential to influence the functioning abilities of the SLR.

Despite this, few studies have worked to understand how landscape and riverscape changes along the SLR influence bacterial processing of DOM and nutrients. In this study, we investigated BR, BP, BGE and BCC and the influence of fluctuations of DOM composition on bacterial activity in order to answer the following questions:

1) How does BR, BP, BGE, and BCC vary spatially and temporally within the SLR?
2) What effects do riverine units, such as fluvial lakes and islands, and hydrology have on BR, BP, BGE, and BCC?
3) How do changes in the carbon pool, in terms of source and composition, effect BR, BP, BGE, and BCC, and overall C turnover in large rivers?
4) Does a river that contains DOM and nutrient depleted headwaters, such as the SLR, function differently than common, headwater stream rivers?

Answering these questions will allow us to better predict the overall response of riverine ecosystems to environmental and anthropogenic disturbances across the riverscape. A greater understanding of the functioning of the St. Lawrence River will also help to strengthen initiatives and remediation projects, benefiting both the environment and the communities living near the river.

1.3 Hypotheses

Based on our knowledge of bacterial metabolism, carbon dynamics, and the SLR, we hypothesize that:

- The bathymetry of the SLR, i.e. a deep, dredged, center channel, and distinct sources will create non-mixing water masses with separate chemical properties. These water masses will each function independently.
  a) The main water mass, with its fast-moving water and oligotrophic characteristics, will produce lower rates of BR and BP compared to the rest of the river.
  b) The northern water mass will have higher rates of BR compared to the main water mass, due to its higher concentrations of DOM and nutrients. In this water mass, there will be an increase in BP downstream, as the DOM inputs shift from forested land to agricultural land.
  c) Between the two water masses, bacterial functional capacities will differ, driven by unique DOM and nutrient compositions.
- Sewage inputs from Montreal will increase BR and BP throughout the river, by providing high concentrations of nutrients and DOM. This spike in bacterial metabolism will quickly decrease downstream as the plume of the effluent is diluted.
- There will be a decrease in BR and BP from the start of the Sorel Islands to the end of the islands and from the start of Lake St. Pierre (LSP) to the end of the lake. This will be driven by an increase in water residence within these riverine units, which will allow for selective removal of labile DOM, with increases of recalcitrant DOM flowing downstream.
- During high flow conditions, increased concentrations of DOM and nutrients from the terrestrial environment will support higher rates of BR compared to baseline flow conditions.

- Terrestrial DOM and nutrients will be the main drivers of BR and BP within the SLR, due to the low DOC and nutrient concentrations at the start of the river (i.e. Lake Ontario).

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Chapter 2

Literature review

2. 1 **What is Bacterial Metabolism?**

Long considered a black box, bacterial metabolism has been identified, more recently, as a multifaceted and dynamic mechanism transforming dissolved organic carbon (DOC) in aquatic systems (Guillemette & del Giorgio, 2011; Koehler, von Wachenfeldt et al., 2012). Through metabolic activities, bacteria modify materials from the surrounding catchments and produce new compounds of organic and inorganic matter, altering the surrounding environment (Creed et al., 2015). Two main components of bacterial metabolism are bacterial respiration (BR) and bacterial biomass production (BP). While prevalent throughout aquatic ecosystems, the degree to which BR and BP occur is highly variable and dependent on factors such as source and composition of the organic carbon, availability of nutrients, and hydrological and morphological changes (Berggren & del Giorgio, 2015; Berggren et al., 2011; Maranger et al., 2005). Due to their sensitivity to environmental changes, a more expansive understanding of the various facets of bacterial metabolism (i.e. BR, BP, Bacterial Growth Efficiency (BGE), Bacterial Carbon

Consumption (BCC)) can help predict changes of material loading and processing in response to changes in land use and climate.

2.1.1 *Bacterial Respiration and Production*

Aquatic BR is a mechanism through which organisms link substrate oxidation (e.g. of glucose) with an electron acceptor, such as oxygen, to produce an energy source, i.e. ATP. The sum of these processes is the consumption of a substrate and oxygen to produce CO_2, water, and energy. The energy produced from this reaction is used for cell maintenance and basic functions. Compared to other functions, BR is less energy intensive, allowing bacteria to allocate more recalcitrant organic matter for this mechanism (Lennon & Cottingham, 2008). Byproducts of BR (i.e. CO_2) are released into the surrounding environment and are transported downstream or escape into the atmosphere. Depending on the substrates available and conditions within the environment, BR can be responsible for a considerable portion of CO_2 released into the environment. In some systems, rates of BR are much higher than algal carbon fixation, leading to net production of CO_2, i.e. creating a heterotrophic system (Cole & Caraco, 2001; Hessen, 1992). In other ecosystems, primary production (e.g. phytoplankton photosynthesis) is higher than BR, producing an excess of O_2, making the system autotrophic (Dodds & Cole, 2007; S. Findlay et al., 1991).

BP, another facet of bacterial metabolism, is the amount of bacterial biomass produced per unit time (Kirchman, 2001; Kritzberg et al., 2004). Unlike BR, which releases inorganic carbon into the system, BP transforms organic carbon substrates into biomass that may be utilized by other organisms (e.g. heterotrophic phytoplankton and zooplankton) (Faithfull et al., 2011; Hessen, 1992; Lennon & Pfaff, 2005). The novel compounds produced by BP supply the base of food webs with energy, allowing bacteria to act as an important source of transformed organic materials (Fasching et al., 2014; Findlay et al., 1991; Kritzberg et al., 2004).

2.1.2 *Indices for Bacterial Activity*

Rates of BR and BP can be assessed in unison to obtain additional information about bacterial activities. BCC and BGE are two metrics used to further analyze bacterial metabolism within aquatic systems.

Equation 1 $$BCC = Bacterial\ Production + Bacterial\ Respiration$$

Equation 2 $$BGE = \frac{(Bacterial\ Production)}{(Bacterial\ Production + Bacterial\ Respiration)}$$

BCC is the sum of BR and BP (equation 1) and can be used to determine the amount of carbon required to support the bacterial activity within a system (i.e. bacterial carbon demand; Fig. 2.1). BCC is also used as a proxy to quantify the amount of organic matter available to bacteria. While BCC is important for understanding how much carbon is being processed within a system, this index does not show how the DOM is being allocated (del Giorgio et al., 2006).

BGE, on the other hand, gives insight into how bacteria are utilizing organic materials and into the possible quality of these substrates (Berggren et al., 2007). BGE is the ratio of BP to BCC (equation 2) and determines the amount of bacterial biomass produced for each unit of carbon consumed (del Giorgio & Cole, 1998). Higher rates of BGE (i.e. values approaching 0.5) suggest that bacteria are utilizing carbon efficiently, with a larger percentage of the carbon consumed going to BP. As the BGE decreases, however, less of the carbon consumed goes towards the production of biomass and more energy is allocated for cell maintenance or BR (del Giorgio & Cole, 1998). Therefore, BGE indicates whether BR or BP is the dominate metabolic pathway within a system (Findlay et al., 1991).

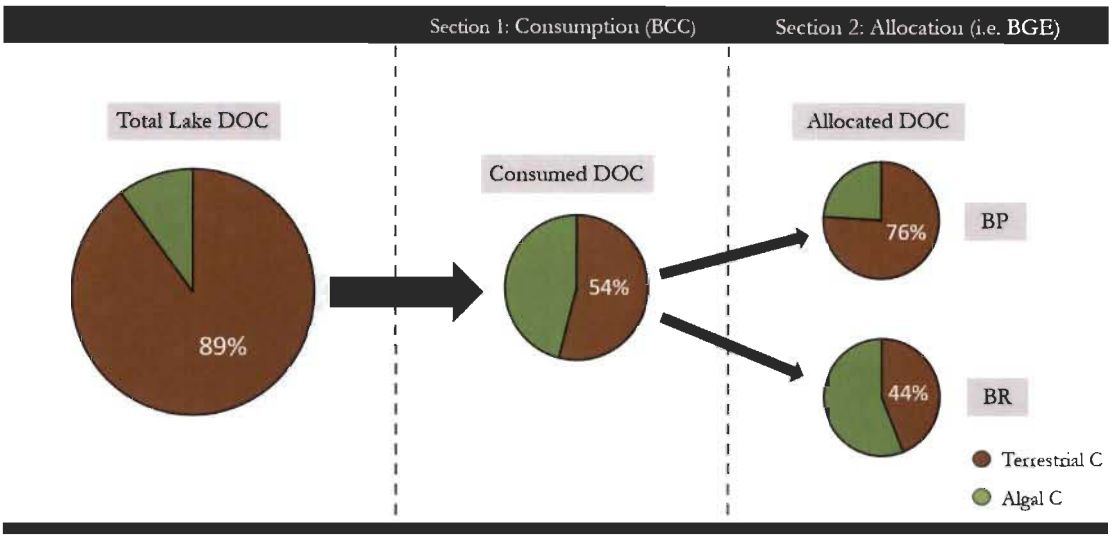

Figure 2.1: The preferential consumption and metabolic allocation of dissolved organic carbon within bacterial communities in lakes of southeastern Quebec (adapted from Guillemette et al., (2016)).

By studying both BGE and BCC, we can conduct a more thorough investigation of bacterial metabolism and how it is influenced by changing carbon pools and by the surrounding environment. In their study of DOM consumption and allocation, Guillemette et al. (2016) explored the dynamics of BCC and BGE within lake ecosystems. In this study, initial measurements showed that the bulk of the carbon consumed by bacteria was terrestrially derived (Fig. 2.1, section 1). When carbon allocation was considered (i.e. BGE), BR and BP utilized terrestrial and algal pools of carbon differently (Fig. 2.1, section 2), suggesting a uncoupling within bacterial mechanism based on substrate availability (Berggren & del Giorgio, 2015; Findlay et al., 1991). By considering multiple facets of bacterial metabolism at once, i.e. BR, BP, BCC, and BGE, we can determine the fate of DOM downstream and predict the potential of river ecosystems to process materials during the journey from land to ocean.

2. 2 Material Inputs into Aquatic Ecosystems

Dissolved organic matter (DOM) is the largest component within the pool of materials in aquatic systems (Findlay et al., 2003). DOM is defined as organic compounds

within water that pass through a filter pore size of 0.2 to 0.7 μm (Benner et al., 2004). DOM consists of 1) organic compounds, such as degraded substrates from soils, plants, and living organisms, and 2) organic forms of nutrients (Fellman et al., 2010). While not within the pool of DOM, inorganic nutrients, mostly coming from terrestrial environments, also play an important role within aquatic ecosystems. Together, these materials largely determine the functioning of ecosystems by providing nutrients throughout the water column, facilitating the transportation and sequestration of metals, and influencing water clarity (Fellman et al., 2008; Moore et al., 2004). DOM, specifically, is a crucial variable influencing bacterial metabolism, with changes in its quality and quantity determining the degree of bacterial activity within aquatic systems. Because of this, understanding the dynamics of DOM within different ecosystems can give insight into bacterial processing capabilities, physiological responses and broader ecosystem impacts of ecosystem changes.

2.2.1 *Dissolved Organic Carbon*

Of the DOM entering aquatic systems, dissolved organic carbon (DOC) makes up a significant portion (~50%). This carbon not only plays an important part of the global carbon cycle, but it can also influence pH (through the input of humic acids), decrease water clarity (as it absorbs light), and regulate bacterial metabolism (where it acts as a primary substrate) (Spencer et al., 2016).

In freshwater systems, there are two main sources of DOC: locally produced (autochthonous) carbon and carbon imported from the surrounding landscape (allochthonous). Each type possesses unique characteristics and interacts differently with the surrounding environment. Autochthonous DOC is produced through phytoplankton photosynthesis, autotrophic and heterotrophic bacteria, and macrophyte activity (Lapierre & Frenette, 2009). This pool of DOC tends to be less complex and more bioavailable. In some environments, highly labile autochthonous carbon can be degraded by more than 60% in a few days (Nguyen et al., 2005). Allochthonous DOC, on the other hand, comes from terrestrial sources (e.g. soils and plant litter), has a more complex structure, and is often more persistent in aquatic systems (Berggren & del Giorgio, 2015; Wilson &

Xenopoulos, 2008). Due to the size of terrestrial watersheds compared to freshwater systems, inputs of allochthonous carbon tend to be much larger compared to autochthonous sources (Cole & Caraco, 2001). Previously, allochthonous carbon was thought to be much less available for bacterial utilization, but recent studies demonstrate that a small fraction of the allochthonous carbon pool is readily consumed by bacteria (Guillemette et al., 2013; Lennon & Pfaff, 2005; Ward et al., 2013). Because the pool of terrestrial carbon is greater than the autochthonous one, this small portion of labile terrestrial carbon could be an importance source of carbon preferentially consumed by aquatic bacteria (Guillemette et al., 2013).

Within allochthonous and autochthonous DOC pools, the composition of carbon is crucial for understanding the bioavailability and persistence of materials within the ecosystem. Although the proportions of each source vary, both DOC pools are composed of a mixture of aliphatic and aromatic compounds (Stedmon et al., 2003). Aromatic DOC is composed of complex aromatic ring structures that are very stable (Kalbitz et al., 2003). Due to this complexity, it takes more energy for aromatic compounds to be broken up, making it hard for bacteria to utilize them (Fellman et al., 2008). In order to become more bioavailable, these compounds can be broken down through mechanisms such as photo, chemical, and/or physical processing (Kalbitz et al., 2003). Aliphatic compounds, however, are simpler structures of carbon that do not contain aromatic rings and tend to be more bioavailable. While they can also be cyclical, these compounds are lighter and smaller. Thus, they are more labile and need less processing before they can be utilized by bacteria (D'Andrilli et al., 2015; Sun et al., 1997). Since it takes less energy to consume these compounds, bacteria selectively utilize aliphatic DOC for processes such as BP (Sun et al., 1997).

2.2.2 *Composition of DOM*

Throughout the past two decades, scientists have been using fluorescence and absorbance analysis as an effective way to study the composition of DOM. This technique uses excitation and emission wavelengths to characterize the fluorescence properties of the colored-DOM (CDOM) (Gabor et al., 2014). This CDOM can be split into humic-like

and protein-like components, with each containing their own excitation and emission peaks (Gabor et al., 2014). Humic-like CDOM is the dominant component found in allochthonous sources and tends to have a more complex, aromatic structure. Because humic-like CDOM originates from a diversity of terrestrial environments, it's composition is often highly variable (Gabor et al., 2014). Protein-like CDOM, however, comes from biological activity, such as primary production, and is often autochthonous. These compounds tend to be simpler and more aliphatic (Gabor et al., 2014). Figure 2.2 and Table 1 show the different peaks within the CDOM pools and what sources they are related to (Gabor et al., 2014).

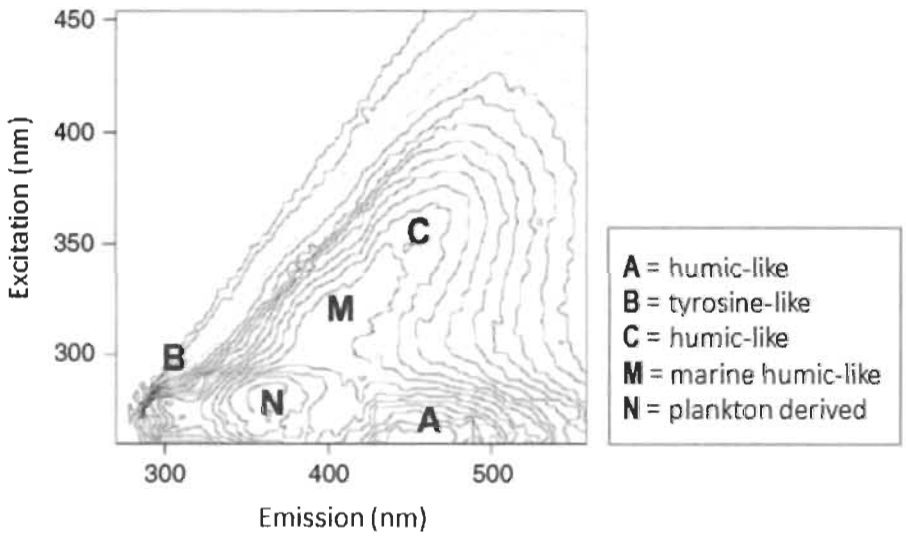

Figure 2.2: An EEM example with the main peaks identified, adapted from Gabor et al. (2014).

2.2.3 *Nutrients*

Nutrients are another key ingredient within aquatic ecosystems (Findlay et al., 2003; Wiegner & Seitzinger, 2004). Nitrogen and phosphorus are the most abundant nutrients within aquatic ecosystems and are essential for the growth of organisms. Nitrogen, though abundant in the atmosphere, is largely unavailable to organisms. Before it can be utilized, nitrogen gas (N_2) must be fixed into an accessible form of nitrogen. In aquatic environments, the nitrogen pool is composed of inorganic nitrogen (i.e. nitrate,

nitrite and ammonium), dissolved organic nitrogen, and total particulate nitrogen, which is mostly organic (Worsfold et al., 2008). Phosphorus, like nitrogen, is plentiful globally, but is greatly unavailable to organisms. In order to become bioavailable, inorganic phosphorus, found in rocks, must be released by weathering and transformed by plants and bacteria before it can be widely used. Both inorganic and organic forms of phosphorus are important to bacteria within aquatic systems and have been associated with increases in bacterial growth efficiency (Jansson et al., 2006; Smith & Prairie, 2004). While inorganic phosphorus was initially thought to be the most bioavailable form of phosphorus, recent studies have emphasized the importance and abundance of organic phosphorus (Worsfold et al., 2008).

Table 2.1: A breakdown of the PARAFAC components and the sources that they are related to (adapted from Gabor et al. (2014).

Component Type	Ex/Em Wavelength	Source
Tyrosine-like protein-like	230/305 274/305	*Autochthonous, resembles tyrosine but may be free or combined amino acids*
Tryptophan-like protein-like	230/340 275/305	*Autochthonous*
Unknown	280/370	*Autochthonous*
Humic–like M	240/350–400 290-310/370-420	*Autochthonous, microbial*
Humic–like C	260/400–460 320–365/420–470	*Humic, terrestrial, allochthonous*

2.2.4 *DOM, Nutrients, and Bacterial Metabolism*

Changes in source and composition of DOM significantly influence bacterial activity within aquatic systems. Inputs of bioavailable carbon are preferentially consumed by bacteria, leaving the recalcitrant material to flow downstream (Guillemette et al., 2013). Labile, autochthonous carbon has been shown to be consumed at rates up to 10 times higher than terrestrial pools of carbon (Guillemette et al., 2013), but the allocation this carbon is not equal between BR and BP. While BR can be supported by aromatic terrestrial carbon, BP is more tightly associated with autochthonous carbon (Berggren et al., 2007; Kamjunke et al., 2015; Kritzberg et al., 2004). Because BP is a higher energy function compared to BR, it requires a larger proportion of labile and energy-rich materials (Lennon & Cottingham, 2008). Similarly, systems with higher amounts of autochthonous compounds often contain higher BGEs, above or around 0.4 (i.e. more efficient). Allochthonous carbon pools, on the other hand, are more strongly correlated with lower BGEs, around 0.1 or less (i.e. less efficient) (Berggren & del Giorgio, 2015; Fasching et al., 2014). While this correlation between BP and BGE and autochthonous carbon is widely accepted by freshwater scientists, recent studies suggest that there is a small pool of high efficiency terrestrial carbon that can be readily allocated for BP (Berggren & del Giorgio, 2015; Fasching et al., 2014; Guillemette et al., 2016).

On a narrow scale, specific changes in carbon substrates within the DOC pool can also influence BCC and BGE. Varying amounts of carbohydrates (CH), amino acids (AA), and carboxylic acids (CA) within natural systems have been shown to control BGE, suggesting that metabolism is affected by the availability of certain carbon substrates (Berggren et al., 2010). In one study, CA and CH from allochthonous inputs supported 30% of the annual bacterial carbon demand (Jansson et al., 2007). In another study, different proportions of CH, AA, and CA compounds were shown to cause variations in BGE (Berggren et al., 2010). Tracking the metabolic capacities of bacteria to utilize these carbon substrates provides additional information regarding carbon availability within ecosystems. One study, comparing bacterial metabolism and bacterioplankton community structure between a variety of ecosystems found that bacterial metabolic capacity was able

to link bacterial carbon consumption with changes in resources and habitats (Comte and del Giorgio, 2009). While there is limited research on the connection between carbon substrates, bacterial metabolic capacities, and bacterial metabolism, an increased understanding of this relationship can help to develop predictive models of how bacterial metabolism could be influenced by natural and anthropogenic land changes (i.e. changes in carbon compounds).

Changes in concentration and composition of nitrogen and phosphorus also influence BR, BP, and BGE (Wiegner & Seitzinger, 2004). While BR is primarily driven by DOC fluctuations, BP is strongly limited by nutrients (Wiegner & Seitzinger, 2004). Increases in nitrogen and phosphorus concentrations can significantly increase rates of BP (Farjalla et al., 2009). In eutrophic systems, excess nutrients and bioavailable substances allow bacteria to allocate more DOM towards BP, increasing the BGE (Smith & Prairie, 2004). In oligotrophic systems, limited resources stimulate bacteria to allocate more energy towards cell maintenance, lowering the BGE in these environments (del Giorgio & Cole, 1998; McTammany et al., 2003).

Between these two nutrients, phosphorus has come forward as the leading limiting factor of BP and BGE (del Giorgio & Cole, 1998). As the amount of total phosphorus within a terrestrially rich system increases, bacterial consumption of DOC also increases (Farjalla et al., 2009; Guillemette et al., 2013; Smith & Prairie, 2004; Wiegner & Seitzinger, 2004). On the contrary, as a system becomes more phosphorus limited, excess carbon is consumed but cannot be utilized for BP. Instead, this carbon is allocated for BR, decreasing the BGE of the system (Hessen, 1992). As nutrient concentrations increase around the world due to human land alterations, it is important to understand how bacterial activities respond to these changes (Wiegner & Seitzinger, 2004). This will allow us to better predict and anticipate the responses of aquatic systems to those alterations.

2.2.5 *Effects of Land Use on DOM and Bacterial Metabolism*

Within freshwater systems, land use and land cover are considered the strongest factors controlling DOM composition (Parr et al., 2015). Natural catchments, containing

an abundance of vegetation and low anthropogenic disturbances, supply aquatic ecosystems with high concentrations of aromatic DOM (Fig. 2.3) (Butman et al., 2012; Hanley et al., 2013). Runoff from forested systems, for instance, contribute heavily humic-like compounds into surrounding ecosystems (Berggren & del Giorgio, 2015; Kamjunke et al., 2015). As the watershed becomes less vegetated, the DOM pool becomes more protein-like, dominated by microbially-derived compounds (Wilson & Xenopoulos, 2008).

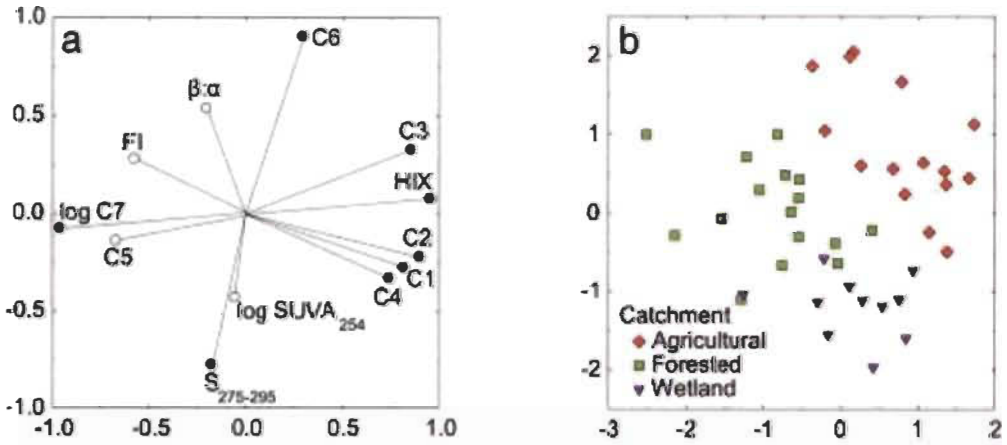

Figure 2.3: Results from the principal component analysis (PCA) of the DOM composition data within three catchment types: agricultural land (red diamond), forested land (green square), and wetlands (purple triangle). Panel a. the loadings of the DOM components and indices; panel b. the scores for the catchments of the spatial-variation dataset (adapted from Grauber et al., 2012).

Similarly, increased urbanization within a catchment leads to an increase in bioavailable autochthonous DOM and a decrease in humic-like allochthonous DOM being exported to freshwater systems (Parr et al., 2015). Urbanized areas are designed to create a homogeneous landscape, with uniform grasses and impervious surfaces replacing vegetation, biodiversity, and connectivity within the catchment. These dramatic modifications in the flow path of surface water throughout cities not only creates fragmentation in the environment, but also influences the diversity and composition of the DOM pool (Bernot et al., 2010; Parr et al., 2015).

Like urban watersheds, agricultural lands often decrease diversity and increase land alteration, leading to distinct DOM pools entering aquatic systems (Fig. 2.3). Compared to other land use types, agricultural lands tend to export high concentrations of DOC into surrounding aquatic systems, due to the poor soil structure and lack of stable root systems (Grauber et al, 2012; Wilson & Xenopoulos, 2008). In these systems, the DOM pool within surface waters tend to contain a higher proportion of aliphatic carbon, with protein-like compounds dominating (Fig. 2.3) (Delprat et al., 1997; Kamjunke et al., 2015; Parr et al., 2015; Wilson & Xenopoulos, 2008). As anthropogenic land changes increase, human inputs of novel DOM components will be introduced into natural systems (Stedmon et al., 2003), causing ramification for the functioning of aquatic bacterial communities.

Anthropogenic land use changes also dramatically influence nutrient concentrations in many freshwater ecosystems. In forested and other natural catchments, the composition of nutrients is dominated by organic nitrogen over inorganic nitrogen and concentrations of phosphorus are low (Stedmon et al., 2006). As the percentage of agriculture increases in a catchment area, the loading of nitrogen and phosphorus often increases exponentially (Hecky et al., 2003; Stedmon et al., 2006). This anthropogenic pool of nutrients is composed of higher levels of organic phosphorus and inorganic nitrogen. In agricultural fields, much of the nutrient-rich fertilizers added to the soil do not stay on the land, but flow into nearby aquatic ecosystems instead (Beckert et al., 2011; Carpenter et al., 1998). Urban point and nonpoint source pollution as well as animal waste from large scale industries also increase the concentrations of nitrogen and phosphorus in surrounding aquatic ecosystems (Carpenter et al., 1998).

As the composition of DOM and nutrient levels change along land use gradients, bacterial metabolic capabilities also fluctuate. In one study, for instance, forested catchments and agriculturally dominated lands showed significantly different percentages of bacterial consumption of DOC, 6% and 14% respectively (Wiegner & Seitzinger, 2001). In streams containing high amounts of complex terrestrial materials, for instance, most of the DOM consumed is allocated for BR, with only small amounts going towards

BP (Fasching et al., 2014). In oligotrophic systems as well, most of the carbon consumed is allocated for BR rather than BP, due to the limited amount of bioavailable dissolved organic matter (Smith & Prairie, 2004). In ecosystems containing large pulses of labile DOM (e.g. freshets, anthropogenic inputs), however, BP has been shown to increase at significantly higher rates then BR (Lennon & Cottingham, 2008). Inputs of high-quality DOM and nutrients, from sources such as urban sewage effluents, have been shown to increase bacterial metabolism by up to 90% (Wassenaar et al., 2010). BP, specifically, has been positively correlated with phosphorus concentrations, suggesting that nutrients could be a critical driver of bacterial activity within anthropogenic watersheds (Williams et al., 2012). While we broadly understand the influence of DOM and nutrients on bacterial metabolism, more in-depth studies of each facet of bacterial metabolism (i.e. BR, BP, BCC, and BGE) and their interactions with changes in land use, DOM composition, and nutrients concentrations are needed to understand the functioning of aquatic systems.

2. 3 **Bacteria and DOM within the Larger Ecosystem Context**

Within the large repertoire of knowledge surrounding bacterial metabolism and its connection to organic materials, most studies have targeted lakes, streams, or smaller biogeochemically active sections within rivers, often neglecting large river systems. Because of their expansive reaches throughout a variety of catchments, complex structures, and dynamic hydrological fluctuations, rivers are difficult to study and are often avoided. As the anthropogenic pressures near fluvial systems increase, it is predicted that there will be greater fluxes of DOM entering rivers (Raymond & Spencer, 2015). Because of this, expanding our knowledge of river processing is needed to predict future impacts on ecosystem functioning (Aufdenkampe et al., 2011).

Despite only making up a small surface area globally, rivers process large quantities of materials and have a significant impact on carbon fluxes within the environment (Cole et al., 2007). Due to their vast reaches, these complex heterogenous systems contain a variety of riverine units, such as tributaries, archipelagos, fluvial lakes, and anthropogenic inputs (Barnes et al., 2018; Battin et al., 2008; Maranger et al., 2005;

Wiens, 2002). These different ecosystems, while physically connected, act as sinks and sources for materials (Battin et al., 2008). For instance, tributaries within the Amazon River basin are responsible for most of the inputs of DOC into the Amazon River and, therefore, are important sources of material (Moreira-Turcq et al., 2003). The extent to which rivers remove and transport materials is highly dependent on their characteristics, such as flow rates, inputs, and connectivity (Battin et al., 2008; del Giorgio & Pace, 2008; Hotchkiss et al., 2015). Environmental changes, such as hydrology and land use, also play a significant role in the structure and functioning of large rivers. For example, increases in discharge, from spring freshets, have been shown to account for up to 68% of the annual exports of DOC to boreal streams (Laudon et al., 2004). These large inputs of materials have the potential to influence the rates of river processing and determine how much DOM flows downstream.

2.3.1 *Conceptualizing Rivers within the Environment*

Due to their unique structure and relatively small width, rivers receive a sizable volume of terrestrially derived DOM (Raymond et al., 2004). The Mississippi River, for instance, makes up a small proportion of the United states, yet drains approximately 40% of the continent within its watershed and accounts for almost 65% of all suspended and dissolved materials entering the oceans from the United States (Dagg et al., 2004). Despite the large load of materials entering rivers, this DOM does not accumulate within oceans (Cole & Caraco, 2001). Instead, rivers act not only as a pipe transporting DOM but also as a reactor, with over three times more terrestrial organic matter decomposing within rivers than is exported by rivers to the ocean (Cole & Caraco, 2001; del Giorgio & Pace, 2008). Processes, such as photo, chemical, and bacterial degradation, transform complex terrestrially derived materials into smaller compounds.

For decades, scientists have tried to make predictions about the functioning capabilities of rivers by developing conceptualizations of fluvial systems. The River Continuum Concept (RCC), one of the earlier river concepts, argues that rivers are highly connected longitudinally, with downstream sections of the river relying heavily on upstream processing (Sedell et al., 1989; Vannote et al., 1980). This concept states that as

you go downstream, river size increases, and the pool of DOM becomes less connected to headwater streams and terrestrial environments. Processing and autochthonous carbon production via bacterial metabolism, phytoplankton, and aquatic plants increase downstream, shifting the pool of carbon to progressively smaller compounds (Berggren et al., 2009). In response to the changing carbon pool, bacterial communities downstream are adapted to smaller particulate matter compared to the bacteria upstream (Vannote et al., 1980). Creed et al. (2015) builds upon this idea, arguing that as stream order increases, there is less influence from the catchment and an increase in homogenization due to selective removed of materials. The River Discontinuity Concept, however, argues that rivers are mosaics of "discontinuities", each with unique characteristics (Sedell et al., 1989). In this concept, abrupt changes within the landscape and riverscape produce distinct units that are geomorphically and biotically independent from the other river units. Each section of the river contains individualized ecosystems with different pools of materials and processing abilities (Sedell et al., 1989). The third concept is a Refined River Continuum Concept which states that, while there is a continuum between the upstream and downstream sections of rivers, there are also riverine units and inputs that are biogeochemically significant as well (Houser et al., 2015; Minshall et al., 1985). Other researchers have built off this idea, arguing that rivers are both continuous and discontinuous systems. The Biogeochemical Hotspots and Hot moments concept (McClain et al., 2003) and the Ecosystem Control Points concept (Bernhardt et al., 2017) both argue that rivers are connected longitudinally, with spatial, temporal, and hydrological changes significantly influencing the biogeochemical processing. These riverine units, inputs, and/or seasonal changes perform differently than the steady state of the river and are important to consider when examining the capacities of the river to process and transport DOM. Together, these concepts identify the importance of considering the entire stretch of a river, with its discontinuities and unique features, in order to fully understand river carbon processing.

2.3.2 *Riverine Units*

Outside the conceptualization of rivers, hydrology and morphology determine much of the processing from headwaters downstream. Residence time, the amount of time something stays within a given stretch of river, is one of the leading drivers determining whether a river acts more as a pipe, passively transporting DOM, or a reactor, actively transforming material (Kothawala et al., 2015). As the residence time of the river increases, the transformation of DOM also increases through processes such as photodegradation, flocculation, and bacterial metabolism (Houser et al., 2010). As residence time decreases, the processing ability of rivers also decrease (del Giorgio & Pace, 2008). Riverine units, such as archipelagos and fluvial lakes, alter the base flows of the river and increase residence time (Barnes et al., 2018; Battin et al., 2008; Maranger et al., 2005; Wiens, 2002). Within archipelagos, complex braided structures slow down the speed of the water, allowing for more material processing to occur. At these sites, a river can act more as a reactor than a pipe (Barnes et al., 2018; del Giorgio & Pace, 2008). Within fluvial lakes and flood plains, the width of the river dramatically increases, while the depth decreases. These sections of the river increase the residence time, as well, allowing for increased photodegradation and interactions between the benthic and pelagic river communities in shallow nearshore regions. In floodplains, increased drying/flooding within this section of the river tends to lead to increased inputs of terrestrial materials. Tributaries, another important riverine unit, deposit large amounts of sediments and dissolved materials into rivers (Frenette et al., 2012; Patoine et al., 2017). These smaller rivers or streams are often strongly connected to the terrestrial environment and contain significantly higher loads of material compared to the main river (Benda et al., 2004; Patoine et al., 2017). Frequently, there is a noticeable change in morphology at the junction between the main river and tributary due to inputs of sediments onto the river floor. This can create a fan effect of materials, flattening the gradient of the main water mass, and increasing turbidity (Benda et al., 2004). The outcome is the production of a dramatically different carbon pool flowing downstream (Frenette et al., 2012). Each of these riverine units alter river functions differentially, and thus should be considered when studying overall C processing within rivers.

2.3.3 *Hydrology*

Hydrological changes, in surface flow and inputs, also influence the pipe and reactor functions of rivers. Higher river flows, from increased precipitation or spring freshets for instance, decrease the residence time and lead a river to act more as a pipe, with less biological activity occurring within the water (del Giorgio & Pace, 2008; Kothawala et al., 2015). In these high flow systems, the carbon composition tends to be more reflective of the watershed, due to an increase in terrestrial water sources (Berggren et al., 2009; Farjalla et al., 2006). In low flow systems, there is an increase in residence time and a decrease in terrestrial inputs. In one study, low flows were positively related to BGE and the amount of bioavailable DOC within the system. This was driven by the higher contribution of autochthonous carbon derived from phytoplankton (Farjalla et al., 2006), illustrating how key aspects of river hydrology may directly influence bacterial metabolism and C processing in riverine systems.

2. 4 **Conclusion**

Within fluvial systems, bacterial metabolism is one of the most prominent transformers of materials. Through BR and BP, bacteria not only process and alter carbon within rivers, but also contribute to global gas emissions, influence the composition of DOM, and produce energy sources for the base of the food web. Many factors, such as changes in composition of DOM and nutrients, water residence time, flow paths, and hydrology within a river can each have a significant impact on the capacities of bacterial metabolism. In the face of increasing water discharge due to climate change or land use management, that predict higher fluxes of DOM into rivers, it is important to understand how bacterial processing capabilities react in response to changes in DOM inputs and hydrology.

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Chapter 3

DOWNSTREAM ENRICHMENT OF DISSOLVED ORGANIC MATTER AND NUTRIENTS DRIVE BACTERIAL METABOLISM WITHIN A LARGE LAKE-FED RIVER

Manuscript in preparation for Environmental Microbiology.

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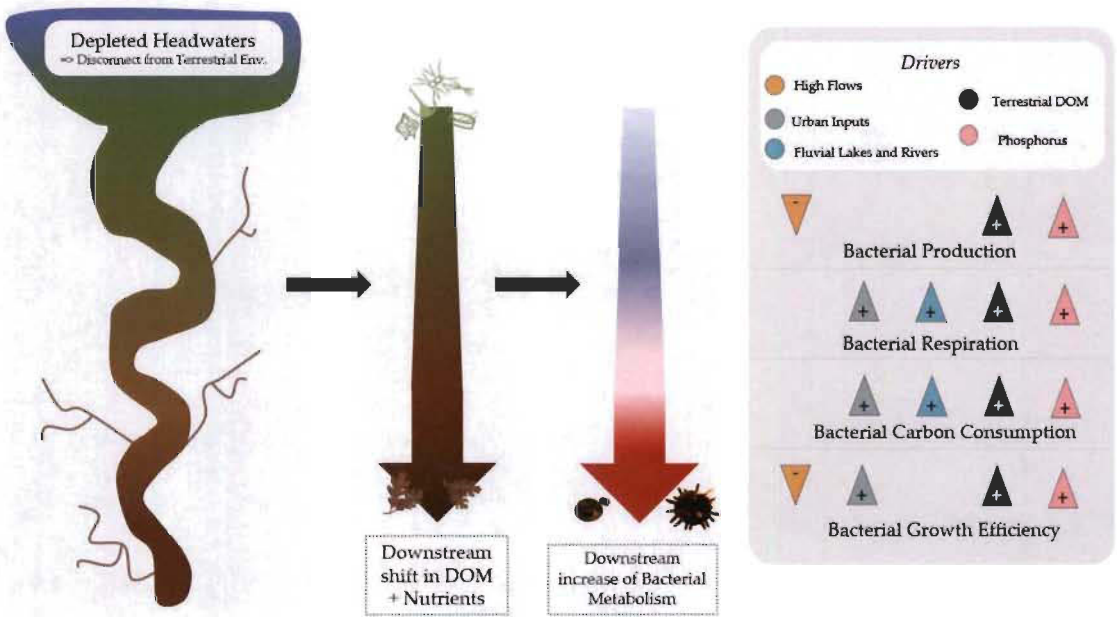
3. 1 Author's Contributions

Elizabeth Grater performed field and laboratory manipulations, carried out statistical analyses of the data, created figures, and wrote the paper. Paul del Giorgio participated in developing the theme of this paper, provided guidance on experimental setup and statistical analyses, and edited this paper. François Tanguay assisted and carried out field and laboratory manipulations and edited this article. Gilbert Cabana provided useful guidance regarding the St. Lawrence River and provided revisions for this article. François Guillemette conceived the project, aiding in idea development, organized field campaigns, provided thorough feedback and advice in the development this paper, and edited the paper.

3. 2 Full article in English: Downstream Enrichment of Dissolved Organic Matter and Nutrients Drive Bacterial Metabolism Within A Large Lake-Fed River

Graphical Abstract



Abstract

Large rivers are complex heterogeneous systems transforming and transporting large amounts of materials from the land to the ocean. Within these systems, bacterial metabolism plays a central role in driving the functioning of rivers, by consuming and producing carbon (C) through bacterial respiration (BR) and bacterial production (BP). Despite this, few studies have conducted a thorough investigation of large rivers in order to understand the processing capacities of bacteria and the drivers of this activity. In order to fill in these gaps, we assessed the rates of bacterial metabolism (i.e. BR, BP, bacterial carbon consumption (BCC), and bacterial growth efficiency (BGE)) in response to dissolved organic matter (DOM) and nutrient dynamics within the St. Lawrence River (SLR), a large river originating from a large oligotrophic lake. To better understand the role of riverine features (i.e. laterally distinct water masses, fluvial lakes, islands, and urban inputs) in shaping bacterial C processing, we sampled the SLR longitudinally and laterally over two summers, each with distinct hydrological conditions (high vs. normal flows). Our results showed that BP, BR, BCC, and BGE increased steadily downstream, corresponding with an increase in terrestrial DOM and phosphorus, contradicting what is commonly found in large rivers. At the discharge point of the Montreal sewage effluent, bacterial metabolism spiked, likely in response to high inputs of nutrients and DOM. Downstream of the fluvial lakes and islands, bacterial metabolism decreased, likely driven by an increase in water residence time and DOM removal within the riverine units. DOC concentrations, however, remained relatively stable (~ 3 mg L^{-1}) throughout the river, suggesting rapid bacterial processing of external C inputs and, ultimately, an uncoupling between the transport and transformation of materials within the river. Hydrology also played a role in driving bacterial metabolism, with high flows corresponding to higher concentrations of DOM and nutrients yet lower rates of bacterial activity, on average, suggesting that more materials are being transported downstream, unprocessed, during these conditions. Our study of the SLR opened the black box of bacterial metabolism, revealing the strong influence of DOM composition, nutrient concentrations, riverine structure, and hydrology on bacterial processing capabilities.

Key Words: Bacterial metabolism, carbon processing, river functioning, riverscape, dissolved organic matter, urban effluent

Introduction

Despite only making up a small surface area globally, inland waters receive ~ 5.1 Pg of terrestrial carbon per year (Drake et al., 2018). Of this, only ~ 0.9 Pg C yr^{-1} ends up in the oceans, with the rest being transformed and sequestered (Drake et al., 2018). Rivers play a crucial role as both a passive pipe and an active reactor transporting and transforming carbon and other materials from land to ocean (Aufdenkampe et al., 2011; Battin et al., 2008; Cole et al., 2007). The extent of the pipe and reactor functions within fluvial systems is dependent on factors such as inputs of materials, biological activity, river morphology, and hydrology. Throughout their vast reaches, rivers are often heterogenous, containing archipelagos, fluvial lakes, and tributary confluence zones, all of which have the potential to influence their functioning (Barnes et al., 2018; Battin et al., 2008; Maranger et al., 2005; Wiens, 2002). Given these complexities, it is often difficult to accurately quantify the processing abilities of these important systems.

Within river systems, heterotrophic bacteria play a central role in transforming organic materials (Guillemette & del Giorgio, 2011; Koehler, von Wachenfeldt et al., 2012). Bacterial respiration (BR), the consumption of carbon for cell maintenance, and bacterial production (BP), the consumption of carbon for biomass production, transform dissolved organic carbon (DOC) into CO_2, affecting gas dynamics and budgets, and introduce biomass into the surrounding food web (Berggren & del Giorgio, 2015; Maranger et al., 2005). Taken together, the sum of BR and BP, i.e. bacterial carbon

consumption (BCC), quantifies the processing capabilities of bacteria within the system. Of the total carbon consumed, only a small, more labile proportion is allocated to BP, while the bulk of the carbon consumed going towards BR, often leading to larger rates of BR compared to BP and to a decrease in bacterial growth efficiency (BGE) (del Giorgio et al., 2006; Rodibaugh et al., 2020). This uncoupling between BR and BP within aquatic systems can be driven by factors such as changes in DOM and nutrient composition and riverscape characteristics.

Currently, it is widely accepted that shifts in the quality and quantity of DOM, driven by land use changes or upstream processing, significantly control the various aspects of bacterial metabolism (Berggren & del Giorgio, 2015; del Giorgio & Cole, 1998; del Giorgio et al., 2006; Lennon & Cottingham, 2008). For instance, labile, autochthonous carbon has been shown to be consumed at rates up to 10 times higher than terrestrial pools of carbon (Guillemette et al., 2013). This labile carbon is often utilized more efficiently for BP (Berggren & del Giorgio, 2015), whereas aromatic terrestrial carbon is allocated, more often, for BR (Kritzberg et al., 2004). Tracking bacterial metabolic capacities to consume carbon substrates have been shown to provide a useful link between changes in DOM, due to habitat changes, and rates of bacterial metabolism (Berggren et al., 2010; Comte and del Giorgio, 2009). Other environmental conditions, such as water residence time (WRT) and river structure have also been shown to influence bacterial processing capabilities (Barnes et al., 2018; Battin et al., 2008; del Giorgio & Pace, 2008; Kothawala et al., 2015; Maranger et al., 2005). Because of their sensitivity to environmental changes and C inputs, bacteria have the potential to be an insightful tool for assessing river

functioning in response to hydrological regime and human disturbance gradients (von Schiller et al., 2017; Young & Townsend, 2008).

Much of what we know surrounding bacterial C processing in rivers has been substantiated from one type of framework, in which vegetated headwater steams, through their course and enlargement downstream, shift in composition from terrestrial, aromatic DOM to autochthonous DOM (Creed et al., 2015; Sedell et al., 1989; Vannote et al., 1980). Within these systems, BCC and DOM availability often decrease downstream, leading to a decrease in BP and BGE (del Giorgio et al., 2006; Maranger et al., 2005). BR, on the other hand, is often more sensitive to local conditions throughout the river, showing weaker longitudinal trends (Rodibaugh et al., 2020). Many rivers, however, depart from this commonly accepted conceptual framework, containing, instead, a headwater source depleted in DOM and nutrients and receive terrestrial inputs downstream, e.g. lake-headwater rivers, spring fed rivers, glacier fed rivers, and oligotrophic rivers (Aiken et al., & Raymond, 2014; Chowanski et al., 2020; Duarte et al., 2010; Vincent & Laybourn-Parry, 2008). One such system is the St. Lawrence River (SLR), a large river that flows along the border of Canada and the United States. The SLR originates at a large lake (i.e. Lake Ontario) that is characterized by low concentrations of DOM and nutrients (Hudon et al., 2017; Massicotte & Frenette, 2011). As it flows downstream, the SLR receives inputs of DOM from surrounding tributaries, shifting the DOM composition from autochthonous to allochthonous (Frenette et al., 2012; Massicotte & Frenette, 2011). The SLR also contains distinct lateral separation between the northern, main, and southern water masses, driven by dredging of the center of the river and changes in bathymetry. A variety of riverine units (e.g. fluvial lakes, islands, and the sewage inputs from the Greater

Montreal area) along the river also impact the functioning of this ecosystem (Massicotte & Frenette, 2011; Massicotte et al., 2014). This lateral and longitudinal complexity within the SLR provides a unique macrocosm in which to study, simultaneously, multiple influences on bacterial metabolism and river functioning.

The main objectives of this study were to 1) assess spatial and temporal variation of BR, BP, BCC, and BGE within the SLR and 2) identify possible drivers of bacterial metabolism within the river. We hypothesized that, contrary to many river functioning models, we should see an enrichment in DOM and nutrients from up- to downstream leading to increased rates of BP, BR, BCC and BGE. We also hypothesized that inputs of sewage from the City of Montreal will create a hotspot of biogeochemical activity within the river, caused by large inputs of nutrients and DOM, and that bacterial metabolism will decrease within fluvial lakes and islands as DOM is selectively removed. We tested these hypotheses by conducting two scientific cruises along a 450 km stretch of the SLR, encompassing different water masses, cities, riverine units, and hydrological conditions.

Methods

Study Site

The St. Lawrence River (SLR), running along the eastern boarder of the United States and Canada (Fig. 1), is the second largest river in North America in terms of discharge (Butman et al., 2012; Hudon et al., 2017; Thorp et al., 2010). From the headwaters at Lake Ontario, the SLR flows for 450 km before reaching the Gulf of the St. Lawrence and the Atlantic Ocean (Frenette et al., 2012). Unlike most large rivers that start

off as headwater streams, the main input of water for the St. Lawrence River is Lake Ontario (Fig. 1), which supplies oligotrophic water containing small amounts of suspended particulate matter, DOM, nutrients, and chlorophyll a (Frenette et al., 2012; Hudon et al., 2017; Rondeau et al., 2000; Thorp et al., 2010). As it flows downstream, the SLR becomes increasingly enriched in DOM and nutrients as tributaries enter the river (Hudon et al., 2017).

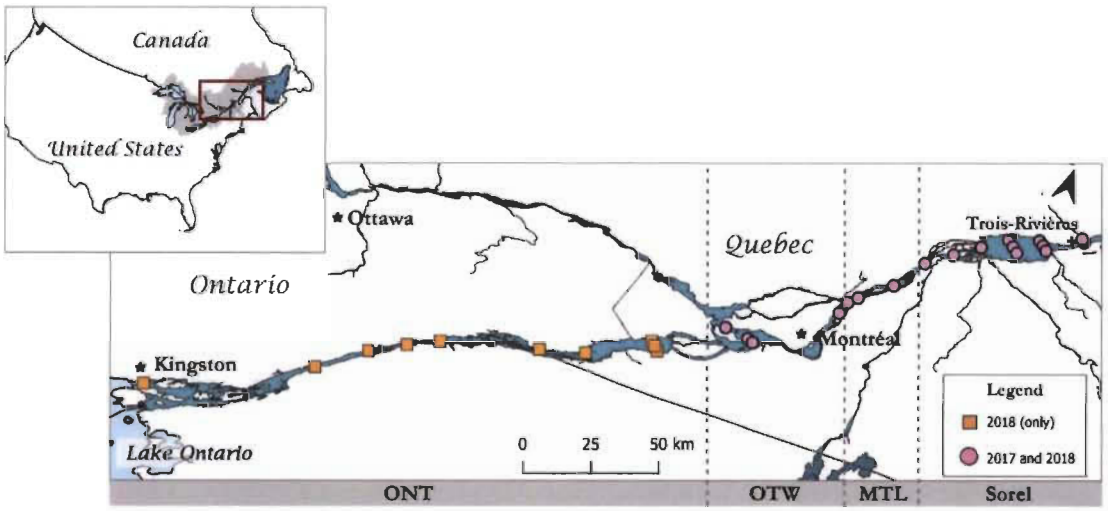


Fig. 1 Sampling stations for the 2017 and 2018 field campaigns aboard the R/V Lampsilis. The pink circles represent sampling transects from 2017 and 2018 and orange squares represent sample transects from 2018 only. The river is broken up into four sections based on riverine units. Section 1 (ONT) is from Lake Ontario to Lake St. Louis, section 2 (OTW) is from the confluence of the Ottawa River to Montreal, section 3 (MTL) is from the input of the Montreal effluent to the Sorel Islands, section 4 (Sorel) is from the Sorel Islands to Trois-Rivières.

The average width of the SLR is 1 to 2 km, widening dramatically at the fluvial lakes (5 to 15 km) (Frenette et al., 2012). Depths vary greatly throughout the river, with the deepest sections located within the main water mass (~ 12 m) and shallowest sections located within the fluvial lakes (< 6m) (Frenette et al., 2012; Hudon et al., 2006). Islands break up the river at Kingston, Montreal, and Sorel, influencing the water residence time.

Due to historical and ongoing dredging in the center of the river, the topography of the river floor restricts lateral mixing. Because of this, the river naturally splits into water masses (e.g. the northern, main, and southern water mass), each characterized by distinct physical and chemical properties (Frenette et al., 2012; Rice et al., 2006). The main water mass of the river, originating at Lake Ontario, accounts for ~70% of the SLR, with a mean annual discharge of around 7,000 m^3 s^{-1} (Hudon et al., 2017). Throughout its reach, the SLR receives large loads of organic matter from 23 tributaries, providing a connection to the terrestrial environment (Frenette et al., 2012). The watershed of the Ottawa River, containing the largest tributary along the SLR with a mean annual discharge varying between 1,500 and 2,500 m^3 s^{-1}, contains a forested, relatively pristine catchment, contributing dark, humic water into the SLR (Fig. 1) (Hudon et al., 2017; Patoine et al., 2017). This water is mass is largely flowing in the northern section of the SLR.

Throughout the river, anthropogenic pressures are prevalent. Within the watershed between Lake Ontario and Quebec, 7 million people reside, over half of whom live along the shores of the SLR (Hudon et al., 2017). Much of the wastewater from these communities is discharged into the river, with varying levels of treatment occurring (Hudon et al., 2017). From the greater area of Montreal alone, primary-treated effluent from over 3 million people is dumped into the SLR (Hudon et al., 2017). This wastewater is one of the largest inputs of nutrients along the river and has a discharge rate of about 35 m^3 s^{-1}, a size comparable to that of several tributaries (Frenette et al., 2012; Morin & Bouchard, 2001). Once released, the plume of the Montreal effluent is trapped between the main water mass and the northern water mass, creating a new, smaller water mass (i.e.

the mixed water mass) with elevated concentrations of nutrients and DOM that consists of a mixture of the main and northern water masses as well as the plume of Montreal.

Water Sampling

Water samples were collected during two field campaigns (July 8th to July 16th, 2017 and July 14th to July 24th, 2018.) aboard the research vessel (R/V) *Lampsilis* (Fig. 1). Each campaign had distinctly different river discharges: 1) high flow conditions during 2017 and 2) average flows in 2018 (Fig. 2). We hereby refer to the 2018 campaign as "baseline flow conditions" and the 2017 campaign as "high flow conditions". During both years, we sampled evenly spaced transects along the freshwater section of the river (Fig. 1). Additional transects were added to pinpoint previously identified riverine units containing distinct biogeochemical activity along land use and riverscape changes (Frenette et al., 2012). These areas include the city of Montreal, the Ottawa River, the archipelagos at Sorel, and four fluvial lakes. At each lateral transect (16 in 2017, 21 in 2018), samples were collected across the width of the river to capture the distinct water masses. At each sample site (44 sites for year 1, 51 sites for year 2), water was collected using a Go-Flo water sampler at 1 m depth and transferred to pre-rinsed 10 L buckets. Out of the samples collected, a subset of samples (23 in 2017, 40 in 2018) were used for this study. This subset included the sites where all the bacterial analyses were performed.

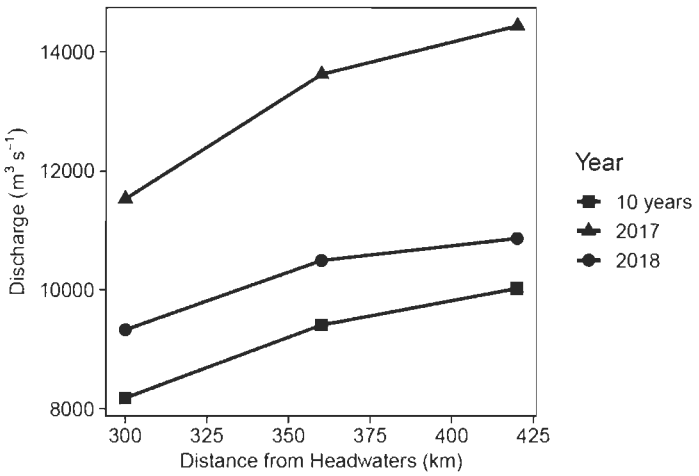


Fig. 2 Flow rates within the St. Lawrence River at LaSalle (300 km from headwaters), Sorel (360 km from headwaters), and Trois-Rivieres (420 km from headwaters). The triangle points represent 2017, the square points represent 2018, and the circular points represent the average flows between 2008 and 2018. The river was in high flow conditions during 2017 and in average flow conditions in 2018. (Data from Environment Canada, unpublished).

Immediately after collection, water was filtered for carbon analysis using 0.7 μm glass microfiber GF/F filters to remove any particulate organic matter and bacteria, then refrigerated in acid-washed polycarbonate bottles until further analysis. Water was also filtered for bacterial metabolism analysis using 2.7 μm glass microfiber GF/D filters to remove large grazers and other predator organisms that could affect rates of bacterial metabolism (Guillemette & del Giorgio, 2011). Finally, raw water was collected for total nitrogen (TN) and total phosphorus (TP) analysis and kept at 4°C until further processing. Ambient dissolved O_2, water temperature, and pH were measured using a ProDSS multi-probe YSI.

Chemical Analysis

The concentration of DOC was measured on an Aurora 1030 TOC Analyzer using a wet persulfate method and a five-point calibration procedure using potassium hydrogen phthalate as standard. TN was measured as nitrates after potassium persulfate digestion following the EPA Method (Method 353.2). TP was measured using the standard molybdenum-blue method after persulfate digestion following the EPA method (Method 365.3). TN and TP concentrations were determined using a colorimetric Lachat FIA Automated Ion analyzer. For sites with high concentrations of TN or TP, dilutions were made before analysis and the data were adjusted accordingly.

DOM Optical Characterization and PARAFAC analysis

Using a 1 cm quartz cuvette, absorbance and fluorescence measurements were collected on an Agilent Cary Eclipse Spectrophotometer. From the absorbance spectra generated (200-800 nm), specific ultraviolet absorbance at the wavelength 254 nm ($SUVA_{254}$), an index of aromaticity, was calculated by dividing the decadic absorption coefficient at 254 nm by DOC concentration (Weishaar et al., 2003). Fluorescence excitation emission matrices (EEMs) were created following the procedure described in (Fellman et al., 2010) with excitation intervals between 250 and 350 and emission intervals between 300 and 450. The EEMS were corrected for inner-filter effect (Kothawala et al., 2013), instrument biases (Cory et al., 2010), and normalized to Raman units (McKnight et al., 2001; Stedmon et al., 2003). All corrections were performed using the FDOMcorr toolbox version 1.6 (Murphy, 2011). The Freshness Index (FRESH), the humification index (HIX), and the fluorescence index (FI) were also calculated (Cory et al., 2010; Fellman et al., 2010)

EEMs were analyzed using Parallel Factor Analysis (PARAFAC) to extract peaks that correspond to different chemical characteristics of dissolved organic matter. For this analysis, we used the drEEM toolbox (Murphy, Stedmon, Graeber, & Bro, 2013) for MatLab (Mathworks, Natick, MA, USA) and extracted five fluorescent components (Fig. S2 and Table S1) following a split-half validation of the model. The model contained a total of 198 EEMs, including surrounding tributaries, island channels and the St. Lawrence itself from parallel studies. Of these components, three were characterized as humic-like (C1, C2, and C3; ex/em: 345/474, 310/412, and 275,435/514, respectively) and two were characterized as protein-like components (C4 and C5; ex/em: 285/352, 280/324, respectively; see Table S1 for addition information regarding the components). All components have been previously identified in the online library OpenFluor (www.openfluor.org) and reported in riverine and wastewater studies (Massicotte & Frenette, 2011; Stedmon et al., 2006; Yang et al., 2015).

Bacterial Respiration

To measure rates of BR, 500 mL flasks equipped with oxygen optical sensors (PreSens) were rinsed and filled with GF/D filtered sample water, sealed with a silicone stopper, and secured with parafilm. Once the flasks were properly sealed, they were immediately run on a FIBOX 3 to obtain initial readings of oxygen concentrations, then placed in a 20°C, water bath in the dark for the duration of the incubations. Measurements of O_2 concentration were taken 2 times per day for 4 to 6 days to capture linear rates of O_2 consumption. The flasks remained sealed for the duration of the incubations to avoid introduction of O_2 (Berggren et al., 2011; Marchand et al., 2009). The rate of change in

O_2 concentration was converted into a rate of carbon consumption per day by using a respiratory quotient of 1. For more details about this method, see Berggren et al. (2011) and Marchand et al. (2009).

Bacterial Production

To measure rates of BP, the radioactive leucine tagging method was used onboard the ship, based on methods developed by Pace et al. (2004) and Smith and Azam (1992). Briefly, 1.5 mL of GF/D filtered sample water was radiolabeled with leucine (3H-leucine) then incubated for one hour. After, bacterial activity was stopped with 100 µg of TCA (5%) and refrigerated until further processing in the lab. Cells were then washed and isolated by centrifugation, then radioactivity counts were taken using a scintillation counter. These values were related to the cell production to obtain BP rates. The data from this analysis were used to calculate total bacterial carbon consumption ($BCC = BR + BP$), and bacterial growth efficiency ($BGE\% = \frac{BP}{BR+BP} * 100$) (del Giorgio & Cole, 1998).

Bacterial Metabolic Capacities

Biolog Ecoplates™ containing a tetrazolium dye and carbon substrates were used to measure the carbon degradation capacity of the bacterial communities at each site. Each Ecoplate™ was composed of 96 wells containing one of 31 different compounds in triplicates, including carboxylic & acetic acids, amino acids, carbohydrates, polymers, and blanks (Weber & Legge, 2009). For each site, 125 µg of GF/D filtered water were pipetted into each well of an Ecoplate™ and incubated in the dark. As the communities consumed

a substrate, the dye was reduced, causing the well to develop color. This color development was measured optically 3 times per day for at least 4 days using a Tecan Genios microplate reader until average color development plateaued. The mean color development values for each class of compounds was calculated based on the an "average well color development" of 0.5 (Berggren & del Giorgio, 2015; Garland & Mills, 1991). The mean color development values for each site were separated into four substrate groups (i.e. carbohydrates, carboxylic acids, amino acids, and polymers) and averaged within the given groups following Berggren and del Giorgio (2015).

Statistical Analyses

Before performing the following statistical analyses, the data were transformed using logarithmic or square root transformations to achieve normal distributions, then scaled using the scale function in RStudio Team (2020) in order to accurately compare the diverse data set. To observe patterns within the pool of DOM and nutrients, principle component analysis (PCA) of the carbon concentration and composition (percentage contribution of each PARAFAC component, and fluorescence indices) and the concentration of nutrients was conducted. The Wilcox Test package in RStudio Team (2020) was used to run a Mann-Whitney U test between the two hydrological conditions (2017 vs. 2018) for each variable. Partial least square regression (PLS) analysis was performed, using the plsdepot package in RStudio Team (2020), on x-variables of DOM (concentration, PARAFAC components, and fluorescence indices) and y-variables (rates of BR, BP, BCC, and percentage of BGE) to single out the variables driving bacterial metabolism within the SLR. For this, PLS correlations plots were created to visualize the

relationships between the variables and PLS linear models were used to further investigate multivariate correlations between DOM and bacterial metabolism. Principle component analysis (PCA) was performed on bacterial community carbon consumption preferences (i.e. the four substrate groups) to determine temporal and spatial trends in terms of bacterial substrate preferences. RStudio Team (2020) was used for all statistical analysis and graph building.

Results

Spatial Patterns of Carbon and Nutrients Across the SLR riverscape

Throughout the SLR, concentrations of DOC remained relatively stable during baseline conditions (Fig. 3). In the main water mass, the DOC concentrations hovered around 2.5 mg L-1 (±0.73) from the headwaters downstream, with jumps in concentrations near the exit of Lake Ontario at Kingston, Ontario (3.78 mg L^{-1}), in the plume of the MTL effluent (3.90 mg L^{-1}), and in Lake St. Pierre (LSP; 5.42 mg L^{-1}; Fig. 3, A). The northern water mass had different spatial trends, with DOC concentrations doubling from 3.19 mg L^{-1} at the exit of the Ottawa River to 6.73 mg L^{-1} at the entrance of the Montreal effluent, then sharply decreased to 4 mg L^{-1} at the Sorel Islands (Fig. 3, B). In the mixed water mass, DOC concentrations remained stable throughout the river, with a mean of 3.0 mg L^{-1} (±0.60; Fig. 3, C). During high flow conditions, DOC concentrations were significantly higher on average in all three water masses, by 24%, 74%, and 50% in the main, northern, and mixed water masses, respectively (Table S2).

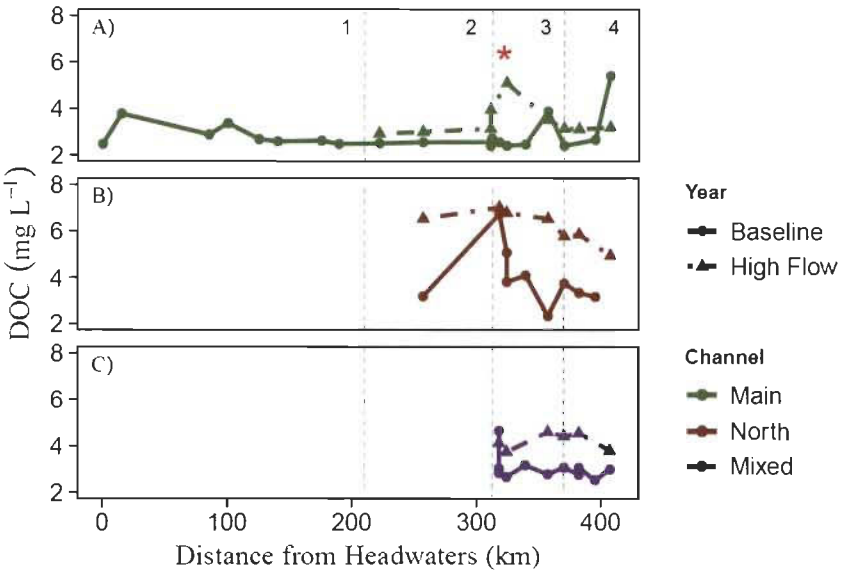


Fig. 3 The concentration of dissolved organic carbon from Lake Ontario to Trois-Rivieres in A) the main water mass, B) the northern water mass, and C) the plume of Montreal in the SLR. The four sections of the river are explained in Fig. 1. Each point represents a sample point, with the solid line representing baseline conditions and the dashed line representing high flow conditions. The red star indicates the input of the Montreal Effluent into the river.

During baseline conditions, TN and TP exhibited different patterns both laterally and longitudinally (Fig. S1). In the main water mass, TN concentrations were similar to DOC concentrations, with little variation from Lake Ontario to Montreal (0.5 mg L^{-1} ±0.04), with a small decrease in concentration from Montreal to LSP (0.3 mg L^{-1} ±0.10; Fig. S1, D). Concentrations of TP in the main water mass, however, deviated from those of DOC and TN, increasing from Lake Ontario to LSP (2.5 to 9.8 ug L^{-1}), with only slight oscillations along the way (Fig. S1, A). In the northern water mass, concentrations of TN and TP diverged very little from their mean of 0.57 mg L-1 and 10 ug L^{-1}, respectively (Fig. S1, B and E). In the mixed water mass, however, TN and TP clearly identified the Montreal effluent, with a peak in concentration at the sewage input of 4.6 mg L^{-1} and 90 ug L^{-1} respectively, followed by a sudden decrease by an order of magnitude within 20

km (Fig. S1, C and F). During high flow conditions, TP concentrations were significantly higher on average than baseline conditions in the main, northern, and mixed water masses, by 1.6, 2, and 1.8 times respectively (Table S2). TN concentrations, however, were not significantly different during high flow conditions compared to baseline conditions (Table S2).

Characterizing the DOM Pool

Although the concentrations of DOC in the SLR remained relatively stable, there were changes in DOM characteristics that suggested shifts in source and composition during transit. Distributions of the PARAFAC components were distinct laterally and longitudinally within the St. Lawrence (Fig. 4). During baseline conditions, there were patterns in PARAFAC components between the main and northern water masses (Fig. 4, A and B). In the main water mass, there was a strong transition downstream from a heavily protein-like DOM pool, dominated by C4, to one containing a larger percentage of humic-like DOM, dominated by C1 and C2 (Fig. 4, A). In the northern water mass, percentages of each component remained stable, with the humic-like components (C1, C2, and C3), dominating the water mass (Fig. 4 B). The mixed water mass contained similar amounts of C1, C2, C4, and C5, with a decrease in C4 and C5 downstream (Fig. 4, C).

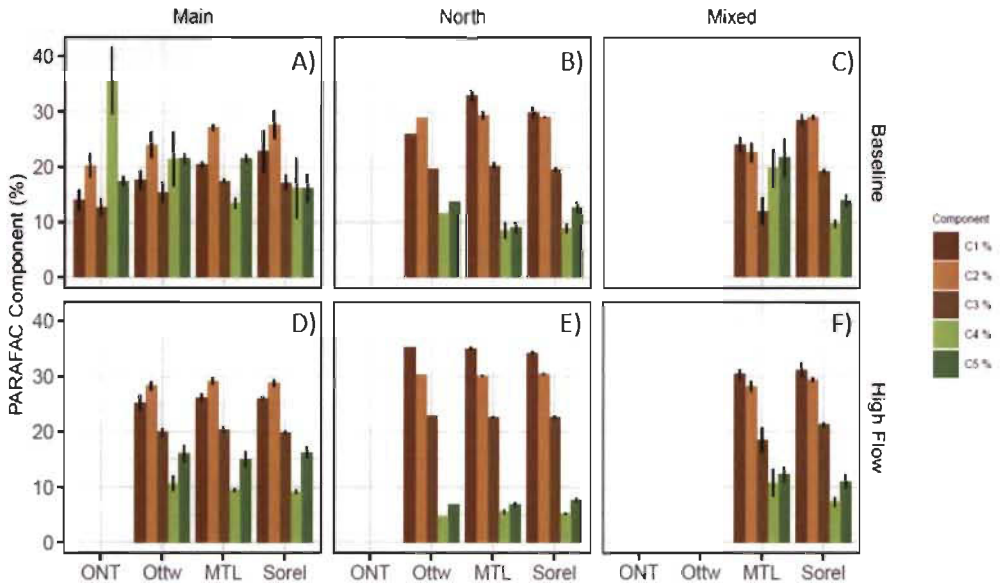


Fig. 4 Bar charts of the average percentage of each PARAFAC component with standard error bars within the four sections of the river. The data is split by water mass (main, northern, and mixed) and by hydrological conditions (baseline and high flows).

Unlike the unique distribution of PARAFAC components within the water masses during baseline flows, all three water masses during high flow conditions were dominated by C1, C2, C3 (Fig. 4, D-F). Although the main water mass was predominantly composed of humic-like components, there were still large amounts of C4 and C5 in the upstream section of the river, with the percentage of these components decreasing downstream (Fig. 4, D). In the northern water mass, the protein-like components were present in very small amounts, with the humic-like components being dramatically higher (Fig. 4, E). The mixed water mass contained a DOM composition representative of a mixture of the northern and main water mass (Fig. 4, F).

PCAs (Fig. 5) performed on the PARAFAC components, concentrations of DOC, TDN, and TP, and well as the fluorescence indices (FRESH, SUVA, and HIX) further

supported the compositional separation between the main and northern water masses. PC1 during baseline and high flow conditions (Fig. 5 A and B), explaining 59% and 80% of the variance respectively, further identified the clear lateral patterns, relating the main water mass sites to protein-like compounds (C4 and C5) and the Fresh index on the left side and northern water mass sites to humic-like compounds (C1, C2, C3), SUVA, concentration of DOC, and concentrations of TDN and TP on the right side.

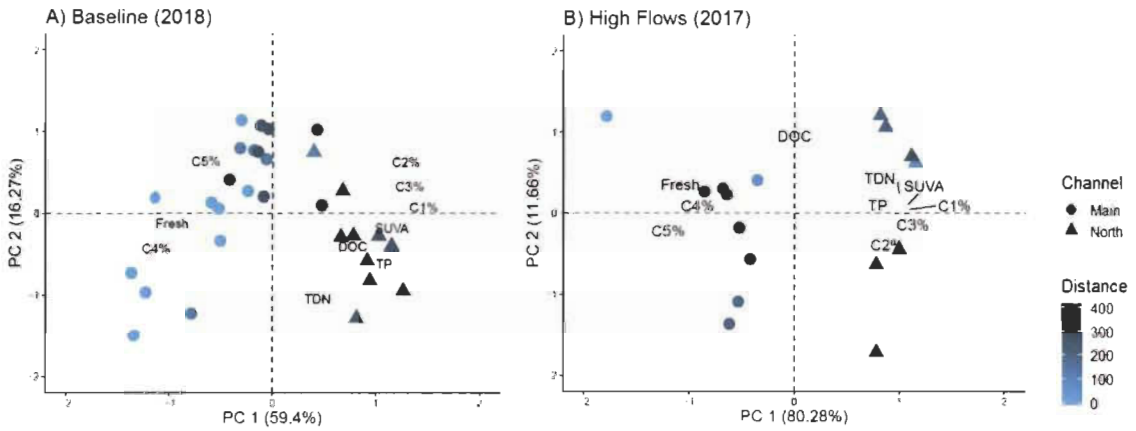


Fig. 5 Principal Component Analysis of the variables of dissolved organic carbon and nutrients in A) baseline conditions (2018) and B) high flow conditions (2017). The circular points represent the main water mass, and the triangle points represent the northern water mass, with a color gradient from light to dark indicating upstream to downstream of the river, respectively.

During baseline conditions, PC1 and PC2 exposed a longitudinal trend from upstream (light colored points) to downstream (darker color points; Fig. 5 A). Along PC1 (Fig. 5 A), increased similarity between the two water masses came forward, with downstream main and northern water mass sites converging towards the center of the PCA. PC2 (Fig. 5, A), explaining 16.2% of the variation, also showed longitudinal patterns within both water masses, with upstream sites having negative scores and downstream

sites having positive scores along this axis. High flow conditions (Fig. 5, B) showed no convergence in characteristics downstream between the two water masses.

Spatial Trends in Bacterial Metabolism

During baseline conditions, significant shifts in bacterial metabolism were apparent from the outlet of Lake Ontario to LSP (Fig. 6). In the main water mass, BP and BGE increased steadily from Lake Ontario to Montreal. BP increased from 4 μgC L^{-1} day^{-1} (outlet of Lake Ontario) to 30 μgC L^{-1} day^{-1} at LSP (Fig. 6, A). BGE increase from 8% (Lake Ontario) to 37% (input of the Montreal Effluent), decreasing and stabilizing after the Montreal effluent, to 25% towards LSP (Fig. 6, J). BR and BCC varied more in the main water mass but followed similar trend to each other (Fig. 6, D and G). Initial rates of BR were 43 μgC L^{-1} day^{-1} at the outlet of Lake Ontario, oscillating 30 μgC L^{-1} day^{-1} within the first three fluvial lakes, then reached a peak rate of 146 μgC L^{-1} day^{-1} at the input of the Montreal Effluent, before stabilizing around 90 μgC L^{-1} day^{-1} at LSP (Fig. 6, D). BCC increased steadily from 50 μgC L^{-1} day^{-1} to 100 μgC L^{-1} day^{-1}, with oscillations of 50 μgC L^{-1} day^{-1} occurring at the start and end of each fluvial lake (Fig. 6, G, section 1). After the inputs of the Montreal effluent, BCC peaked at 130 μgC L^{-1} day^{-1}, decreasing in the Sorel Islands and LSP, returning to a rate of 100 μgC L^{-1} (Fig. 6, G).

In the northern water mass, BP remained around 30 μgC L^{-1} day^{-1} (Fig. 6, B) while BR oscillated between 50 μgC L^{-1} day^{-1} and 100 μgC L^{-1} day^{-1} from the entrance of the Ottawa River to LSP (Fig. 6, E). BCC increased slightly from upstream to downstream, with a peak of 130 μgC L^{-1} day^{-1} at the input of Montreal (Fig. 6, H). BGE showed more variation than the other facets, increasing progressively from 24% at the Ottawa River to

a peak of 50% right before the Sorel Islands, then stabilized in LSP around 25% (Fig. 6, K). In the mixed water mass, there were high rates of bacterial activity. At the input of the effluent, BP, BR, and BCC started high, at 157 μgC L^{-1} day^{-1}, 336 μgC L^{-1} day^{-1}, and 490 μgC L^{-1} day^{-1} respectively, with each decreasing by a factor of 5 downstream to 35 μgC L^{-1} day^{-1}, 74 μgC L^{-1} day^{-1}, and 120 μgC L^{-1} day^{-1} at LSP, respectively (Fig. 6, C, F, I, respectively). BGE decreased consistently downstream from 46% at Montreal to 17% at LSP, with oscillations occurring sporadically (Fig. 6, L).

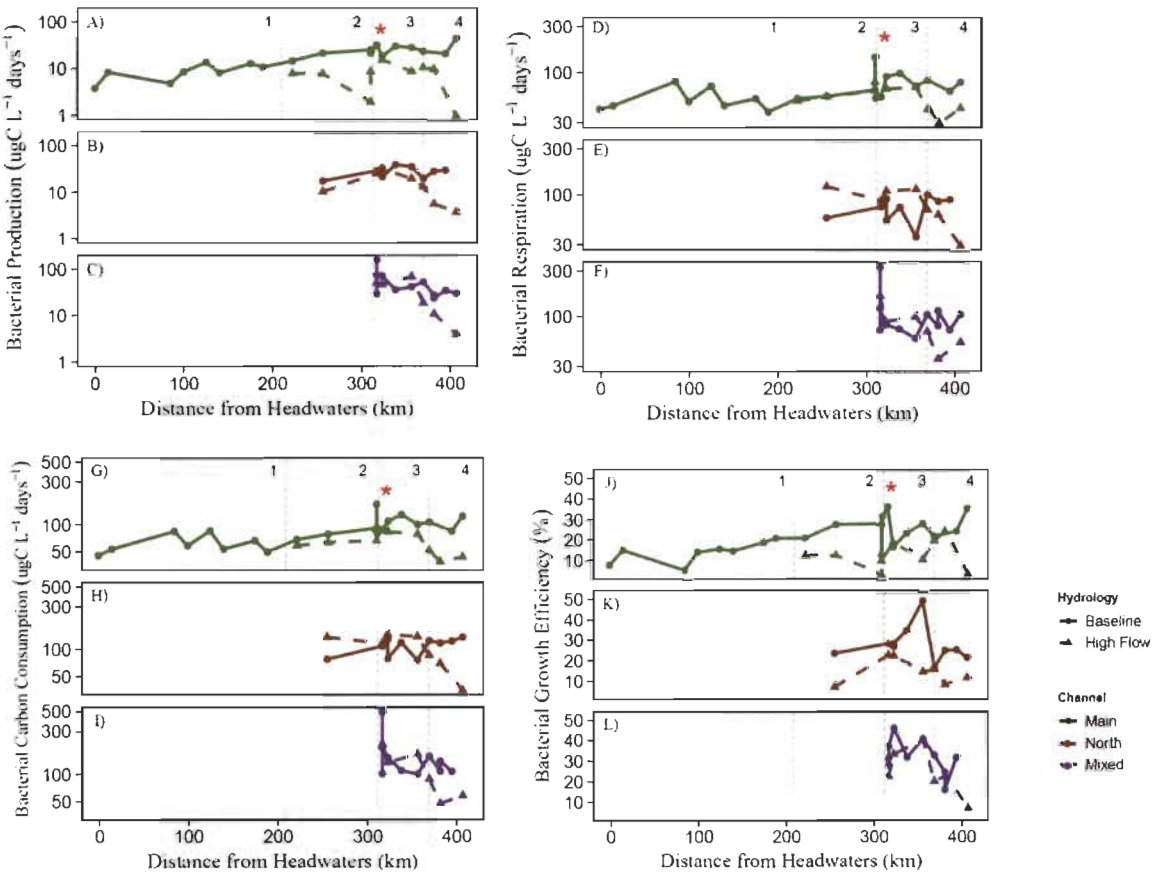


Fig. 6 Bacterial production, bacterial respiration, bacterial carbon consumption, and bacterial growth efficiency (%) from Lake Ontario to Trois-Rivieres in the main water mass (A, D, G, and J), the northern water mass (B, E, H, K), and the mixed water mass, containing the plume of Montreal (C, F, I, L) in the SLR. Each point represents a sample point, with the solid line representing baseline conditions and the dashed line representing high flow conditions. The red star indicates the input of the Montreal Effluent into the river.

During high flow conditions, average rates of BP, BR, BCC, and BGE in the main water mass were each significantly lower compared to baseline conditions, by 62%, 21%, 33%, and 43% respectively (Table S2). Within the northern water mass, average rates of BP and BGE were also significantly lower during high flow conditions compared to baseline conditions, by 43% and 41% respectively (Table S2). Rates of BR and BCC, however, were not significantly different between the hydrological conditions in the northern water mass and none of the facets of bacterial metabolism were significantly different during high flow conditions compared to baseline conditions within the mixed water mass (Table S2).

Linking Bacterial Metabolism to DOM Composition and Nutrient Concentrations

Partial least squares (PLS) analysis (Fig. 7, A and C) revealed that bacterial metabolic variables (BP, BR, BCC, and BGE) were positively correlated with humic-like components of DOM (C1, C2, C3), SUVA, HIX, and nutrients concentrations (TP and TN), and negatively correlated with protein-like carbon (C4 and C5) and the FRESH index along the SLR riverscape. A linear regression (Fig. 7, B.) of the x-scores and y-scores from the PSL component 1 (Fig. 7, A.) revealed a strong positive linear relationship (r^2 = 0.56) between the humic-like components and TP and bacterial metabolism during baseline conditions. Along the linear regression, sites were distributed based on water mass type and distance from headwaters, with upstream main water mass sites clustered near the lower left corner, while downstream main water mass and northern water mass sites were distributed towards the upper right section, highlighting different trends depending on the section of the river. During high flow conditions, the linear relationship

between the x-scores and y-scores from the PSL component 1 (Fig. 7, D) was weaker (r^2 = 0.37), suggesting a weaker correlation between DOM/nutrients and bacterial metabolism. Comparisons between the scores from other components of the PLS analysis showed no clear correlations when the same exercise was conducted.

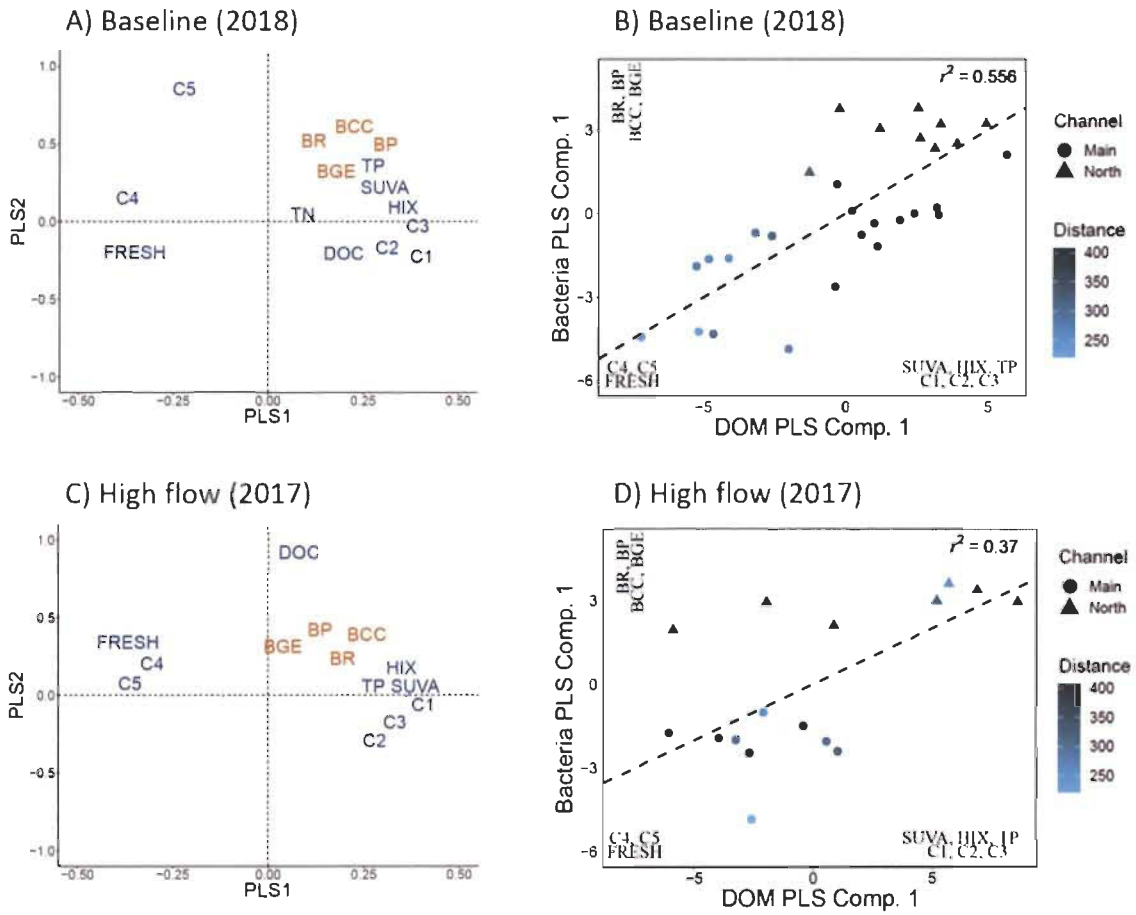


Fig. 7 Partial least squares (PLS) correlations plot (A and C) and linear model of component 1 from each PLS (B and D) during baseline and high flow conditions. DOM x-variable scores (PARAFAC components, fluorescence indices, TN, TP, and DOC) are along the x-axis and bacterial metabolic y-variable scores (BR, BP, BCC, and BGE) are along the y-axis in plot B and D. Variable labels where added, based on their direction of influence, to assist with PLS interpretation.

Bacterial Carbon Substrate Degradation Potential

Despite changes in the composition of the DOM pool and various facets of bacterial metabolism throughout the river, bacterial carbon substrate preferences showed only minimal longitudinal trends (Fig. 8). Indeed, PC1 of the PCA based on the patterns of substrate utilization across sites at baseline conditions, explaining 52.11% of the variation, separating sites with preferential use of amino acids and carboxylic acids from sites with preferential consumption of polymers and carbohydrates, with no marked upstream-downstream pattern (Fig. 8A). PC 2, explaining 27.81% of the variation, defined a slight longitudinal pattern, with upstream communities preferentially utilizing amino acids and polymers, and downstream communities preferentially utilizing carbohydrates and carboxylic acids (Fig. 8, A). Northern water mass sites were mainly restricted to negative regions on both PC1 and PC2, suggesting that the patterns of substrate consumption by bacterial communities within this water mass were narrower and more stable than in the main water mass (Fig. 8, A).

During high flow conditions (Fig. 8, B), bacterial substrate preferences reflected the trends found in the PCA of DOM variables (Fig. 5, B), with a clear separation between the main and northern water masses. Along PC 1 (explaining 44%) of the PCA (Fig. 8, B), sites were separated by bacterial substrate preferences, with main water mass sites showing strong bacterial community utilization of the four substrate groups, while the northern water mass sites contained bacterial communities with no strong substrate preference. PC 2, explaining 23%, showed longitudinal trends during high flow conditions, with upstream bacterial community preferentially utilizing amino acids and

carboxylic acids and downstream bacterial communities preferentially utilizing carbohydrates and polymers (Fig. 8, B).

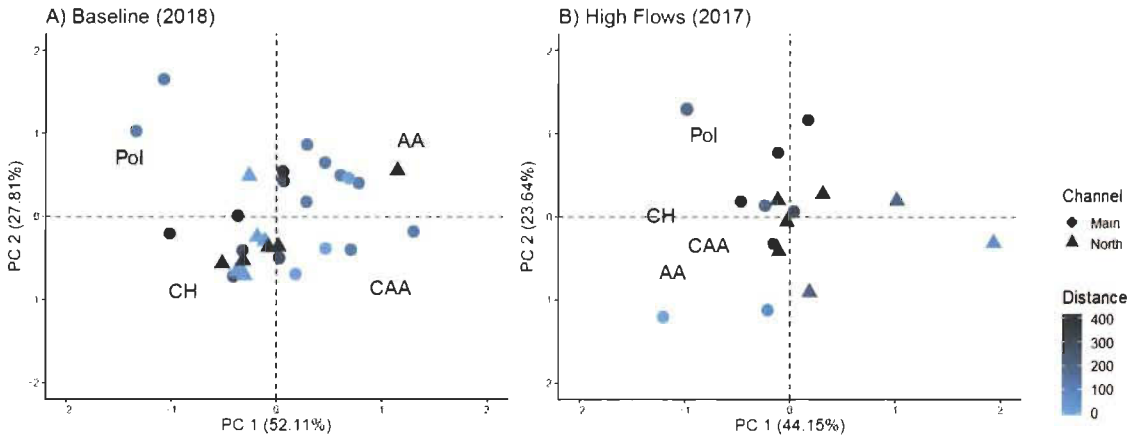


Fig. 8 Principal component analysis (PCA) of the average values in the four main types of substrates within the Biolog Ecoplates during A) baseline conditions (2018) and B) high flow conditions (2017). The Biolog substrates, in black, are Carbohydrates (CH), Carboxylic Acids (CAA), Amino Acids (AA), and Polymers (Pol).

Discussion

If taken alone, the relatively stable concentrations of DOC within the SLR (Fig. 3) suggest that the river is mostly inactive, passively transporting materials downstream. Looking deeper into this pipe, however, we found that the composition of DOM as well as bacterial metabolism varied both laterally and longitudinally, indicating, instead, that the river was highly active, with a high degree of DOM processing occurring during transit. Moreover, our results showed that riverine features within the river, such as the fluvial lakes and the sewage inputs of Montreal, lead to elevated bacterial activity, driven by specific changes in flow rates and DOM composition. Due to their dynamic nature, the

composition of DOM and bacterial metabolism provided important information about river dynamics that would be missed if we only considered DOC concentrations.

Spatial Trends within the SLR

Within the SLR, longitudinal trends of DOM composition found in our study followed those observed by Massicotte (2011), with a general increase in humic-like DOM downstream within the main water mass (Fig. 4). This contradicts previous studies that find a general decrease in terrestrial materials and an increase in autochthonous DOM downstream within large rivers (Creed et al., 2015; Lambert et al., 2016). Unlike more commonly observed river systems, the SLR does not originate with small headwater streams, but instead receives the bulk of its water from the Great Lakes (Hudon et al., 2017). Therefore, this shift towards more humic-like DOM is expected as the influence of terrestrial sources, likely from runoff and inputs from tributaries, increase downstream, (Frenette et al., 2012; Hudon et al., 2017; Massicotte & Frenette, 2011). Our study also showed a clear divide between the highly autochthonous, protein-like DOM within the main water mass and the terrestrial, humic-like DOM within the northern water mass (Fig. 5), similar as well to what Massicotte (2011) has reported. Distinct lateral bathymetric patterns and inputs of materials (i.e. from the Great Lakes and the Ottawa River) encourage the two water masses to flow parallel to each other, with little mixing occurring (Frenette, 2012). Another study, from the Congo River, found similar separations between water masses, with distinct compositional changes occurring laterally based on source and morphology (Lambert et al., 2016). Thus, while land use is considered a main driver of DOM dynamics within rivers (Parr et al., 2015; Wilson & Xenopoulos, 2008), our study

suggests that morphology, hydrology, and the origin of the water mass also play considerable roles in determining patterns of DOM within the SLR (Fig. 4 and Fig. 5).

Bacterial activity within the SLR further emphasized that DOC processing occurred, despite the relatively stable DOC concentrations. Longitudinally, BP, BR, BCC, and BGE increased steadily downstream within the bulk of the river (i.e. the main water mass; Fig. 6). BP and BGE increased at the highest rates, by 7.5-fold and 3-fold respectively, suggesting that the pool of bioavailable DOC was also increasing downstream (Fig. 6, A and J). This departs from other studies that found a decrease in BGE and BP downstream as bioavailable materials are selectively consumed, leaving a more stable C pool downstream (del Giorgio et al., 2006; Maranger et al., 2005). In our study, inputs of terrestrial DOM and TP downstream within the SLR likely drove these unique trends in bacterial metabolism, suggesting that C processing within SLR is different from that of other large rivers.

Compared to BP and BGE, BR and BCC increased at a relatively slower rate (both by 2-fold) but were able to pinpoint specific hot spots of biogeochemical activity, such as fluvial lakes and the Montreal effluent (Fig. 6, D and G). Within the main water mass, BR and BCC decreased within each fluvial lake, with high rates of BR and BCC at the beginning of each lake and lower rates at the end of the lake. At the input of the Montreal effluent, as well, rates of BR and BCC increased dramatically. These trends, however, were not visible within the rates of BP and BGE. Similarly, other studies of BR have found that it is more sensitive to local conditions compared to BP (del Giorgio et al., 2006; Rodibaugh et al., 2020) and together with our own, suggest that BR is a better tracer of the presence and role of riverine units in terms of C processing within rivers.

Laterally, rates of BP, BR, BCC, and BGE were higher on average in the northern water mass compared to the main water mass (Fig. 6), likely driven by increased inputs of terrestrial DOM and nutrients coming from the northern shore of the river (Fig. 4). This difference in DOM composition laterally also explains the trends in bacterial metabolic capacities between the water masses. As seen in Figure 8A, there was a clear division between the northern and main water masses based on bacterial metabolic capacities during baseline conditions, suggesting that the bacterial communities shifted in response to the substrate availability. Other studies have found similar trends, with bacterial community structure adapting in order to maximize their capacity to consume the carbon present (Comte & Giorgio, 2010; Findlay et al., 1998; Sinsabaugh & Shah, 2010).

The mixed water mass showed unique trends of bacterial activity compared to the other two water masses (Fig. 6). Extremely high rates of BP, BR, BCC, and BGE followed by a dramatic decrease in activity within this water mass suggests that the Montreal effluent may represent a biogeochemical hotspot of C processing (Fig. 6, C, F, I, L). While DOC concentrations and DOM composition did not contain different trends within the mixed water mass compared to the other water masses (Fig. 3 and 4), phosphorus and nitrogen concentrations were an order of magnitude higher at the start of the mixed water mass compared to the rest of the river (Fig. S1). This suggests that an increase in nutrients may drive the trends in bacterial metabolism within the mixed water mass. Sewage inputs and overflows into other large rivers have led to spikes in BP and metabolic functioning, driven by increasing in bioavailable DOM and nutrients (Berger et al., 1995; Velimirov et al., 2011). While only making up a small portion of the river in terms of discharge, the impacts of this plume were strong enough to also cause shifts in bacterial metabolism in

the main and northern water masses (Fig. 6). This hotspot of biogeochemistry outside of Montreal did not extend down the river, but quickly stabilized within 50 km, suggesting that consumption of nutrients and/or dilution from other water masses played an important role in managing this plume.

Environmental and Hydrological Drivers of Bacterial Metabolism

Within the main and northern water masses, we found that terrestrial inputs, likely from tributaries and runoff, are the main drivers of bacterial metabolism along the SLR river. As revealed in Figure 7, BR, BP, BCC, and BGE were strongly correlated with DOC, the three humic-like components of DOM indicative of a terrestrial origin, aromaticity (SUVA), and HIX. While terrestrial DOM was historically considered relatively unavailable to bacteria (Kritzberg et al., 2004; Lennon & Cottingham, 2008), recent studies have proposed that, within the larger pool of complex terrestrial materials, there is a smaller pool of terrestrial carbon that is highly labile, which may, alone, strongly stimulate bacterial metabolism (Berggren & del Giorgio, 2015; Fasching et al., 2014; Guillemette et al., 2016). However, our results also suggest that nutrient levels, phosphorus especially, may both stimulate BP and BGE (Guillemette et al., 2013; Jansson et al., 2006; Smith & Prairie, 2004) and promote bacterial metabolism of complex carbon structures (Cimbleris & Kalff, 1998; Hitchcock & Mitrovic, 2015), suggesting that TP could help bacteria utilize the complex terrestrial materials entering the river. Therefore, large inputs of nutrients, from urban and agricultural areas, could stimulate internal production and change the fate of C within rivers.

Hydrology also came forward as a key variable influencing DOM dynamics and bacterial metabolic activity. With significantly higher concentrations of both DOC and phosphorus in the main and northern water masses during high flow conditions (Table S2), higher rates of BP, BR, and BCC would be expected (Cimbleris & Kalff, 1998; Farjalla et al., 2009; Maranger et al., 2005; Smith & Prairie, 2004). Instead, BP, BR, BGE, and BCC were all significantly lower during high flows within the main water mass and BP was significantly lower in the northern water mass, suggesting that less bacterial processing occurred during high flow conditions (Table S2). Weaker correlations between DOM composition, nutrients, and bacterial activity during high flows (Fig. 8, D) further suggests an uncoupling between bacterial metabolism and DOM and nutrient dynamics during these conditions. It is likely that high velocities within the river prevented bacteria from interacting with the DOM present in the water. Other fast-moving systems have shown similar trends, with DOM moving rapidly through the system, restricting bacterial metabolism (del Giorgio & Pace, 2008; Kothawala et al., 2015; Massicotte & Frenette, 2013). An overarching reduction of bacterial processing along with an increase in DOC and nutrients implies that, during high flow conditions, the SLR could act more like a pipe, transporting terrestrial materials from the land to the ocean, with less biological processing occurring (Raymond et al., 2016). During baseline conditions, however, bacteria have more time to interact and breakdown the complex, terrestrial materials before they reach the ocean.

During high flow conditions, we also found unique lateral trends. Unlike during baseline conditions, there was no convergence downstream in DOM composition or nutrients in between the main and northern water masses, suggesting that there was limited

lateral mixing occurring during high flow conditions (Fig. 5). This lateral separation was also shown in the bacterial metabolic capacities, with clear bacterial carbon substrate preferences between the water masses (Fig. 8). Together, this suggests that, during high flow conditions, the northern and main water masses act as two distinct rivers, running side by side, with their own composition and functioning.

The Pipe and Reactor of the St. Lawrence River

To further examine the disconnect between the pipe and reactor function within the St. Lawrence River, we calculated the carbon removal by bacteria within the river in relation to the DOC loss downstream during baseline and high flow conditions. This was done by calculating the total BCC and delta DOC concentrations from the start to the end of the SLR within the main water mass, based on an average water flow velocity of 0.60 m s^{-1} (baseline) and 1.25 m s^{-1} (high flows) (Foubert, 2017; Massicotte, 2012); given this velocity, we estimated a water residence time of ~ 7.9 days (baseline) and ~ 3.8 days (high flow) to cover the entire stretch of the freshwater SLR. From there, we calculated the average percentage of DOC consumed by bacteria within the river (total DOC consumption estimated from BCC divided by initial concentration of DOC) and compared it to the actual change in DOC along the stretch of the river.

During baseline conditions, we found that BCC accounted for 20% to 25% of carbon removal within the river. Yet, as we mentioned previously, the DOC concentration did not decrease or increase downstream during baseline conditions, and instead varied minimally throughout the river, showing little evidence of the large amount of bacterial processing. Therefore, while additional inputs of DOC are necessary to sustain an increase

in BCC during baseline conditions, this pool of DOC does not impact the broad DOC dynamics within the river, further stressing the importance of measuring bacterial metabolism, not just DOC concentrations, when assessing C processing within river systems. During high flows conditions, however, BCC accounted for ~ 10% of the carbon removal within the river. The increase in DOC downstream during higher flow conditions was enough to subsidize this amount of BCC, suggesting that a portion of the DOC entering the river flows downstream, with less processing occurring. Because water residence time has a significant influence on BCC, a more refined model of water velocity throughout the river and during multiple hydrological conditions would provide useful insight into the processing capabilities of the SLR.

The Need for a New River Framework

Unlike other commonly studied large rivers, the SLR begins with inputs from an immense, highly processed lake, becoming gradually more enriched in DOM and nutrients from the terrestrial environment downstream (Fig. 5). We have shown that, as this shift occurs, bacterial metabolism follows suit, with an increase in BP, BR, BCC, and BGE downstream (Fig. 6). Commonly accepted frameworks, such as the River Continuum Concept and the River as a Chemostat Concept, have predicted inverse trends, with DOM composition shifting from a terrestrial DOM pool upstream to an autochthonously dominated DOM pool downstream (Creed et al., 2015, Sedell et al., 1989; Vannote et al., 1980). Within these more frequently studied rivers, there is often a decrease in BP and BGE downstream as bioavailable DOM is selectively utilized (del Giorgio et al., 2006; Lennon & Pfaff, 2005; Rodibaugh et al., 2020). Because the SLR does not follow this

upstream-downstream pattern, we propose that an adjusted framework is needed to include rivers that originate with low DOM and nutrient headwaters sources. Such rivers include spring-fed rivers that originate at ground-water outlets (Duarte et al., 2010), glacier fed rivers that originate with glacial meltwater streams and headwaters (Aiken et al., 2014; Füreder et al., 2001), and other rivers originating at large lakes, such as the McKenzie River in the Northwest Territories. This framework would predict 1) an increase in terrestrial DOM and nutrients downstream as the river transitions from a highly terrestrially-disconnected headwater source to a river channel strongly connected to the terrestrial environment and 2) an increase in BCC, including BR and BP, as well as an increase in BGE as the pool of labile DOM and nutrients accumulate downstream. Adjusting common frameworks to include these types of rivers would help to predict DOM dynamics, bacterial metabolism, and overall C processing within a variety of river systems.

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Supplementary Figures:

Figures

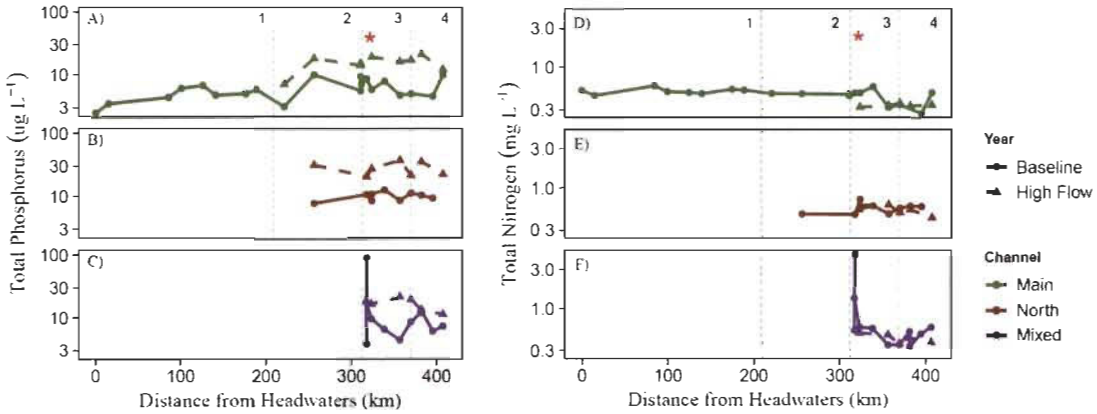

Fig. S1 Total Phosphorus (left) and Total Nitrogen (right) concentrations from Lake Ontario to Trois-Rivieres in the main water mass (top panel), the northern water mass (middle panel), and the mixed water mass, containing the plume of Montreal (bottom panel) in the SLR. Each point represents a sample point, with the solid line representing baseline conditions and the dashed line representing high flow conditions. The red star indicates the input of the Montreal Effluent into the river.

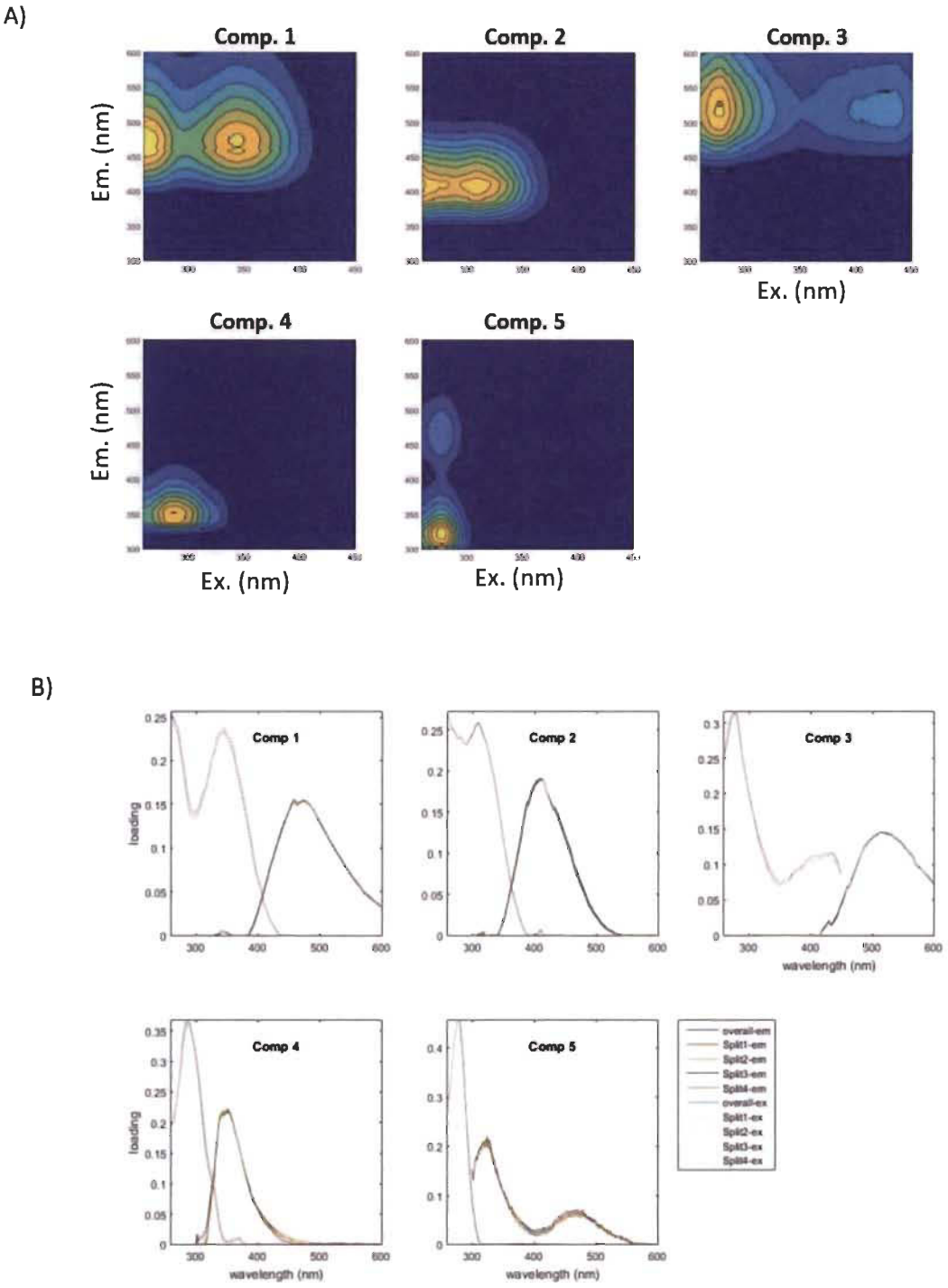

Fig. S2 Excitation and emission spectra from the 5 components extracted from the PARAFAC model. A) The loadings of the fluorescence components identified; B) The split half validation plots from the PARAFAC model. Component 1, 2, and 3 are characterized as humic-like peaks, whereas Component 4 and 5 are protein-like peaks.

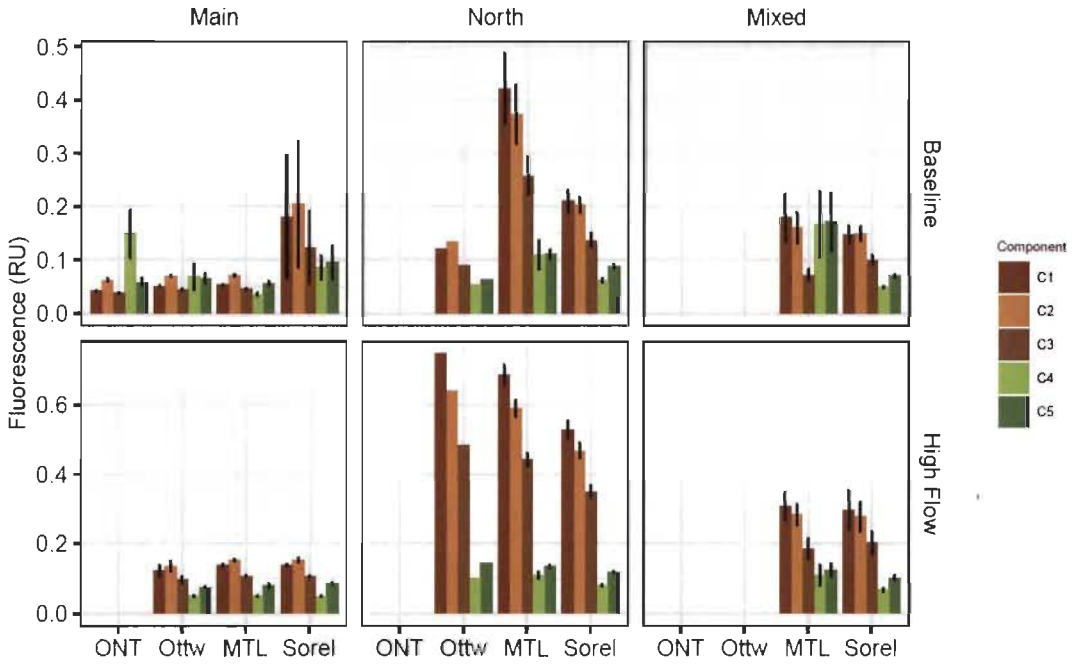

Fig. S3 Bar charts of the fluorescence (R.U.) of each PARAFAC component with standard error bars within the four sections of the river. The data is split by water mass (main, northern, and mixed) and by hydrological conditions (baseline and high flows).

Tables

Table S1: The PARAFAC components from the fluorescence and absorbance analysis. Five components were extracted from the analysis. Component 1, 2, and 3 are characterized as humic-like peaks, whereas Component 4 and 5 are protein-like peaks.

PARAFAC Components	Peak Type	Excitation and emission wavelengths (nm)	Peak Name	Potential Source**	Definition**
C1	UVC Humic-like	ex 345 em 474	A, C	T	High-molecular-weight humic, widespread, but highest in wetlands and forested environments
C2	UVA Humic-like	ex 310 em 412	M	T, A, M	Low molecular weight, common in marine environments associated with biological activity but can be found in wastewater, wetland, and agricultural environments
C3	UVC Humic-like	ex 275, 435 em 514	A, C	T	High molecular weight and aromatic humic, widespread, but highest in wetlands and forested environments
C4	Tryptophane - like	ex 285 em 352	T	T, A, M	Amino acids, free or bound in proteins, fluorescence resembles free tryptophan, may indicate intact proteins or less degraded peptide material
C5	Tyrosine - like	ex 280 em 324	B	T, A, M	Amino acids, free or bound in proteins, fluorescence resembles free tyrosine, may indicate more degraded peptide material

em, emission; ex, excitation
T, terrestrial plant or soil organic matter; A, autochthonous production; M, microbial processing
** (Fellman et al. 2010)

Table S2: Differences (in %) for DOC, BP, BR, BCC, BGE, TN, and TP between the two hydrological conditions (2018 and 2017), including the significance thresholds. Positive values = 2018 is higher; negative values = 2017 is higher.

	%DOC	%BP	%BR	%BCC	%BGE	%TN	%TP
Main	-24*	62***	21*	33**	43***	5.8	-163***
North	-74**	43*	-33	-5	41**	6.5	-214***
Mixed	-50**	19	-15	-4	28	3.5	-176

Significance thresholds: *<0.05, **<0.01, ***<0.001

Chapter 4

Conclusions and Recommendations

4. 1 **Summary of Findings**

In their study of river ecosystem processes, von Schiller et al. (2017) argue that, despite being critical for management and conservation, ecosystem functioning of large rivers is understudied and rarely applied beyond the scientific field. Instead, many scientists have attempted to conceptualize rivers, without conducting a thorough investigation of their processing capabilities (Creed et al., 2015; Sedell et al., 1989; Vannote et al., 1980). Our study represented an important step towards understanding the functioning and significance of rivers globally by providing an in-depth investigation of bacterial processing and of drivers of bacterial metabolism within the SLR.

Unlike many large rivers, the SLR begins at a large lake (Lake Ontario) and contains headwater inputs that are heavily depleted in DOM and nutrients. As it flows downstream, the SLR changes in depth and width as it weaves through disconnected locks, shallow lakes, complex islands, and past urban and agricultural lands. We found that this unique configuration of the SLR had a significant influence on its functioning. As the SLR became more connected to the terrestrial environment downstream, driven by increased runoff and tributary inputs, the composition of DOM became more terrestrial and aromatic and the concentration of phosphorus increased. In response, bacterial metabolism increased downstream, the reverse of what is commonly found in large rivers. Despite the changes in DOM, nutrients, and bacterial metabolism, DOC concentrations remained stable, suggesting an uncoupling between the pipe and reactor capacities of the SLR.

Along its flow path, bacterial metabolic activity identified biogeochemical hotspots, including Montreal and the fluvial lakes. Hydrology also played a significant role, with more bacterial processing occurring during baseline conditions compared to high flow conditions. During high flow conditions, we showed that the northern and main water masses stayed separate, both in terms of DOM composition and bacterial activity, suggesting that high river flows created two side-by-side rivers that functioned differently.

Between the four facets of bacterial metabolism, we found an interesting separation. BR and BCC were able to identify biogeochemical hotspots and local environmental changes (i.e. riverine units). BP and BGE, however, were influenced more strongly by hydrological changes and reflected different processing abilities based on river speeds. Past studies within rivers have focused on one measurement of bacterial metabolism, obtaining the others from it. Our study argues, however, that this could lead to a misrepresentation of the processing capabilities, since each facet gives unique information about the ecosystem. Instead, we suggest that multiple facets of bacterial metabolism are needed to accurately determine the C processing abilities of rivers.

Based on the unique DOM compositional patterns and bacterial activity within the SLR, we observed that not all rivers fit into the commonly accepted framework proposed by Vannote et al. (1980) and adapted by many others (Bernhardt et al., 2017; Creed et al.,

2015; McClain et al., 2003; Raymond et al., 2016; Sedell et al., 1989). We propose that this framework should be adjusted to include rivers that do not originate with headwater streams. This modified framework is needed to predict the processing capabilities of a variety of river types and should explicitly address the dynamics of DOM composition and bacterial metabolism longitudinally and laterally. Our findings create a starting point for this framework, showing that rivers with DOM and nutrient depleted headwaters contain 1) an upstream to downstream shift in DOM composition and nutrients driven by an increase in terrestrial inputs and 2) an increase in BR, BP, BCC, and BGE as the river becomes more enriched in labile DOM and nutrients downstream.

4. 2 **Areas of Improvement**

As they say, hindsight is 20/20. After reflecting on my two-year study, there are limitations of the methods used that need to be addressed. These factors constrain our findings, and should be considered when interpreting our results and for future studies:

1. **Limited Sampling**: As an initial study of bacterial metabolism within the SLR, our field campaigns were limited to one, two-week period in the summer of 2017 and 2018. This allowed us to perform a more thorough investigation within the river and create a base on which to build off. While our sampling route along the river was comprehensive, our results only provide a snapshot of the functioning within the SLR during summer conditions. Additional sampling campaigns during different seasons would allow us to expand our understanding of the overall functioning of the SLR throughout the year.
2. **Filtered vs unfiltered water:** For our analysis of bacterial metabolic activity, we used GF/D filtered water for every experiment. By using this filter type, we were able to maintain the bacterial community structure, while removing the other organism that could impact our measurements of bacterial metabolism. However, by filtering our samples, we risked removing a portion of bacteria that are attached to particles (del Giorgio et al., 2006). With that in mind, our measurements of bacterial metabolism

only include free-floating bacteria and could slightly under-estimate overall bacterial activity.

3. **Sensitivity of Method**: For most of the sites we sampled in this study, our procedure and instrument sensitive were enough to differentiate clear patterns of DOM composition and bacterial metabolism within the river. For BR, specifically, our procedure for tracking bacterial respiration, collecting data at 6 to 12-hour intervals for multiple days, was precise enough to pinpoint many local variations within the river. Some sites, however, mostly located downstream of the Montreal effluent, had incredibly high rates of BR, with much of the carbon being consumed within one day. At these sites, continuous measurements of BR would be needed to track this rapid consumption of carbon.

4. 3 Future Direction

As we continue to conduct research along the SLR, there is a plethora of topics that would be worth exploring in future studies. Each would help us expand our understanding of large river systems and would provide additional support for creating beneficial management and initiatives. We have identified a few possible directions for future research. They are as follows:

1. ***Gathering more data from 0 km to 450 km:*** The addition of sample sites within the upper SLR in 2018 provided crucial information regarding the change in river function longitudinally within the SLR. Because its headwaters are so unique, gathering data from this section of the river provides a baseline in which to compare downstream activity to. Much of the research conducted on the SLR has been restricted to each province or country, and few studies have crossed borders to follow the true path of the river (Hudon et al., 2017; Massicotte & Frenette, 2011). But, in order to establish a deep understanding of the functioning of the entire SLR, we must conduct research from the headwaters to the base of the river. Afterall, the river does not care about human boundaries.

2. ***Seasonality***: During the year, the SLR is constantly changing, as the atmospheric temperatures transitioning from 40 C° in the summer to – 40 C° in the winter. While both of our campaigns were in July, sampling throughout the year is necessary to conduct a more thorough examination of the river. Though it would be difficult, sampling throughout a gradient of temperatures and seasons would provide an extensive data set and help to build a more accurate model of material processing and transport within the SLR. Also, targeting extreme hydrological events, such as spring freshets and inputs from fall storms, would allow us to further develop our predictions of how DOM composition and bacterial metabolism change in response to changes in flows. These data could be incorporated into climate change models, that currently predict higher fluxed of DOM into rivers, due to increased runoff and land use changes.

3. ***Targeting Land Use Changes***: Fertile land along the shores of the SLR have historically been prime areas for agriculture. Tributaries with strong connection to these degraded ecosystems deposit large amounts of sediments, DOM, and nutrients into the river. A study connecting watershed dynamics to SLR functioning could identify anthropogenic drivers of bacterial metabolism within the river. This information is necessary for managing the influence of agriculture on water quality in the region.

4. ***A closer examination of fluvial lakes and islands:*** Our study showed that fluvial lakes and islands are areas of increased DOC processing (Fig. 3 and Fig. 6). In 2018, we conducted a brief sampling campaign throughout the Sorel islands and LSP (unpublished data). The goal of this study was to explore the DOC processing and DOM compositional changes throughout the complex island channels and within the expansive lake. We found that each channel within the Sorel islands had a unique composition of DOM as well as unique rates of bacterial respiration. Within LSP, bacterial respiration pinpointed additional water masses within the lake, each of which were small but contained unique DOM composition and bacterial activity. This brief

study, and well as our study here, suggest together that a more thorough investigation of these riverine units is needed in order to understand the processing capacity of the pool of DOM with the SLR.

5. ***Improved estimates of the flow speeds of the SLR:*** As we saw in our study, flow rates are a driver of bacterial activity within the SLR. Because of the complexities within the river, it is hard to calculate the largescale processing abilities of the river. With more exact estimates of flow rates, we could model bacterial processing within the entire SLR and predict downstream transport of materials.

By targeting these topics in the future, we will have a better understanding of the functioning of the SLR, from its headwaters at Lake Ontario down to the St. Lawrence Estuary.

4. 4 Implications for River Management

Ongoing initiatives throughout the SLR watershed, such as Plan d'action Saint-Laurent (i.e. St. Lawrence Action Plan), the World Wildlife Fund-Canada, and the River Institute at Cornwall, are currently collaborating with researchers, politicians, and community members to improve the health of the SLR. In their 2016-2021 project goals, Plan d'action Saint-Laurent (i.e. St. Lawrence Action Plan) identified "Improving Water Quality" as one of the three main areas of focus, highlighting a need to better understand the transport of organic materials into the St. Lawrence Estuary (Plan d'action Saint-Laurent, 2017). WWF-Canada, as well, produced an assessment of the SLR watershed, identifying pollution and alterations of flow as two main threats to the health of the river (WWF-Canada, 2015). Both organizations emphasized a need for additional data surrounding water quality within the SLR in order to improve local conditions and river management initiatives. Our study provided important information about river functioning, drivers of DOM processing, and the effects of riverine units and hydrological changes that should be incorporated into these river management plans. Given their role

at the base of food webs and as the largest processor of DOM within the SLR (Maisonneuve, unpublished data), bacteria could be used as an effective bioindicator of changes in river processing due to land use and riverscape changes. By collaborating with these organizations and others, we can ensure that we not only expand our knowledge within the scientific field, but that we also apply that knowledge for meaningful environmental restoration.

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