DISSOLVED ORGANIC PHOSPHORUS AND DISSOLVED ORGANIC MATTER IN LAKE ERIE AND ITS TRIBUTARIES

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Abstract

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Phosphorus is the growth-limiting nutrient in freshwater environments. Dissolved organic phosphorus (DOP) refers to phosphorus within dissolved organic matter (DOM). Much of DOP is bioavailable, but it is poorly understood due to its complexity. This thesis explores the export DOP to Lake Erie from its tributaries, by investigating its speciation and concentration seasonally through weekly sampling of two tributaries and spatially across a river to lake transect. The rivermouth was a site of rapid transition, with lower concentrations of DOP in the lake than in the river and a greater proportion of P as DOP in the lake. Phosphomonoesters and aromatic DOM were coupled in the medium-sized Grand River, but not in the Upper Great Lakes-influenced Detroit River. Phosphodiesters and highly processed DOM were coupled in the Detroit River, but only during periods of low terrestrial inputs. Finally, we found that DOP is a large contributor to tributary phosphorus exports.

Keywords: Dissolved organic phosphorus; enzymatic hydrolysis; dissolved organic matter; parallel factor analysis modelling; Lake Erie; rivermouths; riverine export; large lakes; eutrophication.

Preface

This thesis has been prepared in manuscript format, with chapters 2 and 3 prepared for publication and chapters 1 and 4 (general introduction and conclusion) providing context for the research. As a result, some concepts are repeated in more than one chapter. The plural pronoun “we” has been used in this thesis to acknowledge the contributions of co-authors and collaborators to this work.

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Chapter 1. General Introduction

Phosphorus

Phosphorus (P) is an essential macronutrient required by all living things and is the growth-limiting nutrient in most freshwater systems (Schindler, 1974). When water bodies are enriched with P through natural sources or human activities, primary production is stimulated, and algal blooms can form. Blooms have extensive and often harmful effects on water bodies, including increased turbidity, reduced light penetration, unappealing odour and taste, algal toxin production, anoxia and fish kills.

Elemental P is not found in nature; instead, P mainly exists as phosphates or in organic matter. Orthophosphate is the only form of P that can be used directly by organisms for growth; however, P is present in natural waters in a large variety of forms and is typically measured in a number of operationally-defined fractions. Total P (TP) is commonly used for P monitoring programs and load targets (e.g., Dolan & McGunagle, 2005; Grand River Conservation Authority, 2020) as it can be measured fairly easily, but provides no information on bioavailability (Baker et al., 2014). Soluble reactive P (SRP) is also commonly measured and approximately corresponds with orthophosphate (Murphy & Riley, 1962); this fraction is highly reactive and often present in small concentrations and is therefore difficult to measure accurately. In addition, many other forms of phosphorus are indirectly bioavailable so measuring only SRP underestimates the amount of P available for growth (McKelvie, 2005).

One form of P that is still poorly understood is dissolved organic P (DOP). DOP refers to phosphorus-containing organic compounds that can pass through a filter of a specified pore size (typically 0.2 – 0.5 μm), including nucleic acids (DNA and RNA), other nucleotides, inositol phosphates, phospholipids and phosphonates (Baldwin, 2013). DOP can constitute a significant proportion of the TP pool in freshwater environments (e.g., Mohr et al., 2015; Monbet et al., 2009; Selig et al., 2002) and has been recorded as the dominant form of P (62% of TP) in the Western basin of Lake Erie in early summer (Bridgeman et al., 2012).

DOP encompasses a diverse set of compounds of differing origins and properties and it is often grouped into functional classes such as phosphomonoesters (MP), phosphodiesters (DP) and phosphonates (Bell et al., 2020). Our work focuses on MP and DP, two groups of bioavailable DOP compounds that are abundant in aquatic environments (Bell et al., 2020; Read et al., 2014). MP and DP also contain a variety of compounds from different sources, but some generalizations have been made about each class. For example, in freshwater systems dissolved DNA (phosphodiesters) comes primarily from aquatic bacteria (Ishii et al., 1998)(Riemann et al., 2009) and algae (Siuda et al., 1998; Titelman et al., 2008), while glucose phosphates (phosphomonoesters) are produced by plants (Turner et al., 2005). DP is believed to be more persistent in the environment than MP (Turner, McKelvie, et al., 2002), but the diversity of compounds in each class means that this is not always the case (Bell et al., 2020).

Phytoplankton and heterotrophic bacteria are capable of using DOP as a source of P, through the production of hydrolytic enzymes that release orthophosphate from DOP (e.g., Ilikchyan et al., 2009; W. Lin et al., 2018; X. Lin et al., 2011; Siuda & Chróst, 2001; Vahtera et al., 2007). The influence of DOP goes beyond providing P to stimulate productivity, however. For example, DOP may play a role in changing algal community composition. Algal species differ in their ability to use DOP for growth (Donald et al., 1997; Shi et al., 2011); this may give some species a competitive advantage when orthophosphate is scarce but DOP is abundant. The dominance of *Microcystis aeruginosa* in eutrophic waters (Shi et al., 2011) and the spread of invasive *Cylindrospermopsis raciborskii* (F. Bai et al., 2014) may be linked to the abilities of these species to effectively use DOP as a P source. DOP can act as source of C as well as P; carbon-rich DOP (e.g., nucleic acid) stimulates bacterial growth even in the presence of abundant orthophosphate (Siuda & Chróst, 2001).

The complexity of DOP means that the definition and classification of DOP is highly varied in the literature, depending on analytical methods and study design. The simplest way to measure DOP is to subtract SRP from total dissolved P (TDP), as measured with the molybdenum blue colourimetric assay with and without digestion (e.g., Bridgeman et al., 2012); however, this approach does not provide information on structure, source or bioavailability. The enzymatic hydrolysis method uses substrate-specific phosphatases to release P from different DOP compounds, which can then be measured as orthophosphate (Monbet et al., 2007). Enzymatic hydrolysis can be used to quantify broad functional classes (e.g., phosphomonoesters and phosphodiesters) and bioavailability can be inferred when the enzymes used are produced by aquatic microbes and the incubation conditions are environmentally relevant (Monbet et al., 2007). More detailed characterization of DOP can be done using 31-phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR; Baldwin, 2013), however the pre-concentration required by this method can alter DOP, leading to over- or underestimation of different compounds (Turner & Newman, 2005).

While MP and DP are used as classes in both enzymatic hydrolysis and ^{31}P NMR studies, the compounds included in each class vary by method. For example, MP as determined by enzymatic hydrolysis includes inorganic pyrophosphates (Monbet et al., 2007; Table 1.1), but excludes monoester inositol phosphates that can make up a substantial proportion of MP as measured by ^{31}P NMR (X. Bai et al., 2015; Table 1.1). This limitation of enzymatic hydrolysis does not negate its usefulness as a technique for measuring bioavailable DOP, however, as the compounds it can measure (e.g., ATP, DNA) tend to be highly labile, while compounds it cannot measure tend to be refractory (e.g., phytic acid; Li & Brett, 2013) or in low abundance in the dissolved fraction (e.g., phospholipids; Baldwin, 2013). The studies contained herein refer to MP and DP as determined using enzymatic hydrolysis.

Table 1.1. Examples of naturally-occurring phosphorus-containing compounds and their susceptibility to hydrolysis by alkaline phosphatase (APase, equivalent to MP in this thesis) and phosphodiesterase I (PDase, equivalent to DP) according to previous studies using the enzymatic hydrolysis method.

	APase (MP)	PDase (DP)	Not hydrolyzed	Comments
Pyrophosphates	1, 2, 3			Inorganic polyphosphate found in soils and sediments.
Phosphonates			1, 3, 4	Organophosphorus compounds with C-P bond.
Sugar phosphates	1, 2, 3, 4, 5			Monoesters commonly found in plants (Rende et al., 2019).
Adenosine triphosphate (ATP), Adenosine monophosphate (AMP)	1, 2, 3, 4, 5			Ubiquitous, highly labile monoesters involved in biochemical energy transfer. (Björkman & Karl, 2005)
Glycerol phosphates	4,6			Monoesters found in soil extracts after break down of phospholipids (Turner, Mahieu, et al., 2003). Also contained in bacterial cell walls (Vinogradov et al., 2018).
Inositol hexakisphosphates (IP_6)			1	Phosphomonoesters originating from plants and microbes (Giles et al., 2011; Turner, Papházy, et al., 2002).
Phytic acid			2, 3, 4	Refractory isomer of IP_6 that is abundant in higher plants and plant seeds.
Deoxyribonucleic acid (DNA)		1, 3, 5		Phosphodiester polynucleotide
Phospholipids			1	Phosphodiesters present in cell membranes

[1] Monbet et al., 2007; [2] He & Honeycutt, 2001; [3] Turner et al., 2002; [4] Annaheim et al., 2013; [5] Suzumura et al., 1998; [6] Hashihama et al., 2013

Dissolved organic matter

DOP is a component of the broader dissolved organic matter (DOM) pool. DOM refers to all organic compounds that can pass through a 0.2 – 0.5 µm pore size filter. DOM is produced during the decay of plant, animal and microbial detritus. It is also released by living aquatic microorganisms (Guillemette & del Giorgio, 2011). Like DOP, DOM consists of a large assortment of compounds with widely varying structural complexity, properties and bioavailability (Wetzel, 2001; Xenopoulos et al., 2021). The influence of DOM on aquatic ecosystems is extensive: it acts as a source of energy for aquatic organisms (Klug, 2005), limits light penetration (Morris et al., 1995), affects the availability of toxic metals (Aiken et al., 2011) and causes drinking water safety concerns (Weishaar et al., 2003). In rivers DOM is transported or transformed and released or stored depending on conditions such as light, hydrological and redox conditions, nutrient availability and microbial activity (Casas-Ruiz et al., 2017; Fovet et al., 2020; Raymond et al., 2016).

The quantity of DOM is measured as dissolved organic carbon (DOC), but the influence of DOM is not only determined by its quantity; the physical and chemical properties of DOM also affect its reactivity and role in the aquatic environment (e.g., Burford et al., 2022; Calderó-Pascual et al., 2022; D’Amario & Xenopoulos, 2015). The characteristics of DOM are often explored using absorbance and fluorescence spectroscopy (Osburn & Bianchi, 2016). The amount of light absorbed and fluoresced by DOM at different wavelengths can be measured and used to calculate indices of certain characteristics like molecular weight (Helms et al., 2008), humic content (Zsolnay et al., 1999), degree of microbial alteration (Parlanti et al., 2000; Wilson & Xenopoulos, 2009), and aromaticity (Weishaar et al., 2003) More advanced models using Parallel Factor Analysis (PARAFAC) can be created to further characterize DOM by decomposing the fluorescence excitation emission matrix (EEM) of a sample into individual fluorescent components (Stedmon et al., 2003). By comparing the fluorescence characteristics of these components to previously-studied types of DOM, information on origin (e.g., terrestrial, microbial) and structure (e.g., humic, protein-like) can be elucidated. Some components can also be linked with more specific factors like agricultural land use, wastewater and human activity (Stedmon & Markager, 2005; Williams et al., 2016).

As DOP is a component of the DOM pool, studying DOM in tandem with DOP can provide insight into DOP sources and dynamics. The bioavailability of DOM and the ratio of DOC to P in water bodies have impacts on heterotrophic respiration and nutrient sequestration, which in turn mediate eutrophication (Stutter et al., 2018). DOM from different sources can vary in DOP content; in a study by Brooker et al., (2018), DOM from manure contained more P and more diverse DOP compounds than DOM from wastewater treatment plant effluent, agricultural field runoff or the Sandusky River. DOM also influences remineralization of DOP by phosphatases by inhibiting them or protecting them from photoinactivation (Janssen & McNeill, 2015).

Lake Erie

The nutrient status of Lake Erie has been closely monitored for decades. The shallowest of the Laurentian Great Lakes, Lake Erie's warm temperature and highly agricultural and urbanized watershed have made it particularly susceptible to anthropogenic eutrophication (Lake Erie LaMP, 2011). By the 1960s, years of excessive P loading resulted in severe eutrophication of Lake Erie. In response, governments drastically reduced P loading through managing point sources (e.g., improvements in wastewater treatment, bans on phosphates in detergents) and particulate P (e.g., tillage practices that minimize erosion). By the 1980s the restoration efforts were considered a great success but the lake's recovery was short-lived. Lake Erie has been experiencing worsening eutrophication since the 1990s and harmful algal blooms are now an annual occurrence. There are a number of factors that may impede management of eutrophication in Lake Erie, in particular non-point sources of bioavailable dissolved P (Baker et al., 2014), climate change (Michalak et al., 2013), conservation tillage (Jarvie et al., 2017) and invasive species like dreissenid mussels (Hecky et al., 2004).

Lake Erie is composed of three distinct basins on a spectrum of trophic status. The Western basin is the warmest and shallowest with an average depth of 7.4 m (Lake Erie LaMP, 2011). It receives over 50% of Lake Erie's TP and SRP loads from the Upper Great Lakes via the Detroit River and from the lake's largest and most heavily-polluted tributaries (e.g., Maumee River; Maccoux et al., 2016). It is the most eutrophic basin and has experienced record-breaking harmful cyanobacterial blooms in recent years, such as a toxic microcystin-producing bloom in 2014 that led to a drinking water ban affecting over 400,000 residents of Toledo, Ohio (Steffen et al., 2017). The Central basin is intermediate in depth and trophic status. It is susceptible to hypoxia and anoxia in the late summer when it is thermally stratified, which can result in internal P loading from sediment (Hawley et al., 2006). The Eastern basin is the deepest and is oligotrophic. Despite this, its shorelines experience nuisance growth of the green algae *Cladophora* due to the effects of dreissenid mussels on nearshore nutrient retention and water clarity.

DOP and DOM along the river-to-lake continuum

Lakes and rivers differ in their hydrological properties, including connectivity with the catchment, direction of flow and residence time (Essington & Carpenter, 2000). These differences are expected to influence cycling of nutrients, including DOP and DOM. Within rivers, headwaters have high hydrological connectivity with their catchments (Freeman et al., 2007), and their DOM is strongly influenced by terrestrial and wetland sources (Creed et al., 2015; Gergel et al., 1999). Moving downstream, in-stream processing and sources of DOM become more significant (Creed et al., 2015). In lakes, the longer water residence time allows for greater loss and conversion of DOM through microbial processing, photodegradation and flocculation (Bertilsson & Tranvik, 2000; Biddanda & Cotner, 2002; Larson et al., 2007; Von Wachenfeldt & Tranvik, 2008). Rivermouths are unique, biogeochemically active transition zones between river and lake systems that can have outsized effects on the DOM and nutrients that are exported to lakes (Larson et al., 2020; Pearce et al., 2023). Rivermouths combine features of both ecosystems, such as the higher sediment and nutrient content of rivers and the increased residence time of lakes (Larson et al., 2013). Chapter 2 examines DOP and DOM across the river-to-lake continuum in Kettle Creek, a Lake Erie central basin tributary. We measured water quality including DOP and DOM at twelve river sites, the river mouth, a ten kilometer transect from nearshore to offshore Lake Erie and an offshore central basin reference site. We found that DOM character and DOC concentration were relatively invariant within Kettle Creek, while MP and DP were more variable, particularly in lower Kettle Creek. Both DOC and DOP were lower in the lake than in the river. DOM character and DP:MP ratios indicate a shift to more processed, microbial forms moving from river to lake.

Export of DOM and DOP from two catchments over three seasons

In temperate regions, seasonal variation in temperature, precipitation and solar irradiation influence water quality through changes in nutrient export from the catchment and biogeochemical processing within soils and waterbodies. The influence of season and hydrological events on DOM is established (e.g., Raymond et al., 2016; Spencer et al., 2008), but less is known about their effect on DOP. Chapter three addresses the connection between seasonal/hydrological factors, DOM and DOP in Lake Erie tributaries over the course of three seasons, and compares a large connecting channel to a medium-sized tributary. We sampled the Detroit and Grand Rivers weekly from March to November of 2015 and measured different fractions of P and C including DOP. We investigated the coupling between DOP form and DOM character over the course of the sampling period using generalized additive models. Using nutrient export models we also estimated the contribution of DOP to P loading and DOC to C loading in these rivers. In the Grand River we observed an association between MP and terrestrial DOM. In the Detroit River we found an association between DP and low molecular weight, highly processed DOM; however, this relationship dissipated during periods of high terrestrial inputs. We also found that DOP is a substantial contributor to P export, particularly in the Detroit River.

Chapter 2. Transitions in Dissolved Organic Phosphorus and Dissolved Organic Carbon Across a Freshwater Estuary Gradient

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Abstract

Despite the significant role that dissolved organic phosphorus (DOP) plays in ecosystem productivity, efforts to characterize inputs of phosphorus (P) into lakes have largely ignored P fractions complexed to dissolved organic matter (DOM). To address this gap, we characterized DOP and DOM along a transect of a Lake Erie tributary (Kettle Creek) from the headwaters to the rivermouth and into the nearshore and offshore central basin. DOM and DOP characteristics impart a chemical fingerprint that is useful for determining source and production in aquatic ecosystems. We analyzed DOM composition and concentration (DOC) in addition to DOP as phosphomonoesters (MP; predominantly terrestrial in origin) and phosphodiesters (DP; microbially-produced), along with other water quality parameters. DOM and DOC within the river were relatively invariant. While there were no consistent trends in riverine MP and DP, an impoundment on the river appeared to act as a sink for some soluble P forms and a potential source of DP. At the rivermouth, we observed a rapid decrease in DOC, DOP, and total P and a shift to more autochthonous-like DOM, though the decrease in DP was weaker. Relative to in-flowing river water, P pools in nearshore and offshore Lake Erie were enriched in DOP, especially DP. DOP accounted for up to 42% of total P in Kettle Creek and up to 92% in Lake Erie's central basin. Our work shows the importance of considering DOP in P management efforts as its dynamics differ from those of other, more commonly measured P forms.

Introduction

The physical, chemical, and biological properties of rivers change as they flow from headwaters to downstream lakes and oceans (e.g., Vannote et al., 1980; Xenopoulos et al., 2017). Cycling of carbon (C) and phosphorus (P) are connected to these river properties, which leads to changes in their concentration and speciation across the river continuum. The processing of C during transport depends on conditions such as solar radiation, water residence time, hydrological and redox conditions, nutrient availability, and microbial activity (e.g., Casas-Ruiz et al., 2017; Maavara et al., 2021). P cycling in transit through lotic systems is equally complex and varies with catchment land cover and geology, hydrology, stream geomorphology, redox chemistry, and the activities of aquatic organisms (Withers & Jarvie, 2008).

Moving beyond the river continuum, the river to lake transition zone (i.e., the rivermouth, also known in the Laurentian Great Lakes as freshwater estuary) can also be an area of changing physico-chemical conditions. While freshwater estuaries lack the strong salinity gradient of marine estuaries, the systems exhibit many similarities such as chemical gradients, tide/seiche regimes, and nutrient export to receiving waterbodies. Changes in nutrient dynamics, alongside dissolved organic carbon (DOC, measured in units of carbon) and dissolved organic matter (DOM) have been documented in freshwater rivermouths (Larson et al., 2014, 2020, 2021; Pearce et al., 2021, 2023). Nutrient and carbon dynamics in Great Lakes nearshore and rivermouth zones are influenced by seiche-driven mixing, outwelling from coastal wetlands, precipitation and wind patterns, alongshore currents, thermal bars, gyres, upwelling/downwelling and storms (Bouchard, 2007; Gächter et al., 1974; He et al., 2006; León et al., 2005; Morrice et al., 2004; Rao & Schwab, 2007). For example, seiche-driven mixing and flow reversals (similar to tides) occur at the mouths of major rivers flowing into the Great Lakes, which produces fluctuating chemical and thermal gradients across the river to lake transition (Bedford, 1992; Larson et al., 2013). Nearshore and rivermouth communities (attached algae, macrophytes, invertebrates) further influence these transitional areas, and in particular, the widespread colonization of the Great Lakes by dreissenid mussels have a profound effect on nearshore water quality, nutrient cycling and inshore-offshore exchange (Hecky et al., 2004). Lastly, riverbanks and estuaries are often valued as places to live and recreate, and as such, experience human-induced pressures (Elliott & Whitfield, 2011; Larson et al., 2013). The effects of rivers extend into receiving waterbodies, which can be seen in the Western basin of Lake Erie where *Microcystis* blooms can be linked to nutrients and seed colonies provided by the Maumee River (Bridgeman et al., 2012).

DOM pools include dissolved organic phosphorus (DOP) and are heterogeneous in nature, composed of various proportions of aromatic and aliphatic carbon compounds that may differentially affect key biogeochemical processes (Xenopoulos et al., 2021). DOP consists of a complex assortment of P-containing organic compounds that can be broadly grouped into several functional classes, including labile phosphomonoesters (MP) and phosphodiesters (DP), each with varied sources and structures. Aquatic MP generally originates from terrestrial sources such as leachate and overland flow (Bourke et al., 2009; McDowell & Koopmans, 2006) and can be hydrolyzed by extracellular alkaline phosphatases, which are produced by a large number of aquatic organisms (Baldwin, 2013). DP is derived largely from microbial sources (Siuda et al., 1998; Siuda

& Chróst, 2000). Dissolved DNA, often the dominant type of DP, can be hydrolyzed by bacterial and algal enzymes (Baldwin, 2013). DP appears to be more persistent than MP in the aquatic environment (Monbet et al., 2009; Pant et al., 2002). As a bioavailable source of both C and P, DOP compounds like MP and DP are believed to play a complex role in macronutrient cycles and the productivity of aquatic systems (Mitchell & Baldwin, 2005).

DOP can provide a significant pool of P that is available, after transformation, to primary producers (Baldwin, 2013). For example, DOP constitutes approximately 56-88% of total P in early summer in Lake Erie's productive Western basin (Bridgeman et al., 2012). When readily-available inorganic P (usually measured as soluble reactive phosphorus, SRP) is depleted, bioavailable DOP becomes a significant fraction of total P in lakes (Thompson & Cotner, 2018), and may become the main source of P sustaining primary production (Frost et al., 2019). Some harmful cyanobacterial such as *Microcystis aeruginosa* are able to access DOP more readily than other phytoplankton; this combined with their ability to store luxury P for future growth gives them an advantage during periods of low inorganic P (Carey et al., 2012; Shi et al., 2011). Despite the abundance and ecological relevance of DOP, it is rarely included in monitoring efforts due to analytical challenges. P monitoring programs typically focus mostly on total P (TP) and occasionally on SRP, both of which are easier to measure using simple colorimetric techniques. Since SRP is rapidly consumed, it is undetectable in most freshwater systems, while TP does not distinguish between bioavailable and recalcitrant P. As such adding DOP measurements to monitoring programs can be helpful to improve our understanding of P dynamics and algal blooms.

Additionally, the relative amounts of DOC and DOP can determine how these elements are processed, retained, and transported along the freshwater continuum (Stutter et al., 2018) but these two elements are very seldom studied together. When bioavailable DOC:P is high, organic C drives heterotrophic microbial activity, allowing P to be processed and sequestered. When bioavailable DOC:P is low, organic C deficiency inhibits microbial growth and allows P to be exported downstream. As such, DOM that is highly enriched with DOP could contribute to eutrophication of receiving waterbodies by modifying nutrient uptake and cycling. The properties of rivers, their catchments and their rivermouths all can affect the supply and chemistry of major nutrients and organic material moving from the watershed to the Great Lakes. Yet as noted earlier, there is a critical gap in our understanding of the relationships between spatial-temporal changes in solutes from headwater to river-lake interface. As DOP consists of organic matter, its concentration and variability across the river to lake continuum may co-vary more with DOM than with other P fractions. Alternatively, its role as a P source may mean that the behaviour of DOP may be distinct from that of DOM or inorganic P. Here, we quantified changes in DOC, DOM and DOP from the headwater to the mouth and along nearshore-offshore transects of a tributary into Lake Erie. We predicted that along these transects, the influence of terrestrial inputs would decrease relative to internal processing, and result in a downstream progression towards less allochthonous DOM and DOP. We predicted a dominance of MP and high molecular weight, aromatic and humic DOM in the headwaters due to a stronger hydrological connection to the land, and a shift in the DOM and DOP pool towards a predominance of DP and microbially-processed and DOM downstream. We anticipated that the lake sites would be dominated by DP with minimal MP, and show both low molecular weight, autochthonous-like DOM and photo-refractory and bio-refractory compounds and possible evidence of a plume of terrestrial DOM and DOP near the rivermouth.

Methods

Site Description and Sample Collection

Kettle Creek (42.6644 N, 81.2136 W) is a 5th order tributary in Ontario, Canada draining a 440 km^2 catchment directly into the northern part of the central basin of Lake Erie. Its catchment land cover is predominantly intensive agriculture and rural (~75%) with a smaller amount of forested area (~12%) and sparse wetlands (Ontario Ministry of Natural Resources, 2007). An artificial reservoir, used for water level management, is located upstream from a small town, St. Thomas, at approximately river kilometer 35 (measured in km from the rivermouth). The reservoir is surrounded by a protected area containing 25 ha of wetlands. Dodd's Creek, the river's largest tributary, joins Kettle Creek just above river kilometer 29. The main branch of the river passes through St. Thomas (population 41,688; river kilometer 32 – 22) and two villages (Belmont and Port Stanley, populations 1,840 and 2,229; river kilometer 65 and 0, respectively). The St. Thomas Water Pollution Control Plant discharges into Kettle Creek at river kilometer 27 and the Belmont sewage lagoon is located at river kilometer 59. The upper Kettle Creek sub-watershed is dominated by till plain and till moraine, while the Dodd's Creek and lower Kettle Creek sub-watersheds are comprised of clay plain and sand plain (Chapman & Putnam, 2007).

Twelve sampling locations were selected along Kettle Creek from river kilometer 0 – 81 (Figure 2.1). Sites were chosen to be approximately evenly spaced and at points where access to the stream was available. Two of the sites (R50 and R29) have streamflow gauges operated by the Water Survey of Canada. Discharge at non-gauged sites was measured whenever possible by the velocity-area method using a SonTek Flow Tracker handheld acoustic Doppler velocimeter (YSI Incorporated). The 12 river sites were sampled in August and October of 2014. In 2015, a single river site, R29, was sampled again in May, August, and October. This site was chosen because it has a Water Survey of Canada gauge and is below the confluence of Upper Kettle Creek and Dodd's Creek but above the expected reach of lake water influence. River and rivermouth samples were collected approximately 10 cm below the surface and near the stream center either in stream or from a bridge using a Van Dorn sampler.

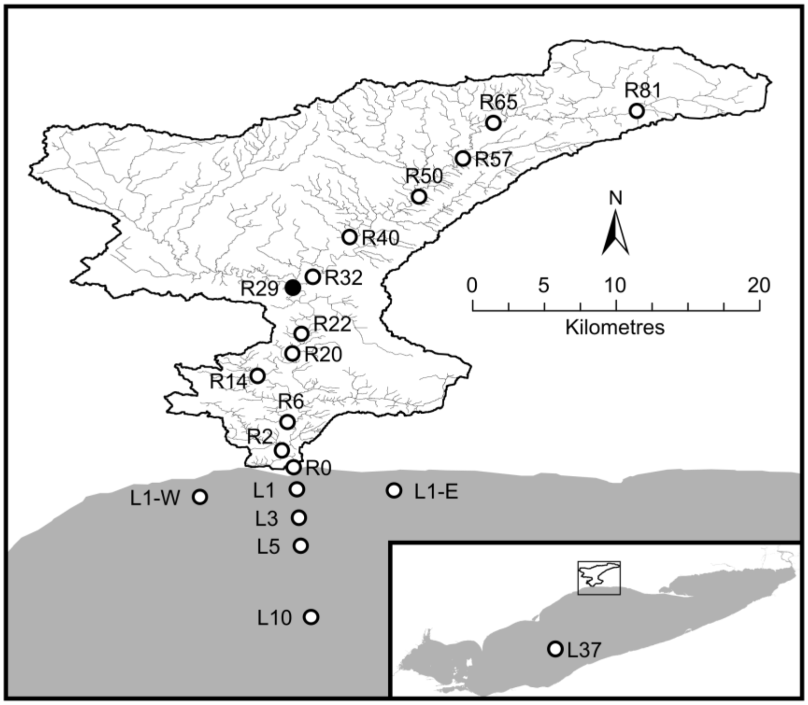

Figure 2.1. Map of Kettle Creek watershed indicating sampling locations (○). Sites are numbered by the distance from the mouth in km, with river sites indicated as R(km) and lake sites indicated as L(km). Lake Erie offshore site L37 is shown on locator map, bottom right. Site R29 (at the confluence of Dodd's Creek and Kettle Creek) is represented by a black point (●).

An offshore site in the central basin approximately 37 km southwest from the rivermouth was sampled in May, August, September and October 2014 and May, August and October 2015. Samples were collected along a transect at the mouth, and at 1, 3, 5, and 10 km from the rivermouth into Lake Erie, and from a site farther offshore in the central basin. In this part of the Lake Erie coast, wind-driven coastal currents are expected to push the Kettle Creek plume to the East (León et al., 2005; Rao & Schwab, 2007). As such, additional sites were sampled 3 km to the East of the 1 km transect site (L1-E) to capture the plume from Kettle Creek and 3 km to the West (L1-W) for comparison. There are no significant tributaries discharging into the Central Basin west of Kettle Creek. Lake samples were collected from 1 meter below the surface.

Whole water was collected at each sampling location in acid-washed HDPE bottles for analysis of total phosphorus (TP). Water for dissolved and particulate nutrients was transported on ice in acid-washed HDPE bottles and was filtered within 12 hours of collection. Whole water was pre-screened through a 60 μm nylon mesh to remove large metazoans (no large algal colonies were visible when water was collected) and filtered onto ashed GF/F filters for chlorophyll *a* (chl *a*) and particulate P (PP), C, and N. Filters were kept on ice until their return to Trent, where the chl *a* filters were stored at −20°C and the other filters were dried overnight at 60°C. For dissolved nutrients, water was filtered sequentially through a pre-rinsed and combusted Whatman GF/F filter and a 0.2 μm pore size Isopore polycarbonate membrane filter (EMD Millipore). Filtered samples were stored at 4°C in acid-washed and ashed amber bottles (DOP and DOM) or in acid-washed HDPE bottles (total dissolved P; TDP).

Sample Processing and Nutrient Analysis

TP, PP and TDP concentrations were measured using the molybdenum blue colorimetric assay (Murphy & Riley, 1962) following persulfate digestion. Absorbance was measured at 885 nm using a Cary 50 spectrophotometer (Agilent Technologies). Chl *a* was extracted from filters for 24 hours in 95% ethanol at 4°C. Chl *a* concentration was measured as fluorescence using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies).

DOP was measured using an enzymatic hydrolysis technique to selectively release P from certain DOP functional classes (Song et al., 2015; Turner et al., 2002). The enzyme preparation for MP consisted of 1 unit/mL alkaline phosphatase (Sigma Aldrich P6774) in 0.1 M Tris-HCl buffer (pH 8.0), while the preparation for MP + DP consisted of alkaline phosphatase and 0.03 units/mL phosphodiesterase (Sigma Aldrich P4506) in 0.1 M Tris-HCl buffer (pH 8.8). Both preparations contained 2 mM $MgCl_2$ as an enzyme activator.

The DOP assay procedure involved adding 0.75 mL of 0.1 M sodium azide (microbial inhibitor) and 0.75 mL of enzyme preparation to 13.5 mL of filtered sample, then incubating in the dark for 10 h at 37°C. Samples containing buffer instead of the enzyme preparation were incubated simultaneously to account for existing phosphate and chemically-hydrolyzed P. After incubation, released phosphate was measured using the molybdate blue colorimetric assay. Two sets of phosphate standards were incubated with either the enzyme preparation or buffer only to account for differences in absorbance caused by protein interference (Shand & Smith, 1997). The P concentration in the buffer only samples was subtracted from the enzyme samples to yield the concentration of enzymatically-hydrolyzed P. The concentration of P released from DP in each sample was obtained by subtracting MP from MP + DP.

DOC Measurements and DOM Characterization

DOC concentration was measured using an Aurora 1030 total organic carbon analyzer (O.I. Analytical) (Wilson and Xenopoulos 2008). DOM was characterized by calculating spectral slope ratio (S_R), specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), absorbance at 350 nm (A_{350}), humification index (HIX), fluorescence index (FI), and freshness index ($\beta:\alpha$) from absorbance and fluorescence data (Table 2.1). DOM absorbance from 800-230 nm was measured using a Cary 50 spectrophotometer. Absorbance scans were corrected for a milliQ blank and turbidity before determining the S_R, $SUVA_{254}$ and A_{350}. Excitation emission matrices (EEMs) were produced using a Cary

Eclipse Fluorometric Spectrophotometer (Agilent Technologies) for excitation wavelengths of 230-500 nm at 5 nm intervals and emission wavelengths of 270-600 nm at 2 nm intervals. The sample and milliQ blank EEMs were first corrected for instrument bias with spectral correction factors provided by the manufacturer. Next the sample EEMs were corrected for primary and secondary inner-filtering effects (absorption by the sample of excitation light before the emission window, or of emitted light before collection; Tucker et al., 1992) using the method from Ohno (2002). The milliQ blank was subtracted and the blank corrected EEMs were Raman normalized (Stedmon et al., 2003). These corrected sample EEMs were used to derive HIX, FI, and β:α (Table 2.1).

Table 2.1. Description of the dissolved organic indices calculated using absorbance and fluorescence spectroscopy data.

Index	**Name**	**Description**	**Reference**
$SUVA_{254}$	Specific UV absorbance at 254 nm	Higher values indicate greater aromaticity	Weishaar et al., 2003
A_{350}	Corrected absorbance at 350 nm	Higher values indicate greater coloured dissolved organic matter content	Moran et al., 2000
S_R	Spectral slope ratio	Inversely correlated with molecular weight; higher values suggest photobleaching	Helms et al., 2008
$\beta:\alpha$	Freshness index	Ratio of two fluorescent components: proportion of recently microbially derived carbon (β) to highly decomposed carbon (α)	Parlanti et al., 2000; Wilson & Xenopoulos, 2009
FI	Fluorescence index	Indicator of DOM source; high values ~1.8 indicate microbial source and low values ~1.2 indicate terrestrial source.	Cory et al., 2010; McKnight et al., 2001
HIX	Humification index	Higher values indicate greater degree of humification.	Ohno, 2002; Zsolnay et al., 1999

Corrected EEMs were also analyzed using the DOMFluorv1_7 toolbox (Stedmon & Bro, 2008) in MATLAB R2007b (Mathworks). The EEMs were fitted to a previously published seven-component parallel factor analysis (PARAFAC) model (Williams et al., 2013, 2016; Williams and Xenopoulos 2023). After fitting the new EEMs to the existing model, graphs of the residuals were evaluated to ensure good model fit. The seven components identified in this model are: C1, ubiquitous humic-like; C2 , terrestrial humic-like and presumed biorefractory; C3, terrestrial humic-like and presumed photorefractory; C4, soil fulvic-like; C5, microbial humic-like; C6, microbial humic-like component correlated with population density; and C7, protein-like (Williams et al., 2010, 2013, 2016; Williams & Xenopoulos, 2023; Yamashita et al., 2010).

Composite Index of DOM Composition

To reduce the DOM composition variables to a composite quality index, we conducted a PCA using the DOM indices and PARAFAC components and extracted PC1 scores. Positive loadings for PC1 represent terrestrial-like DOM (high molecular weight, aromatic, and humic) and negative values represent microbial-derived (low molecular weight, more recently produced, more protein-like; Appendix C: Figure C.1, Figure C.2). Scores on PC1 were separated by river, mouth and lake site types (Appendix C: Figure C.1). For PC2, higher values indicate biorefractory humic-like DOM associated with forest streams and wetlands (terrestrial humic-like C2) and aromatic DOM (soil fulvic-like C4) while lower values indicate photorefractory DOM (terrestrial humic-like C3) and anthropogenic DOM found in wastewater (urban microbial humic-like C6). Within the river sites, PC2 scores were significantly lower in August (mean = −1.25) than in October (mean = 0.41; $P < 0.001$) and were negatively correlated with chl *a* concentration ($r = -0.41$, $P < 0.01$); however, no seasonal difference was seen within the lake sites.

GIS and Statistical Methods

The Ontario Flow Assessment Tool version 3 was used for watershed delineation and to calculate length of the channel (distance from the mouth) at each site. Watershed characterization (land cover, physiography) was performed using ArcMap 10.2.2 with the Southern Ontario Interim Landcover Dataset (Ontario Ministry of Natural Resources, 2007) and data from the Ontario Geological Survey (Chapman & Putnam, 2007).

Analyses were conducted using R statistical software (v4.0.3; R Core Team, 2020) using the *car* (Fox & Weisberg, 2019) and *stats* packages. As some variables were non-normally distributed or right-skewed, data was transformed using logarithmic, square root, or quarter power transformations when necessary to meet assumptions of normality and heteroscedasticity. DOM quality indices were reduced to a single composite index using principal components analysis (PCA) with the function *prcomp* (R Core Team, 2020). The relationships between water quality and location within Kettle Creek and across the entire river to lake transect were evaluated using Pearson correlation coefficients. The river to lake transect was also further divided into five zones (Upper Kettle Creek, R32 – R81; Lower Kettle Creek, R2 – R29; Mouth, R0; Nearshore, L1 – L5; Offshore, L10 – L37). Seasonal differences (August versus October) were tested using paired t-tests. Molar ratios of phosphorus concentrations to DOC concentration and the aromaticity index ($SUVA_{254}$) were used to examine the connection between DOM and DOP. A significance level of $\alpha = 0.05$ was used for all statistical tests.

Results

Phosphorus Fractions and Chl a

We observed different spatial and seasonal patterns for the different P fractions we analyzed, which appear to be related to source. TP, TDP and PP were all higher in the river than in the lake, with intermediate concentrations in the rivermouth (Figure 2.2). In the river samples, PP was higher in August (mean = 80.6 μg P l^{-1}) than in October (mean = 44.3 μg P l^{-1}; $P < 0.001$). No other form of P exhibited significant seasonal differences.

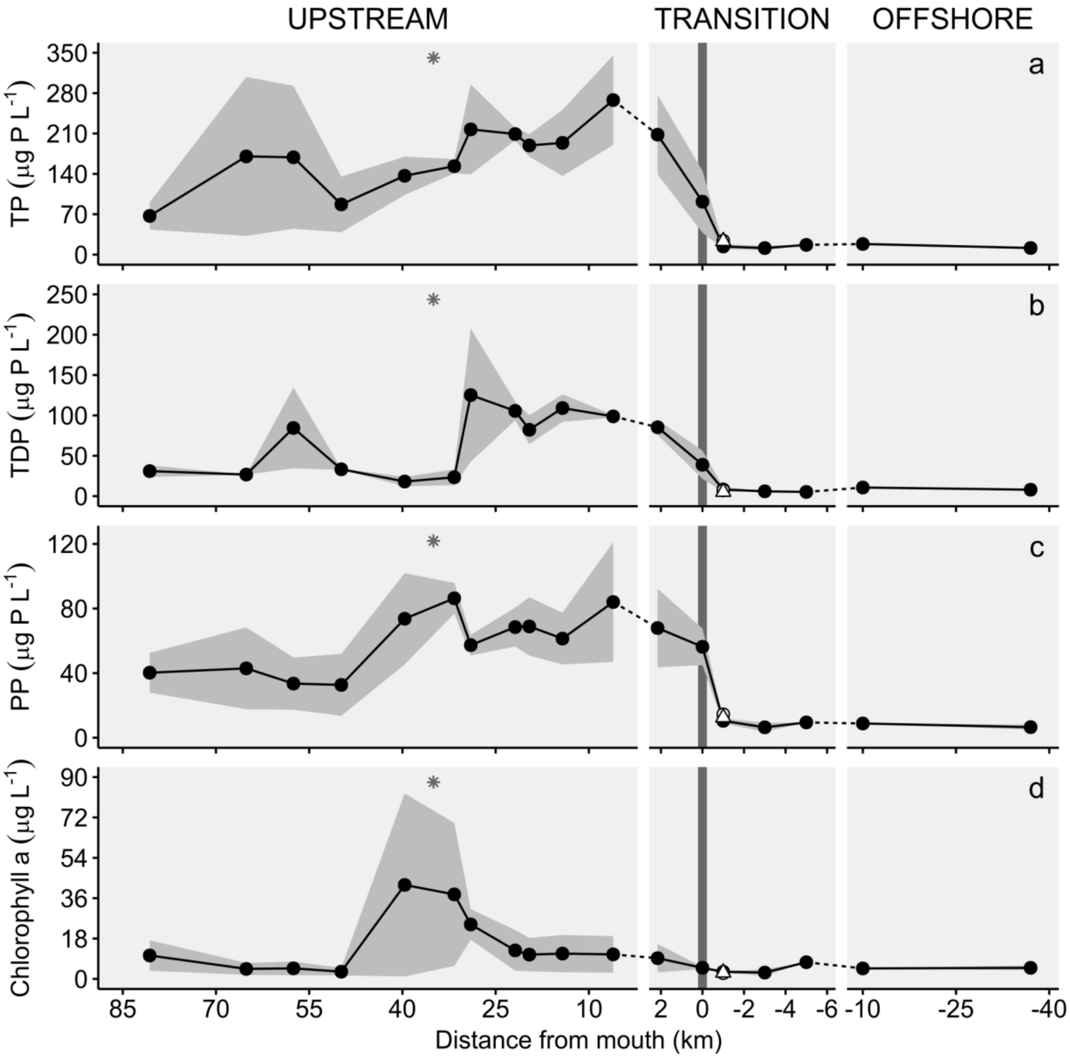

Figure 2.2. Mean total phosphorus concentration (a), mean total dissolved phosphorus concentration (b), mean particulate phosphorus concentration (c) and mean chlorophyll *a* concentration (d) from upstream Kettle Creek (−80 km from mouth) to offshore Lake Erie (30 km from mouth). The transition zone is on an expanded *x*-axis to show rapid changes occurring at the mouth and nearshore. Main sites are represented by black circles, East (within Kettle Creek plume) site by a white circle, and West (outside Kettle Creek plume) site by a white triangle. Rivermouth is marked with a grey bar and location of reservoir is indicated by a grey star. Error ribbon indicates the mean ± standard error; error ribbon is not visible in the offshore panel due to scale and low standard error at the offshore site.

Enzymatically-hydrolyzed DOP (MP + DP) concentrations ranged from 6.1 to 34.0 μg P l^{-1} in the river and from 4.9 to 9.6 μg P l^{-1} in the lake, with the lowest concentration of DOP (4.0 μg P l^{-1}) at the rivermouth. DOP made up 3.9 to ~100% of TDP and 2.4 to 92.0% of TP and comprised a larger proportion of TDP in the lake than in the river. MP concentrations were generally very low at all sites and were lower in the lake than in Kettle Creek (Table 2.2, Figure 2.3). In nearshore Lake Erie, MP was higher at the plume site (L1-E) than the main transect site (L1) and was undetectable at the site west of the Kettle Creek plume (L1-W). DP concentrations ranged from 5.4 to 31.7 μg P l^{-1} in the river and from 4.9 to 8.9 μg P l^{-1} in the lake and were higher than MP concentrations in all samples except at the rivermouth, where DP was very low (Table 2.2). DP experienced a less abrupt decrease between Lower Kettle Creek and the nearshore than other P forms (Figure 2.3).

Table 2.2. *DOM Properties, Concentrations of Phosphorus Fractions and DP:MP Ratios*

	River	**Mouth**	**Lake**
DOC (mg C l^{-1})	6.1 ± 0.2	4.6 ± 0.9	2.2 ± 0.1
HIX (humification index)	0.89 ± 0.01	0.83 ± 0.07	0.69 ± 0.02
SUVA$_{254}$ (aromaticity; L mg^{-1} C m^{-1})	2.54 ± 0.04	2.11 ± 0.09	1.26 ± 0.05
β:α (freshness index; unitless)	0.77 ± 0.01	0.80 ± 0.03	0.92 ± 0.03
FI (fluorescence index)	1.46 ± 0.01	1.58 ± 0.03	1.47 ± 0.11
A$_{350}$ (colored DOM; m^{-1})	3.45 ± 0.13	1.96 ± 0.26	0.36 ± 0.02
S$_{R}$ (spectral slope ratio)	0.89 ± 0.02	0.95 ± 0.10	1.62 ± 0.06
C1 (ubiquitous humic-like; %)	22.2 ± 0.7	21.7 ± 1.3	14.6 ± 1.1
C2 (terrestrial humic-like; %)	22.5 ± 0.9	24.5 ± 0.1	19.0 ± 6.21
C3 (terrestrial humic-like; %)	14.1 ± 0.4	14.4 ± 1.2	18.7 ± 2.7
C4 (soil fulvic-like; %)	5.3 ± 0.2	3.7 ± 1.3	4.7 ± 1.0
C5 (microbial humic-like; %)	8.6 ± 0.3	8.2 ± 0.3	4.1 ± 0.5
C6 (urban microbial humic-like; %)	18.9 ± 1.6	12.6 ± 2.9	11.4 ± 2.1
C7 (protein-like; %)	8.4 ± 0.5	15.0 ± 7.1	27.6 ± 1.9
Fmax/DOC (specific Fmax, RU mg^{-1} l)	0.74 ± 0.03	0.53 ± 0.05	0.18 ± 0.01
TP (µg P l^{-1})	177.3 ± 20.7	91.7 ± 53.7	14.2 ± 1.5
TDP (µg P l^{-1})	74.9 ± 16.2	38.9 ± 17.5	7.6 ± 0.8
PP (µg P l^{-1})	59.5 ± 5.1	56.3 ± 11.4	8.4 ± 1.0
MP (µg P l^{-1})	1.7 ± 0.3	1.9	0.5 ± 0.1
DP (µg P l^{-1})	13.3 ± 1.0	2.2	6.8 ± 0.4
DP:MP	16.8 ± 3.6	0.1	224.3 ± 196.7

Note. Values averaged across all sampling events (± SE) for river (12 Sites), mouth (1 Site) and lake (7 sites). RU = Raman units.

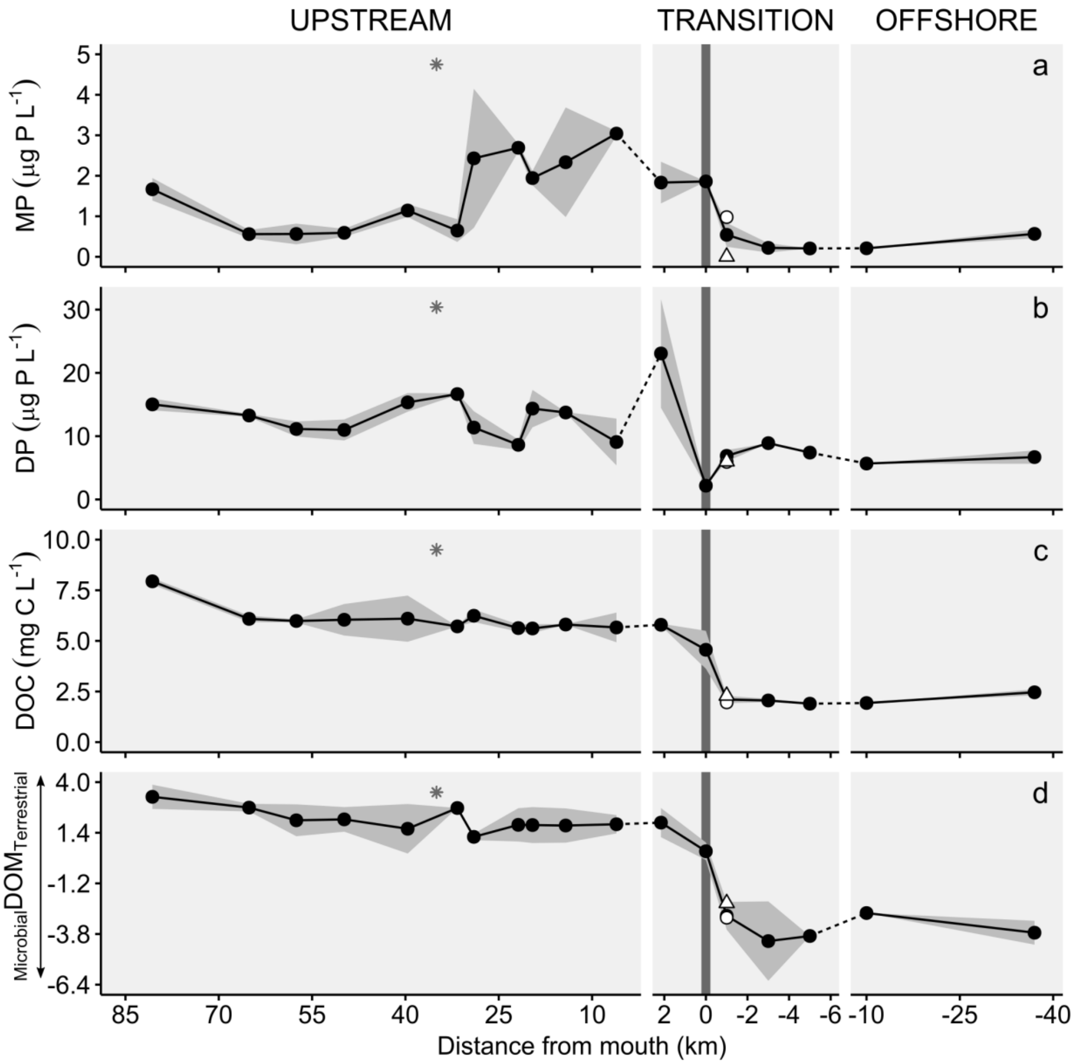

Figure 2.3. Mean phosphomonoester concentration (a), mean phosphodiester concentration (b), mean dissolved organic carbon concentration (c) and composite dissolved organic matter quality index (PC1 object scores) (d) from upstream Kettle Creek (−80 km from mouth) to offshore Lake Erie (30 km from mouth). The transition zone is on an expanded *x*-axis to show rapid changes occurring at the mouth and nearshore. Main sites are represented by black circles, East (within Kettle Creek plume) site by a white circle, and West (outside Kettle Creek plume) site by a white triangle. Rivermouth is marked with a grey bar and location of reservoir is indicated by a grey star. Error ribbon indicates the mean ± standard error.

Within Kettle Creek, MP concentrations increased downstream and were positively correlated with cumulative watershed area ($r = 0.44$, $P < 0.05$). Phosphorus concentrations were generally higher at lower Kettle Creek sites (river kilometer 2 – 29) than in upper sites (river kilometer 32 – 81). TDP in particular experienced a sharp increase at river kilometer 29, just below the confluence of Upper Kettle and Dodd's creeks. This increase was seen to a lesser extent with TP, PP, and MP but not with DP.

Chl *a* concentrations were low from river kilometer 81 (headwaters) to river kilometer 50 (mean = 6.5 μg l^{-1}, SD = 1.0 μg l^{-1}) with the highest concentration seen during an apparent phytoplankton bloom at river kilometer 40 during August 2014 (82.7 μg l^{-1}). Lake chl *a* concentrations were generally low (mean = 4.2 μg l^{-1}, SD = 2.3 μg l^{-1}), with the highest concentration recorded at L37 in August 2015 (11.0 μg l^{-1}). Across all sites, chl *a* was generally correlated with TP (r = 0.49, P < 0.001) and weakly correlated with enzyme-hydrolyzable DOP (r = 0.35, P < 0.05). As with PP, chl *a* at the river sites was significantly higher in August (mean = 22.7 μg l^{-1}) than in October (mean = 3.9 μg l^{-1}; P < 0.001).

DOC and DOM Composition

Consistent with previous studies our results suggest that the predominantly terrestrial signal of river DOM is rapidly lost in the nearshore zone with the mixing of river water into lake water containing less humic, lower molecular weight, more recently-derived and less aromatic DOM (Figure 2.4). Across all sampling events, DOC concentrations ranged from 4.9 to 8.1 mg C l^{-1} in Kettle Creek and from 1.7 to 3.0 mg C l^{-1} in Lake Erie. DOC concentrations were less variable and significantly higher in the river sites than in the lake (Table 2.2, Appendix D: Figure D.1). Similar patterns of higher levels in the river were seen with measures of colored DOM (A_{350}), aromaticity ($SUVA_{254}$) and humification (HIX), whereas spectral slope ratio (S_R) and freshness (β:α) values were lower in the river than in the lake (Table 2.2, Appendix D: Figure D.1)

Specific fluorescence (ΣF_{max}/DOC) was greater in the river than in the lake (Table 2.2). The ubiquitous humic-like PARAFAC component C1 was the most abundant component overall, but DOM composition changed along the river to lake transect. Protein-like C7 was a minor component in Kettle Creek, but was important at Lake Erie sites (Table 2.2, Figure 2.4). The terrestrial humic-like components C2 and C3 were abundant in Lake Erie as well (Figure 2.4).

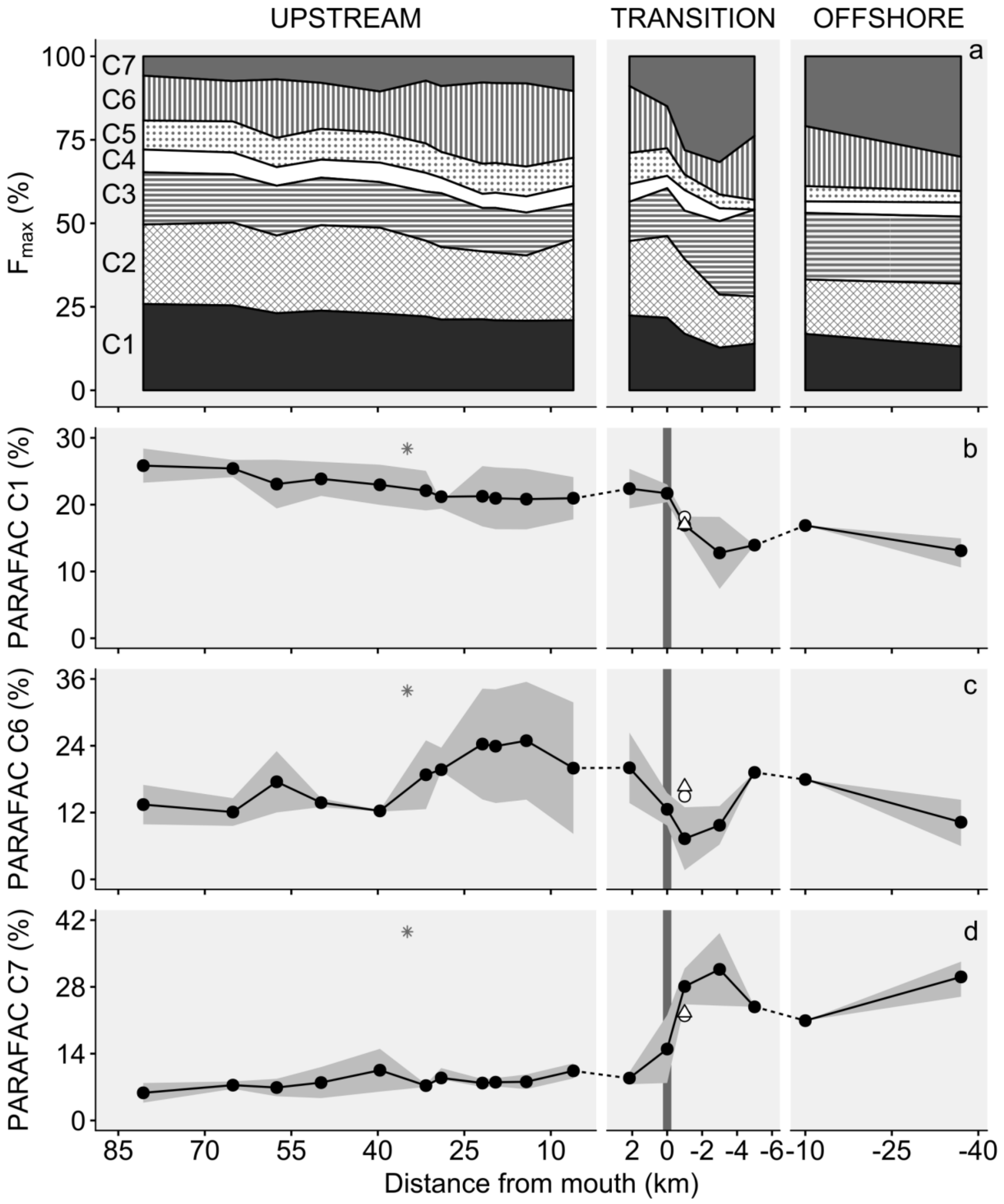

Figure 2.4. Mean percent F_{max} for (a) the seven PARAFAC components (b) C1 (ubiquitous humic-like), (c) C6 (urban microbial humic-like), and (d) C7 (protein-like) from upstream Kettle Creek (−80 km from mouth) to offshore Lake Erie (30 km from mouth). The latter 3 are disparate components that displayed the greatest amount of change in the rivermouth.The transition zone is on an expanded *x*-axis to show rapid changes occurring at the mouth and nearshore. Main sites are represented by black circles, East (within Kettle Creek plume) site by a white circle, and West (outside Kettle Creek plume) site by a white triangle. Rivermouth is marked with a grey bar and location of reservoir is indicated by a grey star. Error ribbon indicates the mean ± standard error.

In addition to the changes seen across the entire river to lake transect, the composition of DOM changed within the river itself. Within Kettle Creek, significant relationships were observed between distance from the mouth and DOC concentration ($r = -0.52$, $P < 0.01$), aromaticity ($SUVA_{254}$; $r = -0.41$, $P < 0.05$), humic content (HIX; $r = -0.43$, $P < 0.05$), and freshness (β:α; $r = 0.64$, $P < 0.001$); however there was no trend in the spectral slope ratio (S_R), an index of molecular weight and photobleaching. While the terrestrial-linked humic-like components C1 and C2 were main contributors throughout the river, the urban microbial humic-like C6 emerged as another important component in lower Kettle Creek (Table 2.2, Figure 2.4).

DOM:P River to Lake Transect

Ratios of DOC:TP were lowest in Lower Kettle Creek, then increased from the rivermouth to offshore Lake Erie (Figure 2.5). A similar trend was seen with ratios of DOC to TDP, PP and SRP (not shown). DOC:MP remained stable across the entire continuum (Figure 2.5). DOC:DP were higher in river sites (mean = 0.52) than in lake sites (mean = 0.33), but the highest DOC:DP value was seen at the rivermouth (Figure 2.5). Similar patterns were seen with $SUVA_{254}$:P ratios (Figure 2.5).

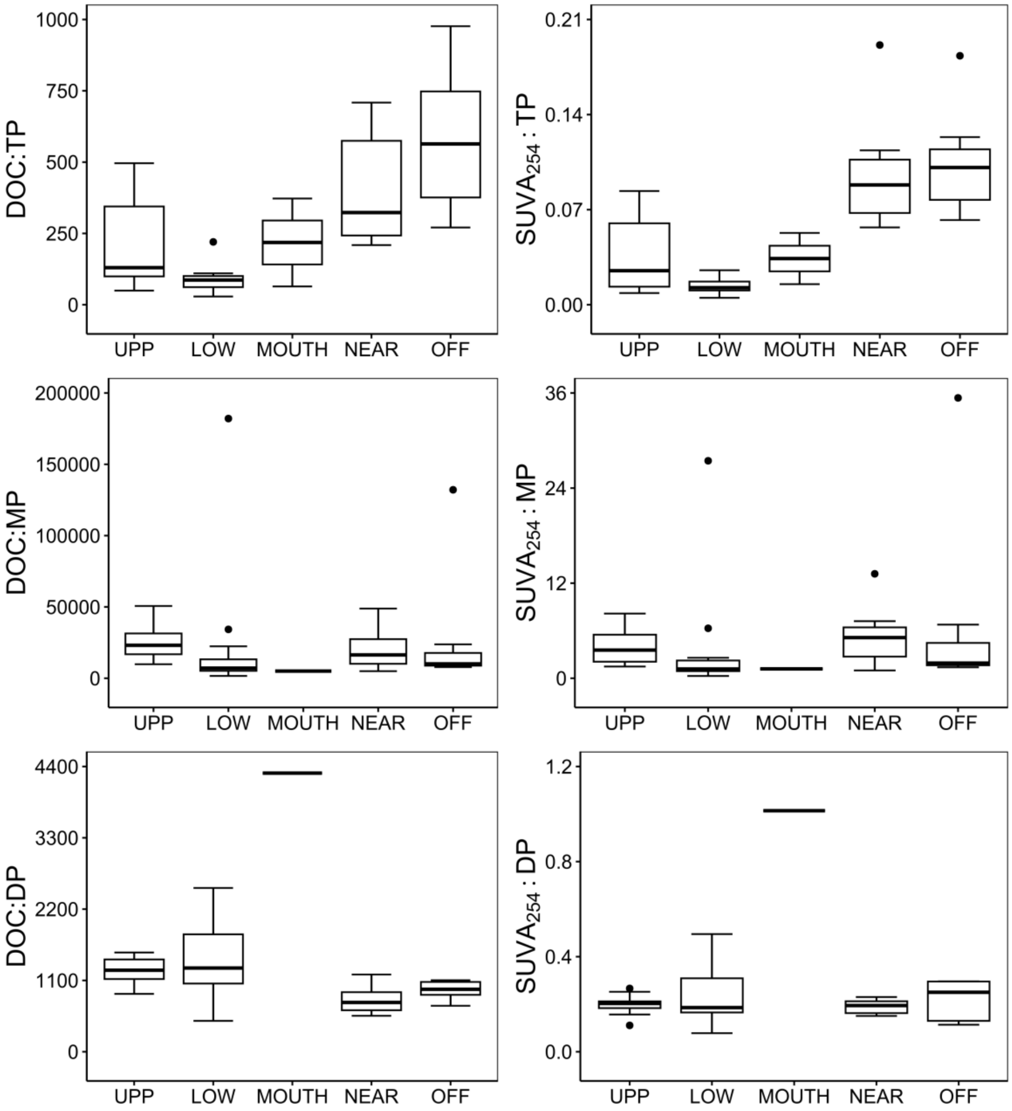

Figure 2.5. Box and whisker plots showing ratios DOC:P and SUVA$_{254}$:P ratios for upper Kettle Creek (UPP), lower Kettle Creek (LOW) rivermouth (MOUTH), nearshore Lake Erie (NEAR) and offshore Lake Erie (OFF) sites. The band indicates the median, lower and upper hinges indicate the first and third quartiles, and whiskers indicate the lowest and highest observations within 1.5 IQR (interquartile range) from the lower and upper quartiles. Points represent observations outside of 1.5 IQR.

Discussion

Our study is the first to simultaneously characterize spatial patterns in DOC, DOM composition and DOP along a river-lake interface. DOC concentration and DOM composition exhibited gradual changes within Kettle Creek, followed by rapid change at the river to lake transition zone. In contrast, concentrations of different P forms including MP and DP were more variable across the river-lake transect, suggesting that phosphorus cycling in Lake Erie and its tributaries is driven by processes different than those influencing DOM composition. It thus appears that DOM is a more conservative constituent whereas some forms of phosphorus are highly dynamic, which is consistent with rapid P cycling (including organic forms) along the river. Our study is also the first to quantify enzymatically-hydrolyzable DOP in a freshwater estuary, an ecologically and biogeochemically significant area that experiences important processes such as turbulent mixing and upwelling. We found that the contribution of DOP to the TP pool can be as high as 92%. This result suggests that the potential contribution of DOP to eutrophication of the lake should not be ignored. Strategies to protect Lake Erie would benefit from better management and monitoring of DOP and DOM in the inflowing rivers and lake.

DOM Across the River to Lake Transect

The spatial patterns of the DOM indices suggest that different processes affect DOM across the river to lake transition, as expected based on previous similar studies across the Laurentian Great Lakes (Larson et al., 2014; Pearce et al., 2023; Stephens & Minor, 2010). The higher freshness index (β:α, recent:decomposed DOM), lower specific fluorescence and the greater relative abundance of protein-like C7 at the lake sites imply internal production of DOM (Stedmon et al., 2003; Williams et al., 2016; Wilson &

Xenopoulos, 2009). Across the continuum, a decrease in $SUVA_{254}$ (aromaticity) and increase in β:α (freshness) imply microbial transformation and production of DOM (Stephens & Minor, 2010; Weishaar et al., 2003; Wilson & Xenopoulos, 2009). The increase in spectral slope ratio (S_R; Appendix D: Figure D.1) and photo-refractory C3 (Figure 2.4, Yamashita et al., 2010) from river to lake indicates that photodegradation of DOM is an important process in Lake Erie but not necessarily in Kettle Creek (Helms et al., 2008; Hiriart-Baer et al., 2008).

DOP Across the River to Lake Transect

All P forms were lower in concentration in the lake than in Kettle Creek. MP concentrations were generally very low and were lower than DP at all sites and dates. This dominance of DOP by DP is generally consistent with other aquatic ecosystem observations (Monbet et al., 2009; Pant et al., 2002; Turner & Newman, 2005). Much greater MP concentrations have been found in stormwater ponds in southern Ontario, Canada (up to 10 times higher than concentrations seen in Kettle Creek), especially at the pond inflows and under high stormflow conditions (Song et al., 2015). Storm runoff is believed to be an important route of transport of MP into surface waters (Turner, 2005) and urban ponds are highly connected to the terrestrial landscape via stormwater flow. We noted the highest MP concentrations and microbial-like processed humic DOM (component C6, urban DOM, Williams et al., 2016) in the lower Kettle Creek sub-watershed, which contains a greater proportion of urban land than upper Kettle Creek (Ontario Ministry of Natural Resources, 2007). MP was especially high during the August 2014 sampling which coincided with heavy rainfall in the region. The relationship between MP and hydrology, stormwater runoff, catchment development and impervious surfaces in the transport merits attention in future studies. On the other hand, while DP increased rapidly 2 km from the rivermouth, the predicted increasing downstream trend in DP concentration was absent in Kettle Creek (Figure 2.3) and DP then declined sharply at the rivermouth. This different spatial pattern in DP indicates that its cycling was less connected to watershed sources and likely more connected to internal river production (e.g., photosynthesis, grazing, viral lysis) or degradation (e.g., enzymatic hydrolysis through microbial activity, photodegradation).

Coupling of DOC to DOP Across the River to Lake Transect

DOC:TP and $SUVA_{254}$:TP ratios were higher in the lake consistent with reduced P relative to DOC and aromatic DOM in the central basin of Lake Erie. We predicted that MP would be depleted relative to DOC because of enzymatic hydrolysis by the planktonic community in the P-limited offshore sites. Conversely, we expected that DP in the lake would be enriched relative to DOC and aromatic DOM ($SUVA_{254}$) due to microbial sources of DP. Instead, ratios of DOC and $SUVA_{254}$ to both MP and DP did not differ between river and lake samples and were stable across the river to lake transect, with the exception of DP depletion at the rivermouth. While P is depleted in central Lake Erie, the invariance of $SUVA_{254}$:MP from river to lake suggests that MP is likely relatively recalcitrant compared to other P forms and likely still connected to terrestrial DOM. In addition, the low variation in $SUVA_{254}$:DP in upper Kettle Creek could denote stronger coupling of terrestrial DOM and DP where terrestrial inputs are most influential, while the high $SUVA_{254}$:DP at the mouth could denote decoupling of DOM and DP in this geochemical hotspot. Taken together, it may be that labile DP is both consumed by heterotrophic bacteria and serves as a nutrient source for primary producers at the rivermouth, whereas the more recalcitrant DOM and MP are simply transported from river to lake (i.e., the rivermouth is acting as a pipe, *sensu* Cole et al., 2007). Nutrient modelling of a Lake Michigan rivermouth (Fox River) suggests that it acts as a sink for SRP through microbial uptake (Larson et al., 2021), and it's possible that similar processes result loss of DP in the rivermouth. This shift in balance between production and consumption of DP and DOC is likely attributable to the distinct conditions that arise from mixing of lake water with river water (Larson et al., 2007, 2021).

Dissolved DNA derived from the breakdown of algal cells can be a large portion of the DOP pool in lakes, especially in eutrophic lakes and during phytoplankton blooms (Baldwin, 2013; Siuda et al., 1998). In the present study, we found no correlation between DP and chl *a* in the river or lake sites; however, we did find some of the highest DP concentrations and lowest DOC:DP ratios immediately above and below the reservoir in Kettle Creek. We observed a conspicuous surface phytoplankton bloom above the reservoir in August, reflected by elevated particulate PP and chl *a*. A smaller bloom was also noted at this site in October. While DP concentrations were high directly above and below the reservoir, the relatively low SRP, TDP and MP measured at these sites suggest that this is an area of high algal activity that acts as a soluble P sink. Compared to rivers, the greater water residence time in reservoirs often increases P retention (Withers & Jarvie, 2008).

Rapid Changes in Water Quality at the Transitional Rivermouth Zone

DOM parameters measured in this study (aromaticity, humification and freshness) changed gradually heading downstream in Kettle Creek, but the most prominent change in DOM and P occurred at the transition zone between the river and lake, at sites R2 and

L1. This abrupt change is likely caused primarily by mixing of lake water into Kettle Creek. While we observed signs of mixing at the most downstream river site, Kettle Creek is only minimally influenced by Lake Erie water. The lake's morphometry and southwest-to-northeast orientation mean that seiches and storm surges are most intense at the either end of the lake's length and are relatively weak in the central area around Kettle Creek (Trebitz, 2006). DP concentration at the rivermouth in October 2015 was far lower than expected; Kettle Creek was at baseflow when the rivermouth sample was collected and the low specific conductivity at the site indicated a large influence of lake water during this sampling event. Our results demonstrate that the lake effect experienced by Kettle Creek is strong but spatially limited.

Conclusions

There are generally few measurements of P fractions other than TP and SRP in lakes and especially in the Great Lakes (e.g., Musial et al., 2021). On average, DOP comprises over a third of the total dissolved P pool in Kettle Creek and over 80% in Lake Erie, yet we are lacking an understanding of its role in the P cycle and its potential to become bioavailable. Our study sought to increase our understanding of DOP and its connection to DOM in a freshwater estuary and along a river to lake transect. We found that the greatest changes in DOP and water quality occur across a small distance at the rivermouth and nearshore of Lake Erie; this is consistent with previous studies on DOM and P and attests to the role of rivermouths as active biogeochemical zones that merit further study. We observed greater amounts of TP, DOP and DOM in Kettle Creek than in Central Lake Erie. As terrestrial sources are generally important for these constituents, these differences may be attributed to the direct hydrologic connectivity of Kettle Creek with the terrestrial catchment compared to Lake Erie, which draws its water primarily from the Upper Great Lakes via the Detroit River (ca. 80%; International Joint Commission, 2014). Within the river itself DOM was relatively conservative, experiencing gradual changes in quantity and quality along its length. In contrast, P was more variable with generally higher concentrations downstream and DOP showed no consistent trends within Kettle Creek. Across the river to lake continuum, patterns of MP and DP concentrations differed from each other and from other P forms. The lake was enriched in DOP relative to TDP, and DP was notably more stable across the continuum than other P forms. With a better understanding of what affects DOP concentration in tributaries and DOP loading to lakes, we will be able to predict and manage its contribution to TP loading. One essential next step would be to characterize these P fractions in other major Lake Erie tributaries, which likely vary considerably due to differences in basin development and loading sources, including the intensively-farmed Maumee River basin and one of North America's largest wastewater treatment plants in Detroit. Our results also highlight a need to examine the potential impacts of storm runoff, urban land use and reservoirs on riverine DOP. Another important research gap is to quantify the turnover rates of DOP, especially DP in Lake Erie. Variation in DOP fractions could also be anticipated across the rest of the lake, which exhibits a strong trophic gradient. Finally, while the differing abilities of algal species to use DOP has been established through genetic studies and growth experiments (e.g., Lin et al., 2018; Shi et al., 2011), it is not yet known whether the large proportion of DOP in Lake Erie is contributing to shifting algal communities or harmful algal blooms. DOP is a nonnegligible component of Lake Erie’s P pool and in some situations will interfere with our ability to meet target P loads in this vulnerable and important lake.

Chapter 3. A Tale of Two Catchments: Dissolved Organic Phosphorus and Dissolved Organic Carbon Export To Lake Erie

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Abstract

The returning eutrophication of Lake Erie after its temporary recovery highlights the need to re-evaluate phosphorus loading. One form of phosphorus that has often been ignored is dissolved organic phosphorus (DOP), phosphorus that is associated with dissolved organic matter (DOM). Here we evaluated the magnitude of DOP and DOM loads and their contribution to Lake Erie phosphorus and carbon loading. We focused on two rivers: the Detroit River – a large river that provides water to Lake Erie from the Upper Great Lakes, and the Grand River – a moderately-sized river with typical characteristics of the Lake Erie basin. By measuring DOC, DOM composition and two bioavailable DOP fractions (phosphomonoesters, MP; phosphodiesters, DP) weekly from March to November 2015, we were able to estimate annual loads of DOM and DOP and compare the export and seasonal dynamics in the two rivers. We found that DOM composition and its relationship with hydrology in the Grand River were typical of temperate rivers, while Detroit River DOM reflected the highly processed and degraded DOM of its lacustrine source. While the coupling of DOM and DOP was not as strong as expected, we found evidence of coupling of MP and terrestrial DOM in the Grand River. There was no consistent relationship between DP and microbial DOM in either river, indicating that internal microbial sources of DP may be less important in rivers than in lentic systems. We estimated that DOP contributed to 10.9 and 50.9 % of annual P export and DOC contributed to 8.5 and 6.4 % of annual C export in the Grand River and Detroit

River, respectively. The contributions of DOP to TP exports indicate that this understudied form of bioavailable phosphorus merits further study.

Introduction

Despite reductions in phosphorus (P) loading that reduced algal production in the 1980s, Lake Erie has experienced episodic harmful algal blooms since the mid-1990s (Scavia et al., 2014). Attempts to reduce P loading through land management practices may have even led to the increased soluble reactive phosphorus (SRP) loads that have been linked Lake Erie's current re-eutrophication (Jarvie et al., 2017). Furthermore, climate change may aggravate high P loadings and excessive algal production in western Lake Erie (Michalak et al., 2013). Both extreme precipitation and drought have been implicated in recent algal blooms and hypoxic events (Y. Zhou et al., 2015). Indeed, years where Lake Erie's P load targets were exceeded were associated with heavier than average rainfall (Baker et al., 2014). It thus remains critical that we further develop our understanding of seasonal P dynamics and export in the Lake Erie watershed.

Even after decades of measuring and studying P dynamics in Lake Erie, one form that has received little attention is dissolved organic phosphorus (DOP). DOP contributes up to 60% of P in the Maumee River, a western Lake Erie tributary (Bridgeman et al., 2012). Of particular importance is enzymatically-hydrolysed DOP, which consists of P-containing organic compounds whose P can be mineralized by enzymes naturally-produced by aquatic microbes. This DOP can be classified into functional classes such as phosphomonoesters (MP) and phosphodiesters (DP) based on their susceptibility to enzymatic hydrolysis (Turner, McKelvie, et al., 2002). Despite its established bioavailability (Thompson & Cotner, 2018), enzymatically-hydrolysable dissolved organic phosphorus (DOP) has not often been studied in streams and rivers (Baldwin, 2013). Past studies have found it is often derived from nonpoint sources such as agricultural runoff (Brooker et al., 2018), is not effectively removed by urban ponds (Song et al., 2015) and can come from internal sources such as microbial production (Hafuka et al., 2021; Ishii et al., 1998) as well as loading from sediment (Paraskova et al., 2014). DOP may also play a role in the changing phytoplankton community structure in Lake Erie, due to the differing ability of species to use DOP as a P source (Harke et al., 2012; Shi et al., 2011). Accounting for DOP is necessary to better plan nutrient management of Lake Erie and other lakes experiencing eutrophication.

Macronutrient (C, N and P) cycles do not occur independently of each other (Kaushal et al., 2018). In particular, the coupling of dissolved organic matter (DOM) with P determines whether, and the extent to which, macronutrients are processed, retained and transported within inland waters with consequences for downstream eutrophication (King et al. submitted; Stutter et al. 2018). An abundance of DOC promotes P sequestration in rivers (Stutter et al., 2018) by increasing heterotrophic microbial activity, which in turn results in elevated microbial extracellular alkaline phosphatase and greater potential for DOP hydrolysis (Anderson, 2018). Climate, weather and land use have effects on the quantity and quality of riverine DOM (Wilson & Xenopoulos, 2008, 2009) and in turn DOM and macronutrient stoichiometry help determine how P is cycled in rivers (Stutter et al., 2018). It is especially important to consider DOM when working with DOP; there is evidence that heterotrophic microbes use DOP as a source of C as well as P (Siuda & Chróst, 2001). DOM, as with DOP, consists of many complex macromolecules, and its composition changes according to its source and processing. The characteristics of a river and its catchment (e.g., flow rate, size, land cover) can affect the composition of DOM (e.g., Autio et al., 2016; Hosen et al., 2021; Williams et al., 2016; Zhang et al., 2022).

We examined DOP and DOC export in two river catchments draining into Lake Erie. Our study has three main objectives: (1) to estimate the contribution of DOP to P loading and DOC to C loading to Lake Erie, (2) to evaluate DOP and DOM coupling from spring to autumn, and (3) to compare export and concentration of DOP and DOC in two catchments that vary in water source and size. We sampled the Detroit River (the largest source of Lake Erie's water) and the Grand River (a smaller Lake Erie tributary) from May to November. We modelled export of MP and DP as well as other forms of P and C in both rivers. We hypothesized that MP, believed to be a primarily terrestrially-derived form of P, is connected to terrestrial DOM as well as factors increasing transport of materials in runoff (elevated precipitation and flow). Conversely, we hypothesized that DP, believed to be a primarily microbially-derived form of P, is more connected to internally-produced DOM and factors promoting greater internal production (warm, low flow conditions). We predicted that these relationships will be stronger in the Grand River than in the Detroit River, as direct runoff is less of a contributor to the Detroit River which receives most of its water from the Upper Great Lakes.

Methods

Site Descriptions

The Grand River is the largest Canadian tributary and the largest river draining into Lake Erie's Eastern basin. This river is characterized as a medium sized tributary, typical of the size of many tributaries draining into Lake Erie. At roughly 6800 km^2, the

Grand River watershed’s land cover consists of approximately 70% agriculture with half of this as monoculture. The Grand River watershed also contains several urban areas with a total population of around one million as of 2016 (Grand River Conservation Authority, 2020). It is known to be important for the delivery of P to nearshore Lake Erie (Higgins et al., 2005) and it has been identified as at risk for P pollution (van Bochove et al., 2011). We sampled the Grand River 43 km upstream from the rivermouth (43.021944, −79.891944). There is a dam between the site and the rivermouth which prevents any lake effect on water quality (MacDougall & Ryan, 2012). We obtained discharge, conductivity and meteorological data from a monitoring station located at the site, operated by the Grand River Conservation Authority.

The Detroit River is a channel connecting Lake Erie to the Upper Great Lakes through Lake St. Clair and the St. Clair River. The Detroit River catchment is several orders of magnitude greater than the Grand River catchment as it encompasses the Upper Great Lakes (Lake Huron, Lake Michigan and Lake Superior). It passes through the cities of Detroit and Windsor with a combined population of nearly one million as of 2018 (Hartig et al., 2021). The Detroit River provides over 90% of the water and about 40 – 50% of the P load to Lake Erie’s western basin (International Joint Commission, 2014). Our sampling site was located on the Canadian side of the river (42.308219, −83.074748). We obtained discharge from the United States Geological Survey gauge at Fort Wayne (#04165710) and meteorological data from the Environment and Climate Change weather station in Windsor, Ontario. In addition, we obtained discharge data from Water Survey of Canada gauges for two of the largest tributaries to the Detroit-St.

Clair System: Thames River (Thames at Thamesville, 02GE003) and Sydenham River (Sydenham at Florence, 02GG003).

Sample Collection

Weekly sampling was conducted from late-March to early-November 2015, for a total of 34 samples for the Grand River and 33 samples from the Detroit River. We collected Grand River samples in-stream approximately mid-channel and Detroit River samples off a dock using a Van Dorn sampler.

Whole water was collected in 50 mL polypropylene centrifuge tubes for total phosphorus (TP). We filtered water for dissolved inorganic carbon (DIC) through 0.2 µm polycarbonate membrane filters immediately after collection into acid-washed 40 mL amber glass vials with no head space, capped with a teflon-coated butyl rubber inner septum and a silicon outer septum. Whole water for dissolved and particulate nutrients was collected in 1– 4 L HDPE containers and transport to Trent University on ice for processing within 24 hours.

Analytical Chemistry

Bottles of whole water were mixed by inversion and filtered sequentially through a pre-combusted 0.7 µm GF/F filter and a milliQ-rinsed 0.2 µm polycarbonate membrane filter for dissolved nutrients. Filtrate was stored in HDPE bottles for total dissolved phosphorus (TDP) and in pre-combusted amber glass bottles for MP, DP and DOM; all samples were stored at 4°C until analysis. For particulate samples, we mixed and filtered whole water onto pre-combusted 0.7 µm pore size GF/F filters. We dried filters overnight at 60°C for particulate carbon (POC) and particulate phosphorus (PP) and stored dried filters at room temperature.

DOM concentration (dissolved organic carbon, DOC; measured in units of C) was analyzed using an Aurora 1030 total organic carbon analyzer (O.I. Analytical). We measured POC using an elemental analyzer (Vario EL III, Elementar). We measured PP, TDP and TP by first converting all P into phosphate by digesting with potassium persulfate in an autoclave for 30 minutes at 121.5°C , then measuring phosphate using the molybdenum blue colourimetric assay (Murphy & Riley, 1962).

DOP was measured using an enzymatic hydrolysis method (Song et al., 2015; Turner, McKelvie, et al., 2002). We prepared two enzyme solutions: (AP) with 1 unit/mL alkaline phosphatase (Sigma Aldrich P6774) in 0.1 M Tris-HCl buffer (pH 8.0); and (AP + PD) with 1 unit/mL alkaline phosphatase and 0.03 units/mL phosphodiesterase (Sigma Aldrich P4506) in 0.1 M Tris-HCl buffer (pH 8.8). We incubated 13.5 mL of samples and P standards in centrifuge tubes in the dark for 10 hours at 37°C with 0.75 mL of the enzyme solution or 0.75 mL of buffer only (BUFFER). All tubes also contained 0.75 mL of 0.1 M sodium azide as a microbial inhibitor. We measured the final phosphate concentrations using the molybdenum blue colourimetric assay. We calculated the concentration of P in MP and DP as follows:

$$[\mathrm{MP}] = (\mathrm{AP}) - \mathrm{BUFFER}$$

$$[\mathrm{DP}] = (\mathrm{AP} + \mathrm{DP}) - \mathrm{BUFFER}$$

DOM Characterization

DOM composition was evaluated using absorbance and fluorescence spectroscopy. We performed absorbance scans from 230–800 nm using a Cary 50 spectrophotometer (Varian). Absorbance scans were corrected for blank (MilliQ) absorbance and turbidity. We calculated the spectral slope ratio (S_R; Helms et al., 2008) and specific ultraviolet absorbance at 254 nm ($SUVA_{254}$; Weishaar et al., 2003) using the corrected scans and DOC concentration. Using a Cary Eclipse Fluorometric Spectrophotometer (Agilent Technologies), we performed fluorescence scans at 5 nm intervals from 230–500 nm (excitation) and at 2 nm intervals from 270–600 nm (emission) to create excitation-emission matrices (EEM). We conducted instrument spectral correction on sample and blank (MilliQ) EEMs. Sample EEMs were further processed by correcting for inner filter effects (Ohno, 2002), subtracting the blank EEM and Raman normalizing. The corrected EEMs were used to calculate the fluorescence index (FI; Cory et al., 2010; McKnight et al., 2001), freshness index (β:α; Parlanti et al., 2000; Wilson & Xenopoulos, 2009) and the humification index (HIX; Ohno, 2002; Zsolnay et al., 1999). Using the DOMFluor Package (Stedmon & Bro, 2008) in Matlab, we fitted the corrected EEMs to a seven component parallel factor analysis (PARAFAC) model previously created from 971 EEMs from Southern Ontario streams, urban ponds, lakes and the Great Lakes (Williams et al., 2013).

Hydrology

We calculated five-day antecedent precipitation by calculating the sum of daily precipitation for the sampling date and the four preceding days. We calculated discharge quantiles Q_{20} (high flow) and Q_{80} (low flow) from 10 years of mean daily discharge from the Grand River gauge and 9 years of mean daily discharge from the Detroit River gauge, using the *quantile* function from the R ‘stats’ package (R Core Team, 2020).

Load Estimation and Statistical Analysis

We used mean daily discharge and our water quality data to construct LOADEST export models with LOADEST (Runkel et al., 2004), using the LoadRunner utility (Booth et al., 2007). For each constituent, LOADEST calibrated nine predefined mathematical models and selected the best model using the Akaike Information Criteria (AIC). We then examined the chosen model by visualising calibration and modelled loads together, checking for multicollinearity of coefficients, residuals analysis (serial correlation, Turnbull-Weiss statistic, distribution of residuals across load values and time), load bias analysis and the Nash-Sutcliffe Efficiency Index (Appendix E: Table E.1). The AIC-selected model yielded unrealistic results for some constituents, such as negative loads or erratic behaviour outside the calibration period. For these constituents (Grand River DOC, TP; Detroit River DIC, POC, TP, PP) we examined the model with the next lowest AIC. Residuals were normal for all constituents so we used the Annual Maximum Likelihood Estimation (AMLE) regression models. We applied the selected model for each constituent to the daily streamflow data for 2015 to calculate daily nutrient loads. For some constituents (e.g., TDP, PARAFAC components) we could not construct an effective and unbiased model using our calibration data; these constituents were omitted from further export analysis.

LOADEST relies on the relationship between discharge and nutrient concentration. Unlike typical rivers, water quality in the Detroit River is heavily influenced by internal processing in the Upper Great Lakes instead of hydrological events, so the use of LOADEST in the Detroit River has been contraindicated (Scavia, Bocaniov, Dagnew, Hu, et al., 2019). Alternative approaches (e.g., Generalized Additive

Models, GAM, with nutrient concentration as a function of time) are more susceptible to extrapolation errors; we did not collect winter samples so LOADEST was a more appropriate option for estimating annual export. We compared our LOADEST results to exports based on GAM models for the sampling period and found differences of 0.13 – 3.36% in total export.

We created GAMs to examine the relationships between DOP and DOM. Each GAM consisted of a DOP form (MP and DP) as the response variable and one DOM index ($SUVA_{254}$ and β:α or S_R) as the explanatory variable, as well as a date term to account for temporal autocorrelation. Models were fitted with the *gam* function from the ‘mgcv’ package (Wood, 2017), using the restricted maximum likelihood method (REML).

Statistical analyses were conducted using R statistical software and the ‘stats’ package (R Core Team, 2020). We tested the differences in individual water quality parameters for the two rivers using the *t.test* function with samples paired by week. Linear correlation was assessed using Pearson correlation with the *cor.test* function. We used a significance level of $\alpha = 0.05$ for all statistical tests.

Results

Hydrology

During our study period, the Grand River was characterized by high flow in the spring in response to snowmelt, followed by relatively low flow for the majority of the sampling period, punctuated by peaks during periods of heavy precipitation in early summer (Figure 3.1). The Grand River high flow (Q_{20}) and low flow (Q_{80}) were 126 and 29 m^3s^{-1} respectively. In contrast, the Detroit River hydrograph did not have peaks or low flow during the sampling period, and experienced its low flow during the winter (Figure 3.2). The Detroit River tributaries (Thames and Sydenham) hydrographs showed more typical seasonal patterns, similar to the Grand River. Detroit River discharge was significantly higher than that of the Grand River ($P < 0.001$; Figure 3.3) with Q_{20} and Q_{80} of 5890 and 4955 m^3s^{-1} respectively. Samples were collected from the Grand River at low, moderate and high flow levels but discharge in the Detroit River was never below Q_{80} during the sampling period (Figure 3.2)). Both sites experienced their greatest amount of cumulative precipitation and largest precipitation events in May – June (Figure 3.1, Figure 3.2). Discharge was positively correlated with five-day antecedent precipitation in the Grand River ($r = 0.50$, $P < 0.01$) but not in the Detroit River.

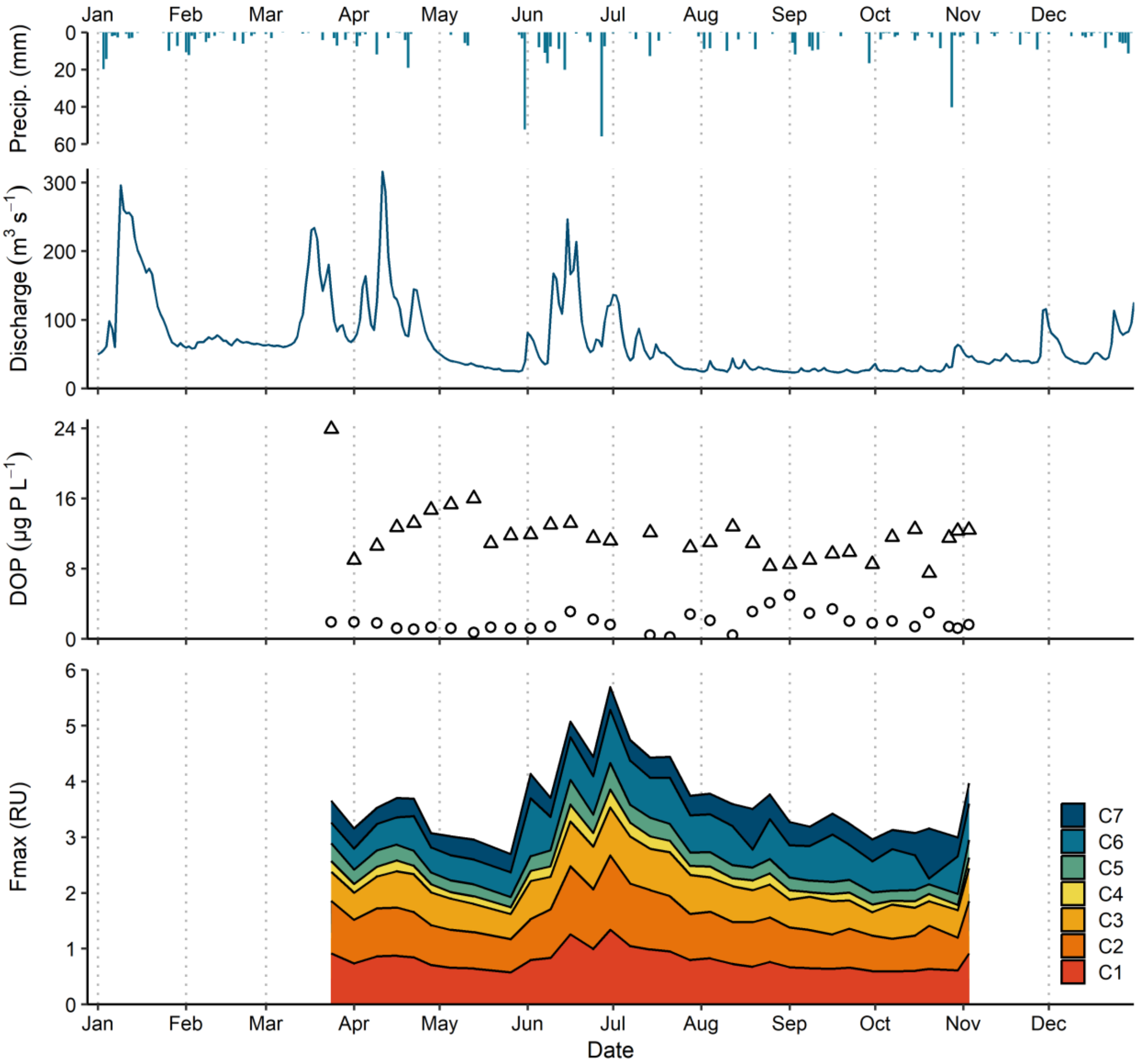

Figure 3.1. Hydrograph for the Grand River in 2015, showing daily precipitation (blue bars) and daily mean discharge rate (blue line). Sampled dissolved organic phosphorus concentrations are represented by open circles (phosphomonoesters, MP) and triangles (phosphodiesters, DP). The bottom panel presents dissolved organic matter PARAFAC component fluorescence for the sampling period in Raman Units (RU).

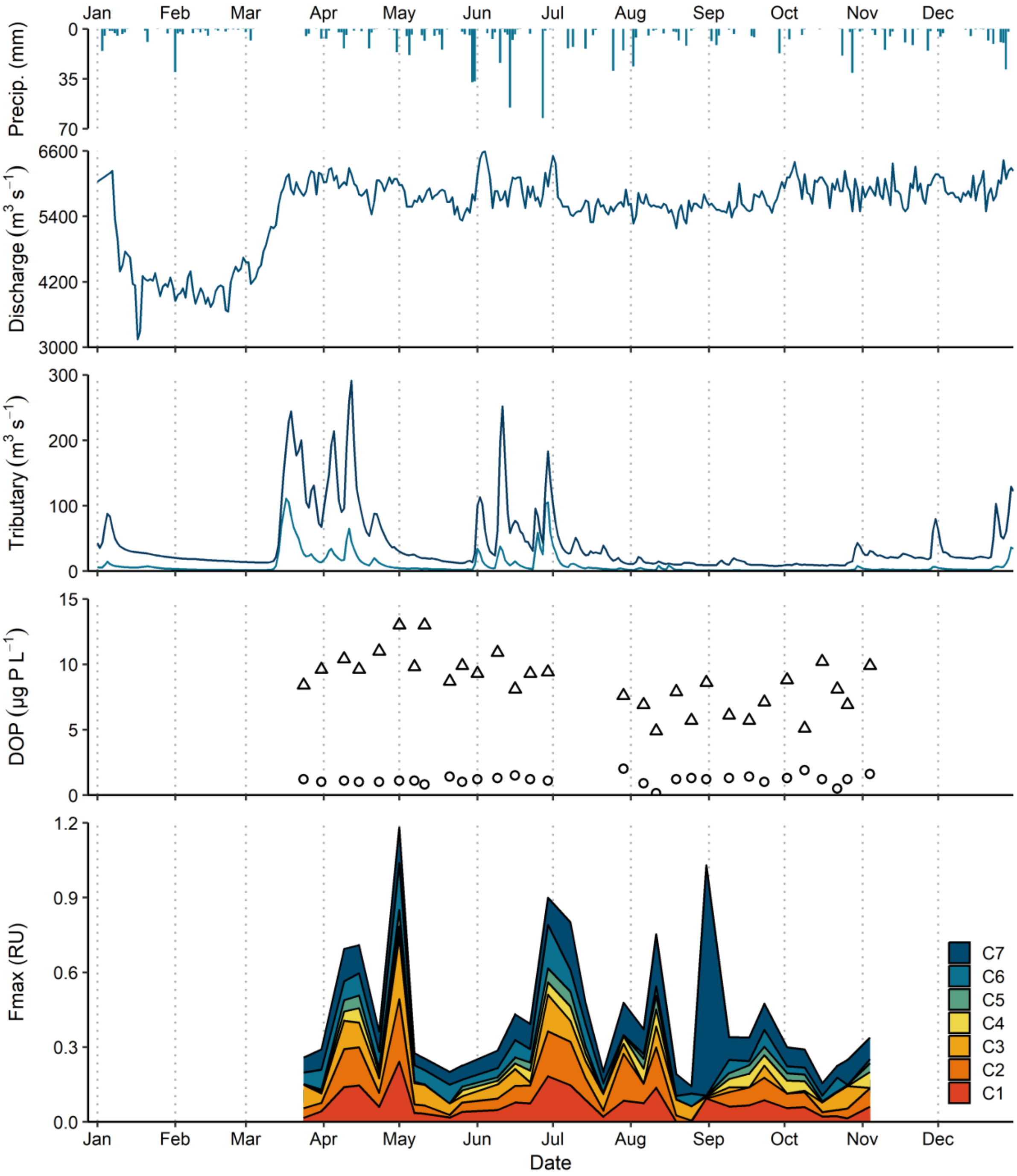

Figure 3.2. Hydrograph for the Detroit River in 2015, showing daily precipitation, Detroit River daily mean discharge rate and daily mean discharge rates for two Detroit River tributaries (Thames in dark blue, Sydenham in light blue). Sampled dissolved organic phosphorus concentrations are represented by open circles (phosphomonoesters, MP) and triangles (phosphodiesters, DP). The bottom panel presents dissolved organic

matter PARAFAC component fluorescence for the sampling period in Raman Units (RU).

DOM and DOP characterization

The Grand and Detroit Rivers differed significantly in both their concentrations and composition of DOM and DOP. DOC in the Detroit River was lower than in the Grand River ($P < 0.001$; Figure 3.3). In addition, the DOM in the Detroit River was less humic (lower HIX; $P < 0.001$), less aromatic (lower $SUVA_{254}$; $P < 0.001$) and lower molecular weight (higher S_R; $P < 0.001$) than Grand River DOM (Figure 3.3). The two rivers differed in their proportions of PARAFAC components: in the Grand River ubiquitous humic-like C1 and terrestrial humic-like C2 dominated, while in the Detroit River protein-like C7 contributed up to 90% of total fluorescence (Figure 3.1, Figure 3.2).

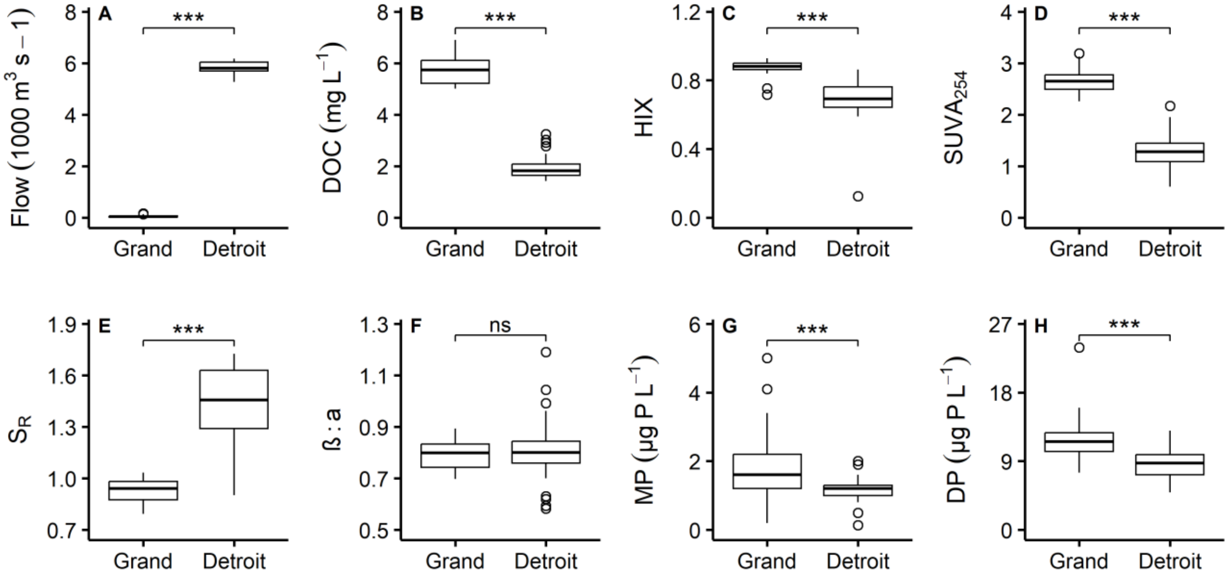

Figure 3.3. Box and whisker plots comparing (A) flow rates, (B) dissolved organic carbon, (C) humification index, (D) specific ultraviolet absorbance at 254 nm, (E) spectral slope ratio, (F) freshness index, (G) phosphomonoesters and (H) phosphodiesters in the Grand River and Detroit River. The band indicates the median, lower and upper hinges indicate first and third quartiles, whiskers indicate the lowest and highest observations within 1.5 interquartile range (IQR) from the first and third quartiles and points indicate observations outside 1.5 IQR. Significance of paired t-test results denoted with *** ($p < 0.0001$) or ns (non-significant).

In the Grand River, DOM fluorescence (ΣFmax) peaked following periods of elevated discharge, regardless of antecedent precipitation (Figure 3.1). The most variable components were the protein-like C7 and the microbial humic-like C6. Most samples collected following heavy rain (five day antecedent precipitation greater than 25 mm) showed an increase in the proportion of C6 and decrease in the proportion of C2. The exception to this was the sample from 30 June, collected following a week of relatively high precipitation (Figure 3.1), which instead exhibited an increase in the proportion of C1 as well as the highest ΣFmax of the study.

Detroit River discharge and DOM fluorescence appeared unrelated; instead, peaks in ΣFmax were preceded by peaks in Thames River discharge approximately five weeks earlier (Figure 3.2). We observed an additional peak in ΣFmax consisting of primarily C7 in late August, when the Thames and Sydenham Rivers were experiencing an extended period of baseflow. In the Detroit River, the most variable components were C7 and the photorefractory, terrestrial humic-like C3.

As with DOC, we observed lower concentrations of MP ($P < 0.001$) and DP ($P < 0.001$) in the Detroit River compared to the Grand River (Figure 3.3). MP concentrations were elevated during mid-August to late October in the Grand River, but relatively invariable in the Detroit River (Figure 3.2). The two rivers exhibited similar seasonal patterns in DP with higher concentrations in the spring and lower in late summer/autumn.

DOP and DOM export

We modelled the annual export of P (TP, PP, MP, DP) and C (DIC, DOC, POC) using LOADEST. Approximately 34.27 megagrams (Mg) of P per year were exported as DOP from the Grand River, and 12100 Mg of C per year were exported as DOC. Export of all P and C forms were closely connected to discharge in the Grand River, with the greatest export rates occurring during three periods of high flow: an early snowmelt in late January, the spring freshet and a rainy period in early summer (Figure 3.4). This pattern is more evident with TP and PP than with DOP and C. High flow periods ($Q > Q_{20}$) accounted for 66% of MP export and 39% of DP export in 2015, even though the Grand River was only at high flow for 13% of the year.

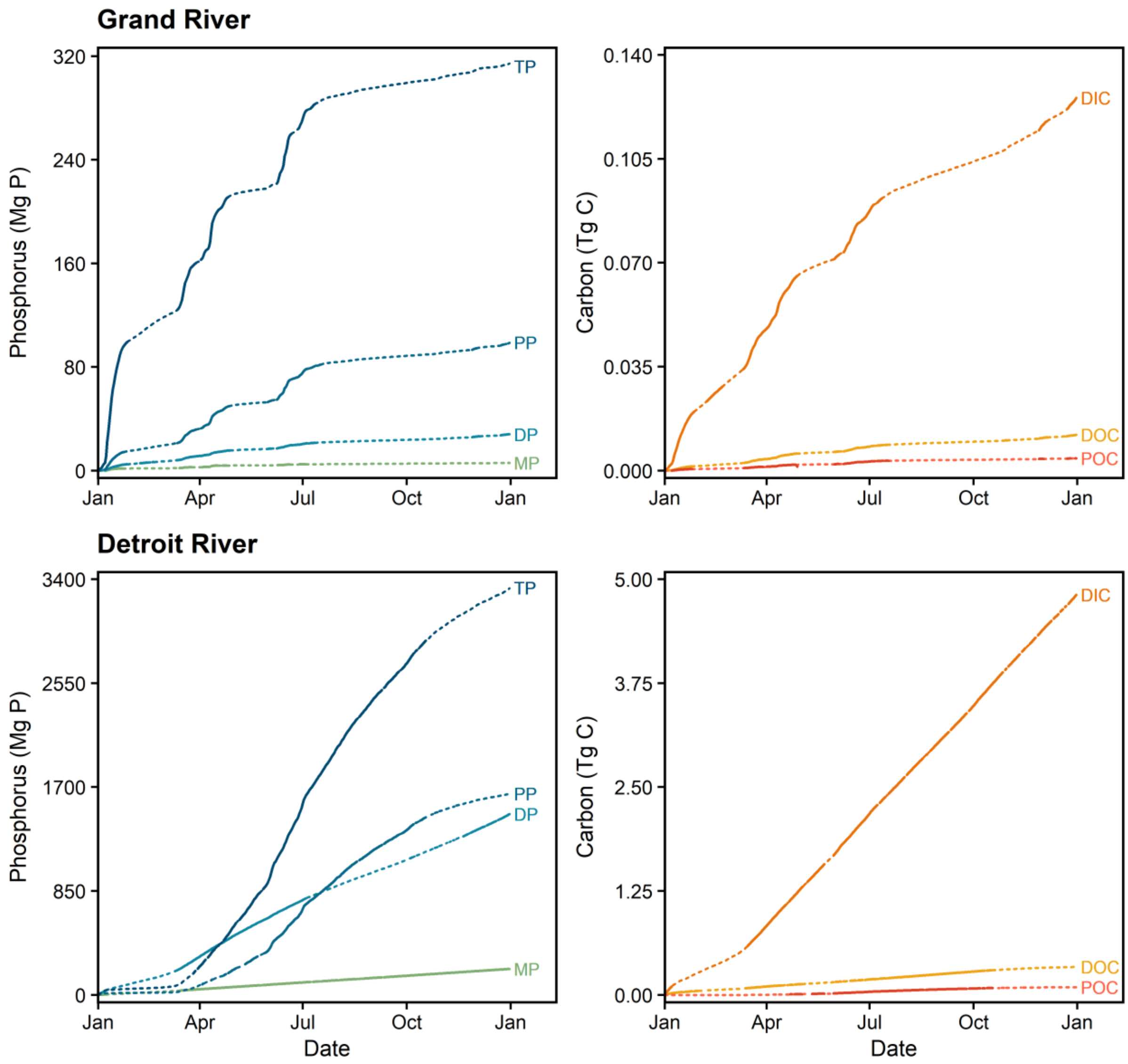

Figure 3.4. Modelled cumulative export of phosphorus and carbon in the Grand and Detroit Rivers through 2015. A greater slope is equal to a greater rate of export. Export above annual average for each constituent is represented by a solid line, while export below annual average is represented by a dashed line. Note the differences in scale between phosphorus and carbon panels as well as between the Grand and Detroit Rivers.

MP, DP and DOC exports were much higher in the Detroit River, with approximately 1693 Mg of P exported as DOP and 338075 Mg of C exported as DOC. MP export in the Detroit River was mostly invariant throughout the year, while DP export was elevated in spring and early summer (Figure 3.2). There were two periods of reduced DP export: during the winter and from late July to October. Detroit River exports of TP and PP were reduced during the winter. Exports of POC and DOC were relatively stable throughout the year, with lower-than-average loads while Detroit River tributaries were at low flow (Figure 3.2). Exports during late summer and early autumn were above average despite low flow in the tributaries. The only period where DIC export was below average was during January – March, when both the Detroit River and its tributaries were at low flow. Only the Detroit River DP model contained "seasonal" terms not solely related to flow (Appendix E: Table E.2; Runkel et al., 2004).

In addition to the difference in magnitude of DOP and DOC exports, the Grand River and Detroit River differed in their organic nutrient export stoichiometry. The cumulative annual molar DOC:DOP in the Grand River was 910, while the Detroit River was more DOP-enriched with a ratio of 515. Regarding individual DOP fractions, DOC:MP in the Grand River and Detroit River were more similar (5179 and 4090, respectively) than DOC:DP (1105 and 589) or overall DOC:DOP.

Correlation of DOP and DOM

The two rivers differed in their associations between DOP and DOM, but overall few correlations were found. In the Grand River, we observed a positive linear association between MP and $SUVA_{254}$ (Figure 3.5). DP in the Grand River and MP in the Detroit River were not significantly associated with any of the DOM optical indices or PARAFAC components. DP in the Detroit River was positively associated with S_R, but only at higher S_R values of approximately 1.4 and above (Figure 3.5). No association between DP and S_R was found below an S_R of 1.4..

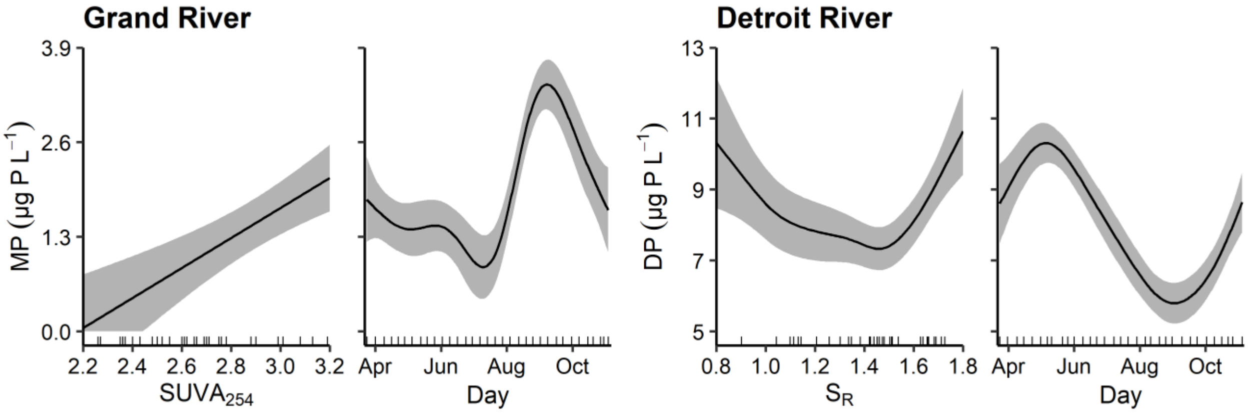

Figure 3.5. GAM smooth plots showing the relationship between DOP and DOM composition as well as DOP trends over time. The Grand River model includes specific ultraviolet absorbance at 254 nm and day as explanatory variables and phosphomonoester concentration as the response variable. The Detroit River model includes spectral slope ratio and day as explanatory variables and phosphodiester concentration as the response variable. Shaded ribbons represent the predicted values ± standard error and ticks above the x-axis indicate the frequency of data.

Discussion

We examined two catchments of contrasting size to assess seasonal and export dynamics of DOM and DOP. Throughout the sampling period we observed differences between Grand River and Detroit River DOC, DOM and DOP dynamics. A combination of internal and external factors influenced both rivers, but the relative importance of each differed. The Grand River was typical of many medium-sized temperate rivers with highly terrestrial DOM and close connection to its terrestrial catchment. The Detroit River derives most of its water from the Upper Great Lakes (Burniston et al., 2018) and its DOM quality reflects its lake source with a smaller contribution of the terrestrial catchment.

Despite the differences between the Grand River and Detroit River, we also found some shared patterns in riverine DOM and DOP. The influence of terrestrial inputs of DOM was strongest in the Grand River, but was present in the Detroit River as well in the lagging connection between tributary flow and DOM fluorescence. We also noted seasonal changes in DOP concentrations in both rivers, with MP peaking in the Grand River in late summer, and DP elevated in the spring in both rivers.

Catchment Differences in DOM

DOM in the Grand River was typical of a temperate river and was comparable to other Great Lake tributaries (Larson et al., 2014) in terms of humic content (HIX), and molecular weight (S_R). Much of riverine DOM is terrestrial in origin (e.g., Larson et al., 2014; Stephens & Minor, 2010; Wilson & Xenopoulos, 2009); this becomes increasingly true at high discharge, especially under storm conditions where reduced residence time means less time for internal processing of DOM and more conservative transport of materials (Casas-Ruiz et al., 2020; Raymond et al., 2016). Periods of elevated discharge coincided with peaks in DOM fluorescence (ΣFmax; Figure 3.1), though there was some variability in relative contribution of different components to total fluorescence during different peaks. Most of the peaks in DOM fluorescence we observed involved proportional increases of all components or a slight increase in the proportions of ubiquitous humic C1 and terrestrial humic C2. These observations demonstrate the increased importance of terrestrial DOM with elevated discharge, either due to greater transfer from the catchment or reduced processing (Casas-Ruiz et al., 2020). The peak at the start of June was driven by a large increase in microbial-processed, humic-like C6; a component that is prevalent in urban environments and dominant in Southern Ontario stormwater ponds (Williams et al. 2016). Indeed, its prominence after intense precipitation could be caused by urban runoff. The impact of allochthonous DOM was evident in the spring, early summer and late autumn, but during an extended period of low flow from August to October we noted spikes in more autochthonous-like, protein-like DOM (C7), likely indicating elevated microbial activity.

DOM composition in the Detroit River was distinct from the Grand River due to the influence of the Upper Great Lakes on the Detroit River. Rivers influenced by upstream lakes display lower DOC concentrations and more processed DOM (Hosen et al., 2021; Larson et al., 2007); the Detroit River is a good example of this. The long residence times of large lakes allow for substantial transformation through bio- and photodegradation or loss through flocculation and precipitation (Stephens & Minor, 2010). Because the Detroit River derives over 95% of its water from Lake Huron and Lake Superior via Lake St. Clair, it had similar DOC and DOM composition as the Upper Great Lakes (Stephens & Minor, 2010; Z. Zhou et al., 2016). Despite this, we did see evidence of small but important terrestrial inputs in the increases in ΣFmax and decrease in S_R following peaks in tributary discharge. This suggests that the autochthonous-like composition of the Detroit River DOM is due to its source rather than high rates of processing within the river itself.

Coupling of MP and DOM

Compared to DOM, very little is understood about DOP dynamics in freshwater systems and the connection between DOP and DOM. We expected that MP would be coupled with terrestrial DOM due to previous findings that MP is a major component of DOP in soil (Toor et al., 2003; Turner, Mahieu, et al., 2003) and urban runoff (Song et al., 2015). We also expected DP to be largely microbial in origin (Ishii et al., 1998; Paraskova et al., 2014) and tightly coupled with microbial DOM. In both catchments, the relationship between DOM and DOP was not as strong as we expected but consistent with our previous findings in a smaller tributary of Lake Erie (King et al. submitted).

After accounting for seasonal variation, we noted a positive relationship between MP and aromatic DOM ($SUVA_{254}$) in the Grand River, suggesting coupling between MP and terrestrial DOM. MP became more variable with peaks in concentration during a period of low discharge in the Grand River from August to October (Figure 3.1). While persistence of less labile MP compounds could have contributed to these MP peaks (Bell et al., 2020), the inverse relationship between MP and DP when the Grand River was at baseflow points towards DP hydrolysis as a contributor of MP during this period (Jørgensen et al., 2015). In the Detroit River we did not observe a significant association between MP and $SUVA_{254}$, which is likely due to the relatively weak terrestrial signal in this river. Song et al. (2015) observed that the high MP in urban pond inlets was followed by a rapid drop in MP, so terrestrial MP appears to be rapidly removed; this may be especially true in the relatively P-poor Upper Great Lakes and Detroit River. We also did not note any significant seasonal trends in Detroit River MP, or the period of inverse relationship between MP and DP that we saw in the Grand River.

Coupling of DP and DOM

DP in the Detroit River was found to be highest from April to June (Figure 3.5). After accounting for seasonal variation, we did not find a correlation between DP and the freshness index (β:α; indicator of microbial processing), but we did find a non-linear association with S_R (inversely correlated with molecular weight, indicator of photodegradation). At S_R above 1.4 (comparable to offshore Upper Great Lakes; Stephens & Minor, 2010), DP was positively associated with S_R. At lower S_R, however, the relationship between DP and S_R dissipated. These samples with S_R below 1.4 were the ones coinciding with the tributary inputs, indicating that the fresh material changes the coupling of DP and DOM. As with the Detroit River, Grand River DP was higher in the spring but the trend was less pronounced (Figure 3.1) and we did not find significant relationships between DP and $\beta:\alpha$ or S_R.

The lack of relationship between DP and microbial DOM in either river suggests that internal microbial sources may be less important in these rivers than in other systems such as urban ponds (Song et al., 2015), constructed wetlands (Jørgensen et al., 2015) and lakes (Hafuka et al., 2021; Paraskova et al., 2014; Siuda et al., 1998). The internal DP sources identified in lentic systems, such as “sloppy” grazing by zooplankton and protozoans (Ishii et al., 1998; Taylor & Lean, 2018), may be diminished by the short residence time of rivers like the Grand River. Instead, the main determinants of riverine DP concentrations seem to be (1) an unresolved source in the spring, and (2) DP compounds accumulated in upstream lakes (Hafuka et al., 2021).

DP accumulation appears to be most pronounced in the Detroit River. The DOC:DP export ratio in the Detroit River was roughly half that of the Grand River, indicating that Detroit River DOM was comparatively enriched with DP. While DP is a bioavailable form of P, its uptake by aquatic microorganisms may have been limited by the supply of bioavailable DOM: DOC was very low in the Detroit River, and much of it is likely recalcitrant. In aquatic ecosystems, highly labile DOM is rapidly consumed (Cory & Kaplan, 2012; Guillemette & del Giorgio, 2011). The remaining DOM fuels a lower level of bacterial activity over a longer timescale, with long term bioavailability decreasing with greater water residence time (Guillemette & del Giorgio, 2011). The

Upper Great Lakes have long residence times of 21 – 173 years, and a scarcity of readily bioavailable DOM could impede bacterial consumption of DP resulting in its preservation and export to Lake Erie (Stutter et al., 2018). Compared to DOC:DP, the DOC:MP in the Detroit River and Grand Rivers were much more similar. This could be due to the coupling of MP and terrestrial DOM and the high lability of the type of MP compounds measured using our methods (Bell et al., 2020; Sato et al., 2013).

DOP and DOM Export to Lake Erie

MP and DP export have not previously been modeled for any rivers. We were, however, able to compare our TP exports to previously reported values. While P export values for the Grand River have not been published recently, our TP export was comparable to estimates from 2003-2013 (Maccoux et al., 2016; Shaker, 2014). Our 2015 Detroit River TP export was higher than other published values. For example, our load estimate (3324 MTA) was 27% higher than that of Burniston et al. (2018) from the same year, and ~30% higher than the 2013-16 average from Scavia et al. (2019). Detroit River nutrient concentrations are spatially variable, with higher concentrations at the shoreline than mid-channel (Burniston et al., 2018), so our nearshore sampling location may be the reason for the difference.

Export of P and C from the Grand River were greatest during periods of high discharge, consistent with observations from other temperate rivers (Banner et al., 2009; Macrae et al., 2007) and from a previous study of a small Grand River subwatershed (Irvine et al., 2019). Hydrological events (snowmelt, precipitation) are especially important to nutrient loading in agricultural watersheds like the Grand River (Stedmon et al., 2006), and this appears to extend to DOP. As a connecting channel between Great

Lakes, the Detroit River's discharge does not experience a large increase in flow following rain events and has relatively consistent discharge outside of winter low flow. As a result, we found that nutrient export in the Detroit River was low during the winter, and relatively invariable during the rest of the year rather than episodic like in the Grand River. The period of elevated export of DP in the Detroit River was earlier and briefer than other P forms, likely due to the high spring DP concentrations. Conversely, PP export in the Detroit River is the greatest during the summer, likely due to the inclusion of phytoplankton in the PP fraction.

Our efforts to conduct comprehensive sampling of DOP yielded good coverage of most of the year under a variety of hydrological conditions; however, we did not sample during the winter season. While many phosphorus export studies focus on the growing season, winter can be an important season for P loading to rivers especially in tile-drained areas like the Grand River watershed (Ball Coelho et al., 2012). Ball Coelho et al. (2012) observed high transfer of particulate and organic P through overland flow during a winter rain event in an agricultural area. While they did not look at DOP composition, it is possible that this included DP from manure or soil bacteria and may be related to the high DP we observed in early spring.

Conclusions

Our work advances our understanding of MP and DP in rivers; however it also highlights important gaps of riverine DOP dynamics. We observed changes in DOP concentrations and coupling with DOM through the sampling period, however the mechanisms behind these changes are still poorly understood. Targeting runoff using high-frequency sampling during spring melt and storm events could help identify the unknown spring DP source. Freeze-thaw cycles on DOP release from soil could also help explain the high spring DP; the effects of drying-rewetting cycles have already been studied (Blackwell et al., 2009; Turner, Baxter, et al., 2003) and a similar approach could be used for freeze-thaw cycles. In addition, this study proposes that DOP should be better incorporated into P studies instead of only TP and SRP to better prepare for controlling eutrophication.

Many studies have documented sources and transformations of DOM; our work has found similar complexity with DOP. We found that the concentration and composition of DOP as well as its coupling with DOM are dependent on river characteristics, season and hydrology. DOP has been largely ignored as a possible contributor to P-loading and eutrophication of sensitive and valuable lakes like Lake Erie, and our work demonstrates that this is an oversight.

Chapter 4. General Conclusion

Compared to other forms of P, DOP is poorly understood in ecosystems due to its complexity, analytical difficulties and the historical oversight of its bioavailability. The goal of this thesis was to improve our understanding of DOP in rivers and lakes. While there is still much work to be done in this area, we have shed some light on DOP concentration and speciation along a river-to-lake continuum, evaluated the coupling of DOP and DOM, and observed how DOP changes seasonally. We also estimated the contribution of DOP to P export in Lake Erie tributaries.

Managing DOP loading to Lake Erie

Our export models for the Grand River and Detroit River suggest that DOP is a large enough contributor to P loading to merit consideration when managing P loading to Lake Erie and other lakes. We also found that the central basin of Lake Erie was enriched with DP relative to other forms of P, sustaining a source of bioavailable P that could stimulate productivity. Management of DOP export is complicated by its largely non-point sources; however, acknowledging its contribution to total and bioavailable P loading will help us create effective regulations. More research into the transfer of DOP from catchment to river would also help to identify management opportunities. For instance, in the highly-agricultural Lake Erie watershed the effects of agricultural practices such as tile drainage, manure application and tilling practices on DOP export are still not fully understood. In more urban catchments, stormwater management should also be considered, as DOP can be exported from stormwater ponds, reducing their P retention efficacy. Finally, our results from Kettle Creek suggest that reservoirs play a role in DOP cycling and export that is worth further investigation. In particular, reservoirs may act as a source of DP similar to urban ponds (Song et al., 2015).

Seasonal patterns and Climate Change

The seasonal trends in MP and DP concentrations that we observed were inconsistent with our expectations that MP (as a terrestrially-linked form) would be elevated in the spring when runoff is high, and DP (as a microbially-linked form) would be elevated in the summer, when low flows and warm temperatures promote microbial activity. Instead, we found that MP in the Grand River peaked in summer and DP in both rivers was higher in the spring. Our methods did not allow us to conclusively attribute the high MP in summer to concentration of recalcitrant compounds or in situ production through the breakdown of DP, two things that have been observed elsewhere. Similarly, we could not identify the source of the elevated spring DP.

Coupling of DOP and DOM

DOP is a component of DOM and the P and C cycles are linked in aquatic ecosystems (Stutter et al., 2018); despite this, many studies on DOP only measure DOC concentrations or do not measure DOM at all. We found that including DOM provides meaningful context for DOP studies. In the Grand River, MP was associated with aromatic DOM ($SUVA_{254}$), providing support for the dominance of terrestrial sources of MP. The invariable MP:$SUVA_{254}$ from Kettle Creek to offshore Lake Erie suggests that the link between MP and terrestrial DOM persists from river to lake. We did not see the same association between aromatic DOM and MP in the Detroit River. The Detroit River is heavily influenced by the Upper Great Lakes, and the link between MP and terrestrial DOM may be eventually weakened after extensive internal processing.

The relationship between DOM and DP is less clear than that of MP. We expected a relationship between DP and either the freshness index or molecular weight (S_R) and neither was seen in the Grand River. In the Detroit River we found a negative relationship between DP and molecular weight, but only when inputs of terrestrial-like material from tributaries to the Detroit River were reduced. Our results suggest that the relationship between DP and processed DOM is absent or minimal in rivers. When combined with our observation of high DP in the spring, it appears that DP in rivers is terrestrially-influenced, perhaps through a terrestrial source of DP such as soil microbes (Turner, Mahieu, et al., 2003), the impacts of nutrient loading from the catchment on DP production by the microbial community (Siuda et al., 1998) or the influence of allochthonous DOM on photolytic or enzymatic hydrolysis of DP (Janssen & McNeill, 2015).

Analytical challenges

Analytical difficulties are a continuing challenge, as there’s no single, standardized way to measure or categorize DOP. Developing a standard approach from the current options may not even be advisable, since the strengths and weaknesses of different analytical techniques and classification approaches make them well suited for different research objectives (Baldwin, 2013; McKelvie, 2005; Worsfold et al., 2008). For now, the importance of considering the analytical methods and exact definitions of different DOP classes is critical when comparing DOP studies.

The enzymatic hydrolysis method we used is advantageous for high throughput studies due to comparatively low financial and time costs, particularly when using flow injection techniques with immobilized enzymes (Omaka et al., 2008; Shan et al., 1994). Enzymatic hydrolysis is also useful when evaluating bioavailability and not just quantity is desired; however, its limitation is that it doesn't provide much information on the structure of the DOM compounds it is contained within. While some broad predictions regarding source can be made from functional class, these generalizations are not necessarily reliable on their own. For example, while MP is often largely terrestrial in origin, they are also produced by the breakdown of internally-produced DP.

Another limitation of enzymatic hydrolysis is that it is dependent on the availability of consistent, high-purity enzyme preparations. Preparations of a given enzyme from different sources and suppliers don't necessarily yield the same results (Annaheim et al., 2013; Bünemann, 2008). We were able to obtain the same preparations of alkaline phosphatase and phosphodiesterase used in previous studies (e.g., Monbet et al., 2007; Suzumura et al., 2012; Turner et al., 2002; Zhu et al., 2013). The phytase enzyme preparation used previously was no longer available, however, precluding the measurement of phytic acid in these studies. Phytase preparations that were available at the time were low purity and inappropriate for this application, as low purity preparations contain excess protein that interfere with the molybdenum blue calorimetric assay used to measure the released phosphate (Z. He & Honeycutt, 2005; Monbet et al., 2007). Even with the enzyme preparations used in our studies, the protein content of the phosphodiesterase preparation meant that the blue complex was less stable than it is with the standard molybdenum blue assay, requiring timely measurement of absorbance to ensure consistent results. Availability of higher purity enzymes (especially phosphodiesterase and phytase) would make enzymatic hydrolysis an easier and more effective technique.

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Appendices

Appendix A: Chapter 2 sampling site information.

Table A.1. Site coordinates in decimal degrees (WGS84 datum) and approximate site depths in metres. For river sites, depth is at the deepest point of the channel. Site depth was not measured at all sites and sampling events.

Site	Zone	Latitude	Longitude	Depth
R81	Upper Kettle Creek	42.8894	− 80.9973	—
R65	Upper Kettle Creek	42.8818	− 81.0877	—
R57	Upper Kettle Creek	42.8594	− 81.1069	5.8
R50	Upper Kettle Creek	42.8352	− 81.1345	5.6
R40	Upper Kettle Creek	42.8098	− 81.1782	—
R32	Upper Kettle Creek	42. 7846	− 81.2015	6.2
R29	Lower Kettle Creek	42.7779	− 81.2138	6.0
R22	Lower Kettle Creek	42.7484	− 81.2081	—
R20	Lower Kettle Creek	42.7361	− 81.2141	—
R14	Lower Kettle Creek	42.7221	− 81.2361	—
R6	Lower Kettle Creek	42.6927	− 81.2175	—
R2	Lower Kettle Creek	42.6751	− 81.2208	—
R0	Mouth	42.6644	− 81.2136	—
L1	Nearshore	42.6503	− 81.2114	8.5
L1-W	Nearshore	42.6455	− 81.2725	—
L1-E	Nearshore (plume)	42.6497	− 81.1503	—
L3	Nearshore	42.6325	− 81.2100	11.8
L5	Nearshore	42.6147	− 81.2089	—
L10	Offshore	42.5697	− 81.2025	—
L37	Offshore	41.9356	− 81.6539	24.3

Appendix B: Summary of Welch's t-tests results.

Table B.1. Results of Welch's t-tests comparing water quality in Kettle Creek and Lake Erie. Positive t-values indicate greater mean in river, negative t-values indicate greater mean in lake. df = degrees of freedom.

	Mean				
Variable	**River**	**Lake**	**t-value**	**df**	**p-value**
DOC (mg C l^{-1})	6.1	2.2	23.06	38.61	< 0.01
S_R	0.89	1.62	− 8.43	16.22	< 0.001
β:α	0.77	0.92	− 4.31	18.47	< 0.01
FI	1.46	1.47	0.46	12.77	0.65
Fmax/DOC (RU mg^{-1} l)	0.74	0.18	23.68	28.16	< 0.001
MP (µg P l^{-1})	1.7	0.5	4.17	31.92	< 0.001
N:P (mol)	10.09	35.60	− 3.75	11.03	< 0.01
DOC:DP	0.52	0.33	4.38	35.98	< 0.001
DOP:TDP	0.38	1.09	− 4.37	15.38	< 0.001

Table B.2. Results of Welchs t-tests comparing water quality in August and October of 2014-15. Positive t-values indicate greater mean in August, negative t-values indicate greater mean in October. df = degrees of freedom.

	Mean				
Variable	**August**	**October**	**t-value**	**df**	**p-value**
[a] PP (µg P l^{-1})	80.6	44.3	4.52	24	< 0.001
[b] Chl a (µg L^{-1})	23.6	3.8	3.46	12	< 0.01

[a] – River sites only

[b] – All site types

Table B.3. Results of Welch's t-tests comparing discharge and water quality in the Grand River and Detroit River, paired by week. Positive t-values indicate greater mean in the Grand River, negative t-values indicate greater mean in the Detroit River. df = degrees of freedom.

	Mean				
Variable	**Grand River**	**Detroit River**	**t-value**	**df**	**p-value**
Discharge (m^3 s^{-1})	57.73	5827.26	− 44.51	32	< 0.001
DOC (mg C l^{-1})	5.76	1.96	25.24	30	< 0.001
HIX	0.87	0.69	7.90	29	< 0.001
$SUVA_{254}$ (L mg^{-1} C m^{-1})	2.67	1.31	18.66	28	< 0.001
S_R	0.93	1.42	− 14.04	29	< 0.001
MP (µg P l^{-1})	2.00	1.17	5.26	29	< 0.001
DP (µg P l^{-1})	11.78	8.66	6.21	29	< 0.001

Appendix C: Composite index of DOM quality in Kettle Creek and Lake Erie.

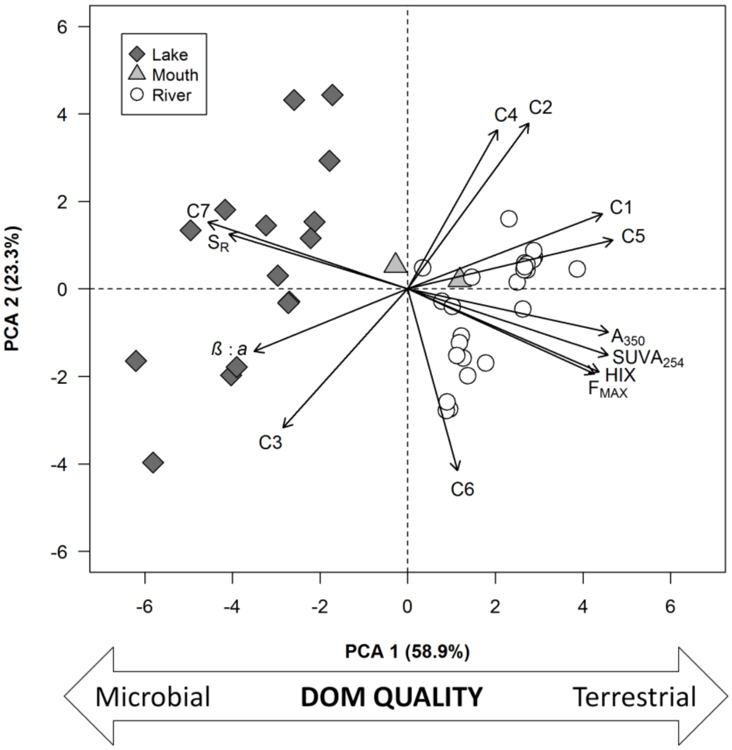

Figure C.1. Biplot for principal component analysis (PCA) of DOM quality indices and PARAFAC components, showing the first two PCA axes. Variable loadings are represented by arrows and individual measurements are indicated by points. PC1 corresponds to the composite index of DOM quality.

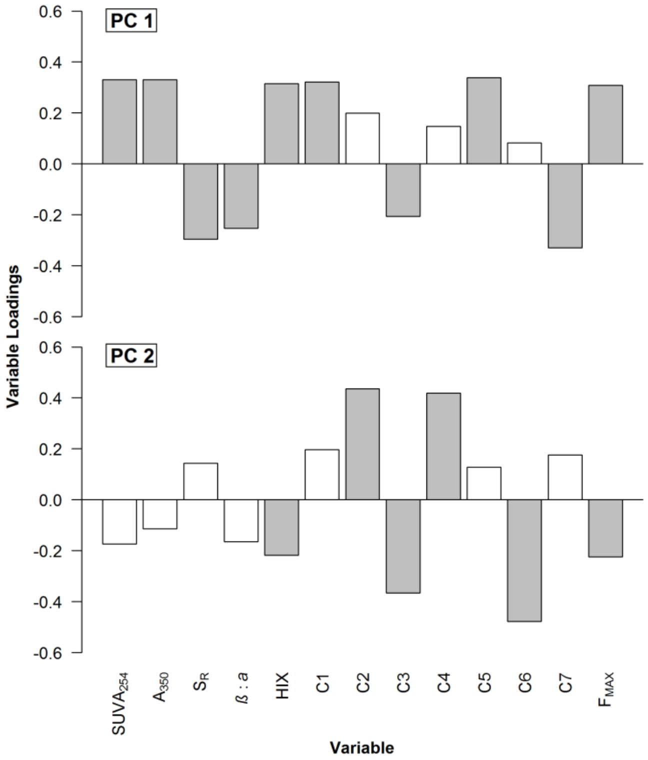

Figure C.2. Variable loadings for the first two axes of the Principal component analysis (PCA) of DOM quality indices and PARAFAC components. Variables with loadings > 0.2 are shaded in grey.

Appendix D: DOC and DOM across the river to lake continuum**.**

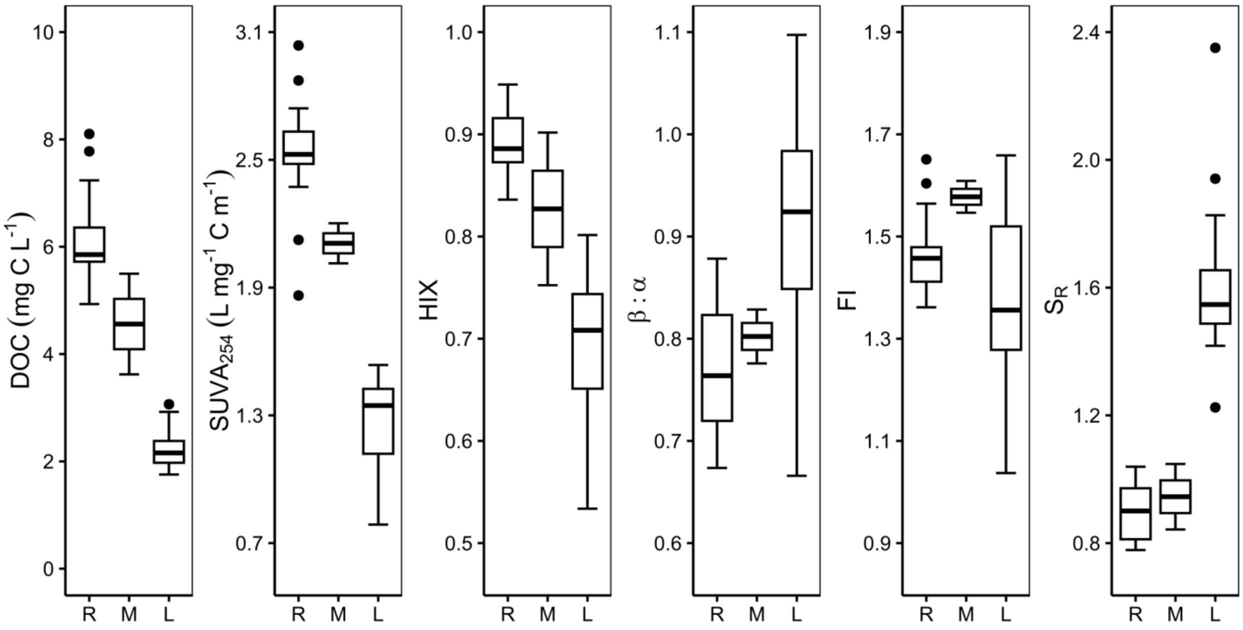

Figure D.1. Box and whisker plots showing concentrations of DOC, aromaticity (SUVA$_{254}$), humification index (HIX), freshness index (β:α; recent:decomposed DOM), fluorescence index (FI) and spectral slope ratio (S$_R$) for river (R), rivermouth (M) and lake (L) sites. The band indicates the median, lower and upper hinges indicate the first and third quartiles, and whiskers indicate the lowest and highest observations within 1.5 IQR (interquartile range) from the lower and upper quartiles. Points represent observations outside of 1.5 IQR.

Appendix E: LOADEST model diagnostic statistics and equations.

Table E.1. Diagnostic statistics for LOADEST load models, including annual maximum likelihood estimate regression R^2 (AMLE R^2), concentration regression R^2 (Conc R^2), Probability Plot Correlation Coefficient (PPCC), load bias (B$_p$) and Nash-Sutcliffe Efficiency Index (E). R^2 value of 100 indicates perfect correlation while value of 0 indicates no correlation. PPCC values above 0.8 indicate normally-distributed residuals. B$_p$ values indicate percentage overestimation (positive) and underestimation (negative) of modelled loads versus observed loads. E value of 1 indicates perfect model fit, value of 0 indicates that the model is no better than the mean of observed loads and negative values indicate observed loads are better than the model.

	Grand River				
	AMLE R^2 (%)	**Conc R^2 (%)**	**PPCC**	**B$_p$ (%)**	**E**
DIC	98.46	0.15	0.9692	2.91	0.98
DOC	98.82	43.75	0.9813	2.64	0.99
POC	84.54	36.26	0.9841	− 1.46	0.71
TP	93.35	72.94	0.9657	− 1.59	0.79
PP	92.17	63.75	0.9940	2.64	0.83
MP	56.46	31.65	0.9676	10.61	0.76
DP	93.86	24.59	0.9747	2.34	0.84

	Detroit River				
	AMLE R^2 (%)	**Conc R^2 (%)**	**PPCC**	**B$_p$ (%)**	**E**
DIC	42.68	17.15	0.9438	2.67	0.39
DOC	23.03	22.75	0.9700	2.53	0.18
POC	73.25	73.09	0.9951	2.21	0.59
TP	44.97	43.53	0.9932	1.56	0.43
PP	67.13	66.66	0.9879	2.71	0.56
MP	1.03	0.54	0.9473	2.80	0.01
DP	63.75	58.75	0.9893	2.75	0.64

Table E.2. Regression model equations for daily nutrient load estimation for the Grand River and Detroit River in 2015

Grand River	
DIC	$\ln(L) = 12.5858 + 0.0.9951 \ln Q$
DOC	$\ln(L) = 10.2700 + 1.1169 \ln Q - 0.1113 \sin(2\pi dtime) + 0.0645 \cos(2\pi dtime) + 0.6248\, dtime$
POC	$\ln(L) = 15.8957 + 1.3776 \ln Q + 0.0134 \sin(2\pi dtime) + 0.4269 \cos(2\pi dtime)$
TP	$\ln(L) = 12.8600 + 1.7941 \ln Q + 0.5743 \sin(2\pi dtime) + 0.4304 \cos(2\pi\, dtime) - 2.1034\, dtime$
PP	$\ln(L) = 12.0567 + 1.6141 \ln Q + 0.0771 \sin(2\pi dtime) + 0.4645 \cos(2\pi dtime)$
MP	$\ln(L) = 8.4138 + 0.8187 \ln Q + 1.1751 \ln Q^2$
DP	$\ln(L) = 10.9985 + 1.1710 \ln Q$
Detroit River	
DIC	$\ln(L) = 16.4426 + 2.0593 \ln Q + 0.0244 \sin(2\pi dtime) + 0.0667 \cos(2\pi dtime)$
DOC	$\ln(L) = 13.6854 + 1.9613 \ln Q + 0.6412 \sin(2\pi dtime) + 0.1438 \cos(2\pi dtime) - 2.5651\, dtime$
POC	$\ln(L) = 19.0482 + 7.1235 \ln Q + 0.1957 \sin(2\pi dtime) + 1.0941 \cos(2\pi dtime)$
TP	$\ln(L) = 15.8262 + 6.0553 \ln Q - 0.0512 \sin(2\pi dtime) + 0.7832 \cos(2\pi dtime)$
PP	$\ln(L) = 15.0174 + 7.1581 \ln Q + 0.1225 \sin(2\pi dtime) + 1.0358 \cos(2\pi dtime)$
MP	$\ln(L) = 13.2760 + 0.5819 \ln Q$
DP	$\ln(L) = 15.2852 + 1.2645 \ln Q - 0.6979 \sin(2\pi dtime) - 0.1144 \cos(2\pi dtime) + 2.1682\, dtime$

L = daily constituent load

$\ln Q$ = ln(streamflow) − center of ln(streamflow)

$dtime$ = decimal time − center of decimal time.

Appendix F: Grand River and Detroit River generalized additive model summaries.

Summary of generalized additive models between dissolved organic phosphorus and dissolved organic matter in the Grand River and Detroit River.

	GRAND RIVER					
	Variable	**edf**	**F-value**	**p-value**	**R^2**	**REML**
MP	Day	5.345	3.875	0.00547	0.481	42.605
	$SUVA_{254}$	1.000	4.690	0.04095		
DP						

	DETROIT RIVER					
	Variable	**edf**	**F-value**	**p-value**	**R^2**	**REML**
MP						
DP	Day	4.307	7.879	0.000237	0.695	51.63
	S_R	3.007	3.324	0.034997		

edf = estimated degrees of freedom

REML = restricted maximum likelihood