Inputs, source apportionment, and transboundary transport of pesticides and other polar organic contaminants along the lower Red River, Manitoba, Canada

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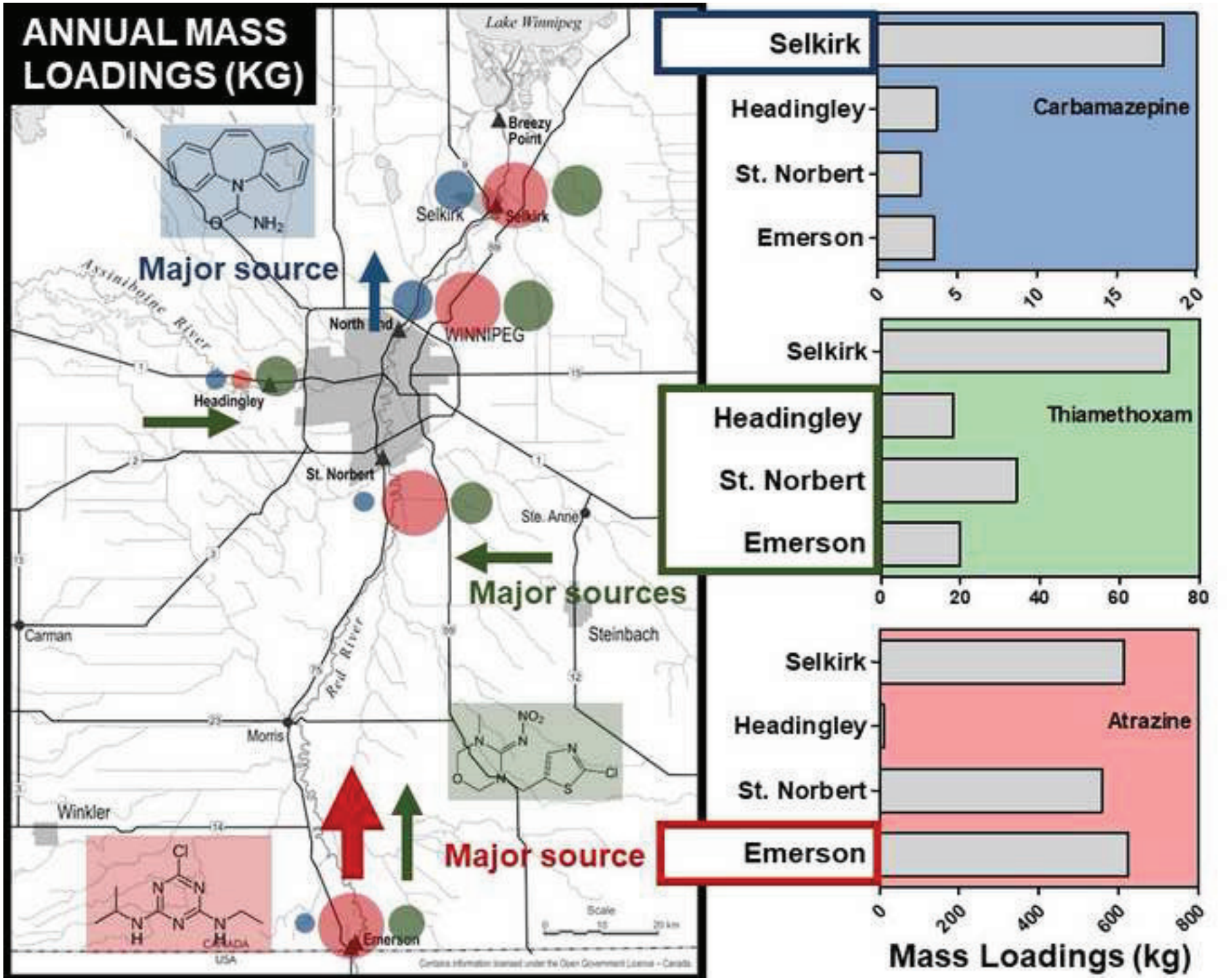
Graphical Abstract



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

- Source apportionment and transboundary flux of contaminants in Red River
- USA was the major source of atrazine into Canada, but not neonicotinoids
- Levels of atrazine and neonicotinoids pose minimal risk to aquatic organisms
- Pharmaceuticals found at elevated levels downstream of wastewater inputs
- PFAS and ARGs found throughout with no evidence of any significant point-sources

ABSTRACT

The Red River originates in the U.S., drains into Lake Winnipeg, and is a significant pathway for nutrients. We investigate its role as a source for pesticides, pharmaceuticals, per- and polyfluoroalkyl substances (PFASs) substances (PFASs), and microbes bearing antibiotic resistance genes (ARGs). We delineate agricultural, urban, and rural land-use for organic contaminants to determine the extent of chemical transboundary riverine fluxes, and characterize levels and trends of organic contaminants and ARGs between spring and fall 2014 and 2015. The herbicide atrazine peaked at over 500 ng/L (14-day time weighted average) near the border, indicating that the U.S. represents the major source into Canada from the Red River. Neonicotinoid insecticides had relatively constant concentrations, suggesting more widespread agricultural use in both countries. Pesticide concentrations were greatest post-application in June and July. Mass loadings of pesticides over the sampling periods, from the river to Lake Winnipeg, ranged from approximately 800 kg of atrazine, to 120 kg of thiamethoxam and clothianidin, to 40 kg of imidacloprid. Exposure distributions for atrazine exceeded benchmark water quality guidelines for protection of aquatic life (0.2% probability of exceeding chronic benchmark) with no exceedances for neonicotinoids. Seven pharmaceuticals were detected, mostly at low ng/L levels downstream of the City of Winnipeg wastewater treatment plant. Carbamazepine, the only pharmaceutical detected consistently at all sites, contributed on average 20 kg each year into Lake Winnipeg. While minor inputs were observed all along the river, city inputs represented the greatest source of pharmaceuticals to the river. Both PFASs and ARGs were observed consistently and ubiquitously, indicative of an anthropogenically-influenced system with no indications of any single point-source signature. While transboundary flux from the U.S. was an important source of pesticides to the Red River, especially for atrazine, observed concentrations of all measured contaminants suggest that known aquatic toxicological risk is minimal.

Keywords: pesticides, POCIS, exposure distribution, source apportionment, contaminant flux

1. INTRODUCTION

Lake Winnipeg is the tenth largest freshwater lake in the world, and the third largest in Canada by surface area (24,000 km^2), with a watershed spanning nearly one million square kilometers over four Canadian provinces and four American states (Environment Canada and Manitoba Water Stewardship, 2011). The Red River is the 3rd largest riverine input into Lake Winnipeg by volume, at approximately 16% of total inflow, and is the largest source of nutrients (Environment Canada and Manitoba Water Stewardship, 2011). From 1994-2001 the Red River contributed 46% of total nitrogen and 73% of total phosphorous to the Lake (Bourne et al., 2002), largely a result of the intensive agricultural land use and high propensity for flooding in the Red River Valley (McCullough et al., 2012). With nutrient run-off, pesticides typically co-occur as the processes that drive their movement into surface waters can be shared, such as rain events (Kewei et al., 2008). Furthermore, precipitation has increased significantly along the lower Red River valley and northeastern Winnipeg River watersheds over the last 20 years, increasing the potential for pesticide inputs via overland runoff. For example, in the 12 monitored tributaries of the Red River, from 1996-2005 runoff was 52–194% greater than the 1946–1995 mean (McCullough et al., 2012). These increases have not been observed throughout the entire Lake Winnipeg watershed, as small to negative changes in precipitation have been documented in the Saskatchewan River watershed and the southeastern half of the Winnipeg River watershed over this same timeframe (McCullough et al., 2012).

Wastewater represents the other major input possibly impacting water quality and contributing to contamination in the Red River. Winnipeg, the major urban centre along the lower Red River Valley, releases its treated wastewater into the Red River either directly (North and South End treatment plants) or indirectly (West End treatment plant) to a major tributary (Assiniboine River). Furthermore, many smaller tributaries in this watershed receive sewage lagoon inputs from rural communities throughout Manitoba (Carlson et al., 2013a; Anderson et

al., 2013; Anderson et al., 2015a). The increase in livestock production, use of synthetic fertilizer, and the frequency and intensity of spring flooding specifically for the Red River Valley, have all been major contributing factors to the rapid eutrophication observed in Lake Winnipeg over the last 20 years (Schindler et al., 2012). Given the concerns around water quality and health in Lake Winnipeg, nutrient loading in the lake's major tributaries has been well characterized (Environment Canada and Manitoba Water Stewardship, 2011; McCullough et al., 2012; Yates et al., 2012), but pesticides and other contaminants remained relatively uncharacterized in this system.

One unique and important aspect of this system is the fact that close to 70% of the contributing land area to the Red River watershed lies in the U.S., making source apportionment and land-use management issues highly complex and important to understand. It was for these reasons that the International Joint Commission was formed and the Great Lakes Water Quality Agreement (GLWQA) was negotiated between Canada and the U.S. (Gilbertson et al., 1998). As a result, many of the pressing transboundary contaminant issues facing the Great Lakes were prioritized (Nisbet 1998). Analogous transboundary and jurisdictional water security issues exist within the Red River-Lake Winnipeg system, yet much less work has been done to characterize and understand these problems. To date, there remains a paucity of data regarding the sources, loadings, and fate of organic contaminants in this watershed. Given known inputs and land-use patterns along the Red River Valley, the presence of pesticides (Rawn et al.,1999; Szocs et al., 2017), pharmaceuticals (Carlson et al., 2013a; Kolpin et al., 2002), and per- and polyfluoroalkyl substances (PFASs) (Scott et al., 2009; Hu et al., 2016) is highly likely based on previous observations here and elsewhere. Pesticides and pharmaceuticals represent the two major inputs (agricultural and municipal waste) into the Red River. Alternatively, PFASs, known to be highly persistent in the environment, are used in a wide variety of industrial and consumer-use products (e.g., cosmetics, firefighting foams, stain-repellent textiles) and thus have

potentially varied inputs, including wastewaters, landfill leachate, and industrial waste (Wang et al., 2017), making their spatial occurrence throughout the Red River less predictable. Studying their major sources, levels, and temporal trends will help estimate risk (if any) posed to aquatic organisms in the Red River and characterize loadings to Lake Winnipeg. Furthermore, this work will help delineate contaminant sources on both sides of the border, and provide important context around contaminant use and land-management in this watershed. In addition to these chemical contaminants, the presence of antibiotic resistance genes (ARGs), a form of bio-pollution, has become a concern due to the widespread occurrence of human and veterinary antibacterial products in natural waters (Kummerer 2009a). As a result, genes encoding for resistance to a variety of antibiotics have become ubiquitous in aquatic environments impacted by human activity (Anderson et al., 2013; Kummerer 2009b) and could act as a vector by which resistance is spread throughout the population, leading to public human health concerns (Huijbers et al., 2015).

Levels of organic contaminants in impacted waters are heavily influenced by the stochastic and often unknown nature of their inputs, which include precipitation and run-off events, wastewater release, and pesticide applications (Carlson et al., 2013a). As a result, accurate characterization through time and space can be challenging. To capture these types of inputs in a natural system high frequency sampling must be conducted either manually (grab sampling) or through automation (auto-sampling), both of which have significant drawbacks and limitations (Vrana et al., 2005). Passive sampling is an alternative method that allows for the continuous *in-situ* monitoring of contaminants without the need of a power source (Miege et al., 2015). The polar organic chemical integrative sampler (POCIS), used here, is the most widely used passive sampling tool for measuring pesticides, pharmaceuticals, and other polar organics in water (Harman et al., 2012; Morin et al., 2012).

115 This study had three primary objectives. First, to characterize the concentrations and
116 fluxes of pesticides, pharmaceuticals, and PFASs and levels of ARGs in the Red River.
117 Second, to determine contributions of these contaminants from the United States, southern
118 Manitoba, and the City of Winnipeg. Finally, to estimate possible risks to aquatic organisms in
119 this system resulting from exposure to these contaminants. Our hypotheses are twofold. First,
120 the presence of pharmaceuticals, PFASs, and ARGs will be indicative of a river system
121 influenced by municipal inputs, with elevated levels observed downstream of the City of
122 Winnipeg. Second, pesticide detections will be a function of both application intensity (e.g.,
123 usage) and runoff events (e.g., timing and intensity of precipitation) in the drainage basin.
124 Specifically, in terms of usage, loadings of atrazine from the Red River will be greater than
125 observed in the Assiniboine River given the intensity of corn cropping and atrazine application in
126 the U.S. Likewise, in terms of runoff, neonicotinoid insecticides, in the Red River will be greater
127 than observed in the Assiniboine River given the more intense and frequent precipitation events
128 (e.g., runoff) characteristic of the lower Red River valley.

129 This work represents the only published data to date describing many of these organic
130 contaminants and ARGs in the Red River to our knowledge, and serves as a baseline for
131 estimating risks and informing management and monitoring around these contaminants.

132 **2. METHODS**

133 **2.1 Sampling sites**

134 Sampling was performed continuously between April and October of 2014 and 2015. With the
135 exception of two extended deployments discussed later, sampling times generally ranged
136 between 14 and 21 days, largely chosen opportunistically based on availability of field personnel
137 and to avoid inclement weather. A major focus of this work is pesticide inputs, which, from
138 November to March are expected to be insignificant compared to that of the growing season.
139 Samplers were not deployed during spring ice melt, as ice and debris in the river at that time

140 can destroy samplers. A total of six sites were sampled: five along the Red River and one on the
141 Assiniboine River (Figure 1). Emerson is a border town and therefore integrates all net inputs
142 coming directly from the United States. The St. Norbert site is south of the city perimeter,
143 upstream of the South End sewage treatment plant and immediately downstream to the St.
144 Norbert floodway diversion. The North End site is downstream of the North End Wastewater
145 Treatment plant (WWTP) and processes approximately 70% of Winnipeg's wastewater.
146 Downstream from the small town of Selkirk and upstream of Lake Winnipeg, both Selkirk and
147 Breezy Point sites respectively, are more removed from known point and non-point sources of
148 pollution and should represent near-final inputs into Lake Winnipeg from the Red River. The
149 Assiniboine River is the largest tributary to the Red River and integrates inputs at Headingley
150 from western Manitoba and eastern Saskatchewan. Small changes to site selection over the
151 two-year study were necessary due to flooding, vandalism, or other logistical factors; however,
152 sites remained in the same general vicinity. Both the Red and Assiniboine Rivers represent
153 watersheds in agricultural intensive regions within Manitoba and for the former, into the U.S.
154 Specific land usage patterns are discussed in section 3.1.

155 Samplers were deployed in triplicate on stainless steel spindles inside stainless steel
156 protective cages (30 cm high $\times$ 16 cm wide) (Environmental Sampling Technologies, St. Joseph,
157 MO) as done elsewhere (Carlson et al., 2013a). Cages were secured to riverbanks using 3/16-
158 inch stainless steel aircraft cable looped around trees. Cages were deployed on the east bank
159 at Emerson, St. Norbert, and Selkirk, the west bank at Breezy Point, and the south bank at
160 Headingley. Major flooding in 2014 compromised or destroyed some samplers, as detailed in
161 Supplementary Information (SI). Eight out of 66 total deployed POCIS sets were destroyed in
162 2014, with 11 out of 138 POCIS lost over the entire two-year study. We do not expect this
163 missing data to have any significant effect on calculated mass loadings or interpretation of
164 contaminant sources or trends given the large data set taken over the two-year period.

2.2 Target chemicals

A total of 32 contaminants were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS): 6 pesticides, 17 pharmaceuticals, and 9 PFASs. The pesticides were: 3 neonicotinoids (imidacloprid, thiamethoxam, and clothianidin); 2 organophosphates (chlorpyrifos and diazinon); and 1 triazine (atrazine). The pharmaceuticals were: 6 sulfonamide antibiotics (sulfapyridine, sulfamethoxazole, sulfisoxazole, sulfamethazine, sulfachloropyridazine, and sulfadimethoxine); 3 macrolide antibiotics (erythromycin, clarithromycin, and roxithromycin); 1 fluoroquinolone antibiotic (enrofloxacin); the antibiotic trimethoprim; 3 beta-blockers (atenolol, metoprolol, and propranolol); 2 selective serotonin reuptake inhibitors (paroxetine and fluoxetine); and 1 sodium channel blocker (carbamazepine). These compounds were measured in both study years. PFASs were measured in 2014 only and included perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS).

2.3 Passive samplers

2.3.1 Polar organic chemical integrative sampler.

POCIS were constructed using 200 mg of Waters OASIS™ HLB (hydrophilic-lipophilic balance) material between polyethersulfone (PES) membranes (0.1 μm pore size) (Environmental Sampling Technologies, St. Joseph, MO). Samplers were secured with two stainless steel rings to allow an exposed total membrane surface area of 41 cm^2. POCIS were stored at -20°C in ashed aluminum foil until use. Twenty-four hours prior to deployment, samplers were soaked in Milli-Q water (18 MΩ cm, Millipore Corporation, Billerica, MA) and stored at 4°C in prewashed plastic sandwich containers until deployment.

189 Pharmaceuticals and pesticides were extracted from organic POCIS according to
190 Carlson et al. (2013b). Upon retrieval, POCIS were rinsed with Milli-Q water, wrapped in ashed
191 aluminum foil, and stored at -20°C until extraction. Extraction was generally performed within
192 two weeks of retrieving the POCIS, during which time loss of compound was deemed
193 insignificant (see Carlson et al., 2013b; Challis et al., 2018). Before extracting, POCIS were
194 soaked in Milli-Q water for 10 minutes. Sorbent was rinsed into a 60 mL glass clean-up column
195 containing 3-5 g anhydrous sodium sulphate (Fisher Scientific, Ottawa, ON) using 30-40 mL of
196 methanol. In this rinsing process, the PES membranes were simultaneously extracted into the
197 column. Internal standard mixture (50 ng) was spiked directly onto the column along with the
198 sorbent from each POCIS sample. The extract was gravity filtered through the column, collected
199 in a 100 mL round bottom flask, rotary evaporated to ~2 mL at 40°C, and then fully evaporated
200 to dryness under nitrogen gas at 40°C. Extracts were reconstituted with 1mL of 50:50 methanol:
201 Milli-Q water and syringe filtered through 0.22 μm polytetrafluoroethylene filters
202 (Chromatographic Specialties, Brockville, ON). Extracts were stored in glass auto-sample vials
203 at 4°C until analysis using liquid chromatography coupled with tandem mass spectrometry (LC-
204 MS/MS).

205 ***2.3.2 PFAS POCIS.***

206 Adapted POCIS samplers for PFAS were prepared using methods developed by Kaserzon et al.
207 (2012). PES membranes with 0.45 μm pore size (Pall Corporation, Ann Arbor, MI) were
208 preconditioned by submerging them in HPLC grade methanol (Fisher Scientific, Ottawa, ON) for
209 20 minutes, followed by Milli-Q water for 10 minutes, according to procedures outlined in
210 Kaserzon et al. (2012). To maintain a 0.0375 g sorbent/cm^2 exposed surface area ratio with 41
211 cm^2 POCIS, 1.5375g of Sepra ZT-WAX sorbent (Phenomenex, Torrance, CA) was used.
212 Maintaining a constant sorbent-to-surface area ratio allowed the use of previously determined
213 sampling rates (Kaserzon et al., 2012). Samplers were secured using stainless steel disks and

214 stored at -20°C in ashed aluminum foil until 24 hours prior to deployment, when they were
215 conditioned for ten minutes in each of 0.1% ammonia in methanol (Fisher Scientific, Ottawa,
216 ON), methanol, and Milli-Q water. POCIS were stored in prewashed Teflon-free containers
217 containing Milli-Q water until deployment.

218 PFAS were extracted from POCIS using a method adapted from Kaserzon et al. (2012).
219 After retrieval, PFAS POCIS were rinsed with Milli-Q water, wrapped in ashed aluminum foil,
220 and stored at -20°C until extraction. Prior to extraction, POCIS were soaked in Milli-Q water for
221 10 minutes. A 70 mL plastic pre-cleaned SPE cartridge (Chromatographic Specialties,
222 Brockville, ON), containing ~2 g anhydrous sodium sulphate and a Grade 413 filter paper
223 (VWR, Mississauga, ON), was pre-rinsed with 15 mL methanol. Sorbent was rinsed from the
224 membranes into the SPE cartridge using 15 mL of 0.1% ammonia in methanol, followed by
225 15mL methanol. Internal standard mix was spiked into each sample along with the sorbent.
226 Extracts were collected in a 50 mL round bottom flask and reduced to ~2 mL using rotary
227 evaporation at 40°C. After evaporation to dryness under a stream of nitrogen gas at 40°C,
228 extracts were reconstituted to 1 mL with 50:50 methanol:Milli-Q water, and syringe filtered
229 through 0.20 μm nylon filters (Chromatographic Specialties, Brockville, ON). Extracts were
230 stored in polypropylene auto-sample vials at 4°C until analysis.

231 **2.4 Antibiotic resistance genes**

232 Protocols for sampling of antibiotic resistance genes (ARGs) followed Anderson et al. (2013).
233 Briefly, 500 mL sterilized polypropylene bottles (Chromatographic Specialties Inc., Brockville,
234 ON) were used for ARG grab samples, collected in 2014 only. Each ARG water sample was
235 filtered using a sterile, disposable Nalgene cup with a pre-installed 0.2 μm filter (Thermo Fisher
236 Scientific Inc., Waltham, MA). The filter was removed using flame-sterilized forceps, folded, and
237 placed into a 1.5 mL sterile polypropylene centrifuge tube. The centrifuge tube was stored
238 frozen and shipped on ice to the University of Strathclyde (Glasgow, UK), where they were

immediately stored at -80°C until further processing. Filters were sterilely quartered and transferred to screw-top centrifuge tubes; DNA were extracted were extracted via MoBio PowerSoil DNA extraction kits (Qiagen, Inc.) according to manufacturer's instructions. Eluted DNA were diluted 1:100 to minimise the presence of PCR inhibitors.

Community DNA were analysed for the presence of 13 tetracycline and 3 sulfonamide resistance determinants via qPCR (iCycler5, BioRad). Tetracycline multiplex assays were based on primers by Ng et al. (2001) and targeted genes associated with efflux pumps (*tet*-A, -B, -C, -D, -E, -G, -K, and –L) and ribosome protection proteins (*tet*-M, -O, -Q and –S) and enzyme deactivation (*tet*X). A high-resolution temperature melt curve (Fan et al., 2007) discerned individual responses for each gene; peak areas were compared to standards (10^1 to 10^6 copies per μL; Peak et al., 2007; McCluskey and Knapp, 2017) for quantification. Sulfonamide resistance genes (*sul*1, *sul*2, and *sul*3; Pei et al., 2006 and Chen et al., 2015) and 16S-rRNA (Caporaso et al., 2011), which represent a surrogate measure of total bacteria, were similarly quantified, but as individual assays. Details around PCR methodology can be found in SI.

2.5 Instrumental Analysis

2.5.1 Analysis of Pharmaceuticals and Pesticides.

Pharmaceutical and pesticide concentrations were determined using an Agilent 6410B LC-MS/MS system (Agilent Technologies, Mississauga, ON). LC mobile phases were 95:5 H_2O:methanol (solvent A) and methanol (solvent B), each containing 0.05% formic acid (Fisher Scientific, Ottawa, ON). Chromatographic separations were achieved using an Agilent Eclipse Plus C18 column (2.1 × 50mm × 1.8μm particle size) (Agilent Technologies, Mississauga, ON) with a Phenomenex HPLC SecurityGuard C18 Guard Cartridge (4 × 3mm) (Phenomenex, Torrance, CA) at 42°C and a flow rate of 0.45 mL/min. Further details of the analytical method, including the LC gradient elution method, source parameters, MRM transitions, and limits of

detection are in Table S1.

2.5.2 Analysis of PFASs.

Concentrations of 9 PFAS were determined using LC-MS/MS. LC mobile phases contained 95:5 H_2O:methanol (solvent A) and 90:10 acetonitrile:H_2O (solvent B), each containing 2 mM ammonium acetate (Sigma-Aldrich, St. Louis, MO). The guard and analytical column setup was the same one used for the pharmaceutical and pesticide method with a temperature and flow-rate of 40°C and 0.5 mL/min, respectively. An Agilent Eclipse Plus C18 column (4.6 × 30mm × 3.5μm particle size) (Agilent Technologies, Mississauga, ON) was attached at the outflow from the aqueous pump head (solvent A) and used as a PFAS trap. Further details of the analytical method, including the LC gradient elution method, source parameters, MRM transitions, and limits of detection are in Table S2.

2.6 Data Analysis

2.6.1 Instrumental analysis.

Agilent MassHunter Workstation Software Quantitative Analysis (Version B.04.01) was used to analyze all LC-MS/MS data. Calibration curve linearity was >0.98 for all analytes and a tolerance ±20% was deemed acceptable for accuracy of individual calibration standards and quantifier:qualifier MRM transition ratios.

2.6.2 Passive sampling data.

Time-weighted average (TWA) concentrations were calculated using POCIS sampling rates specific to each analyte (Table S3). Tables S4A-E, S5, and S6A-H in SI provide the raw data for the target analytes detected in this study, and report the mass of analyte on POCIS for individual samples (M_{POCIS}, mass in ng). The reported TWA concentration (C_{TWA}, ng/L) represents the mean of triplicate POCIS deployed over time (t), calculated using equation 1, where R_S (L/d) is the compound specific POCIS sampling rate:

$$C_{TWA} = \frac{M_{POCIS}}{R_s\, t} \qquad \text{eq. 1}$$

Given that POCIS sampling rates are sensitive to water flow (Harman et al., 2012), it is important to acknowledge the inherent uncertainties (Poulier et al., 2014) associated with POCIS in dynamic and variable flowing system as the Red River. Chemical fluxes (kg/d) were calculated at sites that had Environment Canada gauging stations (Emerson, St. Norbert, Selkirk, Headingley). Daily discharge volumes were obtained from Environment Canada Water Level and Flow website (https://wateroffice.ec.gc.ca/). The calculation of flux data assumed homogenous concentrations at the cross-sectional area of the river where the gauging stations/POCIS samplers were located. Prism v. 5.01 (GraphPad Software, LaJolla, CA) was used for statistical analysis. Specific tests are stated with the specific data set to which they were applied. Significant differences were defined as $p < 0.05$. Errors are presented as standard deviations of the mean, unless otherwise stated.

2.6.3 Exposure distributions.

Exposure distributions were constructed for the four pesticides detected consistently across sampling sites and times, over the two years (raw data in Table S4A-E). Atrazine, thiamethoxam, clothianidin, and imidacloprid concentrations in the Red River at all sites and in the Assiniboine River at Headingley were used to generate exposure distributions for each River. Data were plotted on a logarithmic-probability scale using the Weibull ranking equation (Solomon et al. 2000).

2.6.4 ARG data.

Abundances of genes are presented as log-transformed values; differences between sites was tested using a Kruskal-Wallis test by ranks ($P<0.05$).

2.6.5 Quality Assurance/Quality Control (QA/QC).

310 POCIS lab and field blanks were extracted and analyzed alongside each set of environmental
311 samples. Field blanks were taken to each sampling site and left open to the atmosphere while
312 retrieval and deployment of the POCIS took place. For all analytes reported in this study, levels
313 observed in lab and field blanks were negligible with the exception of PFUnDA, which was
314 detected at elevated levels in all samples, and thus was not considered in this study.

315 **3. RESULTS AND DISCUSSION**

316 The following sections will describe the major findings and implications of this work, organized
317 by contaminant class: pesticides, pharmaceuticals, PFASs, and ARGs, in order. The initial
318 section below summarizes the hydrology and land-use descriptors of the Red River valley,
319 information that is draw upon in the proceeding discussions to help contextualize the observed
320 levels, fluxes, and sources of each contaminant.

321 **3.1 Watershed and Land Use Descriptors**

322 Runoff from treated agricultural fields likely represents the major input of the four pesticides
323 detected in this study. Soil type, topography, and seasonal climate conditions to a large extent
324 control the movement of these pesticides into the Red River (Rawn et al., 1999). The Red River
325 has an effective drainage area just shy of 150,000 km^2, 74% of which is farmed (65% cropland,
326 9% pasture) (Yates et al., 2012; McCullough et al., 2012). Much of this farmland is concentrated
327 along a stretch of low relief terrain characterized by underlying sediments with very low
328 permeability (McCullough et al., 2012). Precipitation and melt events often exceed soil
329 infiltration capacity, leading to a high frequency of seasonal overland flooding and runoff events
330 within the Red River watershed, resulting in significant losses of field applied pesticides (Rawn
331 et al., 1999). Our study sites in Manitoba spanned approximately 250 km from the U.S./Canada
332 border at Emerson to Breezy Point, near the mouth at Lake Winnipeg. On the U.S. side, our
333 sampling at Emerson integrates over 600 km of largely agricultural inputs along the river, from
334 the head waters of the Red River on the North Dakota – Minnesota border at the northern edge

of the Midwestern Corn Belt, to the Canadian border, allowing net inputs from the U.S. to be characterized. The Emerson sampling site on the east bank of the Red River is downstream of two small sewage lagoon releases (on the opposite west bank) from both the U.S. (Pembina) and Canadian (Emerson) border towns, both with combined populations of approximately 1,000. The Assiniboine River originates in Eastern Saskatchewan and flows into the Red River at The Forks in downtown Winnipeg, Manitoba. The Assiniboine River has a drainage basin of approximately 60,000 km^2, which is also predominately agricultural-use (Rawn et al., 1999).

Canada does not have a readily available inventory of regional pesticide-use data, making estimations of annual application volumes difficult to ascertain. Crops in southern Manitoba are primarily grains and cereals. Canola is also a major crop, and corn is grown but in a very limited capacity (Agriculture Manitoba summary statistics, 2016). Atrazine use in Manitoba in the late 1990s was estimated to be around 12,000 kg annually (Rawn et al., 1999). In 2006 an estimated 25,000 kg of atrazine was applied across 17,000 hectares (Wilson, 2012). In contrast, approximately 135,000 kg of atrazine was applied annually in the late 1990s within the U.S. area of the Red River watershed alone (Rawn et al., 1999). Based on raw data from the USGS National Water-Quality Assessment Project (https://water.usgs.gov/nawqa/pnsp/usage/) an estimated 800,000 kg per year were applied on average in North Dakota and Minnesota from 2006-2015. While only a portion of this land area lies in the Red River drainage basin (Fig. 1) these U.S. inputs likely represent the major source of atrazine to the Red River. Neonicotinoids on the other hand are used extensively in southern Manitoba to support canola, soy bean, and grain crops. Canola represented the single largest crop by seeded area in Manitoba at over 1.2 million hectares (Manitoba Agriculture, https://www.gov.mb.ca/agriculture). The most common application of neonicotinoids is seed coatings, and given that 95% of canola grown in Canada is treated with a neonicotinoid active ingredient (Main et al., 2014), we expect extensive inputs of these pesticides from southern

Manitoba. On the U.S. side of the Red River drainage basin (North Dakota, South Dakota, and Minnesota), neonicotinoids are also extensively used. Average annual use across all three states over the 5-year period between 2010 and 2014 was 29000 kg of imidacloprid, 28000 kg of thiamethoxam, and 79000 kg of clothianidin (USGS National Water-Quality Assessment Project, https://water.usgs.gov/nawqa/pnsp/usage/).

Hydrographs shown in Figure 2 describe the daily discharge from the Red River (Emerson, St. Norbert, Selkirk) and Assiniboine River (Headingley) between April and October over the two-year study. Full ice-coverage, normally from December to March, means that many of the hydrological gauging stations are not operational in that time. Both rivers generally see a spike in discharge in spring (usually April) corresponding to snow melt. However, the intensity of this spike depends upon the amount of precipitation in the watershed over the winter, the soil saturation conditions, and the rate of melt. An early melt in 2015 (March) explains the reduced discharge in April and May in the Red River. A second spike in river discharge generally occurs in June/July due to heavy precipitation events characteristic of southern Manitoba at that time. This is observed on both rivers in 2014 and 2015. However, overall it is evident that the Red and Assiniboine River's experienced much greater flows in 2014 compared to 2015. Comparing the mean and total discharge values (Table 1) over each year, 2014 was approximately double that of 2015. In fact, on occasion high water levels in 2014 led to lost samplers or delays in retrieval (details in SI).

3.2 Pesticides

3.2.1 Pesticide concentrations and occurrence.

Of the six pesticides analyzed for in this work, four were measured at detectable levels consistently across sampling year, season, and site. Atrazine, thiamethoxam, and clothianidin were detected in 100% (n=127) of all collected samples, and imidacloprid was detected in >90% (n=116 of 127) of all samples (Fig. 3). Chlorpyrifos and diazinon were not detected in this study

by the methods employed. Table 2 details the mean, median, and maximum environmental concentrations of the four pesticides measured in the Red River (complete data set in SI, Table S4). It is important to note that the concentrations reported here represent time-weighted averages over a given deployment time, typically 14-21d in this study. In the case of extended deployment times caused by flooding in 2014 (42 and 59 days, Table S4), if saturation of the POCIS sorbent occurred during this time analyte concentrations may be underestimated, however this is unlikely given the large capacity and thus linear uptake range generally observed for POCIS (Harman et al., 2012). Regardless, while maximums reported here are lower than if the same concentration spikes had been captured with grab samples, TWA concentration data provides better context around longer-term, chronic exposure scenarios in these systems, a fundamental tenant of many passive samplers (Harman et al., 2012; Poulier et al., 2014; Booij et al., 2016).

The maximums for the three neonicotinoid insecticides thiamethoxam, clothianidin, and imidacloprid in the Red River ranged from 14.1-31.7 ng/L with mean concentrations over the two-year study <8 ng/L (Table 2). On the Assiniboine River at Headingley, mean concentrations of the three neonicotinoids ranged from 0.9-4.4 ng/L. In general, these average concentrations agree with other neonicotinoid occurrence data that generally falls in the low ng/L range, discussed below. However, in a Canadian context, much of the neonicotinoid measurements have been taken with grab samples in small streams, rivers, and wetlands, often representing low-dilution scenarios (Anderson et al., 2015b), making direct comparisons to this study difficult.

Main et al. (2014) measured water concentrations of thiamethoxam, clothianidin, and imidacloprid in 136 pothole wetlands adjacent to grasslands and agricultural fields in central Saskatchewan. Average detection frequencies ranged from 16-91% and mean total (sum of four active ingredients) neonicotinoid concentrations ranged from 4.0-76.8 ng/L, both varying with season, year, and crop type (Main et al., 2014). Struger et al. (2017) studied fifteen streams and

410 rivers in southern Ontario, nine of which had drainage areas <100 km^2 and were in agricultural
411 areas (for context, the drainage area of the Red River is close to 150,000 km^2). Thiamethoxam,
412 clothianidin, and imidacloprid exhibited detection frequencies above 90% at over half the sites
413 sampled over the three-year study (2012-14), with mean concentrations across all 15 sites
414 ranging from 0.21-113 ng/L. In the U.S., similar levels of neonicotinoids are observed as in
415 Canada. Hladik and Kolpin (2015) conducted a nationwide study of 38 U.S. streams between
416 2012 and 2014 and reported a summed (five active ingredients, detections dominated by
417 thiamethoxam, clothianidin, and imidacloprid) neonicotinoid mean concentration of
418 approximately 5 ng/L. Van Metre et al. (2017) used POCIS to monitor pesticides in 100 small
419 streams across the U.S. Midwest and observed a maximum, mean, and median of 275 ng/L,
420 19.3 ng/L, and 4.0 ng/L, respectively for imidacloprid (the single neonicotinoid reported in the
421 study). Year-round grab samples taken between 2015 and 2016 in ten major Great Lake
422 tributaries showed median concentrations of neonicotinoids ranging from non-detect to 10 ng/L
423 with a maximum single measurement of 230 ng/L (Hladik et al., 2018). These values are similar
424 to mean neonicotinoid concentrations observed here in the Red River system.

425 The maximum, mean, and median concentration of atrazine in the Red River over the
426 two-year study was 520, 63.0, and 24.3 ng/L (Fig. 3, Table S4A). Concentrations in the
427 Assiniboine River at Headingley were much lower (maximum = 10.2 ng/L; mean = 3.4 ng/L;
428 median = 2.7 ng/L). This is unsurprising given the limited use of the pesticide in Manitoba
429 (Rawn et al., 1999) and of the amount of corn grown in this part of the system, relative to the
430 Red River proper part of the basin. Atrazine concentrations were reported by Rawn et al. (1999)
431 in their assessment of pesticide loadings in the Red River and its tributaries over a three-year
432 survey from 1993-1995 (Table 3). Concentrations of atrazine measured in the current study
433 were greater (2-3 fold) compared to those reported in 1993-95. Aside from annual and
434 seasonal variability in contaminant levels, fundamental differences between the data sets are

435 expected given that Rawn et al. (1999) used grab samples taken every 2-3 weeks over the
436 study period to characterize atrazine levels compared to our use of passive samplers which
437 provided continuous *in-situ* time-weighted average concentrations.

438 Spatial-temporal trends of each pesticide can be observed in Figure 3 and S1. Atrazine
439 concentrations spiked in early June corresponding with typical field-applications in May. Nowell
440 et al. (2018) observed similar trends in their study of a suite of pesticides in 100 streams
441 throughout the Midwestern U.S. Corn Belt. They detected atrazine at concentrations >100 ng/L
442 at approximately 65% of agricultural impacted sites with peak concentrations observed between
443 May and June, corresponding to major application and runoff events (Nowell et al., 2018). While
444 the major source of atrazine is expected to be coming from use in the U.S., there is not a clear
445 pattern of spatial attenuation moving downstream along the Red River from Emerson to Breezy
446 Point, as indicated by relatively constant mass loading in the Red River (Fig. 4) despite small
447 decreases in concentration with distance downstream from Emerson (Fig. 3). This is likely due
448 to the persistence of atrazine in aquatic systems, with half-lives ranging from 30 to >200d,
449 depending largely on pH conditions and organic matter content of the water (Solomon et al.,
450 1996). Given the relatively recalcitrant nature of the compound, residence times in the Red
451 River are likely too short for degradation processes to be appreciable in the river itself (Schottler
452 et al., 1994). An approximate residence time of 30 days from river source to mouth (885 km)
453 was calculated assuming a mean annual flow of 176 m^3/s, depth of 4 m, and width of 130 m
454 (Kimiaghalam et al., 2015). This equates to a residence time of approximately 7 days between
455 Emerson and Selkirk (218 km). As a result, levels of atrazine in receiving waters can be highly
456 dependent on dilution and hydraulic residence time. In fact, these factors are likely responsible
457 for the temporal attenuation observed here for atrazine, as concentrations decreased following
458 the spikes in June, through to the end of each sampling season in October. Background
459 atrazine levels can be inferred from the beginning (April) and end (September-October) of each

460 sampling year, appearing to reach a steady-state concentration in the range of 1-20 ng/L (Table
461 S4A). Negligible levels (<5 ng/L, average) of atrazine were observed on the Assiniboine River at
462 Headingley, suggesting that usage and thus sources from western Manitoba are minor in
463 comparison to overall contributions to the Red River and ultimately Lake Winnipeg.

464 The three neonicotinoids exhibited very similar concentration profiles across sampling
465 site and time. The spike in neonicotinoid concentrations in May/June was less pronounced than
466 for atrazine. Concentrations between approximately May-August appear to be more consistent
467 across space and time (especially in 2015), including on the Assiniboine River at Headingley.
468 This is indicative of multiple diffuse sources of neonicotinoids all along the Red River Valley and
469 from western Manitoba, which is consistent with known usage throughout the Prairie Provinces
470 (Main et al., 2014).

471 ***3.2.2 Pesticide fluxes and mass loadings.***

472 Daily fluxes (kg/d) were calculated by multiplying measured concentrations by the daily water
473 discharge as shown in Fig. 2. TWA water concentrations measured by POCIS (Table S4A-E)
474 were assumed to be representative of the entire cross-sectional area of the water column at
475 each site. The annual mass loadings presented in Fig. 4 were calculated by summing the daily
476 flux values over the continuous study periods in 2014 (May 28 – October 28; 154d) and 2015
477 (April 29 – October 28; 183d). Absolute errors in flux values are difficult to estimate given that
478 uncertainties in discharge data are not known. However, from the variation in replicate POCIS
479 measurements, we can estimate the uncertainties associated with reported pesticide loadings in
480 Fig. 4. Using the concentration data (mean ± standard deviation) reported in Table S4A-E, the
481 average relative standard deviation for all five compounds in all sampler over the entire study
482 (n=627) is 29%. As an approximation, a 30% relative error was applied to the annual mass
483 loadings. However, there is potential for a large degree of unknown uncertainty with these mass
484 loading calculations as concentrations are measured near the banks of the river, and the extent

485 of transverse mixing in the Red River is not well characterized. While we expect concentrations
486 to be well-mixed, and thus, representative (given the often turbulent flows) the extent of mixing
487 depends also upon the proximity of the POCIS sampler to the input source, which for diffuse
488 agricultural inputs are near impossible to define.

489 While the data in Fig. 4 only represent approximately half of the calendar year,
490 contributions to mass loadings during the fall and winter months are expected to be minimal
491 given reduced water flow, pesticide use, and pesticide inputs via processes such as runoff.
492 Assuming the concentration and water flow remain constant from the final day of each study
493 period (October 28th) to around March of the following year, total mass loadings of atrazine at
494 Emerson, for example, over the remaining 211 days in 2014 and 182 days in 2015, would
495 account for only 4% and 2% of annual loadings respectively. These likely represent
496 conservative estimates given that flows in December, January, and February commonly drop
497 below 30 m^3/s, compared to October and November flows that are typically closer to 60 m^3/s.

498 In 2014 annual atrazine mass loadings were approximately 2-to 3-fold greater than in
499 2015. The mass of atrazine in the Red River is largely conserved within study years from
500 Emerson (2014 mass = 830kg, 2015 mass = 420kg) to St. Norbert (870kg, 245kg) to Selkirk
501 (800kg, 430kg). Given that atrazine is not expected to degrade markedly in the transport time
502 between sites, our data would suggest that inputs from southern Manitoba are minor.

503 Rawn et al. (1999) observed a similar spatial trend for atrazine, although their loading
504 estimates were less, ranging from 100-150 kg annually. Reasons for the 4-8 fold difference in
505 atrazine loadings between studies may include changes in atrazine use over the last 20 years
506 (USGS National Water-Quality Assessment Project, https://water.usgs.gov/nawqa/pnsp/usage/)
507 where increases in regional and state usage patterns (i.e., North Dakota) have been observed,
508 a lack of integrative, continuous sampling by Rawn et al., (1999) and stochastic variation across
509 years and seasons. Rawn et al. (1999) also measured mass loadings of atrazine in a number of

510 Red River tributaries, including the Assiniboine River. They estimated an annual mass of 1.7kg
511 (1994) and 1.4kg (1995) coming from the Assiniboine River, which is smaller than the
512 approximately 16kg (2014) and 7kg (2015) of atrazine estimated here. In seven other Red River
513 tributaries, annual mass loadings of atrazine are much less; ranging from 5-780 g (Rawn et al.,
514 1999, Carlson et al., 2013a), supporting the evidence here that atrazine is largely coming from
515 the U.S. In the Minnesota River, which is more comparable in size to the Red River, Schottler et
516 al., (1994) reported an annual flux for atrazine of 1100kg in 1990 and 2000kg in 1991.

517 The annual mass loadings of neonicotinoids tell a different story than atrazine. In 2014
518 there appears to be a systematic increase in mass loadings of thiamethoxam, clothianidin, and
519 imidacloprid moving downstream from Emerson to Selkirk, indicative of multiple significant
520 sources throughout southern Manitoba. However, in 2015 this pattern is much less pronounced
521 (Fig. 4), for reasons unclear at this time. It may simply be a result of variation between years
522 related to the timing of pesticide applications and precipitation and runoff events. In 2014, a
523 one-month period from mid-June to mid-July accounted for 55-67% of the annual mass loadings
524 of neonicotinoids at the three Red River sites. Such periods of comparatively brief, intense
525 inputs did not occur in 2015, potentially resulting in the more plateaued spatial pattern observed
526 for the neonicotinoids moving downstream from Emerson. In general, mass loadings of the
527 neonicotinoids ranged from 10-120 kg annually (Fig. 4).

528 As noted, there is a 2- to 3-fold difference in loadings for the detected pesticides
529 between 2014 and 2015. This may in part be a result of the 2-fold greater flows observed in
530 2014 in both rivers (Fig. 2). We assume that the bulk of the pesticides are moved into the rivers
531 by surface runoff, and so greater flows would result in greater movement of residual pesticides
532 (Schottler et al., 1994). Additionally, it is estimated that 0.2–3% of applied pesticide reaches
533 surface waters, depending largely on application type (e.g., foliar spray, seed coatings) and
534 timing of rainfall events (Schottler et al., 1994). Assuming application techniques were similar in

535 the Red River valley in 2014 and 2015, a major factor resulting in greater 2014 loadings could
536 be related to the timing of rain events in relation to pesticide applications.

537 ***3.2.3 Risk associated with pesticide exposures***

538 Water concentrations over the two-year study (Table S4A-E) were used to generate exposure
539 distributions for thiamethoxam, clothianidin, imidacloprid, and atrazine (Fig. 5). Exposure
540 distributions can provide exceedance probabilities of specific threshold toxicity benchmarks for
541 exposed organisms (Solomon et al., 2000). To screen for potential adverse impacts of these
542 pesticides, water quality guidelines (WQGs) for the Protection of Aquatic Life were referenced
543 from the Canadian Council of Ministers of the Environment (CCME, 2014). The CCME report
544 long-term freshwater benchmarks of 1800 and 230 ng/L for atrazine and imidacloprid,
545 respectively. As the CCME do not report protection of aquatic life guidelines for thiamethoxam
546 or clothianidin, freshwater aquatic-life benchmarks from the USEPA Office of Pesticide
547 Programs were referenced instead (USEPA Office of Pesticide Programs, 2017a). Toxicity
548 benchmarks of 17500 ng/L (invertebrate, acute) and 1100 ng/L (invertebrate, chronic)
549 represented the most sensitive benchmark values available for thiamethoxam and clothianidin,
550 respectively. The Office of Pesticide Programs compiles acute and/or chronic benchmarks for
551 fish, invertebrates, and vascular and nonvascular plants (USEPA Office of Pesticide Programs,
552 2017a). Where applicable, the chronic toxicity benchmarks were considered given that most of
553 our exposure data represents 14-21d TWA concentrations which represent exposure durations
554 consistent with the USEPA protocols for chronic screening-level risk assessments (Nowell et al.,
555 2018; USEPA Office of Pesticide Programs, 2017b). These threshold values for each pesticide
556 were compared to exposure distributions (Fig. 5) to calculate exceedance probabilities and
557 hence assess risk.

558 The levels of pesticides observed in this study represent no acute risk to aquatic life.
559 Although concentrations reported here represent time-weighted averages and thus likely

underestimate maximum short-term concentration spikes, acute guidelines are in most cases orders-of-magnitude larger than our observed concentrations. For a more conservative estimate of thiamethoxam risk (compared to the benchmark values above) a 35 day chronic no-observed-effect concentration of 300 ng/L was considered (Pickford et al., 2018). The probability of exceeding this chronic end-point in the Red and Assiniboine River was determined to be <0.01%. No other individual neonicotinoid exceeded any toxicity benchmark. The other predicted exceedance was for atrazine with a 0.2% probability of exceeding the CCME long-term WQG of 1800 ng/L. Given the common mode of action for neonicotinoids, assuming a concentration addition model can provide a more conservative estimate of risk (Nowell, et al., 2018). Even based on an exposure distribution from summed neonicotinoid concentrations (data not shown), the CCME long-term protection of aquatic life guideline for imidacloprid (230 ng/L) only has a 0.4% chance of being exceeded. When summed together the maximum for all three neonicotinoids is 73 ng/L, which does not exceed the guideline. Taken together, current exposures of these three insecticides do not appear to pose any acute or chronic risk to non-target aquatic organisms. However, given the varied inputs and resulting suite of chemical classes present in the Red River, as demonstrated here, cumulative risks resulting from exposure to chemical mixtures is important to acknowledge. While this falls outside the scope of this study, this data can contribute to any future efforts to characterize cumulative risks in this system.

3.3 Pharmaceuticals

Of the 17 pharmaceuticals measured in this study, carbamazepine was the only one detected consistently at all sites (Fig. S1 and Table S4E). Carbamazepine is an active pharmaceutical ingredient prescribed globally as an anti-epileptic medication (Cunningham et al., 2010). Given its widespread use and relative persistence in the environment, carbamazepine has become ubiquitous in impacted surface waters (Cunningham et al., 2010). The other six pharmaceuticals

585 of note (antibiotics clarithromycin, sulfapyridine, sulfamethoxazole, trimethoprim and β-blockers
586 metoprolol, propranolol) were only detected at the North End Red River site, downstream of the
587 North End WWTP (Fig. S3 and Table S5). The specific pharmaceuticals detected here are
588 typical of effluent-impacted surface waters (Carlson et al., 2013a; Fairbairn et al., 2016).
589 Concentrations generally ranged from 0.2-35 ng/L with notable spikes in the levels of
590 sulfapyridine (250 ng/L) and clarithromycin (170 ng/L).

591 The mean carbamazepine concentration over the entire study at all sites (Red and
592 Assiniboine, n=127, 11<LOD) was 2.8 ng/L, ranging from 0.3-13.8 ng/L. Concentrations at the
593 North End site immediately downstream of the WWTP were elevated, with a mean of 6.8 ng/L
594 (n=22, 1<LOD). The ten greatest concentrations observed for carbamazepine in this study were
595 measured at the North End site. The levels of carbamazepine observed here were similar to
596 those observed elsewhere in Manitoba and the U.S. For example, concentrations of
597 carbamazepine in Dead Horse Creek, a small tributary to the Red River that receives sewage
598 lagoon effluent from two small towns in southern Manitoba, ranged from non-detect to mean
599 concentrations of 16-24 ng/L (Carlson et al., 2013a). Fairbairn et al. (2016) measured
600 carbamazepine in the Zumbro River watershed in southeastern Minnesota and found
601 concentrations ranging from below detection-0.83 ng/L at upstream sites to 73-150 ng/L at
602 downstream sites. The CCME long-term freshwater WQG for carbamazepine is 10,000 ng/L
603 which is nearly three orders of magnitude above the maximum concentration observed here.
604 The Dutch government adopted an Average-Annual Environmental Quality Standard for
605 carbamazepine of 500 ng/L (Moermond et al., 2016), a much more conservative value.
606 Regardless, even the maximum concentration observed in this study (13.8 ng/L) remains well
607 below these guideline values, suggesting that carbamazepine pose little known risk to
608 organisms in the Red River, or downstream receiving waters.

609 Total average loadings downstream of the North End WWTP over the two years was
610 approximately 20 kg carbamazepine, equating to 0.11 kg/d at Selkirk. Variation in annual
611 loadings of carbamazepine were minimal (Fig. S2) compared to the pesticides measured in this
612 study (Fig. 4), consistent with inputs being completely wastewater derived, and therefore largely
613 independent of variable precipitation patterns and hydrodynamic river conditions. Dead Horse
614 Creek estimated a total load of 0.07 kg carbamazepine over the 2010 summer discharge event
615 (Carlson et al., 2013a), consistent with a much smaller population. Mass loadings <5 kg in the
616 Assiniboine River at Headingley were comparable to the upstream Emerson and St. Norbert
617 sites on the Red River (Fig. S2).

618 The City of Winnipeg is the largest settled population (≈700,000) in the Red River valley,
619 however many smaller cities and municipalities lie along the river on both sides of the border
620 and represent possible sources of municipal effluent. The largest of these, Fargo, ND
621 (≈121,000); Moorhead, MN (42,000); and the Greater Grand Forks area, ND (≈98,500)
622 contribute their municipal wastewater upstream of the Canadian border, and could explain the
623 observed background levels of carbamazepine ranging from ≈0.5-2 ng/L at Emerson and St.
624 Norbert. Additionally, there may be small inputs from the two small border towns of Pembina
625 and Emerson. Given that environmental concentrations of pharmaceuticals typically scale with
626 population (Anderson et al., 2004), based on the lagoon inputs into Dead Horse Creek from two
627 towns totalling ≈18,000 (mentioned above, Carlson et al., 2013a), Pembina and Emerson
628 combined would contribute approximately 0.004 kg of carbamazepine annually to the Red River.
629 This estimate is crude, in that it assumes no transformation or other losses (e.g., sorption to
630 particulate matter, sedimentation) from the water column throughout the river. Nonetheless, it is
631 clear that upstream contributions of carbamazepine to the load of this chemical in the lower Red
632 River are negligible compared to the inputs from the City of Winnipeg.

633 Per capita loadings of the pharmaceuticals downstream of the North End WWTP can be
634 calculated based on the measured chemical concentrations (Table S4E and S5), an estimated
635 average daily Red River discharge at the North End site (8.8×10^6 m^3/d), the WWTP average
636 daily discharge (2.0×10^5 m^3/d), and the served population of 404,000 people. Per capita
637 loadings for the seven pharmaceuticals measured in this study ranged from 7 – 150 µg/person/d
638 (Table S7). These are rough estimates as they are extrapolated from downstream
639 concentrations, and not the raw effluent. That said, our estimates are in general agreement with
640 other Canadian studies reporting per capita loadings estimated from raw effluent (comparison in
641 Table S7). Carlson et al. (2013a) reported loadings ranging from 24 – 203 µg/person/d coming
642 from rural wastewater lagoons (population 18,000) and MacLeod and Wong (2010) observed
643 loadings from 2 to 115 µg/person/d from two WWTPs in Alberta serving 750,000 and 250,000
644 people.

645 **3.4 Per- and polyfluoroalkyl substances (PFASs)**

646 Eight of nine PFASs monitored in this study were detected regularly throughout the duration of
647 the 2014 sampling campaign. PFUnDA was not detected above the elevated background
648 contamination observed in most samples. The raw POCIS TWA concentration data for PFDA,
649 PFHpA, PFHxA, PFNA, PFOA, PFOS, PFHxS, and PFPeA can be found in SI (Table S6A-H).
650 Data for PFHxS and PFPeA is not presented in Fig. 6 because experimentally measured POCIS
651 sampling rates have not been determined for these compounds. Therefore, concentrations of
652 those analytes are presented in Table S6G-H as mass on POCIS per day (ng/d) and represent
653 relative levels of PFHxS and PFPeA. To estimate semi-quantitative TWA water concentrations
654 of PFHxS and PFPeA, the mass/d can be divided by an estimated sampling rate based on the
655 six experimentally measured sampling rates used for PFDA, PFHpA, PFHxA, PFNA, PFOA, and
656 PFOS (Kaserzon et al., 2012). The measured sampling rates reported by Kaserzon et al. (2012)
657 differ maximally by approximately a factor of two, ranging from 0.16-0.36 L/d, which is expected

given the structural similarities between these analytes. Given this, taking the average of the six known sampling rate values (0.26 L/d) offers one approach to estimating a reasonable sampling rate value for PFHxS and PFPeA. Of course, end users of this data should be careful with this approach and may wish to regard the resulting data as semi-qualitative.

The maximum measured concentration was 8.5 ng/L PFOS. Generally, concentrations of the six PFASs ranged from 0.5-2 ng/L (Fig. 6). Additionally, increased concentrations downstream of the NEWPCC at the North End site were not observed, suggesting that the NEWPCC is not a major point-source and that multiple more diffuse inputs along the River may be responsible for these PFAS levels. The PFAS measurements here are consistent with single grab sample concentrations taken by Scott et al. (2009) at two sites on the Red River in 2005 and at a single site on the Assiniboine River close to the Saskatchewan border. Scott et al. (2009) measured 14 PFASs including seven of the compounds measured here (excluding PFPeA). Concentrations of the six common PFASs measured by Scott et al. (2009) on the Red River ranged from 0.15-1.67 ng/L. Notable concentrations of PFHpA (10.5 ng/L), PFOA (6.9 ng/L), and PFHxA (5.8 ng/L) measured in the Assiniboine River by Scott et al. (2009), albeit much further west than our Headingley site, were significantly greater than concentrations observed here (Fig. 6). While there does not seem to be obvious spatial or temporal trends in these systems as they related to PFAS levels, concentrations of PFDA, PFNA, and PFHpA do appear to spike at the Selkirk site during the July 29th deployment. Selkirk, Manitoba is a town of approximately 10,000 that releases its municipal and industrial waste effluent into the Red River, however without further sampling and investigation, it is difficult to know what, if anything, is the cause of these concentration spikes. The Canadian federal water quality guideline for PFOS is 6800 ng/L (Environment Canada, 2017), nearly 1000 times larger than any concentration of PFAS observed here.

3.5 Antibiotic resistance genes

683 Total bacteria levels were not statistically different across all locations (Kruskal-Wallis, H = 7.3,
684 p = 0.20); however, the North End site, immediately downstream of the NEWPCC, generally
685 had greater levels, and upstream at St. Norbert had the least (Table S9). Equally, the sums of
686 tetracycline resistance genes were statistically similar along the river (KW, H = 2.67, p = 0.76),
687 while the sums of sulfonamide resistance genes were different (KW, H = 9.50, p = 0.09) with
688 lower levels at Selkirk. Patterns of "total resistance" are often inadequate to describe the
689 patterns along a river. As such, we examined the trends of individual resistance gene-
690 determinants (Fig. S4). Patterns of "total bacteria" were also included to help visual
691 comparisons (Fig. S4, 16SrRNA plot). It should be noted that gene-determinants *tet*-E, -G, -L, -
692 O, -Q and -X had minimal or non-detected signals, and therefore were subsequently excluded in
693 further analyses (Table S10).

694 Tetracycline resistant gene-determinants *tet*-A, *tet*-B and *tet*-C are related to bacterial
695 efflux pumps, used for detoxification and are relatively common in the samples, with uniform
696 distributions. They are most commonly associated with Gram-negative bacteria (Chopra and
697 Roberts, 2001). *Tet*-A were found in relatively low levels except in Emerson; while *tet*-B and *tet*-
698 C had rather uniform concentrations along the Red River and at Headingley.

699 *Tet*-D, -E, -G, -K, -M were found in greater abundances and frequency downstream from
700 the NEWPCC, north of Winnipeg and Headingley. *Tet*-M encodes for a ribosome-protection
701 protein in both Gram-negative and –positive bacteria. The others represent genes related to
702 efflux-pumps in Gram-negatives. These gene determinants are signatures of human impacts,
703 particularly in wastewater treatment plants and their effluent (Gatica, et al. 2017).

704 Sulfonamide resistance genes have become rather ubiquitous in natural systems
705 (Byrne-Bailey et al., 2009). Many bacterial isolates with the *sul*1 gene have been associated
706 with integrase1 genetic elements in integrons (Byrne-Bailey et al., 2009), which enhances their
707 dissemination of antibiotic resistance in areas of pollution. The presence of *sul*-genes suggest

anthropogenic impact to the waters, agricultural and municipal, especially when all three determinants (*sul*1, *sul*2, *sul*3; Fig. S4) are present (Pei et al., 2006).

4. CONCLUSIONS

This study provides a spatial and temporal assessment of pesticides, pharmaceuticals, PFASs, and ARGs in the Red River of the Lake Winnipeg watershed, measured using POCIS passive samplers in 2014 and 2015. Pesticides represented the major contaminant of interest given the intensive agriculture in the watershed, both in the United States and southern Manitoba. Mass loading estimates helped differentiate chemical sources along the River. For atrazine, inputs to the Red River were largely from the United States, consistent with usage patterns of the herbicide throughout the U.S. side of the Red River watershed compared to in southern Manitoba. As hypothesized, inputs of atrazine from western Manitoba were minor. Neonicotinoid loadings were more indicative of usage all along the Red River valley on both sides of the border, and throughout western Manitoba (Assiniboine River). Annual mass loadings of these pesticides ranged from approximately 40 kg (imidacloprid) to 800 kg (atrazine). Screening for potential toxicity of these pesticides demonstrated no significant concern based on exposure distributions and protection of aquatic life benchmarks. Of the seven pharmaceuticals detected in the Red River, carbamazepine was the single one measured consistently through time and space, at concentrations ranging from 0.5-15 ng/L. Pharmaceutical concentrations were elevated at the North End site, downstream of the City of Winnipeg's major wastewater treatment plant. PFASs and ARGs were found at levels typical of an impacted river system, however exact sources were less clear as profiles were indicative of potentially multiple diffuse sources throughout the watershed. This work will help inform best management practices within the Lake Winnipeg watershed and aid in the efforts to understand contaminant sources and improve water quality of this lake.

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741

742 SUPPLEMENTARY INFORMATION

743 The supplementary information document provides additional details of analytical methods and
744 procedures and the raw Red River contaminant data in the form of tables and figures.

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962

963 **Figure captions**

964 **Figure 1:** Sampling sites (black triangles) from south to north on the Red River: Emerson (N
965 49.008442°, W 97.215310°), St. Norbert (N 49.754725°, W 97.137746°), North End (N
966 49.951508°, W 97.097491°), Selkirk (N 50.142747°, W 96.864826°), and Breezy Point (N
967 50.278267°, W 96.851626°). The site Headingley is located on the Assiniboine River (N
968 49.868906°, W 97.409807°), a tributary to the Red River. Source material for this map includes
969 information licensed under the Open Government Licence – Canada, the U.S. Geological
970 Survey, and NASA/JPL-Caltech.

971 **Figure 2:** Daily flow rate over the entire study period (April to October) in 2014 and 2015 on the
972 Red River (Emerson, St. Norbert, Selkirk) and Assiniboine River (Headingley). Data obtained
973 from Environment Canada Water Office (https://wateroffice.ec.gc.ca/).

974 **Figure 3:** Time weighted average concentrations of thiamethoxam, clothianidin, imidacloprid,
975 and atrazine detected over the two-year study as measured using POCIS. Bars represent the
976 mean and standard deviation (SD) of triplicate measurements. Bar colour corresponds to
977 sampling site in direction of flow (Emerson, St. Norbert, North End, Selkirk, Breezy Point).
978 Headingley on the Assiniboine River is not shown.

979 **Figure 4:** Mass loadings over the duration of each sampling season of thiamethoxam,
980 clothianidin, imidacloprid, and atrazine along the Red River from south to north (flow direction)
981 at Emerson, St. Norbert, and Selkirk and on the Assiniboine River at Headingley. Each bar in
982 the plots represents 11 samples in 2014 and 12 samples in 2015. Error bars represent 30%
983 relative standard deviation, estimated based on the uncertainty observed for replicate POCIS
984 measurements.

985 **Figure 5:** Exposure distributions for TWA concentrations of thiamethoxam, clothianidin,
986 imidacloprid, and atrazine in the Red River (black circles) and Assiniboine River (open circles).

Raw data in these plots from Table S4A-E. Data was plotted on a logarithmic-probability scale. Plotting positions on the y-axis were expressed as percentages calculated using the Weibull ranking equation = $100i/(i+1)$ where i is the ranked datum and n is the total number of data points in the data set. Data below the limit of detection (LOD) was included in the Weibull rankings but not plotted in the probabilistic exposure distributions. Linear regression statistics for each distribution in Table S8.

Figure 6: Concentrations of six PFAS in the Red River (Emerson, St. Norbert, North End, Selkirk, Breezy Point) and Assiniboine River (Headingley) as measured by the adapted PFAS-POCIS. Left to right on each plot is summer (July 22) to fall (September 9) in 2014 and within each group of bars light gray to dark gray represents direction of flow on the Red River. Headingley, on the Assiniboine River, is the white bar. Each bar represents the mean and standard deviation (error bars) of triplicate POCIS.

Table 1: Summary values for the Red and Assiniboine River hydrographs in 2014 and 2015. Mean, median, and total discharge values calculated from April 29 – October 28, 2014 and April 11 – October 28, 2015. Data obtained from Environment Canada Water Office (https://wateroffice.ec.gc.ca/).

Discharge	Emerson		St. Norbert		Selkirk		Headingley	
(m^3/s)	2014	2015	2014	2015	2014	2015	2014	2015
Mean	325	164	400	224	743	403	305	149
Median	259	97	321	160	746	384	307	96
Total (m^3)	$5.1x10^9$	$2.9x10^9$	$6.3x10^9$	$3.9x10^9$	$1.2x10^{10}$	$7.0x10^9$	$4.8x10^9$	$2.6x10^9$

Table 2: Summary concentration statistics for the four pesticides measured in the Red River.

Conc. (ng/L)	Thiamethoxam	Clothianidin	Imidacloprid	Atrazine
Mean	5.4	7.0	2.8	63.0
Median	3.6	5.1	2.1	24.3
Maxiumum (days)[a]	26.9 (14d)	31.7 (7d)	14.1 (7d)	520 (14d)
Site/date[b]	NB/07-2014	EM/07-2014	EM/07-2014	EM/06-2015

a – maximum environmental concentration measured in the Red River with time (d) of POCIS deployment in brackets

b – Sampling site and date (month-year) where maximum was measured (NB = St. Norbert; EM = Emerson)

Table 3: Comparison of atrazine concentrations measured in the current study (2014-15) and by Rawn et al. (1999) (1993-95) in the Red River at Emerson, St. Norbert, and Selkirk sites and in the Assiniboine River at Headingley.

Conc.	Emerson		St. Norbert[a]		Selkirk		Headingley	
(ng/L)	2014-15	1993-95	2014-15	1993-95	2014-15	1993-95	2014-15	1993-95
Mean	98.8	29.4	67.8	36.0	61.3	24.7	3.4	2.1
Median	41.1	11.1	27.8	14.7	27.5	9.2	2.7	1.1
Max.	520	168	356	219	193	169	10.2	8.1

a – sampling site in Rawn et al. (1999) was approximately 25 km south of St. Norbert in Ste. Agathe

Figure 1
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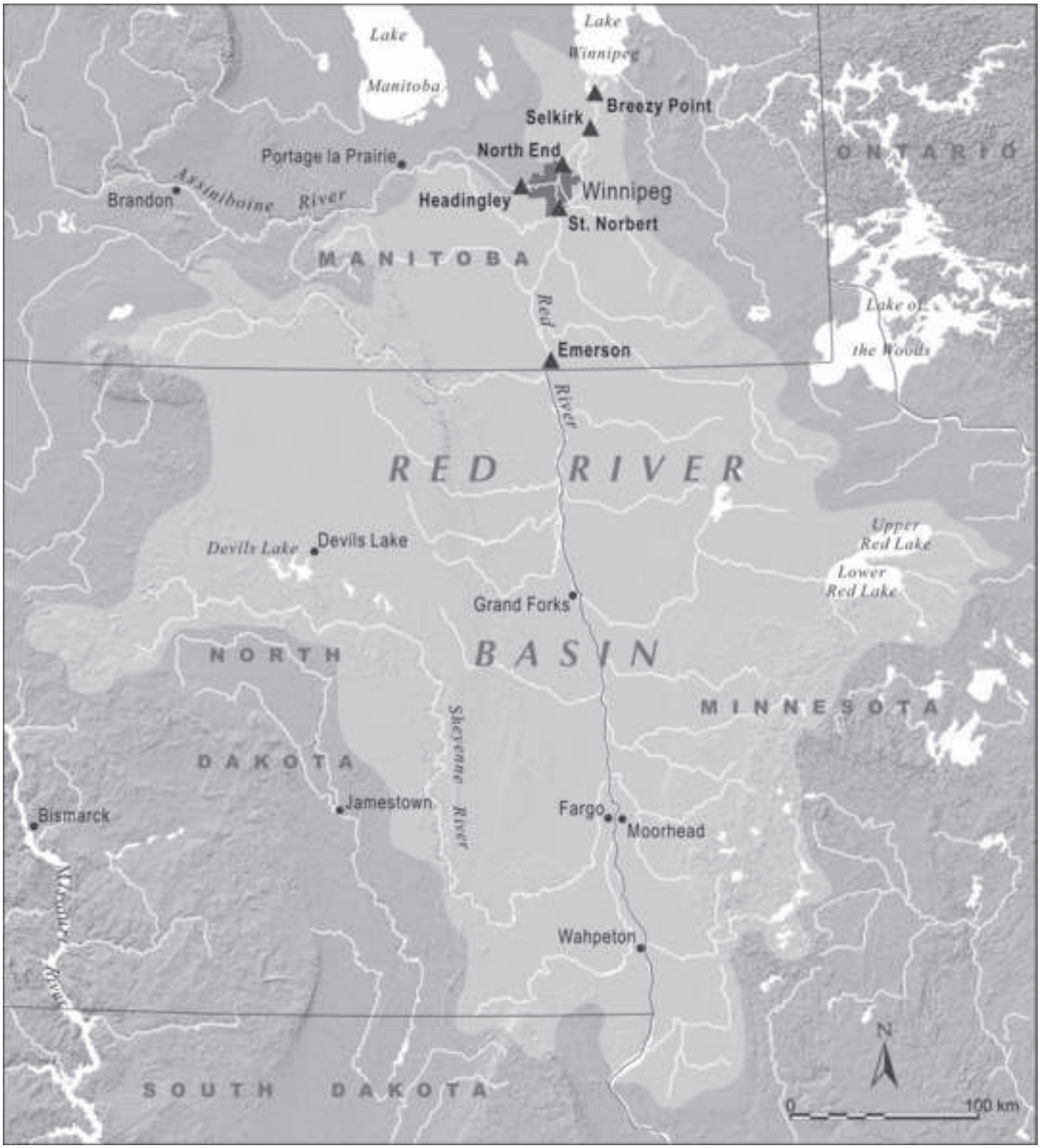

Figure 2

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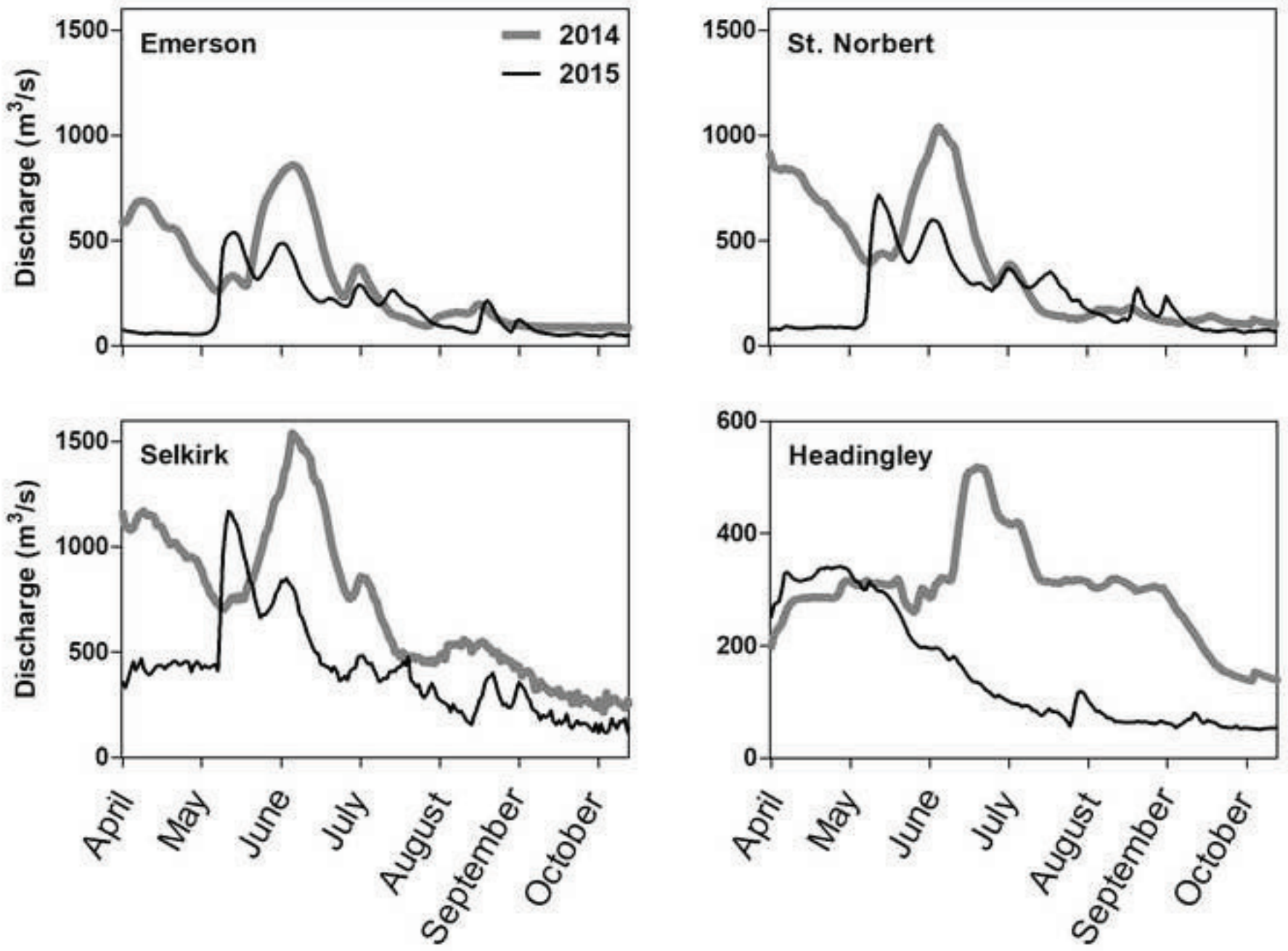

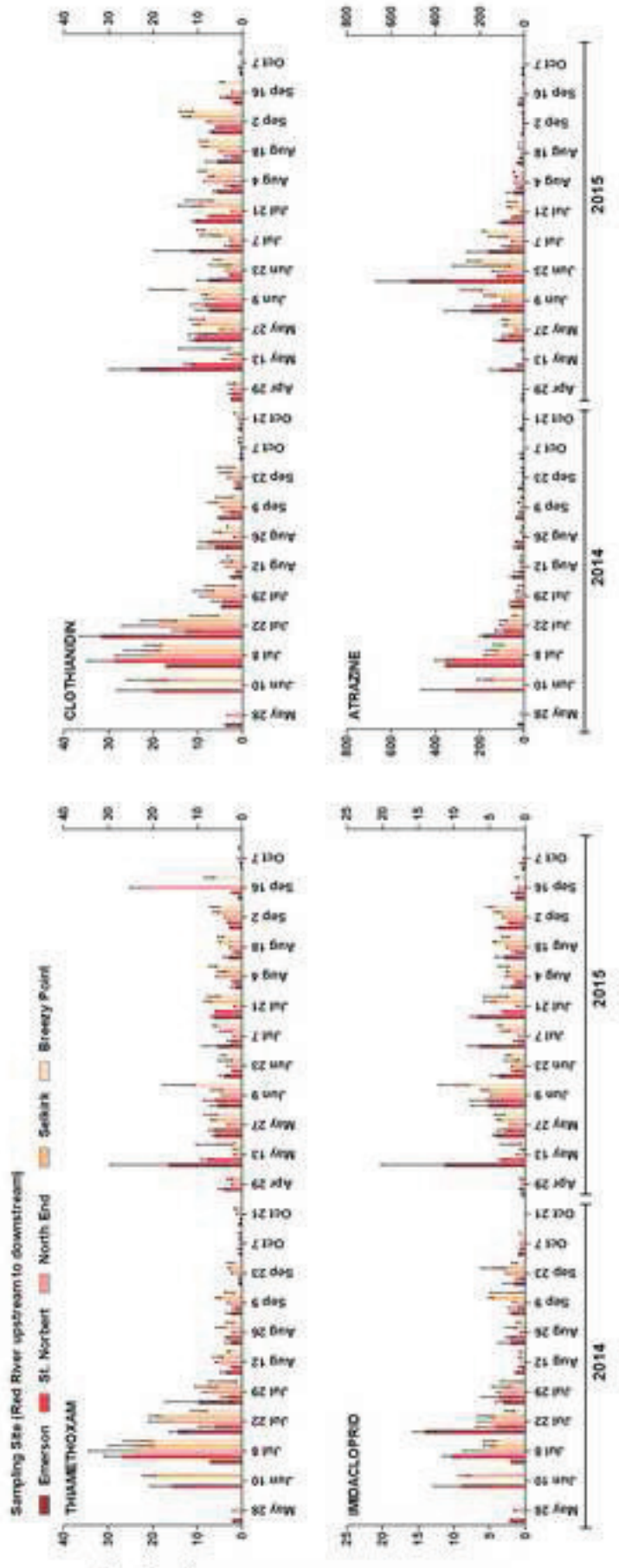

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Figure 4

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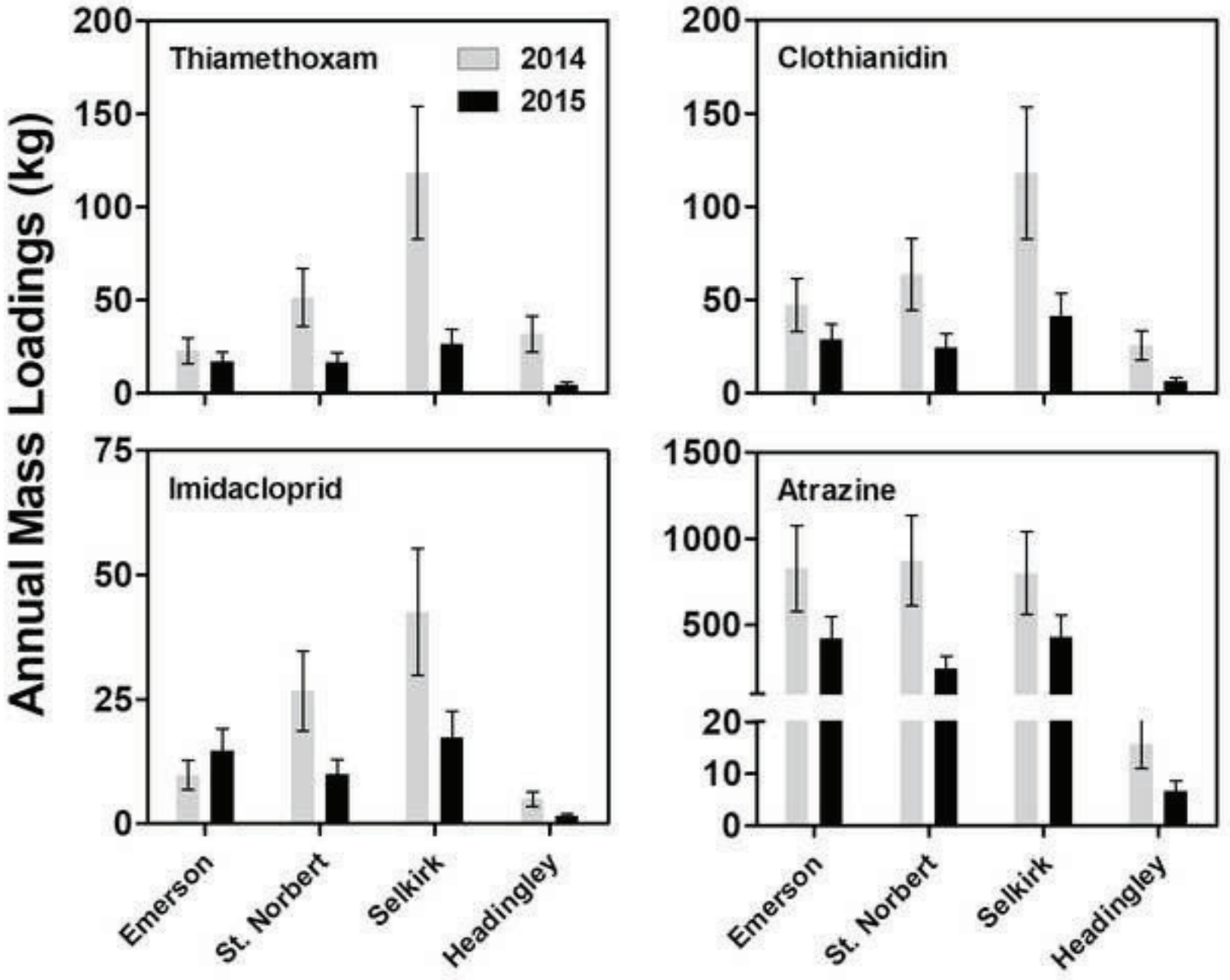

Figure 5

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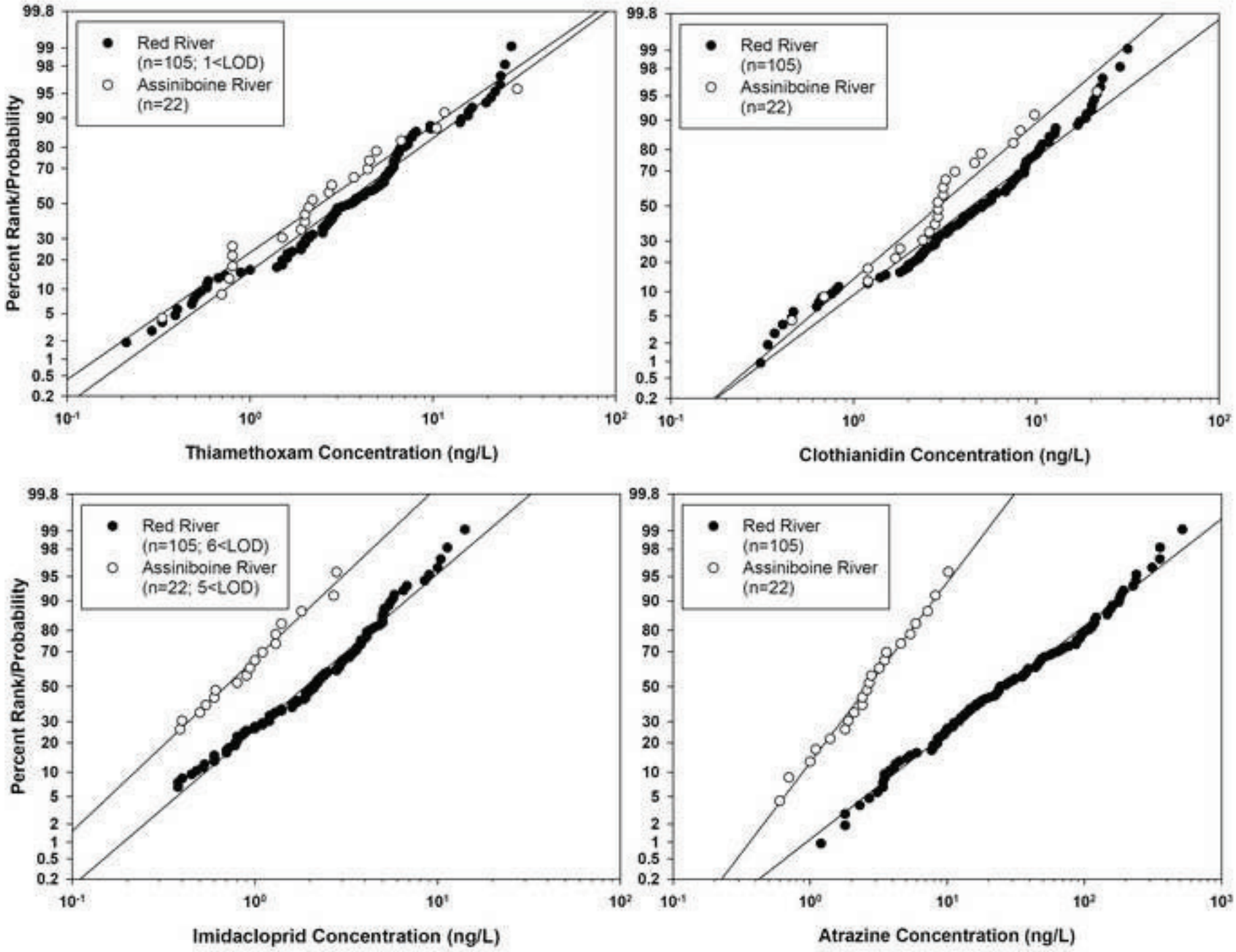

Figure 6

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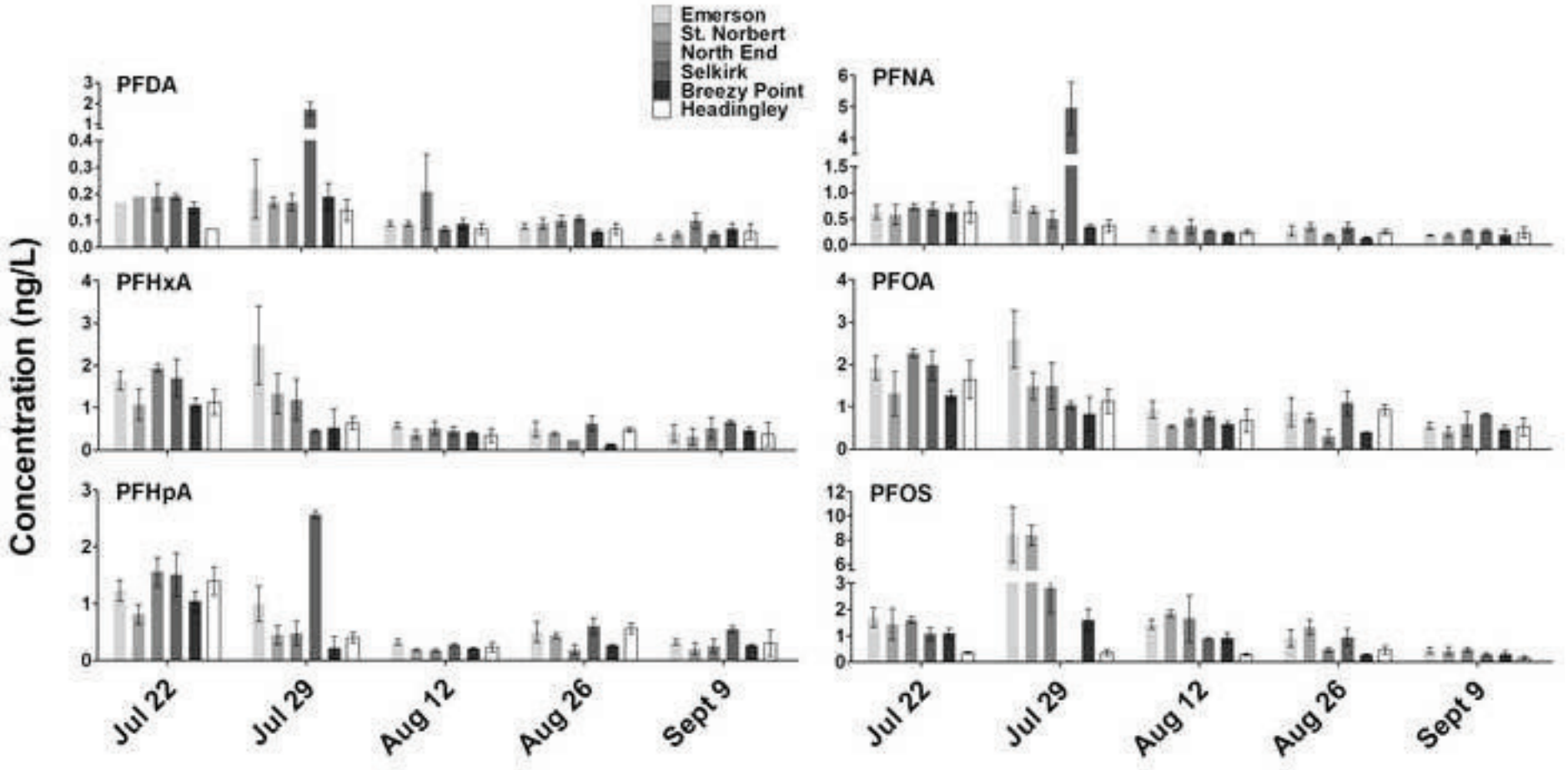

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