Precipitation effects on parasite, indicator bacteria, and wastewater micropollutant loads from a water resource recovery facility influent and effluent

Samira Tolouei, Laurène Autixier, Milad Taghipour, Jean-Baptiste Burnet, Jane Bonsteel, Sung Vo Duy, Sébastien Sauvé, Michèle Prévost and Sarah Dorner IWA

ABSTRACT

The variability of fecal microorganisms and wastewater micropollutants (WWMPs) loads in relation to influent flow rates was evaluated for a water resource recovery facility (WRRF) in support of a vulnerability assessment of a drinking water source. Incomplete treatment and bypass discharges often occur following intense precipitation events that represent conditions that deviate from normal operation. Parasites, fecal indicator bacteria, and WWMPs concentrations and flow rate were measured at the WRRF influent and effluent during dry and wet weather periods. Influent concentrations were measured to characterize potential bypass concentrations that occur during wet weather. Maximum influent *Giardia* and *C. perfringens* loads and maximum effluent *Escherichia coli* and *C. perfringens* loads were observed during wet weather. Influent median loads of *Cryptosporidium* and *Giardia* were 6.8 log oocysts/day and 7.9 log cysts/day per 1,000 people. Effluent median loads were 3.9 log oocysts/day and 6.3 log cysts/day per 1,000 people. High loads of microbial contaminants can occur during WRRF bypasses following wet weather and increase with increasing flow rates; thus, short-term infrequent events such as bypasses should be considered in vulnerability assessments of drinking water sources in addition to the increased effluent loads during normal operation following wet weather.

Key words | bypass discharges, *Cryptosporidium*, *Escherichia coli*, fecal indicator bacteria, wastewater micropollutants, water resource recovery facility

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INTRODUCTION

Waterborne disease outbreaks are of concern for the health, environment, and the economy of a society (Corso *et al.* 2003; Baldursson & Karanis 2011). In order to prevent waterborne disease outbreaks, the application of a source-to-tap multi-barrier approach for drinking water supply systems is recommended (WHO 2011; Health Canada 2012). Characterizing the variability of source water quality and implementing adequate source water protection strategies are essential for the prevention of waterborne disease outbreaks (Signor *et al.* 2005).

The variation of source water quality and contaminant loads depend on many factors, including land use and meteorological conditions (Charron *et al.* 2004; Huang *et al.* 2013; Jalliffier-Verne *et al.* 2015). Meteorological conditions (i.e. rainfall events or snowmelt) influence the quality of stormwater runoff (Parker *et al.* 2010), sediment

transport (Wu *et al.* 2009), resuspension of sewer sediments (Passerat *et al.* 2011), and efficiency rates of water resource recovery facilities (WRRFs, also known as wastewater treatment plants) (Lucas *et al.* 2013). Heavy rainfall events are regularly associated with peak concentrations of pathogens in surface waters (Atherholt *et al.* 1998; Kistemann *et al.* 2002; Signor *et al.* 2007; Burnet *et al.* 2014) and they increase the frequency of bypass discharges and combined or sanitary sewer overflows (CSOs and SSOs). WRRF influent from separate sewer systems includes sewage and additional flows through inflow (during intense storm events and snowmelt) and infiltration (from groundwater through network defects). Inflow/Infiltration (I/I) into sewer lines during wet weather periods is a common cause of bypass discharges from WRRFs served only by separate sewer systems. Bypass discharges are a concern for urban areas as they contribute to beach closures and the contamination of drinking water supplies (EPA 2004).

Several studies have investigated the concentrations, the loadings and removal efficiencies of pathogens, microbial indicators, and wastewater micropollutants (WWMPs) in different stages of WRRFs (Kistemann *et al.* 2008; Fu *et al.* 2010; Ajonina *et al.* 2012; Gallas-Lindemann *et al.* 2013; Burnet *et al.* 2014; Subedi & Kannan 2014) and CSOs (Benotti & Brownawell 2007; Madoux-Humery *et al.* 2013; Al Aukidy & Verlicchi 2017). Concentrations of WWMPs have been shown to be approximately 1 log higher in CSO discharges than in treated effluent discharges (Phillips *et al.* 2012), although continuously discharged effluents remain the main source of environmental contamination of WWMPs (Madoux-Humery *et al.* 2015). Studies examining the variability of pathogens, fecal indicators, and WWMPs loadings from a WRRF (fed by a separate sewer system) under various operating conditions, particularly, bypass discharges are rare even though they are needed for source water protection planning and setting management priorities (Signor *et al.* 2005). Limited attention has been paid to the characterization of pathogen loads from bypass discharges, possibly because of the difficulties in collecting representative samples during these transient events. Åström *et al.* (2009) estimated pathogen and fecal indicator loads from the effluent and emergency discharges of local WRRFs as well as combined and sanitary sewer overflow discharges using the literature data for microbial

concentrations and removals. To our knowledge, similar studies have not been performed on estimating parasites, fecal indicators, WWMPs, and total suspended solids (TSS) loads from bypass discharges. These data are needed to assess microbial loads from potential sources for microbial risk analyses, particularly, wastewater treatment performance data are not relevant when treatment does not occur or is incomplete.

In a WRRF served by a combined sewer system, fecal indicators and WWMP concentrations are influenced by sewer processes such as deposition or resuspension of sewer sediments. Sewer sediments were identified as a reservoir for fecal indicator bacteria (FIB), WWMPs, and TSS, and the importance of their contribution to the loads from CSO discharges has been evaluated (Hajj-Mohamad *et al.* 2014; Madoux-Humery *et al.* 2015). When considering the dilution potential of inflow/infiltration during wet weather in a WRRF fed by a separate sewer system, the concentrations of microorganisms in untreated wastewater were not strongly mass limited and peak contaminant concentrations were observed during wet weather periods (Tolouei *et al.* 2019). However, the contribution of sewer processes as a result of inflow/infiltration following heavy rainfall events to contaminant loadings is still unknown. Data on the variability of contaminant loads during wet weather periods are needed to estimate the vulnerability of drinking water treatment plants influenced by the loads from wastewater effluents as *de facto* or unplanned wastewater reuse is common (Rice *et al.* 2015) and seldom acknowledged. To the authors' best knowledge, this is the first study to examine the relative influence of inflow/infiltration on parasites, FIB, WWMPs, and TSS loads into a WRRF.

In the present study, parasites (*Cryptosporidium* and *Giardia*), FIB (*Escherichia coli* (*E. coli*) and *Clostridium perfringens* (*C. perfringens*)), and WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC, and ASP) were investigated as they are usually present in WRRF discharges (Buerge *et al.* 2003; Kistemann *et al.* 2008; Fu *et al.* 2010; Weyrauch *et al.* 2010; Ajonina *et al.* 2012; Gallas-Lindemann *et al.* 2013; Burnet *et al.* 2014; Subedi & Kannan 2014). The main objective of this study was to investigate the impact of variable weather conditions on contaminants loads from a WRRF serving a separate sewer system. The specific objectives

were to: (1) evaluate the most important factors influencing mass loadings from the WRRF influent and effluent; (2) investigate the importance of sewer processes in the mass loadings arriving at a WRRF under various flow conditions; (3) estimate the variability of parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC, and ASP), and TSS mass loadings from a WRRF in the case of failure, bypass discharges, and normal operation conditions under weather conditions ranging from trace to intense precipitation; and (4) assess the excess loads from a bypass discharge compared with that of final effluent discharges during wet weather conditions. Although the loads estimated are specific to the system under investigation, other similar sewer systems are expected to have similar behavior with regard to wet versus dry weather conditions. Furthermore, data on loads per capita from wastewater discharges are needed for comparing among WRRF loads and their impacts worldwide.

MATERIAL AND METHODS

The study site

The studied WRRF has a capacity of 518,000 m³/day and is fed by a separate sewer system. It receives the sewage from residential (approximately 1 million residents), industrial, and commercial facilities in the Greater Toronto Area, Canada (Kambeitz 2015, personal communication). The WRRF treats raw sewage through primary, secondary treatment (activated sludge processes with phosphorus removal), and chlorine disinfection. Studies from this region (Cole Engineering 2011) and historical data indicate that inflow/infiltration during wet weather periods are a challenge for local WRRFs. Raw sewage is conveyed to primary clarifiers after preliminary treatment (screening and grit removal) by three channels. Plant 3 treats the raw sewage from Channels 1 and 2 and Plants 1 and 2 from Channel 3 (see Supplementary Information, Figure S1, available with the online version of this paper). We sampled Channel 2. The primary treated effluent (with or without disinfection) from Plant 3 is discharged into Lake Ontario when the flow rate exceeds the treatment capacity of the plant. From 2007 to 2014,

the studied WRRF experienced 11 bypass events mostly following heavy rainfall (>80% of bypass events).

Sample collection and analytical methods

WRRF influent (following preliminary screening and grit removal) and effluent were monitored between April 2014 and September 2014. Time-proportional composite samples from the influent (using ISCO 6712FR fiberglass and refrigerated portable auto-samplers in 1 L polypropylene bottles) and grab samples from the effluent (in 10 L collapsible container low density polyethylene bottles) were collected under various weather conditions. Sampling was initiated based on rainfall and flow rate thresholds. The relationship between historical flow rates and rainfall data was determined to establish monthly thresholds that are representative of inflow/infiltration events. Wet weather conditions were defined as 2-day cumulative rainfall prior to sample collection >10 mm and flow rates above the determined threshold from the historical data analysis for each month of the year. Conditions with only trace amounts of rainfall (<3 mm) and flow rate below the threshold (also determined through historical data analysis) were defined as 'dry' weather conditions. Four wet weather events (one in April, two in June, and one in September) and two trace precipitation weather events (May and September) were monitored during the sampling campaign. The 2-day cumulative rainfall prior to sample collection ranged from trace amounts to 32 mm. For the four wet weather events, return periods were below 2 years. Collected samples were analyzed for parasites (*Cryptosporidium* and *Giardia*), FIB (*E. coli* and *C. perfringens*), WWMPs (CAF, CBZ, CBZ-2OH, ACE, SUC, and ASP), and TSS. Detailed information regarding sampling, analytical methods, concentrations, prevalence rates, and recovery efficiencies are provided by Tolouei *et al.* (2019).

Calculations

The contribution of sewer processes to mass loadings arriving at a WRRF

In a WRRF served by a separate sewer system with high level of inflow/infiltration, the total mass loadings into the WRRF during wet weather periods (L_{inf-WW}) is equal to the loadings

during the dry weather period ($L_{inf\text{-}DW}$) and loadings as a result of sewer processes (L_{SP}) and inflow/infiltration ($L_{I/I}$) (Equation (1)). Sewer processes not only include net deposition or net resuspension of particle associated contaminants depending on flow conditions but also include biological activity such as inactivation, predation, and biodegradation. One can assume that parasites, FIB, WWMPs, and TSS concentrations are negligible in inflow/infiltration ($L_{I/I} \approx 0$) as compared with sewage, thus loads as a result of sewer process can be calculated using Equation (2). In this study, the mass loadings into the WRRF during dry weather were calculated using the median values of the observed concentrations and total flow rate at the WRRF influent in the September dry weather event for two reasons: (1) more data were available in this month ($n = 24$) and (2) flow rate data for the September dry weather event (as compared with the May dry weather event) were not affected by other sources of stored water, such as infiltration, as a result of a higher water table (Figure S2, available online). For each wet weather event, loadings were also calculated from the median concentrations and total flow rates (i.e. from all channels) observed at the WRRF influent. Finally, the contribution of sewer processes was estimated for each wet weather event. This calculation was not conducted for *Cryptosporidium* and *Giardia* due to insufficient data.

$$L_{inf-WW} = L_{inf-DW} + L_{SP} + L_{I/I} \tag{1}$$

$$L_{SP} = L_{inf-WW} - L_{inf-DW} \quad \text{if } L_{I/I} \approx 0 \tag{2}$$

$$L_{inf-DW} = C_{inf-DW} \times Q_{inf-DW} \tag{3}$$

$$L_{inf-WW} = C_{inf-WW} \times Q_{inf-WW} \tag{4}$$

where $L_{inf\text{-}DW}$ [log-units/day], $L_{inf\text{-}WW}$ [log-units/day], L_{SP} [log-units/day], and $L_{I/I}$ [log-units/day] are the contaminant's mass loadings from the WRRF influent under dry and wet weather conditions from sewer processes, and from inflow/infiltration, respectively; $C_{inf\text{-}DW}$ [log-units/liter] and $C_{inf\text{-}WW}$ [log-units/liter] are the observed concentrations at the WRRF influent under dry and wet weather conditions, respectively; $Q_{inf\text{-}DW}$ [liter/day] and $Q_{inf\text{-}WW}$ [liter/day] are the total influent flow rates under dry and wet weather conditions, respectively.

Removal efficiency rates and concentrations at the primary effluent (with disinfection)

For the sampling events under dry and wet weather conditions, removal efficiencies were calculated by averaging monitored concentrations in the influent and effluent. Concentrations in the primary effluent (with disinfection) following a bypass discharge were calculated (Equation (5)) based on the log removal values provided in Table 1 and Table S1 (available online). For the contaminants with poor total removal efficiency rates ($\leq 70\%$) (i.e. CBZ, CBZ-2OH, ACE, and SUC), the primary treatment removal efficiencies (with disinfection) were assumed to be negligible.

$$C_{by\text{-}pass} = C_{inf} - R_{pt} + R_{dis} \tag{5}$$

C_{inf} [log-units/liter] and $C_{by\text{-}pass}$ [log-units/liter] are the concentrations in the influent and bypass discharges; R_{pt} and R_{dis} are contaminant log removals by the primary treatment and disinfection processes, respectively. Since the prevalence rate of *Cryptosporidium* in the influent (8.6%) and effluent (30%), as well as ASP in the effluent (0%), was low, calculations were not performed for these contaminants.

Mass loadings from influent, primary effluent (with disinfection), and treated effluent

Bypasses are infrequent as they usually occur when the flow rates are at their highest and it is difficult to predict their occurrence for a sampling campaign. Thus, we estimated mass loadings from the primary effluent using the observed

Table 1 | Estimated removal efficiency rates through primary treatment and disinfection processes[a]

Parameters	Primary treatment	Disinfection
Giardia	0.3 log	0.4 log
E. coli	0.5 log	1.5 log
C. perfringens	0.6 log	0.5 log
CAF	0.12 log	~0
TSS	0.7 log	~0

[a]Based on a reported range of removals (refer to Table S1) and observed total removal efficiency rates.

concentrations in the influent. The daily loads of parasites, FIB, WWMPs, and TSS from the primary effluent (with disinfection) following a bypass discharge were estimated using the median, 10th and 90th percentiles of historical data (2008–2014) for the bypass flow rate (Q_{by}) and duration (D_{by}) as well as estimated concentrations in the primary effluent. Although these bypass flow rates are unique to the WRRF and depend on the plant capacity available, the state of the sewer network, and local hydrometeorological factors, the approach can be generalized to other systems. The percentiles of the historical data ($d10$, $d50$, and $d90$) for the bypass flow rates were 58 ML/day, 136 ML/day, and 240 ML/day for corresponding durations of 3 h, 5 h, and 16.5 h, respectively. Contaminant loadings from primary effluents were estimated considering three scenarios using: (a) 10th percentiles of Q_{by} and D_{by}; (b) the median of Q_{by} and D_{by}; and (c) 90th percentiles of Q_{by} and D_{by}.

Loadings from the influent, bypass, and effluent discharges per 1,000 people were calculated using Equations (6) to (8) and per 1,000 people basis is to compare with other studies (Burnet *et al.* 2014). Two ratios were calculated to (a) compare a bypass discharge to effluent discharge during wet weather (F_1), and (b) compare an effluent discharge during dry weather with the sum of wet weather effluents (effluent and bypass discharges, F_2). The F_1 and F_2 ratios were computed using the median and 10th and 90th percentiles.

$$L_{inf}/1{,}000 \ \text{people} = (C_{inf} \times Q_{inf}) . \ \frac{1{,}000}{\text{pop}} \tag{6}$$

$$L_{by\text{-}pass}/1{,}000 \ \text{people} = (C_{by\text{-}pass} \times Q_{by\text{-}pass}) . \ \frac{1{,}000}{\text{pop}} \tag{7}$$

$$L_{eff}/1{,}000 \ \text{people} = (C_{eff} \times Q_{eff}) . \ \frac{1{,}000}{\text{pop}} \tag{8}$$

$$F_1 = \frac{L_{by\text{-}pass-WW}}{L_{eff-WW}} \tag{9}$$

$$F_2 = \frac{L_{eff-DW}}{L_{by\text{-}pass-WW} + L_{eff-WW}} \tag{10}$$

where L_{inf} [log-units/day], $L_{by\text{-}pass}$ [log-units/day], and L_{eff} [log-units/day] are contaminant loads from the influent, bypass discharge, and effluent per 1,000 people; C_{inf}, $C_{by\text{-}pass}$,

and C_{eff} [log-units/liter] are the concentrations in the influent, bypass, and effluent; Q_{inf}, $Q_{by\text{-}pass}$, and Q_{eff} [liter/day] are the total flow rate in the influent, bypass discharge, and effluent; pop is the population; DW and WW refer to dry and wet weather, respectively. For influent and primary effluent analyses, *Cryptosporidium* and *Giardia* concentrations were not adjusted by the recovery rates in the influent as recovery data were not available for each sample, whereas their concentrations were corrected by the recovery rates for the effluent loading analyses. The sample sizes (illustrated boxplots in Figures 3 and 4) were larger for the influent as compared with the effluent and thus uncertainties with regard to effluent mass loadings were larger.

Statistical analysis

Statistical analyses were performed using the STATISTICA software (Version 12). Given that the majority of loads were neither normally nor log-normally distributed, nonparametric Mann–Whitney U tests were performed to assess the differences between loadings under dry and wet weather conditions. The differences and regressions were considered to be significant at alpha = 5%. EPA's ProUCL software (Singh & Maichle 2013) was used to impute left-censored data (i.e. values below the limit of detection, $n = 4$ below the limit of detection for *Giardia* and ASP). The variation of loadings under dry and wet weather periods were demonstrated in boxplots in which boxes present 10th and 90th percentiles and whiskers illustrate minimum and maximum values, median (square in box), and mean (+ in box).

RESULTS AND DISCUSSION

Treatment removal efficiency rates

Variable removal efficiencies through secondary treatment were observed (Table 2). For all monitored conditions, removal efficiencies for *Giardia* ranged from 72.6% to 99.9% and in most instances (i.e. 80% of the time), it was ≥97%. Removal efficiencies for *E. coli* and *C. perfringens* varied from 99.9% to 99.99% and from 98.2% to 99.7%, respectively. The removal efficiency of pathogens and

Table 2 | Removal efficiencies of parasites, indicator bacteria, total suspended solids, and wastewater micropollutants through secondary treatment

Parameters	Removal efficiency rate	Treatment rank
E. coli	99.9–99.99	High (>99%)
CAF	99.1–99.9	
Giardia	72.6–99.9[a]	Moderate (>90%)
C. perfringens	98.2–99.7	
TSS	94.3–99.5	
CBZ	−48.5–2.52	Poor (≤70%)
CBZ-2OH	−34.5–39.5	
ACE	−5.5–70	
SUC	−59.1–51.7	

[a]*Giardia* removal efficiencies were ≥97% and 80% of the time.

microbial indicators vary with plant sizes and treatment conditions (Fu *et al.* 2010). The observed removal efficiencies for *Giardia*, *E. coli*, and *C. perfringens* are consistent with published removal rates in the literature, which is unsurprising given the wide ranges of reported removal rates (Ottoson *et al.* 2006; Kistemann *et al.* 2008; Fu *et al.* 2010; Kitajima *et al.* 2014).

Treatment processes effectively removed both TSS and CAF (Table 2). This observation is consistent with previous observations showing higher removal of CAF in WRRFs (Miao *et al.* 2005; Lee *et al.* 2011; Sim *et al.* 2011; Gao *et al.* 2012; Lee *et al.* 2013). In contrast, CBZ, CBZ-2OH, ACE, and SUC were not notably removed from this WRRF, and even negative removal efficiencies were observed for these WWMPs (Table 2). In other Canadian and non-Canadian studies, low and even negative removal efficiencies were similarly reported for CBZ, CBZ-2OH, ACE, and SUC (Miao & Metcalfe 2003; Miao *et al.* 2005; Scheurer *et al.* 2009; Hoque *et al.* 2014; Subedi & Kannan 2014). The lower removal efficiency of CBZ concentrations from WRRFs was explained by its poor biodegradability (Kasprzyk-Hordern *et al.* 2009) and the increase of CBZ concentration in the WRRF effluent was attributed to the hydrolysis of carbamazepine glucuronide conjugate and cleavage of the free parent compound (Radjenović *et al.* 2007). The test of biodegradability of ACE and SUC in activated sludge of a typical WRRF under laboratory conditions confirmed their persistence as no degradation was observed within 7 h of incubation at 15 °C (Buerge

et al. 2009). In a fate study, SUC was identified as a resistant compound to microbial degradation, soil sorption, hydrolysis, chlorination, ozonation, and UV-photolysis (Soh *et al.* 2011). ACE and SUC have been suggested as ideal indicators of wastewater contamination in groundwater and surface waters because of their chemical properties (Buerge *et al.* 2009; Oppenheimer *et al.* 2011).

Flow rate influence on concentrations

The influence of flow rate on FIB, WWMP, and TSS concentrations in the influent and effluent of the studied WRRF (served by a separate sewer system) was characterized by log concentration−log flow rate (log C – log Q) plots for all data (wet and dry weather) (Figure 1). This type of analysis was also used for WWMPs, hormones, and indicator bacteria in raw sewage and the treated effluent of WRRFs served by combined sewer systems as well as CSOs (Phillips *et al.* 2012; Madoux-Humery *et al.* 2015). The slope in log C – log Q plots indicates the importance of dilution on concentrations, with slopes greater than −0.7 showing that concentrations decrease at a slower rate than the increase in flow rates. Influent flow rate data are from Channel 2.

In the influent, *Giardia*, *E. coli*, and *C. perfringens* concentrations increased significantly ($p < 0.05$) with the flow rate (Figure 1). The observed patterns suggest that dilution processes did not affect the concentrations of the fecal microorganisms. Higher concentrations with higher flow rates can be explained by several confounding processes: (1) shorter travel times in the sewer network lead to decreased microbial inactivation, (2) higher flow rates occur at times of day that correspond to human defecation patterns, and (3) less sedimentation occurs in the sewer network and higher flow rates may also lead to sewer sediment resuspension.

For WWMPs, the slopes of CAF, CBZ, SUC, and CBZ-2OH in log C – log Q plots were in the range of 0.4–0.44 in the influent (Figure 1 and Figure S3, available with the online version of this paper). ACE, however, displayed a slope of −1.1, indicating that it was strongly influenced by dilution (including from inflow and/or infiltration). Among the studied artificial sweeteners (ACE, SUC, and ASP), dilution processes only affected the concentrations of ACE. The observed behavior may, in part, be explained by
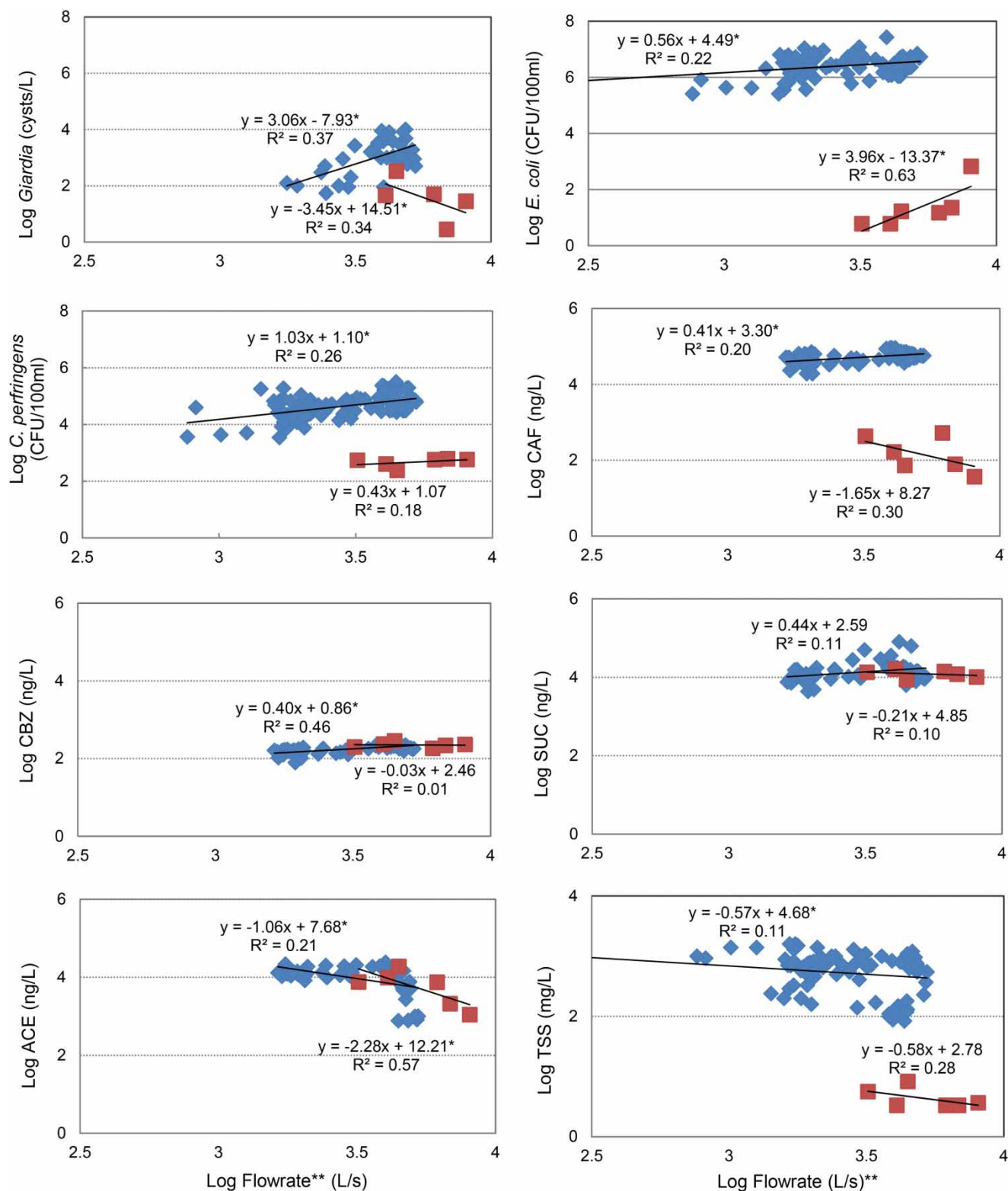
Figure 1 │ Concentrations in the influent (diamonds) and in the effluent (squares); *the regressions were significant at $p < 0.05$; influent flow rates are from Channel 2, which are part of the total flow rate.

the higher solubility of ACE as compared with the other artificial sweeteners studied (SRC 2015). A clear and significant trend was not observed for ASP (Figure S3).

For TSS, the slope (-0.6) in log C – log Q plot remained above -0.7 in the influent, reflecting the contribution of the non-wastewater sources to its loads and reducing the effect of dilution (Phillips *et al.* 2012; Madoux-Humery *et al.* 2015). TSS in sewer lines may originate from wastewater, sewer deposit resuspension, and to a lesser extent, inflow. Solids are deposited in the sewer system during low flows and are mobilized by high flows, which also enhance the transport of particulate compounds (Phillips *et al.* 2012).

In contrast to increasing influent concentrations with flow rate in this study of a separated sewer system, a decrease of hormones, WWMPs, and FIB concentrations with increasing flow rates was reported in combined sewer systems (Phillips *et al.* 2012; Madoux-Humery *et al.* 2015). Although inflow/infiltration causes the flow rate to increase in the influent of a WRRF served by separate sewer systems, combined sewer systems have more potential for dilution. Thus, the dilution of raw sewage could be a more important factor in controlling contaminant concentrations and loads in WRRF influents fed by combined sewer systems and concentrations and loads from separate sewer systems are more strongly influenced by human defecation patterns.

In the effluent, the concentration of *Giardia* cysts was inversely related to flow rate (Figure 1). *Giardia* cysts

are environmentally resistant to degradation; *Giardia* die-off rates in water and sediment are reported to be $0.029 \log_{10}$ day^{-1} and $0.37 \log_{10}$ day^{-1} (Karim *et al.* 2004). Thus, it seems that the concentration of *Giardia* was principally influenced by dilution in the WRRF effluent or that treatment efficiency of *Giardia* was not greatly influenced by higher flow rates. In contrast, the concentrations of *E. coli* and *C. perfringens* in effluents increased with flow rate, suggesting that reduced treatment efficiency was more important than dilution. This trend was previously reported for both *E. coli* and enterococci in Parisian WRRFs that showed decreased treatment efficiency as a result of the decrease of hydraulic retention times during wet weather periods (Lucas *et al.* 2013).

For WWMPs, the slope of the log C – log Q plots for CAF, CBZ, CBZ-2OH, ACE, and SUC ranged from -2.3 to -0.03 in the effluent (Figure 1 and Figure S3), but the regression was only significant for ACE. Dilution appears to influence the concentration of ACE in the effluent as a result of its higher solubility (587,500 mg/L at 25 °C) (SRC 2015).

Source contribution for the contaminant loads into a WRRF

The relative contribution of sewer processes varied among contaminants and events (Figure 2). Depending on flow
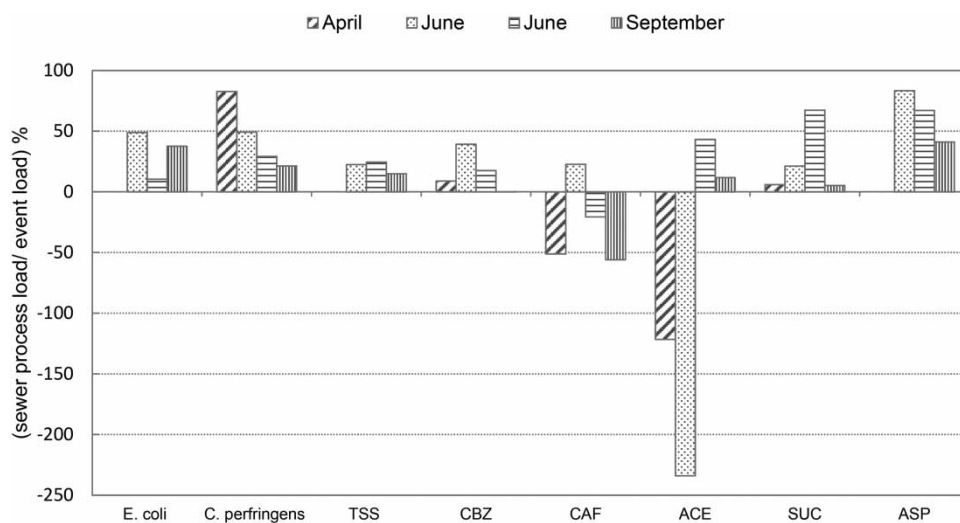
Figure 2 | Relative contribution of the sewer process load to loads of indicator bacteria, wastewater micropollutants, and total suspended solids during four wet weather events. *E. coli*, TSS, and ASP data were not available for the April wet weather event.
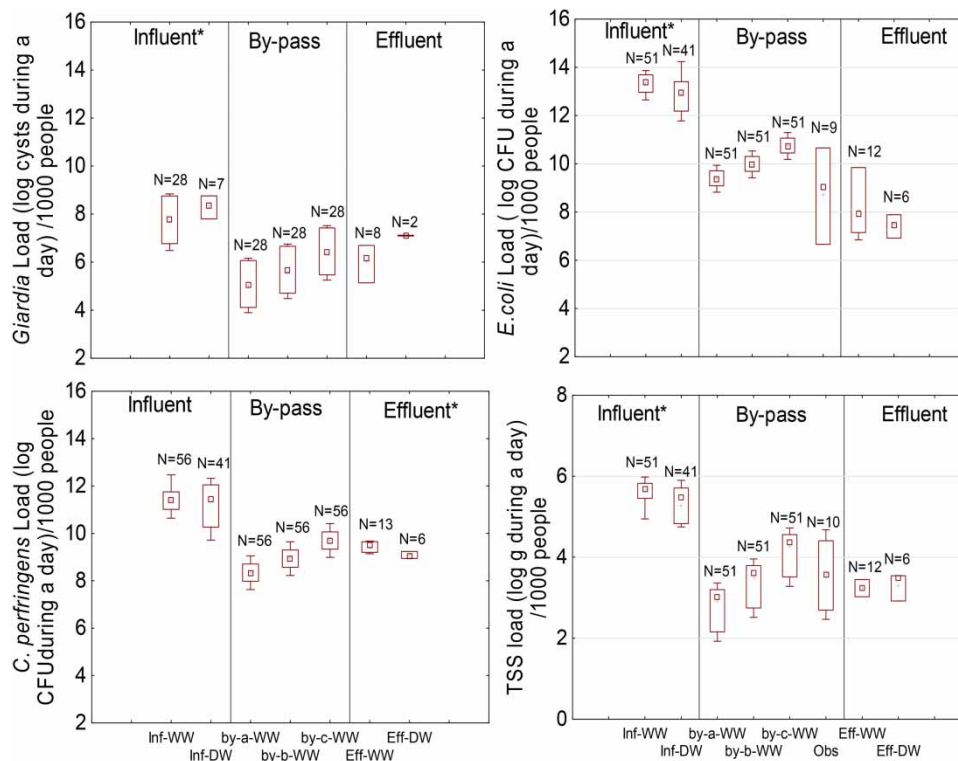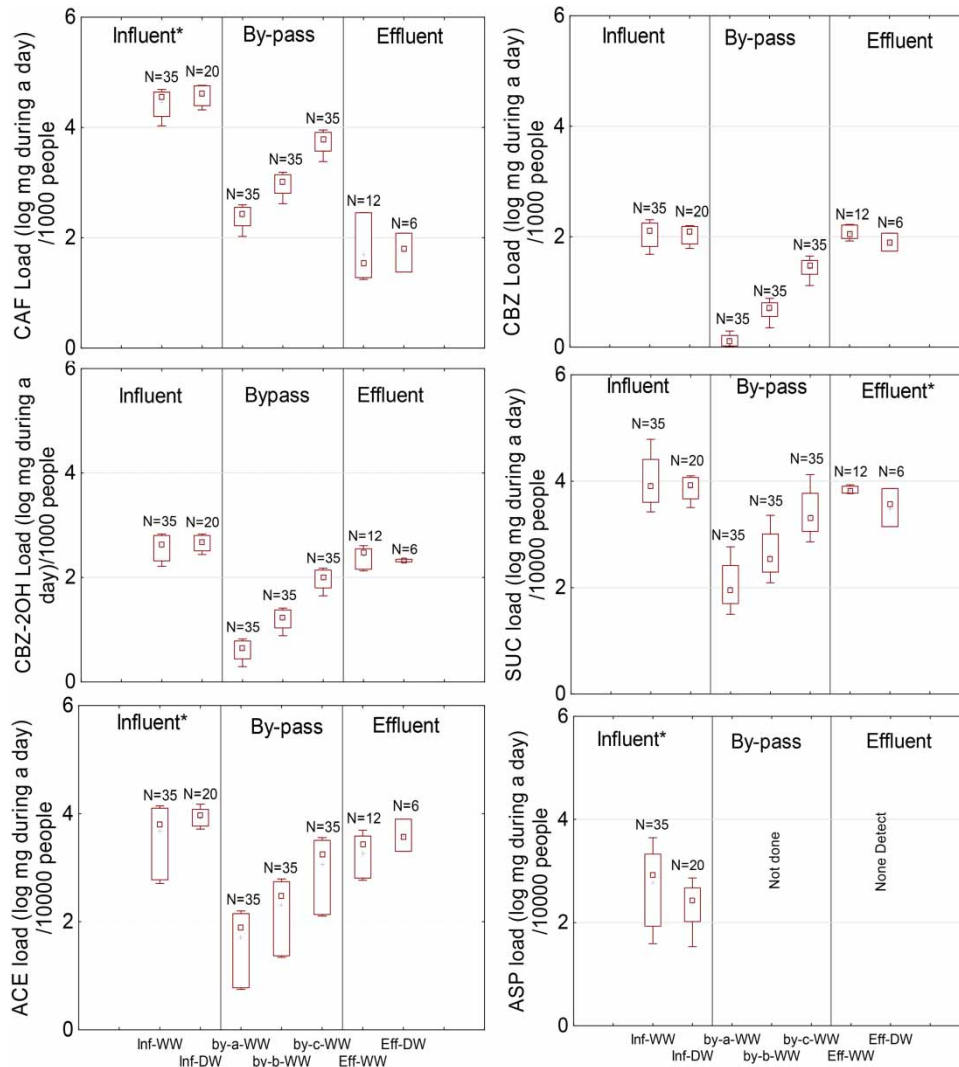
Figure 3 │ Observed *Giardia, E. coli, C. perfringens*, and TSS mass loadings from the influent and effluent, and estimated mass loadings from bypass discharges. Boxplots represent the 10th and 90th percentiles, median values (□), mean (+), and whiskers (minimum and maximum values) by-a-ww: 10th percentile bypasses ($Q_{by} = 58$ ML/day and $D_{by} = 3$ h); by-b-ww: 50th percentile bypasses ($Q_{by} = 136$ ML/day and $D_{by} = 5$ h); by-c-ww: 90th percentile bypasses ($Q_{by} = 240$ ML/day and $D_{by} = 16.5$ h), respectively. Obs. is the observed bypasses through historical data; * indicates a significant difference ($p < 0.05$) between wet and dry weather conditions.

rates, sewer process contribution to *E. coli, C. perfringens*, and TSS loads from the influent varied from 10% to 49%, from 21% to 83%, and from 15% to 24%, respectively. A recent study by Madoux-Humery *et al.* (2015) demonstrated that sediment resuspension contributed to FIB and TSS loads measured in CSOs. The contribution of sewer deposit resuspension ranged from 10% to 70% for *E. coli* loads, from 40% to 80% for enterococci loads, and from 26% to 82% to total suspended solids loads from CSO discharges (Chebbo *et al.* 2001; Gasperi *et al.* 2010; Passerat *et al.* 2011). The contribution of sewer deposit resuspension depends on pollutant type, sewershed type and configuration, rain intensity, and antecedent dry weather period. It has been demonstrated that the contribution of sewer deposit resuspension to the TSS load from CSOs varied significantly among rain events (10–70% for low-intensity events) and were higher for the high intensity events (>60%) (Gasperi *et al.* 2010). In this study of a sewershed with separate storm and sanitary sewers, the effects of sewer sediment

resuspension were less pronounced than in combined sewers likely as a result of lower variability of wet weather flows and a closer association between higher flow rates and human excretion patterns. Increased loads with a higher flow in this study are also the result of less deposition during higher flows and shorter travel times leading to less biodegradation within the sewer network.

In the case of WWMPs, the contribution of sewer processes was observed for CBZ, SUC, and ASP (Figure 2). The contribution of sewer processes to loads of CBZ under the wet weather condition was higher than that of CAF. This can be explained, at least in part, by lower biodegradability of CBZ in comparison to readily biodegradable CAF (Tran *et al.* 2018). Sewer sediments are known to act as a reservoir for CBZ in combined sewer systems (Madoux-Humery *et al.* 2015). Hajj-Mohamad *et al.* (2017) further showed that the sorption coefficient (log k_{dapp}) of native suspended and settled sediments from a combined sewer system were higher for CAF (0.3 ± 0.2 L/kg and

Figure 4 | Observed WWMPs mass loadings from the influent and effluent and estimated mass loadings from bypass discharges. Boxplots represent the 10th and 90th percentiles, median values ($\square$), mean (+), and whiskers (minimum and maximum values) by-a-ww: 10th percentile bypasses ($Q_{by} = 58$ ML/day and $D_{by} = 3$ h); by-b-ww: 50th percentile bypasses ($Q_{by} = 136$ ML/day and $D_{by} = 5$ h); by-c-ww: 90th percentile bypasses ($Q_{by} = 240$ ML/day and $D_{by} = 16.5$ h), respectively. Obs. is the observed bypasses through historical data; * indicates a significant difference ($p < 0.05$) between wet and dry weather conditions.

0.0 ± 0.1 L/kg, respectively) than for CBZ (0.1 ± 0.1 L/kg and -0.1 ± 0.1 L/kg, respectively), whereas desorption constants of CAF were lower than those of CBZ. Among the studied artificial sweeteners, the contribution of sewer processes to the loadings of ACE was limited (negative bars in Figure 2). This can be relatively explained by higher dilution of ACE as a result of its higher solubility following higher flow conditions. Compared with ACE and SUC, the contribution of sewer processes was higher for ASP, as the latter has relatively lower water solubility (10,000 mg/L) and higher log K_{ow} (0.07). Subedi & Kannan (2014) detected

ASP in 92% of influent suspended particles. In their study, the fraction of total ASP sorbed to suspended particulate matter was 50.4% and was higher than that of ACE and SUC. Their sorption coefficient was based on the concentrations measured in influent (ng/L) and suspended particulate matter (ng/kg dw) and reported to be 289, 5.1, and 4,540 L/kg for ACE, SUC, and ASP, respectively.

The flow rate of the WRRF depends on human activities and inflow/infiltration (as a result of precipitation, snowmelt, and groundwater table depths). It usually increases during the day before decreasing at night (Brière 2012) and

during the spring (following the snow melt period, Figure S2). Interestingly, for the majority of the microbial indicators and WWMPs studied, higher contributions of sewer processes were estimated for the Ev1 and Ev3 (events that occurred in spring and daytime, respectively). This could be partly related to higher fecal loads associated with the higher flows (from human defecation patterns) in addition to more resuspension of sewer sediments, less degradation, and less deposition with higher flows.

Mass loadings variability

Mass loadings from a WRRF depend on the population size, water usage and flow rate patterns, type of treatment, and weather conditions. Here, we determined mass loadings during normal operation conditions of the WRRF as well as in the case of failure and bypass discharge during various weather conditions. Daily loads of pathogenic parasites, FIB, WWMPs, and TSS from the influent and effluent as well as estimated daily loads from the primary effluent following scenarios of bypass discharge duration and flow rate (by-a (10th percentile bypass), by-b (median bypass), by-c (90th percentile bypass)) are illustrated in Figures 3 and 4. The impact of WRRFs on receiving waters could possibly be from the treated effluent and bypass discharges during wet weather conditions; thus, contaminant loads from bypasses were quantified to estimate the extra imposed load from WRRFs into receiving waters during wet weather periods compared with the normal operation condition.

Influent mass loadings under dry and wet weather conditions (representing incomplete treatment)

All meteorological conditions considered, influent median loads per 1,000 people were 6.8 log oocysts/day, 7.9 log cysts/day, 13.2 log CFU *E. coli*/day, and 11.4 log CFU *C. perfringens*/day. The median load of *Giardia* was significantly higher during the dry weather events monitored and *E. coli* during wet weather events ($p < 0.05$ in Mann–Whitney *U* test). However, the maximum loads of *Giardia* and *C. perfringens* (8.9 log cysts/day per 1,000 people and 12.5 log CFU/day per 1,000 people) were observed during the wet weather period (Figure 3).

Overall, the median loads of WWMPs per 1,000 people were 4.6 log mg CAF/day, 2.1 log mg CBZ/day, 2.6 log mg CBZ-2OH/day, 3.9 log mg ACE/day, 3.9 log mg SUC/day, and 2.6 log mg ASP/day, respectively. For the studied WWMPs, the maximum loads were generally observed during wet weather periods, CAF and ACE being the exceptions (Figure 4). The median load of TSS from the WRRF influent was 5.5 log g/day and 5.7 log g/day during dry and wet weather conditions, respectively (Figure 3). TSS median loadings from the influent were significantly lower for dry weather events ($p < 0.05$ in Mann–Whitney *U* test). Return periods of monitored events were 2 years or lower, meaning that larger precipitation events with higher return periods could lead to higher loads during bypass events.

Effluent mass loadings under dry and wet weather conditions (representing normal operating conditions)

Overall, the median loads of *Cryptosporidium* and *Giardia* into Lake Ontario per 1,000 people were 3.9 log oocysts/day and 6.3 log cysts/day and indicator bacteria were 7.8 log CFU *E. coli*/day and 9.3 log CFU *C. perfringens*/day. These are similar to the mean loads of *Cryptosporidium*, *Giardia*, and *E. coli* reported from effluent discharges of a WRRF in Luxembourg (4.3 log oocysts/day per 1,000 people, 6.2 log cysts/day per 1,000 people, and 8.7 log MPN/day per 1,000 people, respectively) (Burnet *et al.* 2014). The median *C. perfringens* loads from the effluent were significantly lower during dry weather events as compared with wet weather loads ($p < 0.05$ in Mann–Whitney *U* test) (Figure 3). The difference between *Giardia* and *E. coli* median mass loadings from the effluent under dry and wet weather conditions was insignificant ($p > 0.05$ in Mann–Whitney *U* test). While the low number of data ($n = 2$) for *Giardia* in effluent samples precludes further conclusions; maximum mass loadings were observed during wet weather conditions for *C. perfringens* and *E. coli*.

For WWMPs, the median loads from the effluent discharged into Lake Ontario per 1,000 people were 1.6 log mg CAF/day, 2.0 log mg CBZ/day, 2.4 log mg CBZ-2OH/day, 3.4 log mg ACE/day, and 3.8 log mg SUC/day. As was observed for FIB, the maximum loads of all studied WWMPs into Lake Ontario occurred during wet weather events, ACE being the exception (Figure 4). The median

mass loading of TSS from the effluent discharge was 3.3 log g TSS/day per 1,000 people. Mass loadings of CBZ and CBZ-2OH from the effluent discharge of the studied WRRF were comparable to values 2.3 log mg CBZ/day and 1.7 log mg CBZ-2OH/day per 1,000 people, respectively reported in another Canadian study (Miao *et al.* 2005). Total mass loadings of ACE and SUC from the effluent discharge and sewage sludge of a WRRF in the USA was reported as 3.04–3.13 log mg ACE/day per 1,000 people and 4.23–4.26 log mg SUC/day per 1,000 people, respectively (Subedi & Kannan 2014).

Results demonstrated that loads vary according to the meteorological conditions and hence routine monitoring, which is based on regular sampling dates, does not adequately describe event-based contaminant discharges important for quantifying the risks at drinking water intakes.

Primary effluent mass loadings under wet weather conditions (representing bypass discharges)

Parasite, FIB, WWMPs, and TSS loads from a primary effluent during a bypass discharge were estimated using assumptions adopted for the bypass flow rate and duration (by-a (10th percentile bypass), by-b (median bypass), and by-c (90th percentile bypass)) and are illustrated in Figures 3 and 4. The estimated ranges of *Giardia*, *E. coli*, *C. perfringens*, and TSS daily loads from bypass discharges per 1,000 people were 3.9–7.5 log cysts/day, 8.8–11.3 log CFU *E. coli*/day, 7.6–10.4 log CFU *C. perfringens*/day, and 1.9–4.7 log g TSS/day, respectively. The estimated values for *E. coli* and TSS are in agreement with the observed daily bypass loads (using historical data from 2007 to 2015) which are in the range of 6.7–10.7 log CFU *E. coli* per 1,000 people and 2.5–4.7 log g TSS per 1,000 people, respectively (Figure 3). Historical data were not available for other contaminants.

In order to understand the extra mass loadings that are discharged into Lake Ontario from a primary effluent following a bypass discharge to effluent discharges during the wet weather condition, F_1 ratios were calculated and illustrated in Table 3. The relative loadings from bypass discharges were higher for the microbial contaminants as compared with those of WWMPs that are generally less efficiently removed, an observation that confirms the findings of others for steroid hormones and six WWMPs, including

Table 3 | Relative median (lower limit and upper limit) loads from a bypass discharge to effluent discharge under wet weather conditions (F_1)

Parameters	Median (lower limit to upper limit)	Treatment rank
E. coli	87.4 (0.7–1,989.4)	High (>99%)
CAF	28.6 (1.3–198.3)	
Giardia	0.4 (0.1–5.4)	Moderate (90%)
C. perfringens	0.3 (0.1–2.6)	
TSS	2.2 (0.1–13.4)	
CBZ	0 (0–0.3)	Poor (≤70%)
CBZ-2OH	0.1 (0–0.4)	
ACE	0.1 (0–0.8)	
SUC	0.1 (0–0.7)	

caffeine (Phillips *et al.* 2012). Al Aukidy & Verlicchi (2017) showed that CSOs contributed to >90% of *E. coli* and >77% of enterococci monthly loads in receiving waters despite the fact that flow rates were much lower (9% in June, 17% in July, 2% in August, and 5% in September) in CSO discharges than in WRRF effluents (secondary effluent + bypass). The fractions of effluent loads during dry weather periods relative to effluent loads during wet weather periods (when a bypass discharge occurs) were also evaluated (F_2, Figure 5). For the studied contaminants (except ACE), the values of F_2 were generally <1, suggesting their higher loads into Lake Ontario during wet weather periods than during the dry weather period. For ACE, the amount of $F_2 \geq 1$ can be explained by its relatively higher solubility and poor removal through wastewater treatment.
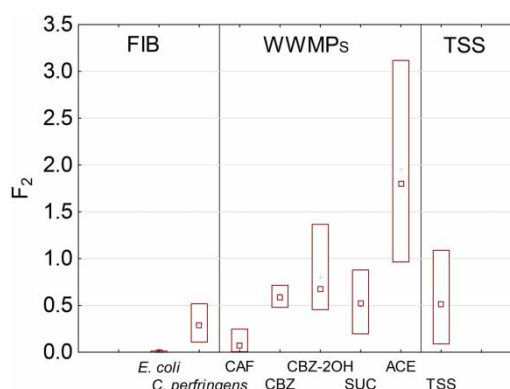
Figure 5 | The ratio of dry weather mass loadings to wet weather mass loadings when a bypass occurs (F_2). Boxplots demonstrate 10th and 90th percentiles, median values (□), mean (+), and whiskers (minimum and maximum values); FIB: fecal indicator bacteria; WWMPs: wastewater micropollutants; and TSS: total suspended solids.

At the studied WRRF, a maximum of two bypass discharges occur yearly and they can last for up to 16.5 h, which suggests that the contribution of bypass loads to total annual loads is insignificant. However, it should be taken into account that maximum mass loadings into Lake Ontario were observed during wet weather periods. Higher amount of parasites and FIB loads have been observed in drinking water reservoirs, at drinking water intakes, and in the influent of a drinking water treatment plant during wet weather periods (Kistemann *et al.* 2002; Burnet *et al.* 2014; Madoux-Humery *et al.* 2016). In Lake Ontario, pathogens have been studied at drinking water intakes (Edge *et al.* 2013) and there is a need to determine the relative importance of their sources for source water protection planning. Bypasses could represent critical events for drinking water treatment plants and communication of bypass events to drinking water treatment plant operators must be ensured.

The methodology applied in this study can be used to estimate the impacts of WRRFs on drinking water sources. Drinking water treatment is more concerned with peak contamination events outside the range of normal operating conditions than average or annual loads from wastewater effluents. This study further provides data for hydrodynamic modeling of the fate and transport of pathogens for quantitative microbial risk assessment of drinking water treatment plants.

The findings of this study were based on four wet weather events with return periods below 2 years and two dry weather events. A greater focus was placed on the influent sampling in order to understand loads of untreated or partially treated sewage during bypass events. Given the relationships observed between precipitation, flow rates, concentrations and loads, it would be useful to collect more samples for precipitation events with higher return periods that are representative of more extreme conditions and larger bypasses. Data for more intense events would improve risk assessments for water users including for drinking water production.

CONCLUSIONS

The present study provided the following key findings:

- In the influent, dilution as a result of inflow/infiltration during wet weather did not lower the loads of studied contaminants, except for ACE. In the effluent, the loads of both *E. coli* and *C. perfringens* were controlled primarily by treatment efficiency, *Giardia* and ACE by dilution processes.

- Sewer processes (deposition/resuspension and inactivation/biodegradation) are important for estimating contaminant loads under wet weather conditions. Considering all wet weather events, the increased loads as a result of sewer processes were in the range of 10–49% and 21–83% for *E. coli* and *C. perfringens*, respectively. Among the studied artificial sweeteners, the importance of sewer processes was more pronounced for ASP loads due to its lower solubility and potential for higher sorption to suspended particulate material in the sewer lines.

- Among the studied contaminants, overall removal efficiencies through wastewater treatment were generally higher for *E. coli* and CAF (>99%), moderate for *Giardia*, *C. perfringens*, and TSS (>90%), and poor for CBZ, CBZ-2OH, ACE, and SUC (≤70%). The contributions of loads from the primary effluent during a bypass discharge relative to the final effluent were higher for microbial contaminants as compared with those of WWMPs with poor total removal efficiency rates. The relative importance of loads from a bypass discharge depends on the removal efficiencies of contaminants through wastewater treatment. Bypass discharges are therefore more important contributors to daily loads of microbial contaminants that generally have high removal efficiencies through secondary wastewater treatment.

- For the studied FIB and WWMPs, the mass loadings during the wet weather period (with a bypass discharge) to mass loadings during the dry weather period were higher (except ACE), indicating their higher loads into Lake Ontario during wet weather periods.

- Emphasis should be placed on characterizing wet weather event discharges upstream of drinking water treatment plants as peak loads were observed during those periods.

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