EPA Public Access

Author manuscript

Environ Sci Pollut Res Int. Author manuscript; available in PMC 2019 September 12.

About author manuscripts

Submit a manuscript

Published in final edited form as:

Environ Sci Pollut Res Int. 2018 May ; 25(13): 12451–12463. doi:10.1007/s11356-018-1505-5.

Comparison of emerging contaminants in receiving waters downstream of a conventional wastewater treatment plant and a forest-water reuse system

Andrew D. McEachran¹, Melanie L. Hedgespeth¹, Seth R. Newton², Rebecca McMahan³, Mark Strynar², Damian Shea⁴, Elizabeth Guthrie Nichols¹

¹Department of Forestry and Environmental Resources, College of Natural Resources North Carolina State University Raleigh USA

²National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency Durham USA

³Oak Ridge Institute for Science and Education (ORISE) Research Participation Program, U.S. Environmental Protection Agency Durham USA

⁴Department of Biological Sciences, College of Science North Carolina State University Raleigh USA

Abstract

Forest-water reuse (FWR) systems treat municipal, industrial, and agricultural wastewaters via land application to forest soils. Previous studies have shown that both large-scale conventional wastewater treatment plants (WWTPs) and FWR systems do not completely remove many contaminants of emerging concern (CECs) before release of treated wastewater. To better characterize CECs and potential for increased implementation of FWR systems, FWR systems need to be directly compared to conventional WWTPs. In this study, both a quantitative, targeted analysis and a nontargeted analysis were utilized to better understand how CECs release to waterways from an FWR system compared to a conventional treatment system. Quantitatively, greater concentrations and total mass load of CECs was exhibited downstream of the conventional WWTP compared to the FWR. Average summed concentrations of 33 targeted CECs downstream of the conventional system were ~ 1000 ng/L and downstream of the FWR were ~ 30 ng/L. From a nontargeted chemical standpoint, more tentatively identified chemicals were present, and at a greater relative abundance, downstream of the conventional system as well. Frequently occurring contaminants included phthalates, pharmaceuticals, and industrial chemicals. These data indicate that FWR systems represent a sustainable wastewater treatment alternative and that emerging contaminant release to waterways was lower at a FWR system than a conventional WWTP.

Keywords

Wastewater; Contaminants of emerging concern (CECs); Forest-water reuse; Nontargeted analysis; Surface water; Water reuse

Introduction

Water availability and quality are expected to be adversely affected by adverse weather events and population pressures (Ingram et al. 2013; Trenberth 1999). Therefore, the ability to regulate water availability and improve water quality will become increasingly important with continued human population growth. One approach to regulating water availability and improving water quality is the use of forest-water reuse (FWR) systems (Braatz and Kandiah 1996; Isosaari et al. 2010). FWR systems are slow-rate irrigation systems that treat municipal, industrial, and agricultural wastewaters through infiltration into forest soils and groundwater (Crites 1984; Crites et al. 2014). These systems are lower cost and less energy intensive than conventional wastewater treatment systems of similar size, provide ecosystem service benefits via wood production and carbon storage, and effectively manage regulated nutrients, metals, and organics (US EPA 2006; Crites 1984; Pound and Crites 1973). However, these systems are dependent on land availability and application rates are subject to precipitation and weather. The management of regulated nutrients in groundwater and receiving waters (Nichols 2016; Pound and Crites 1973; Shifflett et al. 2014) and hydrological implications (Birch et al. 2016; Crites 1984; Hutchins et al. 1985) of FWR systems are more defined than the characterization of contaminants of emerging concern (CECs) released to surface waters from these systems. CECs in irrigated wastewater and receiving waters at FWR systems has only recently been assessed (McEachran et al. 2017a).

Wastewaters are a well-defined source of CECs in the aquatic environment (Bradley et al. 2017; Fick et al. 2009; Kolpin et al. 2002; Petrie et al. 2015; Roberts and Thomas 2006). These contaminants can individually and collectively elicit detrimental effects on aquatic biota (Bringolf et al. 2010; Quinn et al. 2008; Winter et al. 2008) and can propagate antibiotic resistance (Gao et al. 2012; Gatica and Cytryn 2013), among other harmful effects. Conventional, large-scale tertiary wastewater treatment plants (WWTP) are commonplace throughout the USA but do not completely remove many CECs from wastewater before discharge into receiving surface water bodies (Kolpin et al. 2002), resulting in near-continuous influx of CECs into surface waters (Roberts and Thomas 2006). Concentrations of CECs in surface water downstream of conventional wastewater treatment discharges are typically reported between 1 and 1000 ng/L (Petrie et al. 2015), though higher concentrations have been reported for acetylsalicylic acid (90,000 ng/L), carbamazepine (11,500 ng/L), ciprofloxacin (6.5 mg/L), etc. (Fick et al. 2009; Hughes et al. 2012; Kasprzyk-Hordern et al. 2008). Concentrations of CECs in the environment exceeding toxic effect concentrations can have detrimental effects on aquatic biota and have been observed in surface waters and wastewaters for steroid hormones, fluoxetine (Luna et al. 2013), diclofenac, etc. (Fent et al. 2006; Quinn et al. 2008). High levels of antibiotics in the environment may propagate antibiotic resistance (Zhang et al. 2017).

Targeted, quantitative analysis of CECs using analytical standards is well-established in the literature for the quantification of specific chemicals in wastewater and receiving surface waters (Agüera et al. 2013; Kolpin et al. 2002; Petrie et al. 2015). Analytical advances over the last decade have further enabled the detection of previously unidentified or uncharacterized CECs in the environment when analytical standards are either unavailable or not practical for use (Hug et al. 2014; Krauss et al. 2010; Schymanski et al. 2014a). High-

resolution mass spectrometry (HRMS) applications enable users to identify previously unknown or suspected chemical compounds in environmental samples using suspect screening and nontargeted analysis (SSA and NTA, respectively). Both SSA and NTA involve using comprehensive data processing workflows to identify chemical compounds based on observed chemical features (monoisotopic mass, isotope abundance, isotope spacing, etc.) (Schymanski et al. 2014b). SSA includes searching against a database or list of known compounds while true NTA does not. SSA and NTA methods applied to wastewater and surface waters receiving treated wastewater effluent have indicated the presence of thousands of compounds (Agüera et al. 2013; Hug et al. 2014; Schymanski et al. 2014b; Singer et al. 2016), but these methods have yet to be applied to alternative wastewater treatment systems, including FWR systems. SSA/NTA methods have predominantly been qualitative in nature, in contrast to targeted methods which focus on quantitation.

FWR systems release CECs into waterways via irrigation of wastewater, but a prior study showed greater than 90% removal of 33 PPCPs between irrigation to forest soils and surface water discharge (McEachran et al. 2017a). FWR systems have yet to be compared to conventional wastewater treatment systems regarding the release of CECs to surface waters. The study assesses the release of CECs by a FWR system and a conventional, tertiary WWTP to surface waters in order to inform management of wastewater treatment, water quality, and water sustainability. Both a quantitative targeted approach, to quantify the load of select CECs released to waterways, and a qualitative NTA approach, to characterize the overall chemical composition of CECs in wastewater and surface waters, were investigated for these two systems.

Materials and methods

Site description and sampling

Both the FWR system (Birch et al. 2016; McEachran et al. 2016) and conventional WWTP (Bringolf et al. 2010) sampling locations have been previously described. The FWR system land-applies secondary treated wastewater onto 930 ha of forested land after 7–14 days residence time in open-air reservoirs (Electronic Supplementary Material ESM 1). Wastewater is minimally chlorinated before application for worker safety. Land application is permitted such that runoff is minimized and relies on soil to groundwater infiltration before release to receiving waters. This system services a population of 70,000 and treats on average 19 million liters of wastewater per day (5.1 million gallons per day), irrigating 25–75 mm of secondary-treated wastewater weekly (Table 1). A major tributary flows through the FWR system; streamflow and stage data of the creek were collected using a previously installed gage at the subwatershed outlet (Birch et al. 2016). Mean stream discharge during the sampling events at the FWR system was 49 million liters per day. The conventional WWTP serves as one of two WWTPs servicing a population of 150,000 and treats on average 24 million liters per day. Wastewater at the conventional system is tertiary-treated followed by UV-disinfection before discharge of treated effluent into a small channel leading to a larger creek. Streamflow and stage data were obtained from the nearest US Geological Survey gage station. Mean stream discharge during the sampling events was 80 million liters per day downstream of the conventional WWTP. The FWR system services a residential

community while the conventional WWTP receives wastewater from some industrial sources in addition to residential sources.

One-liter surface water and wastewater direct grab samples were collected monthly from October 2015–December 2015 ($n = 3$) from seven locations. At the FWR system, (1) secondary treated wastewater was collected from a central spigot that distributes wastewater to the entire irrigation system. Surface water samples were collected from a major tributary (2) upstream of the land application area (Upstream) and (3) downstream of the irrigation system at the subwatershed outlet (Outlet, ESM 1). Tertiary-treated wastewater from the conventional WWTP system was collected (4) from the effluent discharge pipe before reaching the effluent channel. Surface water samples were collected from the creek (5) upstream of the effluent channel (Upstream) and at two sampling locations (6) 50 m and (7) 100 m downstream of the effluent channel (Down 1 and Down 2, respectively). All water samples were collected in precleaned and baked (300 °C for 24 h) 1-L amber glass bottles transported to the laboratory on ice and stored at 4 °C until extraction within 7 days of sampling (US EPA 2007).

Extraction and analysis

Pharmaceuticals and personal care products (PPCPs) are a subset of CECs and have been previously investigated at the FWR system. Thirty-three PPCPs (Table 2) for targeted analysis were selected for their environmental relevance and presence in wastewater (Bourgin et al. 2018; Kolpin et al. 2002; Metcalfe et al. 2010), previous detection at the FWR system study site (McEachran et al. 2016, 2017a), and status as frequently used PPCPs (Ebele et al. 2017). Extraction and analysis have been previously described (McEachran et al. 2016, 2017a). Briefly, filtered surface water and wastewater samples were spiked with surrogate internal standards ($[^{13}\text{C}]$ carbamazepine and $[^{13}\text{C}]$ 17 β -estradiol) and extracted under light vacuum using Oasis HLB solid-phase extraction (SPE) cartridges (Waters) and eluted using methanol and acidified methanol (0.1% trifluoroacetic acid). Eluents were evaporated to dryness and reconstituted to a final volume of 250 μL with 1:1 (methanol/DI water) and reference internal standards ($[^{13}\text{C}]$ caffeine and $[^{13}\text{C}]$ ibuprofen). Final extracts were split for targeted analysis and NTA.

Targeted analysis via UPLC-MS/MS

Targeted, quantitative analysis was conducted using a Thermo Scientific TSQ Quantum Ultra triple-quadrupole mass spectrometer with a Waters Acquity UPLC separation system and two analytical columns: an Acquity UPLC BEH (Ethylene Bridged Hybrid) Shield RP18 (2.1 mm $\times$ 150 mm, 1.7 μm) and an Acquity UPLC BEH C18 (2.1 mm $\times$ 100 mm, 1.7 μm). Extraction and quantitation has been previously described (McEachran et al. 2016, 2017a). Two separation and instrument methods, for positive and negative electrospray ionization (ESI), were utilized to maximize identification and quantification capabilities. The separation method used for positive mode ESI data collection utilized water and methanol with 0.05% acetic acid as mobile phases while for negative mode ESI, the mobile phases were water and methanol (McEachran et al. 2016). Final calculated concentrations (ng/L) and stream discharge measurements were used to calculate the mass load of total targeted CECs downstream of each system using Eq. 1:

$$\text{Mass load (mg/day}^{-1}\text{)} = \sum \text{CEC} * Q * 1/10^6 \quad (1)$$

where CEC refers to the summed concentration of targeted CECs in downstream samples (in ng/L) and Q is stream discharge at the time of sample collection (in liters/day).

HPLC-TOF/MS

Instrument methods for HRMS analysis are described in detail in Rager et al. (2016) and Newton et al. (2018). Briefly, samples were analyzed using an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) interfaced with an Agilent 6210 Time-of-Flight (TOF) mass spectrometer. Chromatographic separation was accomplished using an Eclipse Plus C8 column (2.1 × 50 mm, 3.5 μm; Agilent Technologies, Palo Alto, CA). The method consisted of the following conditions: 0.2 mL/min flow rate; column at 30 °C; mobile phases: A, ammonium formate buffer (0.4 mM) and DI water/methanol (95:5 v/v), and B, ammonium formate (0.4 mM) and methanol/DI water (95:5 v/v); gradient 0–25 min linear gradient from 75:25 A:B to 15:85 A:B; 25–40 min linear gradient from 15:85 A:B to 100% B; 40–50 hold at 100% B. The total run time was 45 min per sample, and ions from 100 to 1700 m/z were monitored in both positive and negative electrospray ionization (ESI) via separate injections. A reference compound mixture was constantly infused into the ion source for mass correction consisting of purine and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazene. Raw instrument data files were exported for further analysis and compound identification.

HRMS data processing

Data processing, peak alignment, and database matching procedures were modeled after those reported in Rager et al. (2016) with several modifications. Raw instrument files were loaded into MassHunter ProFinder software (Agilent) for molecular feature extraction and alignment across all samples. For data collected under negative ESI, background noise thresholds were set to 1000 counts and feature thresholds to 3000 counts. Intersample feature alignment thresholds were set for retention windows of 1.0 min and mass windows of 30 ppm ± 2.00 mDa. Based on manual observation of background noise, higher thresholds were used for data collected in positive ESI: background thresholds were 2000, counts and feature thresholds were set to 8000 counts. Extracted and aligned features were further interpreted using Mass Profiler Professional (Agilent). Aligned features were imported into Mass Profiler Professional and matched against a desalted and de-duplicated version of the US EPA's Distributed Structure-Searchable Toxicity Version 2 (DSSTox_v2) Database (Richard and Williams 2002). Features were matched to unique formulae within the database based on a mass accuracy, isotope abundance, and isotope spacing of observed features. These factors contribute to an overall match score (0–100).

Aligned features matched to DSSTox were further processed using SAS statistical software. Blank features as observed in a solvent blank and features where peak abundance was less than three times the peak abundance in a blank were removed from subsequent analysis. Features with a formula match score less than 90 were removed, except for those with a negative mass defect and mass less than 500 Da. Negative mass defects indicative of

halogenated compounds (Trier et al. 2011) have been observed to match poorly under the default isotope matching algorithm used (Newton et al. 2018); therefore, these features were kept for subsequent evaluation. Finally, duplicate features within a sample, with mass difference of 0.005 Da and retention time difference of 0.05 min, were identified and removed so that only one remained. Final aligned, database-matched, and filtered data were searched in the US EPA's CompTox Chemistry Dashboard (Williams et al. 2017) (<https://comptox.epa.gov>) following the methods described by McEachran et al. (2017b) to identify the most likely structures based on formulae. No quantitation was performed on data collected via TOF/MS.

Data quality assurance and statistics

One field duplicate sample was collected during every sampling event and processed in the same manner for quality control purposes; median relative percent difference of individual target compound concentrations between duplicates was 12%. Additionally, a deionized water blank and method recovery spike (deionized water fortified with all target analytes) were processed in every batch of samples for background contamination and ongoing method recoveries of targeted compounds. Surrogate recovery standards ($[^{13}\text{C}]$ carbamazepine and $[^{13}\text{C}]$ estradiol) were spiked into every sample to evaluate matrix interferences and extraction efficiency (McEachran et al. 2017a). Mean recovery of surrogate standards was 71%, and average recovery of all targeted compounds was 71%. For the targeted analysis, method quantification limits (LOQ) were defined as the lowest point on the standard curve and ranged from 0.75 ng/L to 7.5 ng/L. The method limit of detection (LOD) was estimated as the LOQ divided by 3.

Statistical analyses were conducted to compare mean concentrations between sampling locations. Concentration and mass load data did not fit the assumptions of normality, and therefore, Mann-Whitney-Wilcoxon tests were used to compare means (with $\alpha = 0.05$). Principal component analyses (PCAs) were performed to assess the variance-covariance structure of observed chemical features from both sites using a matrix of relative abundance of database-matched formulae above a score of 90 in each sample. Statistical analyses and PCA were conducted using R (Version 3.3.2) along with ggplot2 (Wickham 2009).

Results and discussion

Targeted analysis

PPCPs in wastewater and surface water—PPCP concentrations in surface waters upstream of both treatment systems were evaluated to better contextualize PPCP concentrations downstream. Average summed concentrations in surface water upstream of the conventional system and upstream of the FWR system were 120 and 33 ng/L, respectively (Fig. 1, Table 2). Summed concentrations did not vary significantly between systems ($p = 0.1$). At each upstream site, DEET, caffeine, and cotinine were observed among the highest concentrations of all PPCPs. DEET and caffeine are ubiquitous in river systems throughout the US (Bradley et al. 2017; Kolpin et al. 2002), and concentrations observed here are similar to those previously reported in the literature. However, DEET may be

overestimated in environmental sampling due to the occurrence of similarly structured compounds (Merel et al. 2014).

Total summed concentrations of targeted PPCPs in wastewater were similar between the two systems (Fig. 1, $p > 0.5$). Wastewater did vary between the two systems in terms of abundance of individual chemicals: atenolol, cotinine, DEET, bisphenol-A, and ibuprofen were greater at the FWR system while trimethoprim, triamterene, fluoxetine, and sulfamethoxazole were greater at the conventional system (Table 2). Twenty of the 25 PPCPs observed above the LOQ in the wastewater at the FWR system were also observed in the wastewater at the conventional WWTP, indicating the ubiquitous nature of many of the targeted PPCPs (Table 2). Individual PPCP concentrations in wastewater at the FWR system in the present study were similar to concentrations reported throughout a longer term study at the same FWR system (McEachran et al. 2017a) and consistently lower than in literature studies targeting conventional wastewater effluent (Bartelt-Hunt et al. 2009; Fairbairn et al. 2016; Kasprzyk-Hordern et al. 2009). Concentrations in the treated wastewater effluent from the conventional WWTP were within the range of similar studies (Bartelt-Hunt et al. 2009; Sui et al. 2010).

One main difference between the two systems is the level at which wastewater is treated before it is land-applied (FWR) or discharged (conventional WWTP). However, treatment efficacy is beyond the scope of the present study and differences in wastewater influent were not assessed. Nonetheless, it was expected that PPCP concentrations would be greater in wastewater from the FWR system due to the lower level of treatment relative to the conventional WWTP (secondary versus tertiary, respectively) (Luo et al. 2014). However, longer residence times in the open-air reservoir at the FWR system can promote degradation and transformation as well as sorption and settling of PPCPs before irrigation. These processes can potentially affect both individual chemical composition and total concentration. Further, differences in the influent wastewater could affect total PPCP concentrations in the effluent; industrial activity, population dynamics, and agricultural practices all can affect wastewater composition. Specifically, as the WWTP receives wastewater from industrial activities in addition to residential, differences in total PPCPs could be expected between the WWTP and FWR system. Mechanisms such as influent composition, residence times, sorption capacity, and kinetics were not investigated in the current study, and we suggest that they be incorporated into future studies comparing the two treatment types.

Average summed concentrations of PPCPs in surface water downstream (Down 1) of the conventional WWTP were approximately 30% of total wastewater concentrations (Fig. 1). Individual concentrations of PPCPs did not decrease in the 50 m between the two downstream locations at the conventional WWTP, and some concentrations increased, potentially due to release of contaminant stores within the stream basin (Table 2). Summed PPCPs at the outlet of the FWR system were approximately 1% of summed concentrations in irrigated wastewater (Fig. 1) and summed PPCP concentrations downstream of the conventional WWTP were approximately 30 times higher than concentrations at the outlet of the FWR system ($p = 0.1$, Wilcox test) (Fig. 1).

It was expected that individual PPCP concentrations would be greater downstream of the FWR due to lesser initial wastewater treatment; however, all individual PPCP concentrations were greater downstream of the conventional system (Table 2). The most abundant PPCPs downstream of the conventional WWTP were similar to concentrations previously reported in surface waters downstream of WWTPs (Hirsch et al. 1999; Kasprzyk-Hordern et al. 2008). The antidepressant drug fluoxetine was present at greater concentrations in surface waters (330 ± 200 ng/L) than previous research at this system reported (Bringolf et al. 2010), but were still within the range of environmental concentrations reported elsewhere (Martínez Bueno et al. 2007; Webb 2001). The concentration was highest in the month of December, indicating the potential effect of seasonal usage of pharmaceuticals on presence in water sources. Fluoxetine has been reported to cause detrimental effects in aquatic biota, but concentrations shown here were an order of magnitude below reported no observed effect concentration (NOEC) values for aquatic invertebrates (Péry et al. 2008) and vertebrates (*Pimephales promelas*) (Stanley et al. 2007) and multiple orders of magnitude below reported LC₅₀ values (Brooks et al. 2003). Streamflow was higher during this study compared to previous research at the same study site, and it has been documented that CECs can increase in concentration during high flow periods due to potential upstream sources or the release of chemicals from contaminant stores within the watershed (Fairbairn et al. 2016; Veach and Bernot 2011), instead of following more conventional concentration-dilution effects.

Forest-water reuse systems rely on the soil-forest system to remove and/or reduce contaminants before reaching groundwater and ultimately surface water. Previous research at the FWR system demonstrated the role soil serves in this system as a sink for PPCPs (McEachran et al. 2017a). Upstream surface water and wastewater PPCP concentrations were similar between the two treatment systems in this research, but greater concentrations of PPCPs were observed downstream of the conventional WWTP (Fig. 1). This indicates the potential value of the soil-forest system for management and removal of PPCPs before reaching water resources. Wastewater influent prior to treatment was not evaluated at both sites which prohibits an evaluation of treatment efficiency for the two systems, but it is clear that the soil-forest system has a profound effect on PPCPs in receiving waters.

Mass PPCP release to receiving streams—Total PPCP release of targeted chemicals as mass load was calculated in surface waters at each sampling location based on discharge of receiving streams (Eq. 1). Average discharge during sampling events downstream of the conventional WWTP was double the discharge at the FWR system (Table 1). Average summed concentrations of PPCPs were 1400 and 33 ng/L downstream of the conventional WWTP and FWR System, respectively. Total PPCP mass load of targeted PPCPs downstream (location Down 1) of the conventional WWTP was almost 100 times that at the FWR System (93,000 vs 1600 mg/day, respectively). These 33 PPCPs are not indicative of the complete chemical space of wastewater and do not define the entire input of CECs. However, a greater mass load of total measured PPCPs ($p = 0.1$) entered surface waters from the conventional system.

Nontargeted analysis

Molecular feature extraction of raw data files from the TOF acquisition resulted in ~ 10,000 molecular features in ESI+ mode and ~ 3500 features in ESI- mode. After database matching and data filtering, ~ 500 unequivocal formula matches in both ESI+ and ESI- remained, or roughly 7% of the total number of features initially observed via HRMS. However, 23% of the total peak area of observed features in the raw files was captured in the final database-matched formulae, indicating that many of the largest peaks present were captured. There was a substantial overlap in the number of unique formula matches between system types and sampling locations, indicating similarity of observed chemicals in the systems (Table 3) and ubiquity of certain chemicals in water and wastewater systems. A greater number of unique formulae were observed in the wastewater and downstream samples at the conventional WWTP. It was expected that the FWR system would contribute a greater number of chemical compounds to receiving waters due to lesser initial wastewater treatment than the conventional WWTP; however, a greater percentage of formulae found in the wastewater was also found in downstream samples at the conventional WWTP than at the FWR. More than 50% of formulae in the conventional WWTP wastewater were found in the downstream surface water. Only 30% of formulae in FWR wastewater were found in surface waters downstream of the FWR system. The FWR system relies on soil to groundwater infiltration before release to receiving surface waters, potentially decreasing the number of compounds present. More transformation processes (e.g., photolysis, longer residence time) could also result in fewer detections in the system. The NTA findings agree with the targeted data in that soil infiltration of wastewater by the FWR system dissipates a greater percentage of emerging organic compounds from wastewater to surface waters than the conventional WWTP system.

Principal component analysis compared overall chemical compositions of samples between wastewater treatment systems and their sampling locations. The wastewaters from the two locations were distinct from each other (ESM 4). Figure 2 shows that upstream samples were similar to each other from both sites, but downstream samples were distinct between the FWR and WWTP and 1 month in particular (October) was unique. FWR samples were generally collocated, indicating the lack of dramatic inputs between upstream and downstream samples. Figure 3 depicts all matched features observed in both wastewaters and downstream of both treatment systems. The wastewater and downstream samples at the conventional WWTP system contained a greater number of high-abundance chemicals as indicated by increased bubble size (Fig. 3); thus, more chemicals were present in greater relative abundance downstream of the conventional WWTP.

Database matching for identification—To understand the chemical-specific contribution of wastewater on surface waters, the most likely chemical for each matched formula above a match score of 90 was identified based on data source ranking techniques (Little et al. 2012; McEachran et al. 2017b). In this manner, the candidate chemicals with the greatest number of data sources within the US EPA's CompTox Chemistry Dashboard (<https://comptox.epa.gov>) are sorted such that the most likely candidate chemicals rise to the top of a search results list (McEachran et al. 2017b; Williams et al. 2017). These matched

chemicals are generally considered to be at least tentative candidates (Schymanski et al. 2014a).

Tentatively identified chemical compounds included industrial chemicals (diethyl phthalate, diphenyl sulfone), plasticizers, flavorants (butyl acetate), pharmaceuticals (litoxetine), endogenous compounds (purine, phosphoglycolate), and pesticides (anilazine, carboxin). Both diethyl phthalate and diphenyl sulfone were more abundant in the wastewater of the WWTP, which receives waste from industrial processes, than the FWR. The most frequently observed chemicals (present in greatest number of samples) across all samples included many previously reported in wastewaters and surface waters: Tris(2-chloroisopropyl)phosphate, 17 β -estradiol, diethyl phthalate, and butyl acetate (Table 4) (Loos et al. 2013; Loraine and Pettigrove 2006). In ESI+, the most frequently observed compounds were tentatively identified as being present in > 70% of the samples while in ESI-, the most frequently observed compounds were present in only 40% of the samples. Additional chemicals tentatively identified that were expected to be observed in wastewater samples included PFOS, PFOA, and fipronil. Further, PPCPs from the targeted list were also observed: DEET, ibuprofen, diphenhydramine, and fluoxetine, among others. The sensitivity of the nontargeted method is lower than the targeted LC-MS/MS method, but based on their abundance in the targeted method, these PPCPs were expected to be identified via NTA.

The most abundant chemicals (in peak area) identified regardless of frequency of occurrence included sucralose, fexonfenadine, tramadol, and 2-ethylhexyl 4-(dimethylamino)benzoate (ESM 5). The only compound that was one of the most frequently observed and present in high abundance in this study was the fungicide carboxin. Carboxin has been previously reported in wastewater effluent (Quesnel and Nakhla 2005), and its usage across many products is indicative of both high frequency of occurrence and high abundance. However, other compounds such as sucralose and phthalates are ubiquitous in wastewater systems (Kolpin et al. 2002; Loos et al. 2013), and it was expected that these would be both frequently occurring and present at high abundances in this study.

From an NTA perspective, more unique molecular features were present in the conventional WWTP system, including waters upstream of the system. Differences in observed chemical compositions between systems may have resulted from possible differences in influent composition (which was not measured in this study) or treatment methods used at the facilities. Previous research at the FWR system showed that concentrations in wastewaters decreased with increasing temperatures (McEachran et al. 2017a). At the FWR, wastewater is stored in open-air reservoirs for 10 to 14 days prior to irrigation, a physical, chemical, and biological factor not applicable to the conventional WWTP. Different types of disinfection by-products and other transformation products may have been introduced at the two facilities; the conventional WWTP uses UV disinfection whereas the FWR chlorinates wastewater prior to its distribution to the irrigation system. Such differences could explain the number of unique chemical compounds observed at the two systems, but these compounds are unlikely to be tentatively identified in the NTA workflow because they are neither well-characterized nor well-captured in reference databases, including the CompTox Chemistry Dashboard. The FWR system should also contain phytochemicals and endogenous compounds that may not be present at the conventional system due to the

relatively long residence time of treated wastewater at the FWR and its infiltration through soil to groundwater and surface waters. Although the characterization of the chemical space of wastewater is complex, NTA is a promising tool in the analysis of chemical constituents in water whose utility will only increase in the future as reference databases evolve to include more diverse groups of compounds.

Future work for NTA—In total, less than 10% of the molecular features observed in the raw data files were database-matched to unique chemical formulae. Additional data processing and prioritization methods are necessary to deal with the remaining (~ 90%) unmatched molecular features, requiring the integration of many sources to arrive at confident consensus structures. Thus, it is important to determine ways to increase the capabilities of data processing in NTA to ensure better coverage of the chemical space of samples. Additionally, using data source ranking alone is not sufficient to confirm structures—current methods of confirmation involve the use of analytical standards. In the absence of analytical standards, future work will require the integration of fragmentation prediction (Ruttkies et al. 2016), retention time analysis (Aalizadeh et al. 2016; Bade et al. 2015; McEachran et al. 2018), and other means to increase certainty in compound identification. Further data processing and prioritization methods under development include integrating multiple data streams for identification (Blaženović et al. 2017; Ruttkies et al. 2016), compound and sample prioritization using toxicity and bioactivity (Newton et al. 2018; Rager et al. 2016; Sobus et al. 2017), and processing code for filtering, blank subtraction, etc. Without such additional steps the likelihood of identifying false positives increases dramatically.

Conclusions and implications for wastewater treatment

Humans and ecological systems rely on effective wastewater treatment for clean water. For decades, conventional, and alternative treatment systems have been effectively treating nutrients and priority pollutants in wastewater to meet acceptable regulatory standards. The detection of CECs in wastewater and environmental samples coupled with greater water reuse necessitates evaluation of how effective treatment systems remove these chemicals for sustainable wastewater treatment and clean water availability. Our study has documented that over a 3-month period, fewer targeted and nontargeted emerging contaminants, by mass and/or quantity, were released into receiving waters by an FWR system land-applying secondary treated wastewater than by a conventional, tertiary WWTP of comparable treatment size. Further research is necessary to completely define the treatment efficacy and environmental release of CECs of both systems and should include untreated influent wastewater. Additional work should address the toxicity and bioactivity downstream of both systems. This research provides data that can inform the implementation of wastewater land application systems as a sustainable alternative to conventional treatment systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank Wanda Bodnar and Leonard Collins of the UNC Biomarker Mass Spectrometry Center for instrument use and technical assistance. We additionally would like to thank Andrew Birch and Hallie Hartley for field and technical assistance and Jon Sobus, Sarah Laughlin-Toth, and Jarod Grossman for data processing assistance. This study was supported with funding from the North Carolina State University Department of Forestry and Environmental Resources, the North Carolina Department of Agriculture and Consumer Services Bioenergy Research Initiative (G40100278314RSD), the National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program (Grant 5 P42ES005948), and the UNC Center for Environmental Health and Susceptibility (P30ES010126). This work was supported in part by an appointment to the ORISE Research Participation Program at the Office of Research and Development, US EPA, through an interagency agreement between the US EPA and DOE. This work has been internally reviewed at the US EPA and has been approved for publication. The views expressed in this paper are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

References

- Aalizadeh R, Thomaidis NS, Bletsou AA, Gago-Ferrero P (2016) Quantitative structure–retention relationship models to support nontarget high-resolution mass spectrometric screening of emerging contaminants in environmental samples. *J Chem Inf Model* 56: 1384–1398. 10.1021/acs.jcim.5b00752 [PubMed: 27266383]
- Agüera A, Bueno MJ Martínez, Fernández-Alba AR (2013) New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters. *Environ Sci Pollut Res* 20:3496–3515. 10.1007/s11356-013-1586-0
- Bade R, Bijlsma L, Miller TH, Barron LP, Sancho JV, Hernández F (2015) Suspect screening of large numbers of emerging contaminants in environmental waters using artificial neural networks for chromatographic retention time prediction and high resolution mass spectrometry data analysis. *Sci Total Environ* 538:934–941 [PubMed: 26363605]
- Bartelt-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K (2009) The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. *Environ Pollut* 157: 786–791. 10.1016/j.envpol.2008.11.025 [PubMed: 19110357]
- Birch AL, Nichols EG, James AL, Emanuel RE (2016) Hydrologic impacts of municipal wastewater irrigation to a temperate forest watershed. *J Environ Qual* 10.2134/jeq2015.11.0577
- Blaženović I et al. (2017) Comprehensive comparison of in silico MS/MS fragmentation tools of the CASMI contest: database boosting is needed to achieve 93% accuracy. *J Cheminform* 9:32 [PubMed: 29086039]
- Bourgin M et al. (2018) Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: abatement of micropollutants, formation of transformation products and oxidation by-products. *Water Res* 129:486–498. 10.1016/j.watres.2017.10.036 [PubMed: 29190578]
- Braatz S, Kandiah A (1996) The use of municipal waste water for forest and tree irrigation Food and Agriculture Organization of the United Nations. <http://www.fao.org/docrep/w0312e/w0312e09.htm>
- Bradley PM et al. (2017) Expanded target-chemical analysis reveals extensive mixed-organic-contaminant exposure in U.S. streams. *Environ Sci Technol* 51:4792–4802. 10.1021/acs.est.7b00012 [PubMed: 28401767]
- Bringolf RB, Heltsley RM, Newton TJ, Eads CB, Fraley SJ, Shea D, Cope WG (2010) Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. *Environ Toxicol Chem* 29:1311–1318 [PubMed: 20821574]
- Brooks BW et al. (2003) Waterborne and sediment toxicity of fluoxetine to select organisms. *Chemosphere* 52:135–142. 10.1016/S0045-6535(03)00103-6 [PubMed: 12729696]
- Crites RW (1984) Land use of wastewater and sludge. *Environ Sci Technol* 18:140A–147A. 10.1021/es00123a712
- Crites RW, Middlebrooks EJ, Bastian RK (2014) Natural wastewater treatment systems CRC Press, Boca Raton

- Ebele AJ, Abdallah M, Abou-Elwafa, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg Contam* 3:1–16. 10.1016/j.emcon.2016.12.004
- Fairbairn DJ et al. (2016) Contaminants of emerging concern: Mass balance and comparison of wastewater effluent and upstream sources in a mixed-use watershed. *Environ Sci Technol* 50:36–45. 10.1021/acs.est.5b03109 [PubMed: 26605430]
- Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. *Aquat Toxicol* 76:122–159. 10.1016/j.aquatox.2005.09.009 [PubMed: 16257063]
- Fick J, Söderström H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ (2009) Contamination of surface, ground, and drinking water from pharmaceutical production. *Environ Toxicol Chem* 28:2522–2527. 10.1897/09-073.1 [PubMed: 19449981]
- Gao P, Munir M, Xagorarakis I (2012) Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. *Sci Total Environ* 421–422:173–183. 10.1016/j.scitotenv.2012.01.061 [PubMed: 22369865]
- Gatica J, Cytryn E (2013) Impact of treated wastewater irrigation on antibiotic resistance in the soil microbiome. *Environ Sci Pollut Res* 20:3529–3538. 10.1007/s11356-013-1505-4
- Hirsch R, Ternes T, Haberer K, Kratz K-L (1999) Occurrence of antibiotics in the aquatic environment. *Sci Total Environ* 225:109–118. 10.1016/S0048-9697(98)00337-4 [PubMed: 10028708]
- Hug C, Ulrich N, Schulze T, Brack W, Krauss M (2014) Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening. *Environ Pollut* 184:25–32. 10.1016/j.envpol.2013.07.048 [PubMed: 24012788]
- Hughes SR, Kay P, Brown LE (2012) Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. *Environ Sci Technol* 47:661–677 [PubMed: 23227929]
- Hutchins SR, Tomson MB, Bedient PB, Ward CH, Wilson JT (1985) Fate of trace organics during land application of municipal wastewater. *Crit Rev Environ Sci Technol* 15:355–416
- Ingram KT, Dow K, Carter L, Anderson J (2013) *Climate of the Southeast United States: variability, change, impacts, and vulnerability* Springer, Berlin
- Isosaari P, Hermanowicz SW, Rubin Y (2010) Sustainable natural systems for treatment and disposal of food processing wastewater. *Crit Rev Environ Sci Technol* 40:662–697
- Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Res* 42: 3498–3518 [PubMed: 18514758]
- Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res* 43:363–380. 10.1016/j.watres.2008.10.047 [PubMed: 19022470]
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environ Sci Technol* 36:1202–1211 [PubMed: 11944670]
- Krauss M, Singer H, Hollender J (2010) LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Anal Bioanal Chem* 397:943–951. 10.1007/s00216-010-3608-9 [PubMed: 20232059]
- Little J, Williams A, Pshenichnov A, Tkachenko V (2012) Identification of known unknowns utilizing accurate mass data and ChemSpider. *J Am Soc Mass Spectrom* 23 10.1007/s13361-011-0265-y
- Loos R et al. (2013) EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res* 47:6475–6487. 10.1016/j.watres.2013.08.024 [PubMed: 24091184]
- Loraine GA, Pettigrove ME (2006) Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. *Environ Sci Technol* 40:687–695. 10.1021/es051380x [PubMed: 16509304]
- Luna TO, Plautz SC, Salice CJ (2013) Effects of 17 α -ethynylestradiol, fluoxetine, and the mixture on life history traits and population growth rates in a freshwater gastropod. *Environ Toxicol Chem*

- Luo Y et al. (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ* 473:619–641. 10.1016/j.scitotenv.2013.12.065 [PubMed: 24394371]
- Bueno MJ Martínez, Agüera A, Gómez MJ, Hernando MD, García-Reyes JF, Fernández-Alba AR (2007) Application of liquid chromatography/ quadrupole-linear ion trap mass spectrometry and time-of-flight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater. *Anal Chem* 79:9372–9384 [PubMed: 18001124]
- McEachran AD, Shea D, Bodnar W, Nichols EG (2016) Pharmaceutical occurrence in groundwater and surface waters in forests landapplied with municipal wastewater. *Environ Toxicol Chem* 35: 898–905. 10.1002/etc.3216 [PubMed: 26297815]
- McEachran AD, Shea D, Nichols EG (2017a) Pharmaceuticals in a temperate forest-water reuse system. *Sci Total Environ* 581–582:705–714. 10.1016/j.scitotenv.2016.12.185 [PubMed: 28073640]
- McEachran AD, Sobus JR, Williams AJ (2017b) Identifying known unknowns using the US EPA's CompTox Chemistry Dashboard. *Anal Bioanal Chem* 409:1729–1735. 10.1007/s00216-016-0139-z [PubMed: 27987027]
- McEachran AD, Mansouri K, Newton SR, Beverly BEJ, Sobus JR, Williams AJ (2018) A comparison of three liquid chromatography (LC) retention time prediction models. *Talanta* 10.1016/j.talanta.2018.01.022
- Merel S, Nikiforov A, Snyder S (2014) Monitoring DEET in Water: Fundamental Study to Evaluate the Plausibility of Mimics. *Environmental Analysis Techniques Workshop*, pp 18–19
- Metcalfe CD, Chu S, Judt C, Li H, Oakes KD, Servos MR, Andrews DM (2010) Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. *Environ Toxicol Chem* 29:79–89. 10.1002/etc.27 [PubMed: 20821422]
- Newton SR, McMahan RL, Sobus JR, Mansouri K, Williams AJ, McEachran AD, Strynar MJ (2018) Suspect screening and nontargeted analysis of drinking water using point-of-use filters. *Environ Pollut* 234:297–306. 10.1016/j.envpol.2017.11.033 [PubMed: 29182974]
- Nichols EG (2016) Current and future opportunities for forest land application systems of wastewater. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (ed) *Phytoremediation Management of Environmental Contaminants*, Vol III
- Péry ARR et al. (2008) Fluoxetine effects assessment on the life cycle of aquatic invertebrates. *Chemosphere* 73:300–304. 10.1016/j.chemosphere.2008.06.029 [PubMed: 18656226]
- Petrie B, Barden R, Kasprzyk-Hordern B (2015) A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res* 72:3–27. 10.1016/j.watres.2014.08.053 [PubMed: 25267363]
- Pound CE, Crites RW (1973) *Wastewater treatment and reuse by land application*, vol 1 Office of Research and Development, US Environmental Protection Agency, Washington, DC
- Quesnel D, Nakhla G (2005) Characterization and treatability of aerobic bacterial thermophilically treated wastewater by a conventional activated sludge and granular activated carbon. *Water Res* 39:677–687. 10.1016/j.watres.2004.11.010 [PubMed: 15707641]
- Quinn B, Gagné F, Blaise C (2008) An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, *Hydra attenuata*. *Sci Total Environ* 389:306–314. 10.1016/j.scitotenv.2007.08.038 [PubMed: 17931692]
- Rager JE et al. (2016) Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring. *Environ Int* 88 10.1016/j.envint.2015.12.008
- Richard AM, Williams CR (2002) Distributed structure-searchable toxicity (DSSTox) public database network: a proposal. *Mutat Res Fundam Mol Mech Mutagen* 499(1):27–52
- Roberts PH, Thomas KV (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Sci Total Environ* 356:143–153 [PubMed: 15936058]
- Ruttikes C, Schymanski EL, Wolf S, Hollender J, Neumann S (2016) MetFrag relaunched: incorporating strategies beyond in silico fragmentation. *J Cheminform* 8:1–16. 10.1186/s13321-016-0115-9 [PubMed: 26807156]

- Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J (2014a) Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ Sci Technol* 48:2097–2098 [PubMed: 24476540]
- Schymanski EL et al. (2014b) Strategies to characterize polar organic contamination in wastewater: exploring the capability of high resolution mass spectrometry. *Environ Sci Technol* 48:1811–1818. 10.1021/es4044374 [PubMed: 24417318]
- Shifflett SD, Hazel DW, Frederick DJ, Nichols EG (2014) Species trials of short rotation woody crops on two wastewater application sites in North Carolina, USA. *BioEnergy Res* 7:157–173
- Singer HP, Wössner AE, McArdell CS, Fenner K (2016) Rapid screening for exposure to “non-target” pharmaceuticals from wastewater effluents by combining HRMS-based suspect screening and exposure modeling. *Environ Sci Technol* 10.1021/acs.est.5b03332
- Sobus JR et al. (2017) Integrating tools for non-targeted analysis research and chemical safety evaluations at the US EPA. *J Expo Sci Environ Epidemiol* 10.1038/s41370-017-0012-y
- Stanley JK, Ramirez AJ, Chambliss CK, Brooks BW (2007) Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. *Chemosphere* 69:9–16. 10.1016/j.chemosphere.2007.04.080 [PubMed: 17582462]
- Sui Q, Huang J, Deng S, Yu G, Fan Q (2010) Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. *Water Res* 44:417–426. 10.1016/j.watres.2009.07.010 [PubMed: 19674764]
- Trenberth KE (1999) Conceptual framework for changes of extremes of the hydrological cycle with climate change. In: *Weather and Climate Extremes* Springer, Berlin, pp 327–339
- Trier X, Granby K, Christensen JH (2011) Tools to discover anionic and nonionic polyfluorinated alkyl surfactants by liquid chromatography electrospray ionisation mass spectrometry. *J Chromatogr A* 1218: 7094–7104. 10.1016/j.chroma.2011.07.057 [PubMed: 21871631]
- United States Environmental Protection Agency (2006) *Process design manual: Land treatment of municipal wastewater effluents* Cincinnati
- United States Environmental Protection Agency (2007) *Method 1694: pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS* Washington, DC
- Veatch AM, Bernot MJ (2011) Temporal variation of pharmaceuticals in an urban and agriculturally influenced stream. *Sci Total Environ* 409:4553–4563. 10.1016/j.scitotenv.2011.07.022 [PubMed: 21855963]
- Webb SF (2001) A data based perspective on the environmental risk assessment of human pharmaceuticals II—aquatic risk characterisation. In: Kümmerer K (ed) *Pharmaceuticals in the environment: sources, fate, effects and risks* Springer Berlin Heidelberg, Heidelberg, pp 203–219. 10.1007/978-3-662-04634-0_16
- Wickham H (2009) *ggplot2: elegant graphics for data analysis* SpringerVerlag, New York
- Williams AJ et al. (2017) The CompTox Chemistry Dashboard: a community data resource for environmental chemistry. *J Cheminform* 9: 61 10.1186/s13321-017-0247-6 [PubMed: 29185060]
- Winter MJ et al. (2008) Defining the chronic impacts of atenolol on embryo-larval development and reproduction in the fathead minnow (*Pimephales promelas*). *Aquat Toxicol* 86:361–369. 10.1016/j.aquatox.2007.11.017 [PubMed: 18179830]
- Zhang K, Niu ZG, Lv Z, Zhang Y (2017) Occurrence and distribution of antibiotic resistance genes in water supply reservoirs in Jingjinji area, China. *Ecotoxicology* 10.1007/s10646-017-1853-9

Fig. 1.

Mean summed concentrations of the 33 targeted PPCPs over a 3-month sampling period at the conventional WWTP and forest-water reuse (FWR) system. Concentrations on the y-axis are represented as log₁₀. Error bars represent the standard error of the mean ($n=3$).

Downstream data at the conventional WWTP is represented by the site “Down 1”

Fig. 2.

Principal component analysis (PCA) of the total chemical composition of upstream and downstream surface waters from both locations. Inputs for the PCA were all aligned and matched formulae (with match score > 90 as detailed in *HRMS Data Processing*) and the relative abundance of each formula within each sample. $N=3$ per sampling location is depicted (October–December 2015)

Fig. 3. Feature mass (y -axis) versus retention time (x -axis) of observed features with a match score > 90 in wastewater samples (top) and downstream samples (bottom) of the conventional WWTP and FWR System. Bubble size indicates relative abundance, as peak area, of the feature

Table 1

Wastewater treatment system and receiving water statistics

	Forest-water reuse system (FWR)	Tertiary conventional WWTP
Population served	70,000	75,500 ^a
Treatment capacity (L/day)	23×10^6	45×10^6
Mean volume treated (L/day)	19×10^6	24×10^6
Mean stream discharge (L/day)	49×10^6	88×10^6

^aThe municipality has two WWTP for 150,000 people

Table 2

Mean concentrations (ng/L) $\pm$ standard error of the mean ($n = 3$) of targeted PPCPs at sampling locations for both wastewater treatment systems

	Forest-water reuse (FWR) system			Conventional WWTP			
	Upstream	WW	Outlet	Upstream	WW	Down 1	Down 2
17- α -Ethinyl-estradiol	ND	ND	ND	ND	ND	ND	ND
17- β -Estradiol	ND	ND	ND	ND	ND	ND	ND
Acetaminophen	1.2 $\pm$ 0.7	ND	NQ	ND	8.6 $\pm$ 1.3	2.7 $\pm$ 1.9	1.2 $\pm$ 0.7
Atenolol	ND	920 $\pm$ 300	1.9 $\pm$ 1.3	ND	760 $\pm$ 100	160 $\pm$ 70	270 $\pm$ 50
BPA	ND	120 $\pm$ 60	ND	ND	ND	ND	ND
Caffeine	2.3 $\pm$ 0.4	120 $\pm$ 4	4.4 $\pm$ 1.9	43 $\pm$ 3	28 $\pm$ 1	50 $\pm$ 8	44 $\pm$ 6
Carbamazepine	NQ	68 $\pm$ 30	1.6 $\pm$ 0.2	NQ	250 $\pm$ 20	61 $\pm$ 20	91 $\pm$ 30
Cotinine	4.0 $\pm$ 3.0	96 $\pm$ 80	2.1 $\pm$ 1.4	30 $\pm$ 20	33 $\pm$ 10	10 $\pm$ 5	12 $\pm$ 5
DEET	23 $\pm$ 5	230 $\pm$ 100	6.7 $\pm$ 3.0	27 $\pm$ 10	16 $\pm$ 5	36 $\pm$ 20	35 $\pm$ 10
Diltiazem	NQ	28 $\pm$ 10	0.70 $\pm$ 0.3	NQ	120 $\pm$ 20	35 $\pm$ 20	51 $\pm$ 20
Diphenhydramine	NQ	240 $\pm$ 80	1.2 $\pm$ 0.7	NQ	165 $\pm$ 20	26 $\pm$ 10	43 $\pm$ 20
Erythromycin	ND	5.3 $\pm$ 3.7	ND	ND	8.9 $\pm$ 2.0	1.6 $\pm$ 0.8	2.8 $\pm$ 1.4
Estriol	ND	15 $\pm$ 6	3.6 $\pm$ 2.7	NQ	NQ	28 $\pm$ 10	32 $\pm$ 30
Estrone	ND	ND	ND	ND	ND	ND	ND
Fluoxetine	ND	160 $\pm$ 100	ND	ND	1000 $\pm$ 70	300 $\pm$ 180	330 $\pm$ 200
Gemfibrozil	ND	ND	ND	1.6 $\pm$ 1.0	7.0 $\pm$ 1.6	ND	ND
Ibuprofen	ND	200 $\pm$ 70	ND	ND	34 $\pm$ 6	ND	ND
Lincomycin	ND	NQ	ND	ND	NQ	NQ	13 $\pm$ 10
Meprobamate	NQ	83 $\pm$ 40	0.59 $\pm$ 0.2	NQ	140 $\pm$ 10	20 $\pm$ 10	36 $\pm$ 9
Naproxen	ND	ND	ND	5.7 $\pm$ 2.1	3.2 $\pm$ 1.4	13 $\pm$ 10	1.4 $\pm$ 0.6
Paraxanthine	1.0 $\pm$ 0.3	81 $\pm$ 30	1.8 $\pm$ 0.5	4.7 $\pm$ 0.4	91 $\pm$ 4	36 $\pm$ 9	52 $\pm$ 10
Paroxetine	NQ	4.1 $\pm$ 0.6	1.3 $\pm$ 0.7	NQ	16 $\pm$ 4	2.0 $\pm$ 1.3	6.5 $\pm$ 2.6
Progesterone	ND	6.7 $\pm$ 3.9	ND	ND	ND	ND	ND
Salicylic acid	ND	ND	ND	ND	ND	ND	ND
Sulfamethazine	NQ	1.0 $\pm$ 0.5	4.2 $\pm$ 0.5	NQ	NQ	NQ	2.8 $\pm$ 1.6
Sulfamethoxazole	NQ	280 $\pm$ 70	2.6 $\pm$ 0.7	NQ	460 $\pm$ 70	88 $\pm$ 40	160 $\pm$ 70
Testosterone	NQ	1.2 $\pm$ 0.7	ND	NQ	ND	ND	ND
Triamterene	NQ	82 $\pm$ 50	ND	NQ	200 $\pm$ 40	67 $\pm$ 30	83 $\pm$ 20
Triclocarban	ND	5.4 $\pm$ 4.1	0.9 $\pm$ 0.4	ND	ND	14 $\pm$ 10	10 $\pm$ 8
Triclosan	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	2.0 $\pm$ 0.1	250 $\pm$ 90	0.8 $\pm$ 0.2	6.5 $\pm$ 3.0	300 $\pm$ 100	100 $\pm$ 60	140 $\pm$ 60
Tylosin	ND	ND	ND	ND	3.3 $\pm$ 1.1	1.1 $\pm$ 0.6	3.0 $\pm$ 1.2
Valsartan	ND	36 $\pm$ 0.2	0.8 $\pm$ 0.4	ND	15 $\pm$ 1	4.7 $\pm$ 2.0	9.5 $\pm$ 2.4

Monthly data is presented in Electronic Supplementary Material ESM2 and ESM3. Estimations were made to calculate mean and standard error when at least one data point was below LOQ ($ND = 1/2 \text{ LOQ}$, $NQ = \text{LOQ}$). *Upstream* upstream, *WW* wastewater, *Outlet* subwatershed outlet, *Down 1* 50 m downstream, *Down 2* 100 m downstream, *ND* all samples not detected/below LOD, *NQ* all samples not quantified/below LOQ

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Table 3

Mean number of unique unequivocal molecular formulae observed at each location within each type of treatment system, FWR system and conventional WWTP, and the number of common formulae observed at both systems. Formula counts at sampling location and sites are independent. Results from both positive and negative ionization modes are combined

	FWR system	Conventional WWTP	Both
Upstream	25	44	183
Wastewater	127	370	412
Downstream	82	188	154

Table 4

The 15 most frequently observed compounds across both systems (tentatively identified) in ESI+ and ESI– modes, respectively, ordered from most to less frequent. Mass error = (observed mass – exact mass)/exact mass × 1,000,000. DTXSID is the unique DSSTox Substance Identifier (<https://comptox.epa.gov>)

Formula	Mass error (ppm)	Preferred name	DTXSID
ESI+			
C ₅ H ₅ N ₄	0.03	2-Azidopyridine	DTXSID80449938
C ₉ H ₁₈ Cl ₃ O ₄ P	0.22	Tris(2-chloroisopropyl)phosphate	DTXSID5026259
C ₂₂ H ₃₀ N ₂ O ₂	– 2.62	Aspidospermine	DTXSID70196883
C ₁₈ H ₂₄ O ₂	0.99	17β-Estradiol	DTXSID0020573
C ₁₅ H ₃₃ NO	– 1.30	C10–16-Alkyldimethylamines oxides	DTXSID1028992
C ₁₀ H ₉ FO ₄	– 1.82	Dimethyl 2-fluoroterephthalate	DTXSID10200973
C ₁₂ H ₁₄ O ₄	3.11	Diethyl phthalate	DTXSID7021780
C ₁₂ H ₂₂ N ₂ O ₂	– 6.76	Cycloleucylleucine	DTXSID30241782
C ₁₅ H ₃₃ NO	– 2.53	C10–16-Alkyldimethylamines oxides	DTXSID1028992
C ₁₆ H ₃₂ N ₆ O ₂	– 3.74	1H-1,3-Diazepin-2-amine, N,N'-(1,2-ethanediylbis(oxy-2,1-ethanediyl)bis(4,5,6,7-tetrahydro-, dihydriodide	DTXSID80232084
C ₅ H ₄ N ₄	0.03	Purine	DTXSID5074470
C ₁₂ H ₁₀ O ₂ S	0.68	Diphenylsulfone	DTXSID6041892
C ₁₃ H ₂₅ NSi	4.36	N-Triisopropylsilylpyrrole	DTXSID50236539
C ₁₂ H ₁₃ NO ₂ S	0.85	Carboxin	DTXSID0023951
C ₁₆ H ₁₉ NO	– 9.80	Litoxetine	DTXSID30235828
ESI–			
C ₆ H ₁₂ O ₂	– 1.98	Butyl acetate	DTXSID3021982
C ₇ HF ₇ S	– 2.87	2,3,5,6-Tetrafluoro-4-(trifluoromethyl)benzene-1-thiol	DTXSID20347262
C ₈ H ₁₆ Br ₂ O	2.48	Butane, 1,1'-oxybis(4-bromo-)	DTXSID70222691
C ₁₄ H ₈ BrClN ₂	0.51	2-(4-Bromophenyl)-4-chloroquinazoline	DTXSID00358472
C ₁₀ H ₁₀ O ₁₀ S ₃	– 2.36	2-Hydroxy-1,2-dihydronaphthalene-1,2,3-trisulfonic acid	DTXSID10767197
C ₁₈ H ₁₅ Cl ₃ O ₆	– 2.60	Buellolide	DTXSID20320313
C ₂ H ₅ O ₆ P	– 5.61	Phosphoglycolate	DTXSID60157064
C ₅ H ₅ ClN ₂ S	1.27	4-Chloro-2-methylthiopyrimidine	DTXSID60198114
C ₈ H ₆ F ₂ N ₂ O	3.16	5-(Difluoromethoxy)-1H-indazole	DTXSID00743405
C ₈ H ₁₀ O ₃ S	10.40	Methyl toluene-4-sulfonate	DTXSID4052550
C ₉ H ₁₂ O ₃ S	– 1.07	Sodium 4-isopropylbenzenesulfonate	DTXSID6047523
C ₁₂ H ₁₈ O ₃ S	3.86	Diisopropylbenzenesulphonic acid	DTXSID60182513
C ₁₃ H ₂₂ O ₄	– 0.45	Butanedioic acid, methylene-, dibutyl ester	DTXSID5062213
C ₁₀ H ₁₄ O ₅ S	2.05	2-(3,4-dimethoxyphenyl)sulfonylethanol	DTXSID30374342
C ₉ H ₅ Cl ₃ N ₄	– 5.76	Anilazine	DTXSID9020089