ABSTRACT

MCEACHRAN, ANDREW DAVID. Pharmaceutical and Personal Care Product (PPCP) Occurrence, Distribution, and Export at a Forest-Water Reuse System (Under the direction of Elizabeth G. Nichols).

Forest-water reuse systems are sustainable water-reuse systems which infiltrate municipal, industrial, and agricultural wastewater through forest soils to shallow aquifers and then surface waters via slow-rate irrigation. Their ability to mitigate regulated nutrients, metals, and organic chemicals is well known, but the fate of non-regulated chemicals in these systems is largely unstudied. Non-regulated chemicals include contaminants of emerging concern and pharmaceuticals and personal care products, which individually and collectively elicit detrimental effects on aquatic ecosystems and potentially human health. The environmental input of emerging contaminants from conventional wastewater treatment plants is well documented. How forest-water systems provision and regulate water is informative to sustainable technologies that can mitigate climate change variability, provision wood products and bioenergy, preserve forested landscapes, and improve water resources for communities and wildlife. This research investigated pharmaceuticals and personal care products (PPCPs) in soils, groundwater, and surface water at a 2,000 hectare forest irrigated year-around with secondary-treated, municipal wastewater. This temperate, irrigated forest does contribute PPCPs to soils, groundwater, and surface waters. PPCPs were more abundant in soils versus underlying groundwater by an order of magnitude. PPCP concentrations in surface waters were greater at the onset of significant storm events and during low-rainfall periods. Finally, PPCPs and other emerging contaminants were lower at the forest-water reuse system than a conventional treatment system of similar treatment volume. Overall, PPCP concentrations in surface waters and groundwater were at least one

order of magnitude below guideline values for ecological risk and several orders of magnitude below estimated human health risks. This research has shown that in addition to regulating water quality and availability, this forest-water system reduced the environmental input of PPCPs to a greater extent than a conventional treatment system did. This data will inform future implementation of forest-water reuse systems and indicates that these systems can be effective sustainable water-reuse systems with regards to PPCPs in the environment.

Pharmaceutical and Personal Care Product (PPCP) Occurrence, Distribution, and Export at a Forest-Water Reuse System

by
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A dissertation submitted to the Graduate Faculty of
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in partial fulfillment of the
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BIOGRAPHY

Andrew David McEachran was born on May 19, 1989 in Mt. Holly, NJ where he lived until 1997 when his family moved to Hickory, NC. Upon graduation from St. Stephens High School in Hickory, NC, Andrew enrolled at North Carolina State University to pursue a Bachelor of Science in Environmental Technology and Management. Andrew graduated from NC State in 2011 and soon after began pursuing a Master of Science degree in Environmental Toxicology at Texas Tech University in Lubbock, TX. At Texas Tech Andrew conducted novel research on the environmental fate of antibiotics and antibiotic resistance genes under the guidance of Dr. Phil Smith and graduated from Texas Tech in 2013. Andrew then returned to NC State to pursue a Ph.D. working with Dr. Elizabeth Nichols in the Department of Forestry and Environmental Resources.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Climate change and population pressures are expected to place increasing demands on water quality and availability.[1] Temperate climates, in addition to more arid regions of the world, are already experiencing water availability shortages due to competition of resources.[1, 2] Sustainable water reuse systems can provide water security through improvements of water quality and regulation of water availability.[2] Forest-water reuse systems are one type of sustainable water reuse system capable of providing water security. Forest-water reuse systems are slow-rate land application systems that that treat industrial, agricultural, and municipal wastewaters while providing ecosystem services such as provisioning wood products, biodiversity, and carbon storage.[3, 4] Forest-water reuse systems in temperate regions enable year-round wastewater treatment and nutrient storage without detriment to forest integrity or water quality. These systems mitigate regulated nutrients, metals, and organics in wastewaters to acceptable criteria levels before reaching groundwater and surface water[4, 5,20] and are more energy efficient and cost-effective than conventional treatment systems of similar size. Land application of wastewater has effectively mitigated regulated contaminants in the United States for decades.[6] However, the fate and effects of non-regulated wastewater contaminants in these systems remains largely uninvestigated.

The occurrence and fate of contaminants of emerging concern, and specifically pharmaceutical and personal care products (PPCPs), in the environment are of increasing public importance due to their ubiquitous nature and documented environmental effects on wildlife.[7-10] These compounds, which include antibiotics, synthetic hormones, and prescription and non-prescription drugs, enter the environment chiefly through wastewater

but also via agricultural processes and pharmaceutical production. Once in the environment, PPCPs elicit detrimental effects on aquatic wildlife in the manner of negative reproductive effects,[11-13] behavioral changes,[14] and proliferation of antibiotic resistance,[15, 16,21] among others.[17] Conventional, tertiary wastewater treatment plants (WWTPs) are well-documented sources of PPCPs and emerging contaminants in the environment;[18,19] however, alternative wastewater treatment systems have been under-investigated.

This research was conducted to better understand how forest-water systems manage emerging contaminants over time and to define the extent to which forest-water reuse systems input emerging contaminants into the environment. Understanding emerging contaminant input from forest-water reuse systems will inform the future implementation of these systems for sustainable water reuse. This research was carried out through three separate but related studies outlined here as subsequent Chapters 1-3. Chapter 1 is a preliminary investigation of PPCPs at a forest-water reuse system in Jacksonville, NC. This study identifies the occurrence of PPCPs in groundwater and surface water at the forest-water reuse system. Chapter 2 is a year-long study of the environmental fate of a select group of PPCPs at the same forest-water reuse system in Jacksonville, NC. Soil, groundwater, and surface water were investigated in an effort to more completely understand how the forest-water reuse system functions with respect to PPCPs. And finally, Chapter 3 presents an explicit comparison of PPCP and broader emerging contaminant input between a conventional, tertiary wastewater treatment plant and the Jacksonville forest-water reuse system through a three month sampling period of surface water and wastewater. Water reuse systems are likely to play a critical role in stabilizing water resources as climate change and

population pressures place increasing demands on clean water sources. Forest-water reuse systems can provide a sustainable alternative to traditional wastewater treatment but minimizing emerging contaminant input into the environment is critical to their large-scale implementation.

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CHAPTER 1

Title: Pharmaceutical Occurrence in Groundwater and Surface Waters in Forests Land-Applied with Municipal Wastewater

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PHARMACEUTICAL OCCURRENCE IN GROUNDWATER AND SURFACE WATERS IN FORESTS LAND-APPLIED WITH MUNICIPAL WASTEWATER

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Abstract: The occurrence and fate of pharmaceutical and personal care products in the environment are of increasing public importance because of their ubiquitous nature and documented effects on wildlife, ecosystems, and potentially humans. One potential, yet undefined, source of entry of pharmaceuticals into the environment is via the land application of municipal wastewater onto permitted lands. The objective of the present study is to determine the extent to which pharmaceuticals are mitigated by or exported from managed tree plantations irrigated with municipal wastewater. A specific focus of the present study is the presence of pharmaceutical compounds in groundwater and surface water discharge. The study site is a municipality that land-applies secondary treated wastewater onto 930 hectares of a 2000-hectare managed hardwood and pine plantation. A suite of 33 pharmaceuticals and steroid hormones was targeted in the analysis, which consisted of monthly grab sampling of groundwater, surface water, and wastewater, followed by concentration and cleanup via solid phase extraction and separation, detection, and quantification via liquid chromatography coupled with tandem mass spectrometry. More than one-half of all compounds detected in irrigated wastewater were not present in groundwater and subsequent surface water. However, antibiotics, nonsteroidal anti-inflammatory drugs, caffeine, and other prescription and over-the-counter drugs remained in groundwater and were transported into surface water at concentrations up to 10 ng/L. These results provide important documentation for pharmaceutical fate and transport in forest systems irrigated with municipal wastewater, a previously undocumented source of environmental entry. *Environ Toxicol Chem* 2016;35:898–905. © 2015 SETAC

Keywords: Pharmaceuticals Wastewater Groundwater Liquid chromatography–tandem mass spectrometry

INTRODUCTION

As human populations grow, the need for sustainable treatment and reuse of municipal wastewater becomes increasingly essential. Most municipal wastewater generated in the United States is treated through various levels of wastewater treatment plants (WWTPs), and the treated effluent is discharged into receiving waters, which can include streams, rivers, and lakes. Because of the unique chemical nature of many pharmaceuticals and personal care products (PPCPs), these frequently administered and consumed compounds can remain in wastewater throughout the treatment and discharge process and have been detected in ambient rivers and streams throughout the United States [1]. Once in the environment, PPCPs can result in detrimental effects on aquatic wildlife. Natural and synthetic estrogen compounds, including 17β-estradiol, estrone, and 17α-ethynylestradiol, have been reported to cause decreased fecundity in fathead minnows [2] and reduced testicular development in trout [3], among other negative reproductive effects in fish. Furthermore, inhibition of growth and development of secondary sexual characteristics in *Daphnia magna*, an important indicator species in aquatic ecosystems, was reported after exposure to a variety of endocrine-active chemicals [4]. Other commonly used compounds that are persistent in wastewater treatment effluent are nonsteroidal anti-inflammatory drugs. These compounds, including ibuprofen and naproxen, have been reported in the environment at concentrations of 300 ng/L in surface waters [5], and they elicit negative reproductive effects on aquatic organisms at low, but potentially environmentally relevant, concentrations [6]. Furthermore, commonly used antidepressant drugs, once in the environment, can elicit behavioral changes in fish. Antibiotics in the environment lead to the development and propagation of antibiotic resistance [7–9] and have been frequently detected in surface waters and in wastewater lagoons at concentrations exceeding 100 ng/L. These concentrations have been reported to influence the proliferation of antibiotic-resistant bacteria in the environment and thus present potential public health concerns [1]. In addition to the effects of individual classes of chemical compounds on aquatic organisms, exposure to mixtures of chemicals likely leads to compounded and interactive effects on aquatic species and ecosystems. Because pharmaceutical compounds are often found at low levels and in mixtures from wastewater effluent, the potential additive, antagonistic, or synergistic effects of mixtures on aquatic ecosystems is important to understand. Luna et al. [10] reported that mixtures of 17β-estradiol and the antidepressant fluoxetine decreased reproductive success of *D. magna* significantly more than either chemical compound alone.

An alternate solution to direct discharge of treated wastewater is the land application of municipal wastewater onto permitted lands [11]. In the United States, land application technologies were implemented in the 1970s with the introduction of the National Environmental Policy Act and amendments to the Federal Water Pollution Control Act in 1972 [12]. This technology involves the irrigation of municipal wastewater onto agricultural or forested lands; the treatment of wastewater may vary from no treatment to tertiary treatment before actual irrigation onto land. The state of North Carolina has 86 permitted sites for the land application of municipal wastewater. The study

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site of interest in the present study applies secondary treated wastewater onto 930 hectares of mixed hardwood and pine forests with a potential capacity to irrigate 2000 hectares of forest (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). The existing forest and new bioenergy hardwood tree plantations comply with nutrient management requirements for groundwater [13].

Although these forest systems filter nutrients from wastewater to acceptable criteria levels before reaching surface water sources, the fate and transport of PPCPs from wastewater application to groundwaters and surface waters is unknown. Preliminary screening data indicated detectable amounts of the wastewater indicator and plasticizer bisphenol A in groundwater collected at the site via a nontargeted qualitative method. The evaluation of PPCP release from land-applied forest systems to aquatic ecosystems requires characterization of flow paths over time, because the volume of land-applied wastewater can be greater during dry conditions and lower during significant rainfall events. The hydrological characterization of the study site is the focus of a separate research publication and indicated that wastewater makes up an average of 24% of the mean daily discharge of the site (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). The objective of the present study was to determine the presence of PPCPs in the land-applied system and provide preliminary data as to the extent to which PPCPs can be mitigated by or exported from managed forest systems irrigated with municipal wastewater.

METHODS

Site description and sampling

The wastewater land-application facility receives approximately 19 000 m^3/d of municipal wastewater from a city with a population of 70 000 and a daily wastewater outflow of approximately 19 700 m^3 [13]. Secondary treated wastewater is land-applied using 1-m irrigation risers after residence times of 7 d to 14 d in open reservoirs onto 930 hectares, as needed, of forested land. The land application facility has been in operation since 1998, when it was constructed in response to the failure of a traditional WWTP. Soils within the watershed containing the land-application site generally fall under the sandy loam or fine sand soil textural class and are well to moderately well drained. The soils at the groundwater sampling locations are specifically classified as Norfolk loamy sand. The watershed receives an annual average rainfall of 1300 mm. On average, 1200 mm wastewater is land-applied on a yearly basis on this site [13]. During the present study period, the watershed received 130 mm precipitation and 120 mm wastewater irrigation per month. The land-application process is designed such that there is no overland flow of wastewater, indicating that all of the applied wastewater is either retained in the soil vadose zone, is evapotranspired, or becomes groundwater.

Six groundwater wells and 2 surface water points were selected from the 2000-hectare wastewater land application site. Groundwater wells were selected where previous research indicated infiltration of wastewater by means of similar isotopic signatures ($\delta^{18}O$ and $\delta^{2}H$) in the groundwater to that of wastewater (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). The groundwater gradient labeled transect A (TA) connects a large upland area of irrigation (wells 2, 3, and 6) to a small tributary (TA SW) down gradient, providing the capability to analyze PPCP concentrations along a groundwater gradient (Figure 1). Transect B is groundwater unrelated to the gradient in TA, and the final surface water sampling point (outlet) was included to determine PPCP concentrations in surface water leaving the facility's property (this sampling point was not sampled for August and September 2014).

Sampling occurred monthly from August 2014 to January 2015 and followed US Geological Survey field manual protocols for groundwater and surface water [14]. Monthly sampling was conducted to cover greater temporal variation, the lack of which is a common critique of environmental sampling and analysis of PPCPs [15]. For groundwater, wells were purged 3 volumes before collection of 1 L groundwater, using a stainless steel bailer, into precleaned and baked amber glass bottles. The bailer was rinsed with water and acetone between groundwater wells. One-liter depth-integrated and 1-L width-integrated surface water samples were collected by using amber glass liter bottles; wastewater in the irrigation system was collected from a central spigot between the holding reservoirs and the land application irrigation system. A total of 10 samples (6 groundwater samples, 2 surface water samples, 1 wastewater sample, and 1 field duplicate) were collected each sampling event. Samples were transported to the laboratory on ice and stored at 4 °C until extraction, within 10 d of sampling.

Target compounds

The 33 compounds targeted in the present study were selected according to their potential risk to aquatic organisms, or are commonly prescribed or consumed pharmaceuticals in the United States (Table 1). Chemical standards were purchased from Sigma Aldrich, and stable–isotopically labeled internal standards were purchased from Cambridge Isotope Laboratories.

Extraction and analysis

Extraction procedures were similar to those described by Cahill et al. [16], Klosterhaus et al. [17], and Phillips et al. [18] with minor amendments. All water samples were filtered through 2.7-μm Whatman glass fiber filters (Fisher Scientific) and spiked with surrogate internal (recovery) standards before extraction. Samples were extracted and concentrated using Oasis HLB solid phase extraction cartridges (Waters). Cartridges were preconditioned with methanol and water, and prefiltered samples were loaded through solid phase extraction cartridges under vacuum at 10 mL/min to 15 mL/min. The solid phase extraction cartridges were dried under vacuum for 15 min, rinsed with 2 mL water, and dried under vacuum for 15 min again before elution with 2 4-mL aliquots of methanol followed by 4 mL acidified methanol (0.1% trifluoroacetic acid). Eluents were evaporated to near dryness, rinsed and combined in methanol, and evaporated to dryness. Samples were reconstituted in a final volume of 250 μL composed of 200 μL 1:1 methanol:water and 50 μL of stable-isotopically labeled reference internal standard. Processed samples were then stored at −80 °C until instrumental analysis. Immediately before analysis, samples were centrifuge-filtered using Pall Life Sciences Nanosep 3K Omega filters.

Two separation and analysis methods were used to separate, detect, and quantify target compounds (Supplemental Data, Tables S1 and S2). The basic/neutral targeted compounds were separated by reverse-phase liquid chromatography, using an Acquity UPLC BEH (Ethylene Bridged Hybrid) Shield RP18 (2.1 mm × 150 mm, 1.7 μm) column from Waters and analyzed on a Thermo Scientific TSQ Quantum Ultra triple-quadrupole mass spectrometer configured with a Waters Acquity UPLC separation system. The second method was used to target the

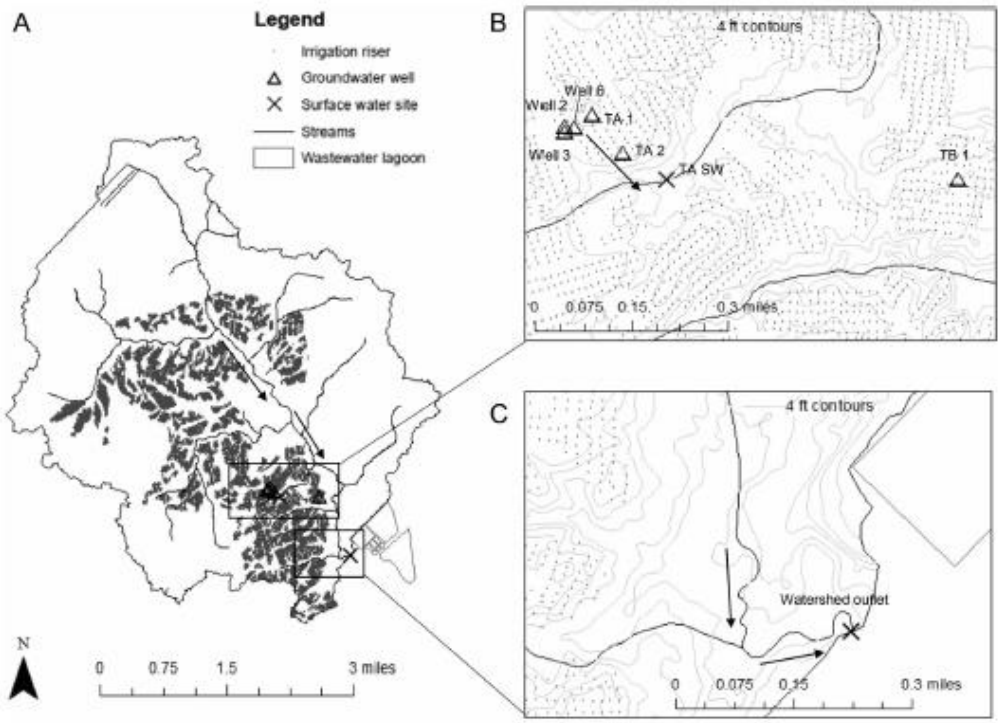

Figure 1. Map of the land-application area, targeted sampling locations, and associated watershed. (**A**) The watershed is represented, including streams, groundwater wells, surface water sites, wastewater irrigation risers, and the wastewater holding lagoons. Areas of sampling are zoomed in from the larger watershed image. (**B**) Groundwater flow direction connecting the upland groundwater wells (wells 2, 3, and 6) and transect A (TA; TA 1, TA 2, TA SW) is depicted. Transect A 1 and TA 2 are groundwater wells, and TA SW is a surface water discharge point at the bottom of the gradient. (**C**) The watershed outlet and stream flow directions are depicted (**C**). TA = transect A; TA SW = transect A surface water.

more acidic analytes, and separation occurred via an Acquity UPLC BEH C18 (2.1 mm × 100 mm, 1.7 μm) column. Compound detection was achieved via electrospray ionization in positive mode for the first method and negative mode for the second. Target compounds were quantified using an internal standard method comprising 7 levels of calibration standards.

Quality assurance

Field duplicate samples were collected for every sampling event and analyzed in the same method. In addition, for each set of samples collected, 1 field blank and 1 standard spiked blank were extracted and analyzed in the same manner to determine background contamination and ongoing method recoveries, respectively. Finally, all samples were spiked with 2 stable-isotopically labeled surrogate recovery standards before extraction. Method recoveries and relative standard deviations (SDs) of recoveries were within the range of other studies targeting a similar number of compounds from water and wastewater [17,19]. Average standard analyte recoveries for all compounds of interest were 67%, and average relative SD of recovery (%RSD) was 26% (Supplemental Data, Table S3). Two surrogate internal standards, [^{13}C] carbamazepine and [^{13}C] 17β-estradiol, were added to every sample before extraction, and ongoing %RSDs of these were 18% and 50%, respectively. In field blanks only, %RSD was 4% ([^{13}C]

Table 1. Pharmaceuticals and personal care products (PPCPs) targeted in chemical analysis

Chemical analysis grouping[a]	PPCP
Steroid hormones	17-α ethynyl estradiol, 17-β estradiol, estrone, estriol, progesterone, testosterone
Antibiotics and antimicrobials	Sulfamethoxazole, lincomycin, sulfamethazine, tylosin, trimethoprim, triclosan, erythromycin
Nonsteroidal anti-inflammatory drugs	Naproxen, ibuprofen, acetaminophen, salycilic acid (aspirin metabolite)
Prescription and nonprescription drugs	Gemfibrozil, meprobamate, fluoxetine, diltiazem, paroxetine, valsartan, carbamazepine, atenolol, diphenhydramine, triamterene
Caffeine	Caffeine, paraxanthine (metabolite)
N,N-diethyl-meta-toluamide (DEET)	DEET
Nicotine metabolite	Cotinine
Plasticizer	Bisphenol-A (BPA)

[a]Specified groupings for chemical analysis based on general chemical class.

carbamazepine and [^{13}C] 17β-estradiol). Only the compound N,N-diethyl-meta-toluamide (the insect repellent DEET) was detected in extracted field blanks above limits of detection. Field duplicates were used to determine the relative percentage difference of targeted compounds. Average relative percentage difference was 37% (±15%) across all PPCPs detected in duplicate samples throughout the sampling period. Method detection limits were compound specific. Most analyte method detection limits values were less than 1 ng/L, and no detection limit was higher than 15 ng/L.

RESULTS

PPCP detection and quantitation

Of the 33 pharmaceutical compounds targeted in analysis, on average 24 were detected in wastewater during each of the 6 sampling months, with 27 being the maximum number of compounds detected in any given month and 20 the minimum. No more than 12 PPCPs were detected in any groundwater well during any given sampling event, and no more than 10 PPCPs were detected in most groundwater samples (Figure 2). Excluding wastewater, the greatest number of compounds were detected in the watershed outlet surface water point. Detections of pharmaceuticals at the watershed outlet exceeded detections at individual groundwater locations throughout the site (Figure 2). With respect to compound-specific prevalence in groundwater and surface water, cotinine, caffeine, carbamazepine, and DEET were detected in more than 80% of all sampling locations (not including wastewater) for nearly every month of sampling (Figure 3). Other frequently observed compounds included sulfamethoxazole (40–70% of all sampling locations, depending on the month) and paraxanthine (40–100%). Estrone and progesterone were the only steroid hormones identified. Progesterone was present only in wastewater, and estrone was intermittently found in all water sample types. No significant patterns were observed in the frequency of detection of compounds by month of the year.

Pharmaceutical concentrations in groundwaters and surface waters were at least 1 order of magnitude lower than land-applied wastewater. The compounds with the highest average concentrations in irrigated wastewater were valsartan (1000 ng/L), sulfamethoxazole (500 ng/L), trimethoprim (900 ng/L), diphenhydramine (400 ng/L), and gemfibrozil (750 ng/L). Of these, only sulfamethoxazole was consistently present in groundwater, and average concentrations were less than 5 ng/L (Supplemental Data, Table S4). The highest concentrations of pharmaceuticals in groundwater were cotinine (mean = 9 ng/L), caffeine (12 ng/L), carbamazepine (5 ng/L), and sulfamethoxazole (4 ng/L; Figure 4). The PPCPs present at the highest average concentration in the watershed outlet surface water point were lincomycin (19 ng/L), cotinine (23 ng/L), DEET (29 ng/L), caffeine (8 ng/L), and trimethoprim (7 ng/L).

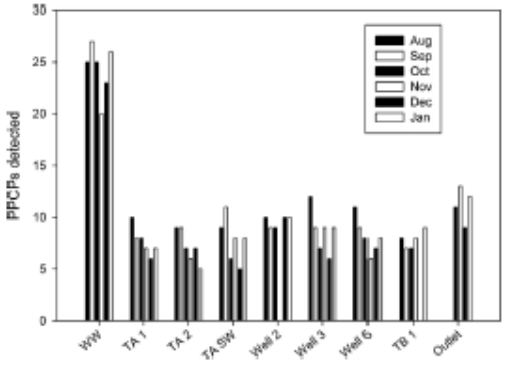

Figure 2. The total number of targeted pharmaceuticals and personal care products (PPCPs) detected in water samples by sampling month (August 2014–January 2015). Samples are wastewater (WW), transect A surface water (TA SW), and watershed outlet (Outlet). The remaining are groundwater wells. Transect A (TA) 1, TA 2, and TA SW represent a gradient of groundwater (TAs 1 and 2) flowing down from an upland area (wells 2, 3, and 6) to a surface water discharge point (TA SW). TB = transect B.

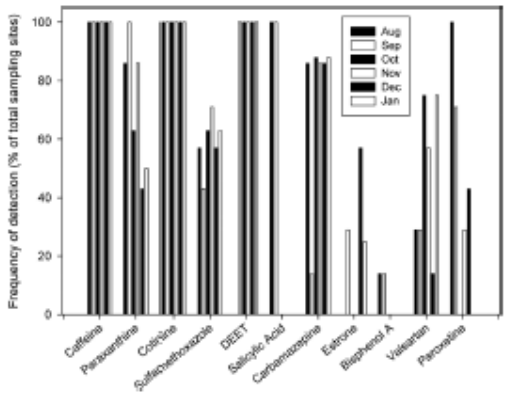

Figure 3. Frequency of detection of selected pharmaceuticals and personal care products (PPCPs) in groundwater and surface water samples by sampling month (August 2014–January 2015). The PPCPs along the *x*-axis are arranged in order of lower to higher octanol-water partition coefficient values, indicating which PPCPs would be more or less likely to persist in water or sorb to soil. Cotinine (a nicotine metabolite), caffeine, N,N-diethyl-meta-toluamide (DEET), and carbamazepine (an antiepileptic prescription drug) were some of the most frequently detected compounds throughout the sampling periods.

In an effort to simplify observations, the targeted pharmaceuticals were grouped according to chemical class (Table 1). This grouping was for observational purposes only and was not meant to indicate mechanistic similarities or additive effects, or to adhere to other groupings in the literature. Summed concentrations of pharmaceuticals for specific sampling points were analyzed to investigate the change in concentration and movement along a gradient to a surface water discharge point, and ultimately out of the watershed through the outlet (Figure 5). Antibiotics and antimicrobials and other prescription and nonprescription drugs make up the greatest summed concentrations in the land-applied wastewater. This pharmaceutical group was present all the way through a groundwater gradient to a surface water discharge point and in the watershed outlet, but caffeine and caffeine's major metabolite paraxanthine were present at similar concentrations throughout groundwater and surface water. Most chemical groups decreased throughout the gradient to the surface water discharge point; however, the watershed outlet surface water point contains markedly higher concentrations than the other surface water point sampled. Antibiotics, DEET, and antimicrobials made up the greatest fraction in the watershed outlet point.

DISCUSSION

Determining effective approaches of municipal wastewater treatment and disposal is critical to the future of societal and

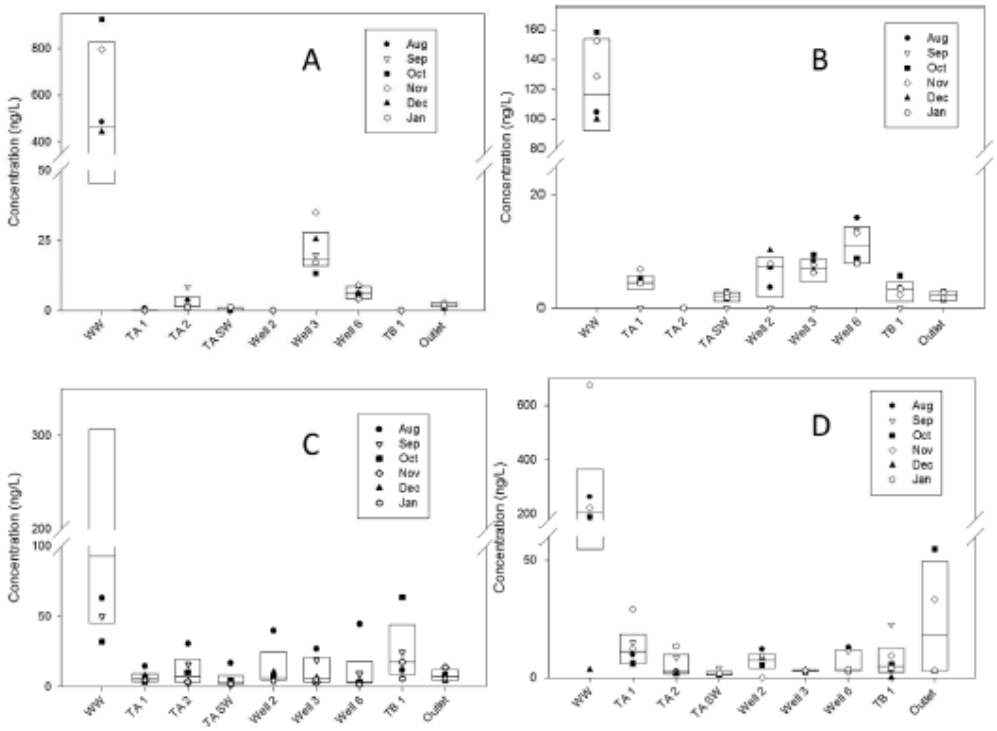

Figure 4. Boxplots depicting median, 25th, and 75th percentiles of pharmaceutical concentrations (ng/L) of selected pharmaceuticals from each of the 6 mo of sampling (August 2014–January 2015): (**A**) cotinine; (**B**) caffeine; (**C**) sulfamethoxazole; (**D**) carbamazepine. Transect A (TA) 1, TA 2, and TA SW represent a gradient of groundwater (TA 1 and 2) flowing down from an upland area (wells 2, 3, and 6) to a surface water discharge point (TA SW). TA SW = transect A surface water; WW = wastewater; TB = transect B.

environmental health. Therefore, determining how land application of minimally treated municipal wastewater may lead to environmental input of pharmaceuticals is crucial to the greater understanding of wastewater treatment options and development. Few studies in the literature have reported concentrations of pharmaceuticals in groundwater. Barnes et al. [20] conducted a study sampling groundwater from 18 states across the country and detected pharmaceuticals, including sulfamethoxazole, caffeine, ibuprofen, cotinine, and fluoxetine. The groundwaters sampled in that study were specifically selected to be of potential concern because of their location proximate to agriculture operations and cities; however, they were not directly related to wastewater or land application. Of the PPCPs observed in the Barnes et al. study [20], sulfamethoxazole was detected in the greatest percentage of groundwater sources and was observed at a similar frequency in the present study area. Caffeine and cotinine were detected in only 13% and 2% of groundwater samples, respectively, whereas these 2 compounds were ubiquitous in the present study area. Overall, the targeted PPCPs were more frequently detected in the present study than in the Barnes et al. study [20]; however, concentrations were not higher. In addition, only 1 such study currently in the literature examines groundwater in relation to wastewater land application. In that study, ibuprofen was not detected in any groundwater samples, and caffeine was detected only intermittently [21]. In addition, these compounds were not consistently detected in wastewater effluent, indicating that these results may vary substantially from the present study, in which high concentrations of pharmaceuticals were present in the wastewater.

By examining a groundwater gradient, it was possible to determine which PPCPs were the most persistent from land application to groundwater transport and surface water export and thus the extent to which infiltration of wastewater occurs. As an additional indicator of wastewater infiltration on the site, chloride concentrations in groundwater directly down gradient of irrigation were significantly higher than those not receiving irrigation (A. Birch, 2015, Master's thesis, North Carolina State University, Raleigh, NC, USA). Surface water data from the watershed outlet represent sample collections from October to January only, when water-use and transpiration of water by the forest system declines because of hardwood and other plant dormancy. Several targeted PPCPs, including caffeine, DEET, sulfamethoxazole, and cotinine, were detected throughout the subsurface pathway and in surface waters (Figure 5). Caffeine and DEET were observed at the highest concentrations throughout groundwater and into surface water, followed by the prescription and nonprescription drugs. These chemical compounds were also present in the watershed outlet surface water point; however, antibiotics and antimicrobials made up greater total concentrations than any other group in this surface water. Even though these chemical compounds were prevalent throughout a groundwater gradient, the concentrations are several orders of magnitude below that which is land-applied in wastewater, likely because of chemical sorption to soil and decomposition before reaching groundwater. However, despite

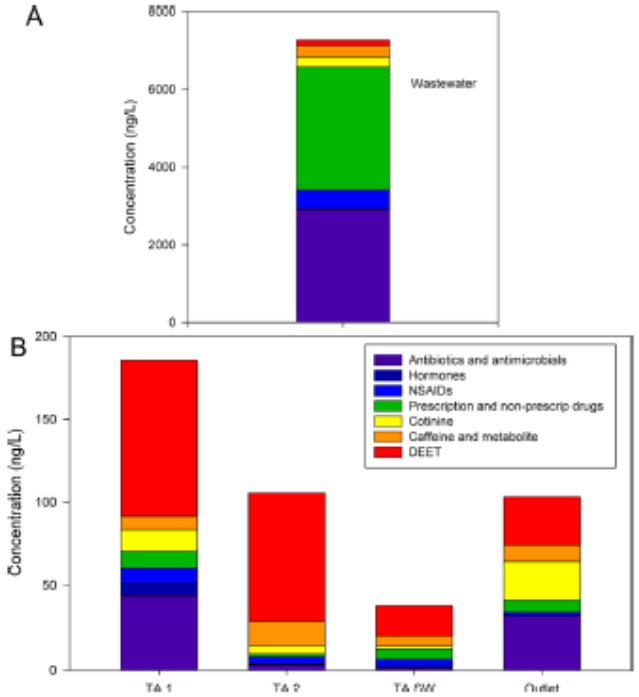

Figure 5. Six-month average concentrations of targeted pharmaceuticals, presented in summed groupings (Table 1): (**A**) wastewater concentrations. (**B**) 2 selected groundwater samples and 2 surface water points. Transect A (TA) 1, TA 2, and TA SW represent a gradient of groundwater (TA 1 and 2) flowing down from an upland area to a surface water tributary (TA SW). Outlet is the watershed outlet surface water point. Caffeine, N,N-diethyl-meta-toluamide (DEET), prescription and nonprescription drugs, and antibiotics and antimicrobials remain the most persistent throughout groundwater into surface water. NSAIDs = non-steroidal anti-inflammatory drugs.

reduction in concentration, these chemical compounds are persistent throughout groundwater and remain so into surface water. With the present data and temporal scope, we are unable to explicitly define the land-application facility as the sole source of PPCPs leaving the watershed via surface water at the outlet, because an upstream surface water point was not included throughout the present study period. However, after the present study period, 1 surface water sample was analyzed from upstream of the entire watershed and indicated little input into the system (7 PPCPs were present, and the highest concentration of any of these, DEET, was 10 ng/L; all others were less than 3 ng/L). A more extensive sampling regimen is underway to characterize PPCPs in surface waters entering and exiting the land application site.

To assess differences in overall environmental input and mitigation, the environmental input of PPCPs from wastewater land application was compared with WWTP effluent discharge into receiving waters. Several studies have quantified pharmaceuticals in WWTP effluent and receiving surface waters to determine environmental input from incomplete removal during the WWTP process [1,19,22]. In general, concentrations detected in surface waters proximate to or downstream from WWTPs in the literature were greater than those detected in groundwater at this forested land application site (Table 2). However, caffeine, sulfamethoxazole, and DEET were quantified in groundwater at similar concentrations to those reported in surface waters, albeit at the low end of these ranges. Similarly, most pharmaceuticals quantified in surface water in the present study were at concentrations below that which has been reported in the literature. However, DEET and trimethoprim concentrations in the watershed outlet on the land-application site were similar to those reported in surface water sites throughout the country and world.

To more thoroughly assess the effectiveness of the forest system as a wastewater treatment technology, the percentage of decrease in concentration of target compounds was calculated. For those compounds detected in the watershed outlet, decreases

Table 2. Selected pharmaceutical concentrations in groundwater and surface water from the present study compared with literature values in surface water, presented in ng/L.

	Present study		Literature values
Pharmaceutical	GW range	SW range	SW
Cotinine	3–13	1–23	20–50[a]
Caffeine	6–25	5–7	105[b]
Carbamazepine	0–11	2	25[a], 157[c]
Sulfamethoxazole	0–21	0–2	60–150[a], 20[b], 1[c]
DEET	6–150	6–80	22[b]
Trimethoprim	0–2	0–20	10–150[a], 71[c]
Ibuprofen	0–2	0–2	200[a], 28[b]
Erythromycin	0	0	3.4[b]
Estrone	0–20	0	3.6[b]
Naproxen	0–12	0–6	11[a], 53[c]

[a]Presented as range of median concentrations [1].
[b]Presented as mean concentration.
[c]Presented as median concentration [19].
GW = groundwater; SW = surface water; DEET = N,N-diethyl-meta-toluamide.

in concentration between that in land-applied wastewater and the watershed outlet were largely greater than 98%, with few exceptions. Lincomycin (19 ng/L) and sulfamethoxazole (4 ng/L) concentrations were higher in the watershed outlet than in land-applied wastewater (9 ng/L and 1 ng/L, respectively); however, these concentrations were still lower than those previously reported in surface waters. Trimethoprim, present at 1 of the highest concentrations in land-applied wastewater, experienced a 99% decrease in concentration before reaching the watershed outlet point. And DEET, which was present at the highest concentration in the watershed outlet, was reduced by 81% compared with land-applied wastewater concentrations. This indicates that the forested system effectively removes significant percentages of PPCPs before reaching the watershed outlet. Changes in concentration of PPCPs along the groundwater gradient to the surface water discharge point were also determined. More than 50% of paraxanthine, caffeine, carbamazepine, and valsartan remained in the water between well TA 1 and surface water site TA SW (Figure 1). This indicates that these compounds persist in groundwater and are likely to remain in groundwater to the watershed outlet. However, 75% to 100% of cotinine, acetaminophen, triclosan, and DEET were removed between well TA 1 and surface water site TA SW, indicating that although these compounds infiltrate to groundwater, they are less persistent and degrade before reaching surface water. The concentrations of the vast majority of targeted PPCP compounds are substantially reduced from concentrations in land-applied wastewater before being exported off of the site in surface water.

When comparing PPCP concentrations at this forested land application system with traditional WWTP effluent, one must qualify several attributes. First, the location of surface water samples relative to a traditional WWTP effluent outfall is critical. Distance downstream from a discharge location can change PPCP concentrations by orders of magnitude. Second, the type of WWTP is critical, because certain WWTPs (traditional tertiary, advanced activated charcoal, and so forth) may remove larger percentages of pharmaceutical compounds before release into surface water. Although several pharmaceuticals were routinely detected in groundwater and surface water at this facility, which land applies secondary treated wastewater, most of these compounds were present at concentrations lower than that reported in surface waters downstream of traditional WWTPs which discharge tertiary-treated effluent.

In general, acute toxicity studies have reported concentrations at which 50% of a population exhibit a response (EC50) and concentrations lethal to 50% of a population (LC50) in the milligram per liter range, several orders of magnitude greater than the concentrations reported in the present study. For example, carbamazepine was acutely toxic to *D. magna* at 17.2 mg/L [23,24], and the mean concentration in groundwater and surface water in the present study was less than 25 ng/L, indicating no acute risk at the measured concentrations. Chronic effects are likely more appropriate to consider because of the continuous input of pharmaceutical compounds at low levels. Rarely are acute effects seen at environmentally relevant concentrations; however, low-level exposure over time can lead to subtle changes in aquatic organisms and populations. Nonsteroidal anti-inflammatory drugs were reported to affect reproduction in *Daphnia* at concentrations of 1.8 mg/L [25], and carbamazepine has been reported to elicit sublethal effects as low as 10 μg/L [26]. In the present study, even when added together, all nonsteroidal anti-inflammatory drugs total less than 50 ng/L, and carbamazepine never exceeded 20 ng/L in groundwater or surface water. Another study examining changes in morphology and feeding in the cnidarian *Hydra attenuata* reported that sulfamethoxazole, trimethoprim, and caffeine all exhibited EC50 values greater than 100 mg/L, and gemfibrozil, ibuprofen, and carbamazepine exhibited EC50 values greater than 1 mg/L [27]. Concentrations of pharmaceuticals in land-applied wastewater are high enough to elicit chronic effects, and perhaps acute effects; however, pharmaceutical concentrations in groundwater and surface water at this land application facility were lower than the range of direct risk to aquatic organisms. The lone exception is the presence of antibiotics at concentrations sufficiently high to influence the proliferation and propagation of antibiotic resistance in aquatic environments. Risk associated with the development of antibiotic resistance is insufficiently addressed when considering WWTP effluent or land application, where continuous input of antibiotics occurs.

CONCLUSIONS

The present preliminary assessment found that PPCPs are present in secondary-treated municipal wastewater after residence times of 7 d to 14 d in open reservoirs before land-application onto a mixed pine–hardwood forest. Select pharmaceuticals were detected in groundwaters and surface waters on site and at the watershed outlet of the site, but concentrations were lower than PPCP concentrations in irrigated wastewater as well as concentrations reported elsewhere in surface waters downstream of conventional WWTPs. Research is ongoing to more fully address how land application systems function with regard to PPCP fate and transport.

Supplemental Data—Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3216.

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Data availability—Data are available on request by contacting A.D. McEachran (admceach@ncsu.edu) or E.G. Nichols (egnichol@ncsue.du) at North Carolina State University.

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SUPPLEMENTAL DATA
for:

Pharmaceutical Occurrence in Groundwater and Surface Waters in Forests Land-Applied with Municipal Wastewater

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Mobile phase conditions for liquid chromatography separation

Supplemental Data Table S1. Mobile Phase gradient composition for Method 1- Positive ESI

Time (min)	Mobile Phase A (%)[a]	Mobile Phase B (%)[b]
0:00	100	0
1:00	95	5
5:00	95	5
8:00	50	50
13:00	50	50
18:00	20	80
23:00	20	80
27:00	0	100
42:00	0	100

[a]Mobile phase A is water

[b]- Mobile Phase B is Methanol with 0.05% Acetic Acid. Flow rate is 0.200 mL/min. After gradient program, mobile phase conditions were restored to time 0:00 and equilibrated for 5 minutes before the next run.

Supplemental Data Table S2. Mobile Phase gradient composition for Method 2- Negative ESI.

Time (min)	Mobile Phase A[a] (%)	Mobile Phase B[b] (%)
0:00	99.9	0.1
2:00	95	5
6:00	95	5
10:00	50	50
13:00	50	50
16:00	20	80
19:00	20	80
21:00	0.1	99.9
31:00	0.1	99.9

[a]-Mobile phase A is water

[b]- Mobile Phase B is Methanol. Flow rate is 0.250 mL/min. After gradient program, mobile phase conditions were restored to time 0:00 and equilibrated for 8 minutes before the next run.

Quality control and method performance

Supplemental Data Table S3. Method Performance. Recovery of targeted analytes (%) and associated relative standard deviation (RSD %) of recovery.

Analyte	Percent Recovery (*n*)	RSD (%)
Lincomycin	174 (4)	22
Cotinine	171 (4)	23
Trimethoprim	165 (5)	21
Valsartan	92 (5)	23
Carbamazepine	90 (9)	13
Ibuprofen	88 (8)	6
DEET	83 (9)	26
Erythromycin	79 (6)	40
Gemfibrozil	77 (5)	15
Sulfamethazine	77 (7)	24
Caffeine	73 (9)	8
Testosterone	72 (9)	19
Estriol	72 (9)	15
Naproxen	69 (9)	27
Progesterone	67 (9)	25
Tylosin	67 (8)	40
Meprobamate	61 (9)	29
BPA	56 (8)	26
Paroxetine	56 (7)	31
Triamterene	54 (5)	32
Diphenhydramine	54 (9)	23
Triclosan	53 (9)	23
Sulfamethoxazole	52 (9)	30
17α- Ethynylestradiol	49 (7)	31
Atenolol	45 (6)	43
Paraxanthine	38 (9)	21
Fluoxetine	37 (8)	34
Estrone	36 (7)	50
17β- Estradiol	33 (8)	29
Acetaminophen	28 (9)	22
Diltiazem	19 (5)	32
Triclocarban	9 (8)	46
Salicylic Acid	8 (6)	19
[^{13}C] Carbamazepine	112 (4)	4
[^{13}C] 17β-Estradiol	22 (8)	50

n= number of replicates included in average calculation

CHAPTER 2

Title: Pharmaceuticals in a Temperate Forest-Water Reuse System

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Submitted and under review for publication

ABSTRACT

Forest-water reuse systems infiltrate municipal, industrial, and agricultural wastewater through forest soils to shallow aquifers and then surface waters via slow-rate irrigation. Their ability to mitigate regulated nutrients, metals, and organic chemicals is well known, but the fate of non-regulated chemicals in these systems is largely unstudied. How these systems provision and regulate water is informative to sustainable technologies that can mitigate climate change variability, provision wood products and bioenergy, preserve forested landscapes, and improve water resources for communities and wildlife. This study quantified 33 pharmaceuticals and personal care products (PPCPs) in soils, groundwater, and surface water at a 2,000 hectare forest irrigated year-around with secondary-treated, municipal wastewater. This temperate, irrigated forest does contribute PPCPs to soils, groundwater, and surface waters. PPCPs were more abundant in soils versus underlying groundwater by an order of magnitude. PPCP concentrations in surface waters were greater at the onset of significant storm events and during low-rainfall periods. Overall, PPCP concentrations in surface waters and groundwater were at least one order of magnitude below guideline values for ecological risk and several orders of magnitude below estimated human health risks.

KEYWORDS: Water reuse, forests, PPCPs

INTRODUCTION

Climate change and population pressures are expected to increase the scarcity and diminish the quality of water resources.[1] While significant attention has been paid to more arid regions of the world, temperate climates are experiencing water availability shortages and competition.[1] As such, managing water security will become increasingly important as urbanization and human population growth continues. One strategy to improve water quality and regulate water availability is the use of forest-water reuse systems.[2, 3] These systems are permitted, slow-rate land application systems that treat municipal, industrial, and agricultural wastewaters via soil infiltration and groundwater recharge prior to surface water discharge.[4, 5] Slow-rate irrigation is the most ubiquitous and oldest wastewater land application technology that can be designed for different natural and managed landscapes such as crop lands, golf courses, and forest ecosystems.[4, 6, 7] Slow-rate infiltration systems are designed to treat the most wastewater on a given amount of land at rates in excess of crop irrigation needs.[8] In temperate regions, forest slow-rate infiltration systems enable year-around wastewater treatment, nutrient storage, and intermittent soil saturation without adverse impacts on forest integrity and water quality. Forest-water reuse systems effectively mitigate regulated nutrients, metals, and organics to acceptable criteria levels before reaching groundwater and surface water[4,7-9] and compared to traditional wastewater treatment plants (WWTP) of similar wastewater volume treatment capacity, these systems are more cost-effective and energy efficient. In addition, they provide various ecosystem services such as provisioning wood products, biodiversity, carbon storage, and bioenergy.[10] However, very little is known about the fate and effects of non-regulated wastewater contaminants in these forest systems.

Contaminants of emerging concern, specifically pharmaceuticals and personal care products (PPCPs), are non-regulated contaminants that have been routinely reported in the environment [11-14] where they can elicit reproductive,[15, 16] behavioral,[17] and endocrinological[18] effects on aquatic organisms. Environmental input of PPCPs is generally associated with municipal wastewater release to surface waters because most wastewater treatment technologies do not completely remove them prior to discharge to surface water bodies.[19-21] Unknown and under-investigated sources of PPCPs into the environment need to be studied due to growing evidence of adverse effects in aquatic organisms from low level exposure to PPCPs. The fate of PPCPs in forest-water reuse systems merit evaluation to understand how these systems fully regulate water quality for groundwater and surface waters.

This study reports on monthly PPCP occurrence in a temperate forest-water reuse system that is irrigated with secondary-treated, municipal wastewater throughout the year. Previous research has documented that forest-water reuse systems effectively manage regulated nutrients for groundwater and surface waters.[10] A recent hydrological study of this system found that municipal wastewater comprised on average 50-76% of groundwater and 23% of surface water in irrigated catchments.[22] Additionally, select PPCPs were identified in forest groundwater and surface water leaving the irrigated subwatershed.[23] However, the temporal dynamics of PPCP concentrations in wastewater and forest groundwater remained uncertain as well as the temporal export of PPCPs from the forest-water reuse watershed via surface waters. Because of their potential contribution to sustainable water reuse, aquifer recharge, and surface water streamflow, the ability of these systems to remove or contribute

non-regulated contaminants across temporal dynamics is important to future forest-water reuse, design, implementation, and optimization.

MATERIALS AND METHODS

Study Area and Sampling

The majority of the study area of interest has been previously described.[10, 22, 23] Briefly, the study site is a 2,000 hectare, wastewater land-application facility which services a municipality of 70,000.[24] Secondary-treated wastewater is land-applied using one meter irrigation risers onto 930 hectares of forested land after 7-14 days residence time in open reservoirs with weekly application rates of 25-75 mm. The 930 hectare land-application area receives approximately 1200 mm of wastewater and 1300 mm of precipitation annually. Regulatory permits require that wastewater is applied only when conditions support infiltration of wastewater into soils; wastewater pooling and run-off at the soil surface is not allowed.[7]

Wastewater, groundwater, and surface water were collected over 12 consecutive months from August 2014 to July 2015 for analysis of PPCPs. Wastewater was collected from a central distribution spigot within the irrigation network and reflects wastewater after secondary treatment and storage in open reservoirs. Surface water samples were collected from Southwest Creek upstream of the land-application facility (Upstream), at a small tributary within the site (TA SW), and at the subwatershed outlet on Southwest Creek (Outlet) shown in Figure S1. Groundwater samples were collected along two separate gradients in two separate catchments, Transect A (TA 1-2, Well 3) and Transect B (TB 1-2),

and from a reference groundwater well in a forested area that is not irrigated with wastewater (Reference). Surface and groundwater sampling procedures followed USGS field manual protocols[25] and have been previously described.[23] All water samples were collected in pre-cleaned and baked 1-Liter amber glass bottles, transported to the laboratory on ice, and stored at 4 $^{\circ}$C until extraction, within 10 days of sampling. A separate surface water sampling event took place during a 24 hour storm event in July 2015. The stage of the watershed outlet surface water was continuously monitored during the event while seven surface water samples were collected in order to evaluate how PPCPs are transported during high flow periods.

Soil samples were collected in two consecutive months (June and July 2015) concurrent with water sampling events along each groundwater transect (Transect A and B, see Figure S1) at two depths: 0-5 cm from the surface and 60-65 cm below the surface. At each location and depth, approximately 100 grams of soil (wet weight) were collected from four locations surrounding the groundwater sampling well. Samples were thoroughly mixed and quartered in the field following US EPA protocols for soil sampling.[26] All soil samples were collected in pre-cleaned and baked glass bottles, transported to the laboratory on ice, and stored at 4 $^{\circ}$C until extraction, within 10 days of sampling. Soils ranged from sandy loam to fine sand and were fully characterized before subsequent analysis (Table S1). The percent dry material was very consistent among locations ranging from 86%-93% (Table S1). Thus data are presented in wet weight because normalization to dry weight would not influence our conclusions.

Sample Extraction and Analysis

Wastewater and water samples. Extraction and pre-concentration of PPCPs in water samples has been previously described[23] and is based on previously published methods.[27, 28] Briefly, Oasis HLB solid phase extraction (SPE) cartridges were used for extraction and pre-concentration, followed by evaporation and reconstitution to a final volume of 250 µL (including 50 µL of reference internal standards [^{13}C] caffeine and [^{13}C] ibuprofen).

Soil samples. Soil samples were extracted following EPA Method 1694[29] with amendments. All samples were both water and solvent extracted to define fractionation of PPCPs within the soil. Briefly, 10 g of undried homogenized soil was mixed with 15 mL of deionized water in 50 mL Oak Ridge Teflon© tubes to create a slurry. Reference internal standards and an additional 10 mL of deionized water was added and the mixture was shaken for 4 hours. Tubes were centrifuged, the water fraction was decanted off, and the process was repeated. Water fractions were kept separate and stored at 4 $^{\circ}$C until SPE cleanup. Proceeding with the solvent extraction, 25 mL of acetonitrile was added to the same Oak Ridge Teflon© tube and shaken for 4 hours, centrifuged, and the solvent decanted off. The process was repeated with 10 mL of methanol plus 15 mL acetonitrile. Solvent extracts were combined, evaporated to 10 mL under a nitrogen stream, and mixed with 200 mL deionized water for SPE cleanup. Cleanup and pre-concentration followed the same method as the water samples and has been previously documented.[23]

Targeted analysis of PPCPs. Thirty-three PPCPs were targeted in the method (Table 1) and include steroid hormones, antibiotics and antimicrobials, prescription and non-prescription drugs, and personal care products including plasticizers, insect repellants, caffeine, and

nicotine metabolites. The targeted analytes were selected due to their ubiquity in wastewater[11, 19, 30] and status as some of the most prescribed/consumed pharmaceuticals in the country. All soil and water samples were analyzed in the same manner.[23] Two separation and instrument methods (positive and negative electrospray) were used to identify and quantify all targeted compounds on a Thermo Scientific TSQ Quantum Ultra triple-quadrupole mass spectrometer configured with a Waters Acquity UPLC separation system. Targeted PPCPs were quantified using a seven-point internal calibration curve.[23]

Data Quality Assurance and Statistics

Data quality assurance and controls consisted of field duplicates, method blanks, method recovery spikes, and surrogate stable isotopically labeled internal standards. One field duplicate sample was collected for every sampling event throughout the study period. Additionally, with every set of samples, both a deionized water blank and method recovery spike was extracted and analyzed to determine background contamination as well as ongoing method recoveries of all targeted pharmaceuticals. A surrogate recovery standard with an intermediate log K_{OW}, [^{13}C] carbamazepine, was spiked into every sample (soil and water) before extraction to evaluate extraction efficiency and matrix interferences. Average recovery was 105% in all water samples and 86% in all soil. Method recovery of targeted compounds was within the range of other similar studies[23, 28] and the average for all targeted compounds was 69% in water samples and 42% in soil. Method detection limits ranged from 0.75 ng/L to 7.5 ng/L in water samples and were 0.075 ng/g wet weight in soil. Statistical differences and relationships in concentrations were determined using, where appropriate,

one-way analysis of variances (ANOVA), Student's t-tests, and Pearsons product-moment correlation coefficient. Data that did not fit assumptions of normality were analyzed using non-parametric statistics, specifically the Kruskal-Wallis Rank Sum, Wilcoxon Rank Sum tests, and Spearmans' rank order correlation test, as denoted. All analyses were conducted in R Computing Software (version 3.0.1).

RESULTS AND DISCUSSION

Wastewater

There are little data on seasonal variation of PPCPs in wastewater and river systems, and no data on PPCP seasonal occurrence in open-air reservoirs and forest water reuse systems. The most abundant PPCPs in wastewater were predominated by the Prescription and Over-the-Counter Drug, NSAIDs, and Antibiotics/Antimicrobials chemical groups (Table 1, Figure 1). Average concentrations of individual PPCPs in the irrigated wastewater ranged from <1 ng/L to 900 ng/L; the most abundant PPCPs were valsartan, fluoxetine, gemfibrozil, trimethoprim, and diphenhydramine. Concentrations of valsartan, fluoxetine, and caffeine in irrigated wastewater were similar to concentrations reported for municipal wastewaters in other studies.[10,14,24,25] Total summed concentrations of all PPCPs in wastewater decreased significantly with respect to increasing daily temperatures for spring and summer (Figure 1, r=-0.74, p=0.003). The decrease in total PPCP concentrations with warmer daily temperatures may reflect chemical, biological, and physical PPCP dissipation in the open-air reservoirs due to increased sunlight intensity (photodegradation), algal growth, and subsequent water quality changes with photosynthetic fluxes. Seasonal variation

in PPCP occurrence in wastewater may also result from use or disuse of certain PPCPs in certain seasons (i.e. cold medicines/NSAIDs in the winter, allergy medicines in the spring). Photodegradation of some PPCPs has been documented; triclosan and steroid hormones[26] can photodegrade and these PPCPs were some of the least abundant PPCPs detected in the irrigated wastewater (Table S2). Algal growth may promote uptake, sorption, and biodegradation of PPCPs in the wastewater lagoons during the 7-14 day residence time before irrigation. Likewise, higher temperatures could lead to higher rates of hydrolysis, volatilization, and microbial degradation. Finally, variations in reservoir volumes due to rainfall may decrease PPCP concentrations due to dilution effects. While exact mechanisms remain unknown and were beyond the scope of this study, PPCP occurrence and concentrations decreased in the open-air reservoirs during the warmer months of the year, representing a PPCP removal mechanism not previously reported in the literature.

PPCPs in Soils and Groundwater

The forest-water reuse system treats wastewater by soil infiltration and eventual groundwater discharge to surface waters. Forest soils are critical to PPCPs and nutrient removal to protect groundwater and surface water quality.[4,5,7] PPCP concentrations were greater in surface soils compared to 60 cm deeper soils (Figure 2A) and total summed PPCP concentrations were greater on irrigated soils than the reference, non-irrigated soil (Figure 2A, 2B). Concentrations of PPCPs ranged from less than 1 ng/g to 5 ng/g wet weight for irrigated soils. Carbamazepine, meprobamate, bisphenol-A, and 17-α ethynyl estradiol were the most abundant PPCPs in soils (Table S2). PPCP concentrations in irrigated forest soils were similar to PPCP concentrations in soils irrigated with reclaimed wastewater (1-15 ng/g)

[31] and soils amended with biosolids (<1 ng/g).[32] Based on more than 20 years of wastewater irrigation at this site and expected half-lives of PPCPs greater than 400 days in soil,[33] PPCP concentrations were lower than predicted. Increased organic matter from wastewater irrigation can enhance biodegradation and sequestration of PPCPs in soil[34] and could have contributed to lower PPCP concentrations.

Municipal wastewater irrigation contributes water and organic matter to surface soils which can impact soil sorption of PPCPs. Surface soils for Transect A had twice the organic carbon content as their deeper soils (Table S1). These surface soils also had greater amounts of total PPCPs and greater concentrations of PPCPs with higher Log K_{OW} values (Figure 2A). These trends were not observed for Transect B soils where surface and deeper soils had similar organic carbon content, PPCP amounts, and PPCP distribution. Transect B soils were more sandy than Transect A. More antibiotics and antimicrobials were quantified in Transect A soils than Transect B soils while Transect B soils contained more prescription and over-the counter drugs (Figure 2A). Physicochemical differences in soil quality likely contribute to differences in PPCP profiles in these different soils.

The composition and distribution of PPCPs by chemical class was markedly different between soils and underlying groundwater; PPCPs with larger log K_{OW} values were found at greater concentrations in soils versus groundwater (Figure 2B & 2C). As relative hydrophobicity (as average log K_{OW}) of PPCPs within chemical groups increased, the abundance of those chemical groups in soils vs underlying groundwater also increased (Figure S2). PPCP concentrations in soils were an order of magnitude higher than PPCP concentrations in groundwater (Figure 2B and 2C). Total PPCP concentrations in soil were

greater than 6 ppb while total PPCP concentrations in underlying groundwater ranged from 60-120 ppt.

Concentrations of PPCPs in groundwater ranged from less than 1 ng/L to greater than 50 ng/L throughout the year (Table S2). The most abundant PPCPs in groundwater were cotinine, caffeine, valsartan, and carbamazepine which generally have lower log K_{OW} values and greater water solubilities than the other targeted PPCPs (Table 1). Total summed concentrations varied significantly by groundwater sampling location (ANOVA, $p=0.03$, Figure 3, differences denoted by letters), and groundwater in irrigated forest areas had significantly greater total PPCP concentrations than the non-irrigated, reference groundwater (t-test and Wilcoxon Rank Sum test, all $p<0.05$) except for well TA 2 (Wilcoxon Rank Sum test, $p=0.38$). In general, the most upland groundwater wells had higher total PPCP concentrations than groundwater wells down gradient (Figure 3B and Figure S1).

Concentrations in groundwater were at least one order of magnitude lower than those in irrigated wastewater. For example, both valsartan and fluoxetine concentrations in groundwater were less than 10 ng/L, and caffeine was less than 20 ng/L while concentrations in wastewater were greater than 1000, 700, and 150 ng/L in wastewater, respectively. In general, as the sum of precipitation and wastewater irrigation increased, total PPCP concentrations in groundwater also increased (Figure 3B). Groundwater with the greatest total PPCP concentrations were dominated by chemical groups with the lowest mean log K_{OW} values indicating that these PPCPs migrated from soils to groundwater more so than the more hydrophobic PPCPs.

PPCPs in Surface Waters

The irrigation of forest soils with wastewater provides a viable pathway for PPCP input to groundwater and surface waters. Surface waters entering the irrigated forest area did contain PPCPs (Figure 4A and 4B). Several PPCPs, including atenolol, acetaminophen, paroxetine, and gemfibrozil, were quantified in upstream surface waters but not the irrigated wastewater, and some PPCPs were present at similar concentrations both upstream and downstream of irrigated forest areas. In general, PPCP concentrations were greater for surface waters in the irrigated forests than upstream of the irrigated forest area (Figure 4B), and concentrations of PPCPs were greatest at the watershed outlet (t-test, $p<0.001$, Figure 4B). The most abundant PPCPs at the Outlet were lincomycin, cotinine, caffeine, DEET, fluoxetine, and valsartan while the most abundant PPCPS in upstream surface waters were atenolol, caffeine, and cotinine (Figure S3). Concentrations of PPCPs in surface waters were similar to and often lower than previously reported values for PPCPs in surface waters.[11, 30, 35, 36]

Monthly irrigation volumes or seasonal rainfall did not significantly affect PPCP concentrations in surface water; however, concentrations at the watershed outlet did vary significantly with respect to precipitation (r=-0.54, p=0.05). As precipitation increased, the total summed concentration decreased due to dilution by non-irrigated catchments within the watershed. PPCPs were quantified in surface waters collected at several time points throughout a major storm event in July 2015 (Figure 4C). As expected, the Southwest Creek stage increased at the watershed outlet in two pulses.[37] Total PPCP concentrations increased until after the first crest of stream stage and then steadily decreased as rainfall continued and a second rise in stage occurred (Figure 4C). Declining PPCP concentrations during the

second stage pulse follows expected dilution effects from rainfall contributions across the entire subwatershed while initial PPCP spikes in the first stage represent initial mobilization and "first flush" of groundwater containing PPCPs. These observations are unique and important to understanding how forest-water reuse systems can contribute PPCPs to surface waters, particularly with expected increased storm intensities due to climate change.

Throughout the year, the most abundant PPCPs at the watershed outlet were consistently below reported ecotoxicological no observed effect concentrations (NOECs), even during low rainfall conditions when land-applied wastewater comprised 74% of the surface water at the subwatershed outlet.[22] Lincomycin and valsartan, two of the more abundant PPCPs in this study, have NOEC values exceeding 70 ug/L[38] and 80 mg/L[39], respectively. Their concentrations in surface water at the forest system subwatershed outlet were less than 20 ng/L. Additionally, while the development of antibiotic resistance is a possibility whenever the long-term presence of antibiotics exists, concentrations at the watershed outlet were an order of magnitude lower than concentrations where sulfonamide antibiotics correlated to sulfonamide resistance.[40] These individual concentrations do not present chronic ecological risk at the detected values and are lower than surface water concentrations downstream of conventional WWTPs.[11, 12, 14] However, wastewater contains hundreds of chemical compounds and exposure to mixtures of PPCPs can increase toxicity;[41] the long term effects of aquatic organismal exposure to complex mixtures of PPCPs remains unknown.

Potential human health risk exposure includes groundwater and surface waters used for drinking sources. In this study, concentrations of PPCPs in groundwater were an order of

magnitude below guideline values indicated in human health risk assessments previously conducted for individual PPCPs in drinking water.[42, 43] Further, risk-based action levels developed by the National Academy of Sciences (NAS) were compared to observed concentrations of caffeine, carbamazepine, sulfamethoxazole, and ibuprofen, four of the most frequently detected PPCPs in groundwater in this study.[44] Concentrations in groundwater in this study were similar to the estimated PPCP concentrations used by the NAS in developing action levels. The risk-based action levels ranged from 70,000,000 ng/L (caffeine) to 280,000,000 (ibuprofen), yielding margins of safety of 3,500,000 to 120,000,000, or several orders of magnitude above the USEPA's margin of safety for a low level of concern. However, action levels using mixtures of pharmaceuticals remain undefined for these systems. Collectively, these comparisons suggest that forest-water reuse systems can mitigate PPCP input to below actionable risk levels of individual PPCPs before reaching current drinking water supplies.

Management Implications

Forest-water reuse systems are one option for sustainable wastewater treatment of nutrients and regulated chemicals such as metals and organics when systems are properly designed and managed. This study shows that non-regulated organic contaminants of concern are also mitigated below levels considered harmful to ecosystems and human health. This temperate forest system has been in operation for 20 years, and it does contribute PPCPs to irrigated soils, groundwater, and surface waters. However, substantial percentages (>90% concentration) of PPCPs were removed through the forest-soil-groundwater system before reaching the watershed outlet at individual concentrations below aquatic and human health

risks. These findings are important to the potential utility of these systems to provide sustainable water reuse alternatives to conventional municipal wastewater treatment for temperate landscapes and can help inform risk evaluation for wastewater irrigation onto human food crops. Although forested systems do not pose a human health risk from ingestion of plant material, exposure to PPCPs via ingestion of wastewater-irrigated crops can occur[45,46] and uptake of PPCPs into plant material has not been completely characterized. Forest-water reuse systems can inform risk assessments and future water reuse applications by providing the opportunity to understand human exposure and risk without direct exposure to PPCPs.

Our findings suggest that land application of municipal wastewater in forest-water reuse systems presents a feasible option for water reuse and wastewater treatment with minimal risk to aquatic wildlife and human health. Other ecosystem benefits can be derived from these systems such as provisioning wood products, regulating soil and biodiversity, and provisioning water availability for surrounding areas. The benefits and tradeoffs of ecosystem services from forest water re-use systems merits quantification to understand their potential utility in both built and rural landscapes now and for the future.

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TABLES

Table 2.1 Pharmaceuticals and personal care products (PPCPs) included in analysis by chemical group and corresponding log K_{OW} values, in order of decreasing mean log K_{OW}.

Chemical Analysis Groups	PPCPs	Log K_{OW}[a] Low-High (Mean)
Steroid Hormones	17-α ethynyl estradiol, 17-β estradiol, estrone, estriol, progesterone, testosterone	2.45-4.01 (3.32)
Plasticizer	Bisphenol-A (BPA)	3.32 (N/A)
Prescription and Non-prescription Drugs	Gemfibrozil, meprobamate, fluoxetine, diltiazem, paroxetine, valsartan, carbamazepine, atenolol, diphenhydramine, triamterene	0.16-4.77 (2.61)
Nonsteroidal Anti-inflammatory Drugs (NSAIDs)	Naproxen, ibuprofen, acetaminophen, salycilic acid (aspirin metabolite)	0.46-3.97 (2.21)
DEET	N,N-Diethyl-meta-toluamide (DEET)	2.02 (N/A)
Antibiotics and Antimicrobials	Sulfamethoxazole, lincomycin, sulfamethazine, tylosin, trimethoprim, triclosan, erythromycin	0.14-4.76 (1.94)
Nicotine Metabolite	Cotinine	0.07 (N/A)
Caffeine	Caffeine, paraxanthine (metabolite)	-0.07 (N/A)

[a] log Kow values compiled from the Hazardous Substances Data Bank. Bethesda (MD): National Library of Medicine (US) [Last Revised 2016-03-17]. Available from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

FIGURES

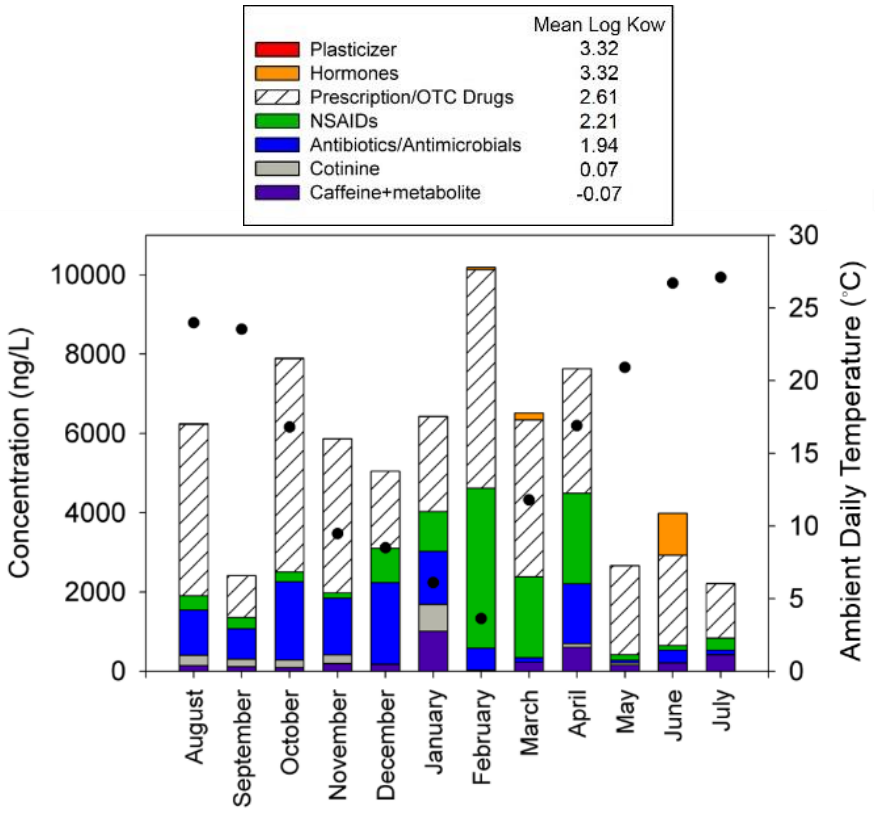

Figure 2.1 Total summed concentrations of PPCPs by chemical group in wastewater (bars) and average monthly daily temperature (filled circles). Chemical groups are indicated by color and are ordered by decreasing mean log K_{OW} of the group. Total PPCP concentration in wastewater decreased with increasing mean daily temperatures.

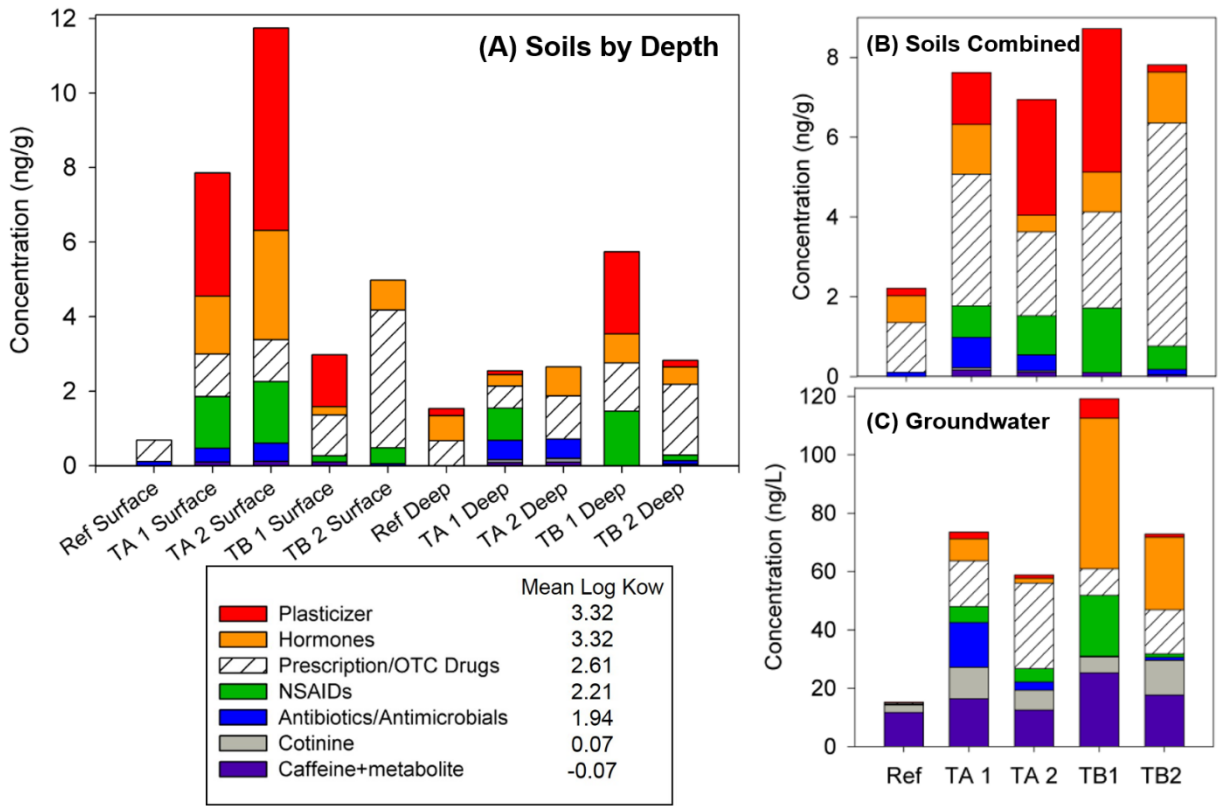

Figure 2.2 (A) Average concentrations of PPCPs in chemical groups for soils at a non-irrigated location (Ref) and at two irrigated forest transects (TA 1-2 and TB 1-2) at two soil depths (0-10 cm and 60-65 cm below surface). Chemical groups are indicated by color and are ordered by decreasing mean log K_{OW} of the group. (B) Average concentrations of PPCPs by group in combined surface and deep soils by location. (C) Average PPCP concentrations by chemical group in groundwater at a non-irrigated location (Ref) and at two irrigated transects (TA 1-2 and TB 1-2).

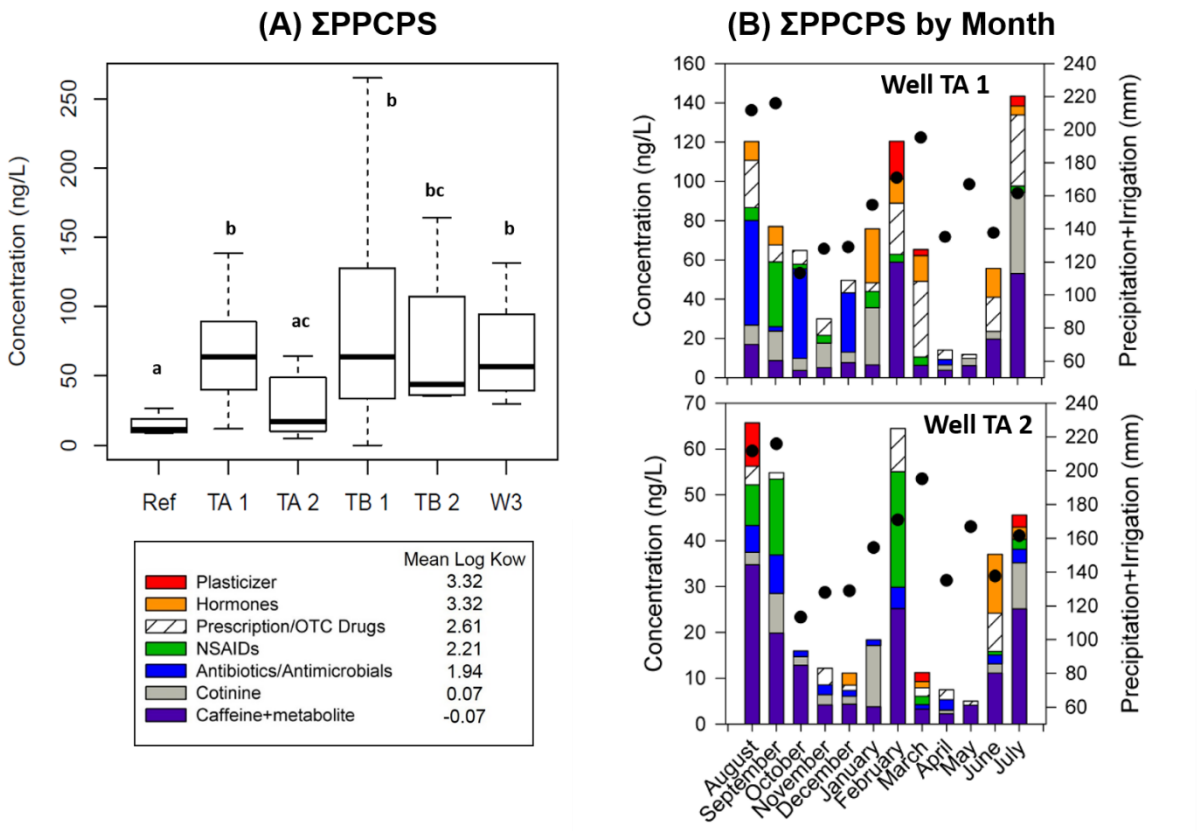

Figure 2.3 (A) Box plots of summed ΣPPCP concentrations at six groundwater sampling locations for one year (n=12). Significant differences of ΣPPCP concentrations between locations are denoted by letter (ANOVA, p=0.03). Boxplots denote the median, 25th percentile, and 75th percentile with whiskers extending to the 5th and 95th percentile. (B) Monthly ΣPPCP concentrations by chemical group with monthly precipitation plus irrigation volumes for irrigated wells TA 1 (top) and well TA 2 (bottom- downgradient of TA 1).

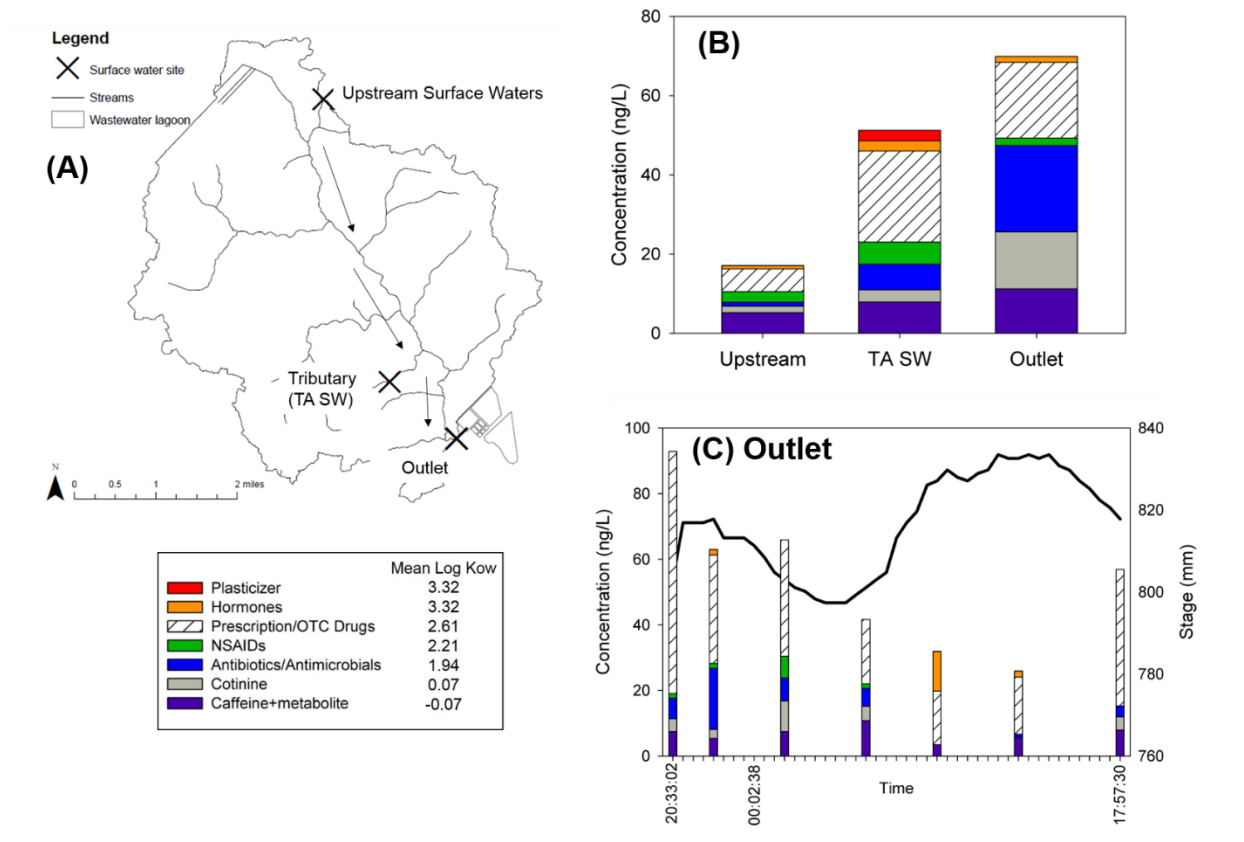

Figure 2.4 (A) Locations of surface waters collected upstream of irrigated areas, within the forest-water reuse site, and at the sub-watershed outlet. (B) Average PPCP concentrations by chemical group at surface water locations. (C) Total summed PPCP concentrations by chemical group at seven sampling times throughout the duration of a storm event in July 2015. The stage (mm) of Southwest Creek is denoted by a black line throughout the duration of the storm event (22 hours).

CHAPTER 3

Title: Comparing the Environmental Input of Pharmaceuticals from Conventional and Alternative Wastewater Treatment Systems: a Targeted and Suspect Screening Study

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ABSTRACT

Wastewaters are a well-defined source of emerging contaminants into the environment. Conventional, tertiary wastewater treatment systems do not effectively remove many emerging contaminants, including pharmaceuticals, endocrine disrupting compounds, and perfluorinated compounds, resulting in the discharge of chemical contaminants into waterways. Forest-water reuse systems infiltrate municipal, industrial, and agricultural wastewater through forest soils via slow-rate irrigation. Forest-water reuse systems stabilize water availability, provide ecosystem service benefits, and are lower cost and energy than conventional treatment systems. Forest-water reuse systems have only recently been investigated as to their pharmaceutical input. To better understand their role as a source of emerging contaminants and outlook for broad-scale implementation, these systems need to be directly compared to conventional wastewater treatment systems. In this study, both a quantitative, targeted analysis and a suspect screening approach were utilized to better understand how emerging contaminant input from a forest-water reuse system compares to a conventional tertiary treatment system. Quantitatively, greater concentrations and total mass flow of pharmaceuticals was exhibited downstream of the conventional treatment system. From a suspect screening standpoint, more confirmed chemicals were present, and at a greater relative abundance, downstream of the conventional system as well. This data shows that increased implementation of sustainable forest-water reuse systems can reduce the environmental input of emerging contaminants to a greater degree than conventional systems.

INTRODUCTION

Wastewaters are a well-defined source of emerging contaminants in the environment [1-4]. Subsets of emerging contaminants widely studied over the last decade include pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds (EDCs), and, more recently, perfluorinated compounds (PFCs). These contaminants can individually and collectively elicit detrimental effects on aquatic biota [5-7] and can propagate antibiotic resistance.[8] Conventional, large-scale tertiary wastewater treatment plants do not effectively remove many emerging contaminants from wastewater before discharge into receiving surface water bodies, resulting in continuous influx of emerging contaminants into surface waters.[3] Concentrations of PPCPs and other emerging contaminants downstream of conventional wastewater treatment effluent discharge are typically reported in the ng/L range [4] but have been reported as high as 6.5 mg/L,[9] or within the range of effect concentrations developed for certain aquatic organisms.[5]

Forest-water reuse (FWR) systems are slow-rate irrigation systems that treat municipal, industrial, and agricultural wastewater through infiltration into forest soils and groundwater.[10, 11] These systems are lower cost and energy than conventional treatment systems of similar size, provide ecosystem service benefits via wood production and carbon storage, and effectively manage regulated nutrients, metals, and organics.[10, 12, 13] However,[13] their role as a source of emerging contaminants in the environment has only recently been investigated.[14] These systems do input PPCPs into the environment via surface water but remove greater than 90% of PPCP concentration in irrigated wastewater before reaching surface water.[14] Alternative wastewater treatment systems, such as FWR systems, have yet

to be explicitly compared to conventional wastewater treatment systems regarding their environmental input of emerging contaminants.

Targeted, quantitative analysis of emerging contaminants using analytical standards is a well-established practice and has been well documented in the literature.[2, 4] Analytical advances have enabled previously unidentified or uncharacterized emerging contaminants to be detected in the environment when analytical standards are either unavailable or not practical.[15, 16] High-resolution mass spectrometry (HRMS) can be used to identify unknown or suspected chemical compounds. Suspect screening involves using existing chemical information databases to identify chemical compounds in samples by utilizing mass, isotope abundance and isotope spacing.[17] Suspect screening methods applied to wastewater and surface waters receiving treated wastewater effluent reported the presence of thousands of compounds,[16-18] but this method has yet to be applied to alternative wastewater treatment systems, including FWR systems. A combined effort utilizing quantitative, targeted analysis and suspect screening methods in surface waters downstream of wastewater discharge systems will enable the determination of both mass flow of pharmaceuticals and an overall estimation of suspected chemicals present within a large-scale inventory search.

Water scarcity and quality are expected to be adversely affected by climate change and population pressures,[19] and as such, the ability to regulate water availability and improve water quality will become increasingly important with growing human populations. Forest-water reuse systems can manage water quality and availability while treating wastewater and present one solution towards sustainable water reuse.[10] Understanding how emerging contaminant input compares between FWR systems and conventional treatment systems is

important for the future implementation of FWR systems. In this study, emerging contaminants were investigated utilizing a quantitative, targeted and qualitative, suspect-screening approach to compare the overall environmental input of emerging contaminants in surface water between a FWR system and a conventional, tertiary wastewater treatment system. Defining the relative environmental burden of emerging contaminants between the two systems will provide data that can improve wastewater treatment, water quality, and water sustainability.

MATERIALS AND METHODS

Site Description and Sampling

Both the FWR system[14, 20] and traditional WWTP[6] sampling locations have been previously described. The FWR system land-applies secondary treated wastewater onto 930 hectares of forested land after 7-14 days residence time in open air reservoirs. 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 wastewater weekly (Table 1). Southwest Creek flows through the system and a streamflow and stage data of the creek was collected using a previously installed gage at the subwatershed outlet. The North Cary Water Reclamation Facility (NCWRF) serves as one of two WWTPs servicing a population of 150,000 and treats on average 24 million liters per day (6.55 million gallons per day). Wastewater at the NCWRF is tertiary-treated followed by UV-disinfection before discharge into a small channel leading to Crabtree Creek. Crabtree Creek is a tributary of the Neuse

River beginning in the town of Cary, NC before emptying into the Neuse. Streamflow and stage data was collected from the nearest USGS gage station.

One-liter surface water and wastewater samples were collected monthly from October 2015-December 2015. At the FWR facility wastewater was collected from a spigot which is part of the central irrigation system that represents wastewater headed for irrigation after residence time in the open reservoirs. Surface water samples were collected from upstream of the land application area on Southwest Creek (Upstream) and downstream of the irrigation system at the subwatershed outlet on Southwest Creek (Outlet). Wastewater from the NCWRF was collected from the effluent discharge pipe and surface water samples were collected upstream of the effluent channel on Crabtree Creek (Upstream) and at two sampling locations 50 meters and 100 meters downstream of the discharge pipe on Crabtree Creek (Downstream 1 and Downstream 2, respectively). All water samples were collected in pre-cleaned and baked 1-Liter amber glass bottles, transported to the laboratory on ice, and stored at 4°C until extraction within 7 days of sampling.

Extraction and Analysis

Thirty-three PPCPs were selected for their ubiquity in wastewater, previous detection at the FWR system study site,[14] and status as frequently used pharmaceuticals (Table 2). Extraction and analysis has been previously described.[14] Briefly, filtered surface water and wastewater samples were extracted under light vacuum using Oasis HLB solid phase extraction (SPE) cartridges. Eluents were evaporated down and reconstituted to a final volume of 250 ul with 1:1 (Methanol:DI Water) and reference internal standards ([^{13}C]

caffeine and [^{13}C] ibuprofen). Final extracts were split for targeted and suspect screening analysis.

Targeted analysis via UPLC-MS/MS

Targeted, quantitative analysis was conducted using a Thermo Scientific TSQ Quantum Ultra triple-quadruople mass spectrometer with a Waters Acquity UPLC separation system and has been previously described.[14] Two separation and instrument methods (positive and negative electrospray ionization) were utilized to maximize identification and quantification capabilities.

Suspect screening via HPLC-TOF/MS

Methods for suspect screening are described in detail in Rager et al. (2016).[21] 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 um; Agilent Technologies, Palo Alto, CA). The run time was 45 minutes per sample and ions from 100-1700 m/z were monitored. Formulas were identified using the Find By Formula tool and the corresponding personal compound database library (PCDL) in MassHunter Workstation Software Qualitative Analysis (Agilent Software, v.B.06.00). The four databases used were the EPA's Distributed Structure-Searchable Toxicity (DSSTox) database, MassHunter Forensics and Toxicology (Agilent), MassHunter Pesticides (Agilent), and an in-house database containing approximately 50 per- and polyfluorinated compounds. The output from this tool includes candidate compounds from each database with a match score that is based on mass, isotope abundance, and isotope spacing. In total, extracted

molecular features were matched against more than 10,000 structures encompassed by all three databases. Strict match criteria indicates scores of ≥ 90 having a high degree of confidence, however match scores ≥80 contain many confidently matched chemical compounds. Thus match scores of both ≥80 and ≥ 90 were evaluated for further analysis.

Data Quality Assurance

One field duplicate sample was collected during every sampling event and processed in the same manner for quality control purposes. Additionally, a deionized water blank and method recovery spike were processed in every batch of samples for background contamination and ongoing method recoveries of targeted compounds. Surrogate recovery standards were spiked into every sample to evaluate matrix interferences and extraction efficiency. Average recovery of surrogate standards was 71% and average recovery of all targeted compounds was 71%. Method quantification limits were compound-specific and ranged from 0.75 ng/L and 7.5 ng/L.

RESULTS AND DISCUSSION

Targeted Analysis

Concentrations of targeted PPCPs upstream of the conventional system on Crabtree Creek were greater than concentrations upstream of the FWRS (Table 1). Average concentrations were all less than 40 ng/L, 24 of the 33 targeted PPCPs were not detected upstream of either system, and caffeine and DEET were present at the greatest concentrations (Table 1). Total summed wastewater concentrations of targeted PPCPs did not differ significantly between the two systems; wastewater did vary between the two systems in

terms of chemical composition (Figure 1). Concentrations of DEET, BPA, and caffeine were greater at the FWR system while the group of prescription and non-prescription drugs were greater at the conventional system. Individual chemical concentrations depended on the system: atenolol, cotinine, DEET, bisphenol-A, and ibuprofen were greatest at the FWR system while trimethoprim, triamterene, fluoxetine, and sulfamethoxazole were greater at the conventional system (Figure 1).

Individual PPCP concentrations in wastewater at the FWR in the present study are within the range of individual concentrations reported throughout a longer term study at the same FWR system (McEachran et al in review). The PPCPs present at the greatest concentrations in irrigated wastewater at the FWR system were atenolol, sulfamethoxazole, and trimethoprim, and diphenhdryamine and, excluding sulfamethoxazole, all were consistently lower in the present study than in literature values in conventional wastewater effluent.[22-24] Residence time in the open-air reservoir at the FWR system can encourage degradation and transformation of PPCPs before irrigation leading to differences in both chemical composition of wastewater and individual PPCP concentrations between the FWR system and a conventional system. PPCP concentrations in the treated wastewater effluent from the NCWRF were within the range of similar studies targeting PPCPs in effluent.[23, 25] DEET has been ubiquitously reported in environmental systems, especially surface waters, [2, 26] and was greater in concentration at the FWR system than the conventional system. DEET is likely more abundantly used in the rural region containing the FWR system than the urban NCWRF. Additionally, employees of the FWR system may contribute DEET to the system.

Total concentrations of PPCPs downstream of the NCWRF were approximately 30% of total wastewater concentrations (Figure 1). Individual concentrations of PPCPs did not decrease in the 50 meters between the two downstream locations at the NCWRF, and some concentrations increased. Summed PPCPs at the Outlet of the FWR system were approximately 1% of summed concentrations in irrigated wastewater (Figure 1) and total summed PPCP concentrations downstream of the conventional system were more than an order of magnitude greater than concentrations at the outlet of the FWRS (Figure 1). All individual PPCP concentrations were greater downstream of the conventional system (Table 1); the most abundant PPCPs at Crabtree Creek were within the range of previous studies in surface waters.[27] Fluoxetine concentrations in downstream surface waters were greater than previous research on Crabtree Creek indicated,[6] but were still within the range of environmental concentrations reported elsewhere[28] and an order of magnitude below indicated no observed effect concentration (NOEC) values for this antidepressant.[29] Streamflow was higher during this study compared to previous research, and it has been documented that emerging contaminants can increase in concentration during high flow periods due to other potential upstream sources or the release of concentrations from contaminant stores within the watershed,[24, 30] instead of following more conventional concentration-dilution effects. Total environmental input of the mass of PPCPs was calculated at each sampling location based on discharge of receiving streams (Southwest Creek that runs through the FWRS and Crabtree Creek that receives treated effluent at the NCWRF). Average discharge during sampling events at Crabtree Creek was double the discharge at Southwest Creek (Table 1). When summed, average environmental input of

targeted PPCPs at Crabtree Creek was almost 100 times that at Southwest Creek (126,000 mg/day vs 1600 mg/day).

Forest-water reuse systems rely on the soil-forest system to remove contaminants before reaching groundwater and ultimately surface water. Wastewater influent was not evaluated and this research cannot speak to treatment efficiency of the two systems. Background upstream and wastewater PPCP concentrations were similar between the two systems, but downstream of the conventional system contained substantially more PPCPs than downstream of the FWR system. Environmental input of targeted pharmaceuticals is considerably lower from the FWR compared to the conventional treatment system.

Suspect screening

Molecular features per sample ranged from 2000 (Upstream at both sites) to 5200 (wastewater at the FWRS). Similar numbers of molecular features were identified upstream of both systems, more unique molecular features were identified in the wastewater from the FWRS, and more features were identified downstream at the conventional system (SI Table). Suspect screening previously conducted on wastewater samples indicated a greater number of molecular features than identified in the present study,[17] but the numbers in this study are similar to recent work conducted using similar instrumentation and molecular feature extraction workflow on house dust.[21] Extracted features database-matched at a score of ≥ 80 in wastewater samples identified, on average, 400 chemical compounds (Figure 2). More compounds were matched in the NCWRF wastewater, indicating there are more unknown or unidentified compounds present in the irrigated wastewater at the FWR system than at the

conventional system. Total molecular features identified downstream of the two systems were similar, but more database-matched compounds were present downstream of the conventional system (Figure 2).

The most abundant chemical compounds identified in database-matching varied by sample (Figure 3), but consistently abundant compounds were glycerol, DEET, insecticides (buprofezin), and herbicides (difenzoquat). The sweetener glycerol (or glycerin) was predominant in all surface waters and wastewaters. Relative abundance of suspected chemical compounds was greater in the FWRS wastewater and upstream of the FWRS compared to the conventional system (Figure 3), but identified compounds were more abundant downstream of the conventional system.

In total, less than 10% of the unique molecular features extracted in all samples were database-matched to individual chemical compounds (using a match score of ≥ 80, SI Table), a common occurrence in suspect screening analysis. Additionally, approximately 15% of the total abundance of the unique molecular features was captured via database matching, therefore further data analysis utilizing non-targeted identification methods is necessary to identify the remaining (>90%) un-matched molecular features. From a suspect screening standpoint, more unique molecular features were present in the FWR system, both in the wastewater and in surface waters upstream. However, a greater abundance of chemical features and suspected chemical compounds were present downstream of the conventional system. This indicates that even though irrigated wastewater contains more potentially emerging contaminants, in terms of presence of suspected compounds, the FWRS inputs a smaller load of emerging contaminants than the conventional system.

Conclusions and implications for wastewater treatment

Humans and ecological systems rely on effective wastewater treatment for clean water sources. Wastewater treatment has been effectively treating primary pollutants, nutrients, and metals for decades. However, with new classes of well-documented contaminants of emerging concern, effectiveness of removal of these classes represents the next step in wastewater treatment and clean water availability. Emerging contaminants in ambient upstream surface waters and wastewaters were relatively similar between an alternative and a conventional treatment system, but a greater abundance and mass of emerging contaminants existed downstream of the conventional system. This research has documented that over a three month period, a forest-water reuse system land applying secondary treated wastewater inputs fewer emerging contaminants, by mass and sheer number, into receiving waters than a conventional, tertiary wastewater treatment system of comparable size. The FWRS lowers the contaminant burden and decreases ecological risk in downstream surface water sources to a greater extent than the conventional system. This research provides evidence for the ecological benefit of increased implementation of wastewater land application systems. The increased implementation of sustainable water reuse systems that remove emerging contaminants more effectively than conventional systems can benefit ecological and human health while stabilizing water availability pressures.

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TABLES

Table 3.1 Treatment system statistics and mass flow of targeted PPCPs in surface water output.

	Jacksonville FWRS	North Cary WRF
Population Served	70000	151000
Treatment Capacity (L/day)	$2.3x10^7$	$4.5x10^7$
Average Volume Treated (L/day)	$1.9x10^7$	$2.4x10^7$
Average Stream Discharge (L/day)	$4.9\ x10^7$	$8.8\ x10^7$
Average Summed Concentration (ng/L)	33.4	1439.1
Average Total Mass Input (mg/day)	1624.98	126,673.9

Table 3.2 Pharmaceuticals and personal care products (PPCPs) included in targeted analysis by chemical group.

Chemical Analysis Groups	PPCPs
Steroid Hormones	17-α ethynyl estradiol, 17-β estradiol, estrone, estriol, progesterone, testosterone
Plasticizer	Bisphenol-A (BPA)
Prescription and Non-prescription Drugs	Gemfibrozil, meprobamate, fluoxetine, diltiazem, paroxetine, valsartan, carbamazepine, atenolol, diphenhydramine, triamterene
Nonsteroidal Anti-inflammatory Drugs (NSAIDs)	Naproxen, ibuprofen, acetaminophen, salycilic acid (aspirin metabolite)
DEET	N,N-Diethyl-meta-toluamide (DEET)
Antibiotics and Antimicrobials	Sulfamethoxazole, lincomycin, sulfamethazine, tylosin, trimethoprim, triclosan, erythromycin
Nicotine Metabolite	Cotinine
Caffeine	Caffeine, paraxanthine (metabolite)

Table 3.3 Mean ± standard deviation in ng/L of sampling locations at both wastewater treatment systems. Up=Upstream, WW=Wastewater, Outlet=subwatershed outlet, Down 1= 50 m downstream, Down 2= 100 m downstream

	Jacksonville Forest-Water Reuse System			North Cary Water Reclamation Facility			
	Up	WW	Outlet	Up	WW	Down 1	Down 2
Atenolol	ND	922 ± 532	1.70 ± 3	0.00 ± 0.0	760±295	156±156	270±107
Lincomycin	ND	ND	ND	ND	ND	ND	12.0±21
Cotinine	3.78 ± 7	95.9 ± 166	1.85 ±3	29.9±31	33.4±24	9.85±10	11.6±11
Trimethoprim	1.97± 0.3	249 ± 184	1.05 ±0.2	6.49 ±6	304±227	102±119	141±118
Triamterene	ND	81.8 ± 78	ND	ND	199±88	67.2±65	83.4±52
Acetaminophen	0.99± 2	0.00 ± 0	ND	ND	8.60±3	2.48±4.30	0.92±2
Paraxanthine	0.87± 1	81.4 ± 62	1.80 ±1	4.71±1	90.6±9	35.8±18	52.0±24
Diphenhydramine	ND	240 ± 160	0.92 ± 2	ND	165±40	26.2±29	42.8±34
Caffeine	2.32± 1	119 ± 9	4.35 ±4	43.4±7	27.6±2	50.1±16	44.0±12
Diltiazem	ND	28.4 ± 25	0.45 ±1	ND	115±36	34.7±35	50.5±32
Sulfamethazine	ND	0.78 ± 1	4.22 ±1	ND	ND	ND	2.27±4
Erythromycin	ND	5.16 ± 8	ND	ND	8.95±4	1.48±2	2.77±3
Tylosin	ND	ND	ND	ND	3.30±2	0.81±1	2.96±3
Paroxetine	ND	4.10 ± 1	1.01 ±2	ND	16.3±9	1.74±3	6.50±5
Fluoxetine	ND	158 ± 269	ND	ND	1092±154	302±383	332±344
Sulfamethoxazole	ND	281 ± 139	2.62 ±1	ND	457±137	88.2±95	162±145
Meprobamate	ND	82.4 ± 84	0.34 ± 0.6	ND	139±22	20.2±26	36.1±43
Carbamazepine	ND	68.0 ± 63	1.60 ± 0.4	ND	251±41	60.8±50	91.0±62
DEET	22.9± 10	225 ± 200	6.69 ± 6	26.9±24	16.4±10	36.4±37	34.5±30
Naproxen	ND	0.00 ± 0	ND	5.71±5	3.22±3	13.3±2	1.25±1
Estriol	ND	14.7 ± 13	3.40 ± 6	ND	ND	27.7±27	31.8±55
Testosterone	ND	0.98 ± 2	ND	ND	ND	ND	ND
Estrone	ND	0.00 ± 0	ND	ND	ND	ND	ND
Progesterone	ND	6.58 ± 8	ND	ND	0.31±0.53	ND	ND
Cholesterol	ND	0.00 ± 0	ND	ND	ND	ND	ND
Gemfibrozil	ND	0.00 ± 0	ND	1.31±2	7.02±3	ND	ND
Triclocarban	ND	5.11 ± 9	0.63 ±1	ND	ND	13.5±23	10.1±18
Valsartan	0.01	100 ± 66	ND	ND	42.5±20	5.92±9	8.69±12
Salicyclic Acid	ND	0.00 ± 0	ND	ND	ND	ND	ND
BPA	ND	124 ± 123	ND	ND	ND	ND	ND
Valsartan	ND	35.96 ± 1	0.81 ±0.7	ND	15.4±2	4.72±4	9.52±5
17-β Estradiol	ND	0.00 ± 0	ND	ND	ND	ND	ND
Estrone	ND	0.00 ± 0	ND	ND	ND	ND	ND
17-α Ethynyl-estradiol	ND	0.00 ± 0	ND	ND	ND	ND	ND
Ibuprofen	ND	196 ± 150	ND	ND	34.0±12	ND	ND
Progesterone	ND	8.14 ± 6	ND	ND	1.73±1	0.43±0.7	ND

Triclosan	ND	ND	ND	ND	ND	ND	ND

FIGURES

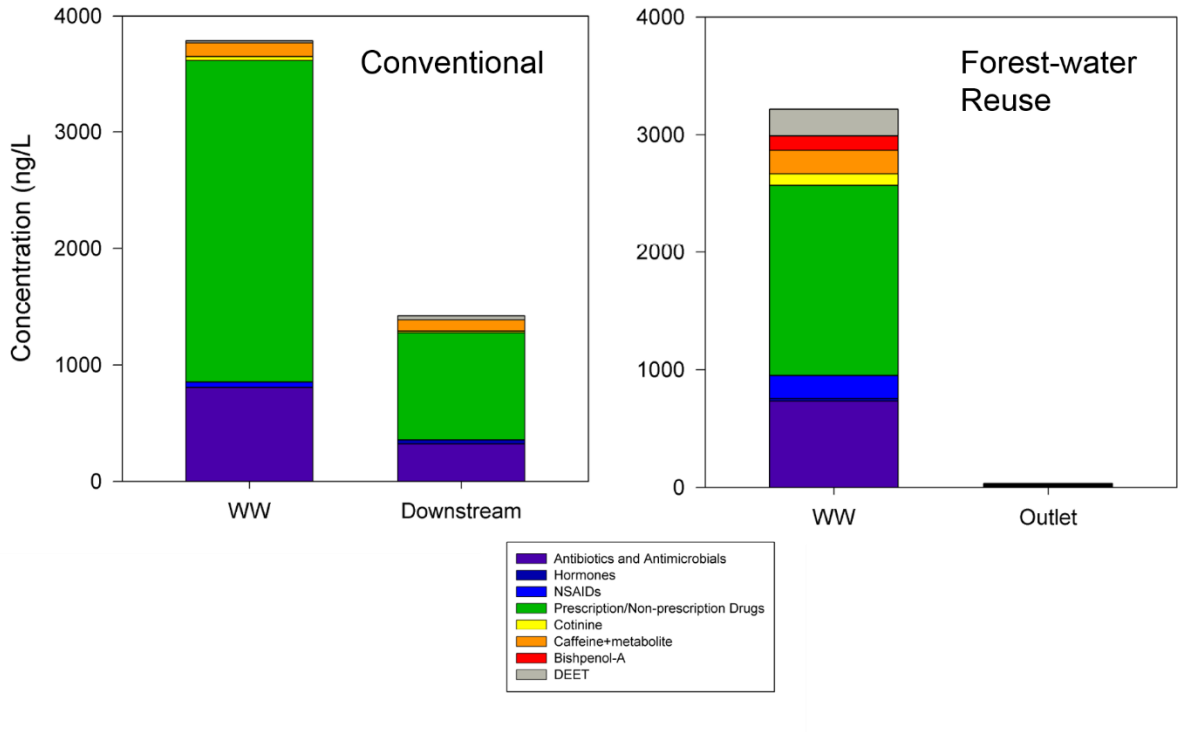

Figure 3.1. Total average summed concentration of targeted PPCPs divided by chemical group at the conventional (North Cary Water Reclamation Facility) and forest-water reuse systems.

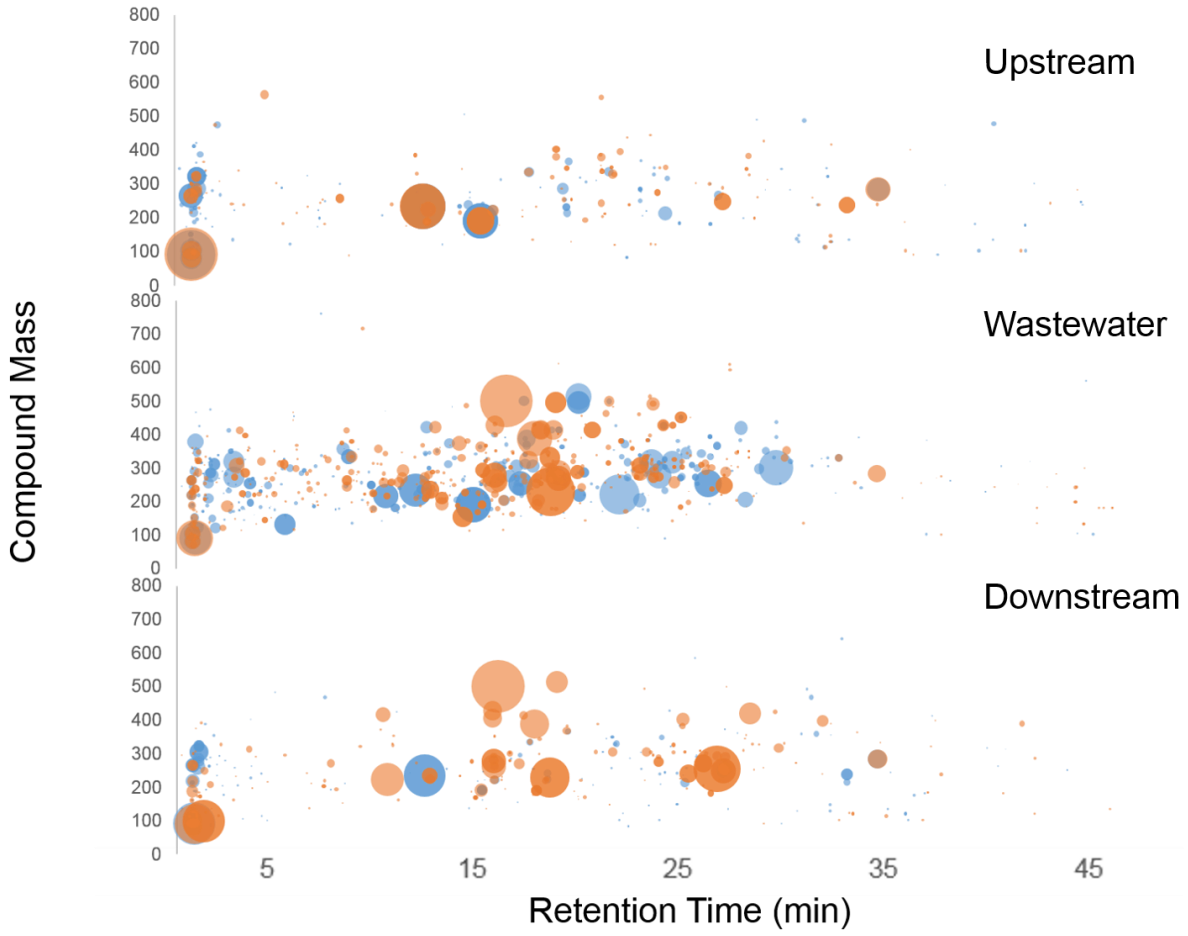

Figure 3.2. Chemical compounds identified from database matching suspect screening analysis in the wastewater, upstream, and downstream at the Jacksonville FWR System (Blue) and North Cary Water Reclamation Facility (Orange). Each dot represents a single compound and bubble size is compound relative abundance.

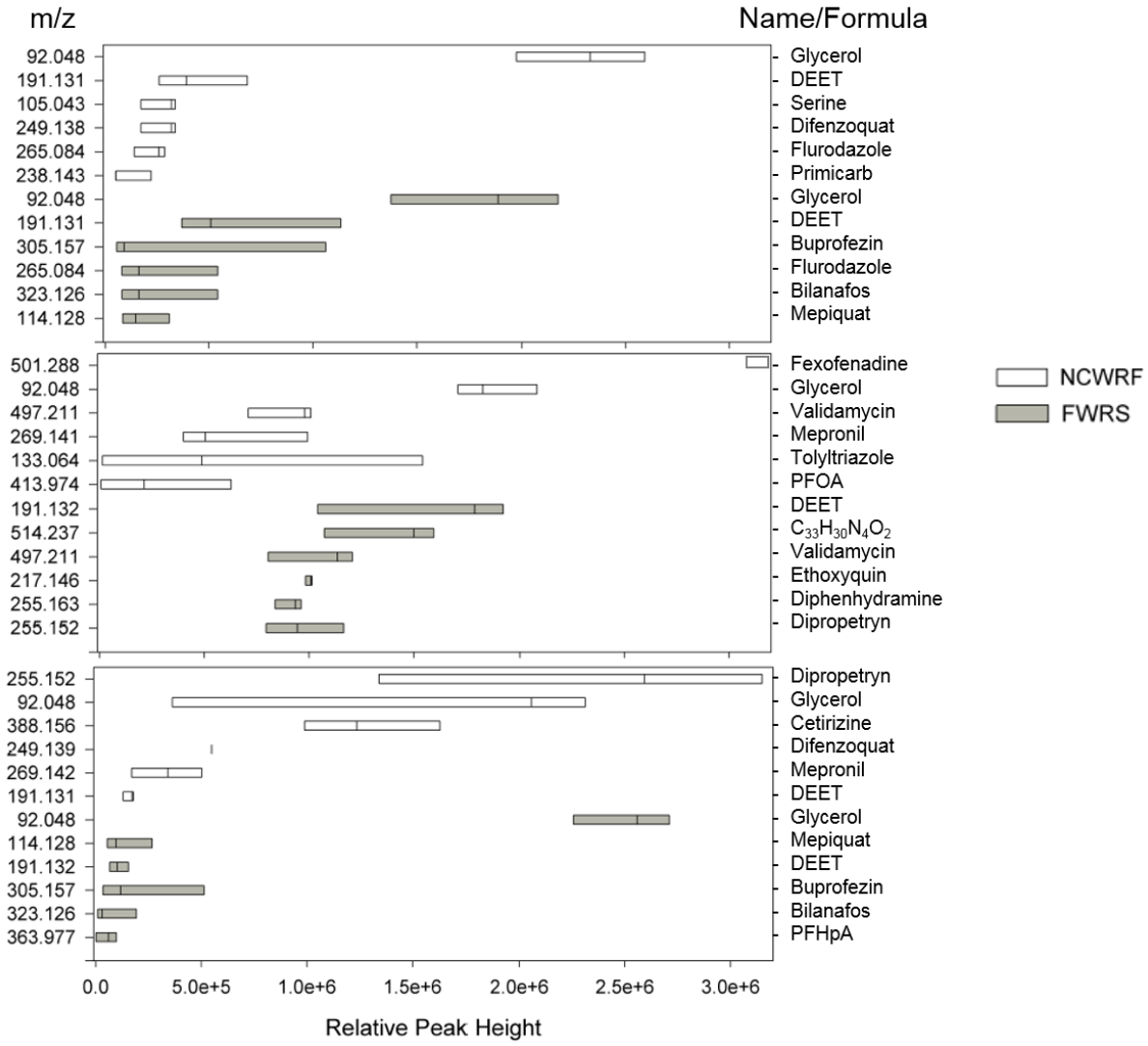

Figure 3.3. Relative abundance of the 6 most abundant suspect chemicals upstream, in the wastewater, and downstream of each treatment facility. The conventional North Cary Water Reclamation Facility (NCWRF) are white boxes and the forest-water reuse system (FWRS) and grey. Boxes depict median, maximum, and minimum abundances.

Supplementary Data for:

Comparing the Environmental Input of Pharmaceuticals from Conventional and Alternative Wastewater Treatment Systems: a Targeted and Suspect Screening Study

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SI Table 1. Total unique molecular features identified per sample and corresponding suspected chemicals matched at a score of 80 and 90.

	Total Molecular Features	Matched at Score ≥ 80	Matched at Score ≥ 90
Jacksonville FWRS			
Upstream	2153	157	37
Wastewater	5254	371	104
Downstream	2282	135	28
North Cary WRF			
Upstream	2094	146	39
Wastewater	4868	418	150
Downstream 1 (50 m)	1955	190	45
Downstream 2 (100 m)	2229	211	56

SUMMARY AND CONCLUSIONS

As water quality and availability continue to be threatened by climate change and population pressures, emerging contaminants in the environment represent the newest threat to water quality. The constant influx of these contaminants and low level exposure to mixtures of emerging contaminants results in detrimental ecotoxicological effects.[1,2, etc.] Finding ways to stabilize water resources while mitigating the input and impact of emerging contaminants is critical to clean water sources throughout the world, especially in areas most likely to be impacted by climate change and population pressures. This research has indicated that the land application of municipal water into a forested system does result in the environmental input of pharmaceuticals and other non-regulated contaminants. However, the majority of the concentrations present in the irrigated wastewater are removed through the forest-soil system before reaching groundwaters and surface waters. The concentrations present in groundwater and surface waters were below documented effect concentrations for both ecotoxicological and human health risk. Additionally, pharmaceutical concentrations in surface water downstream of the land application system were significantly lower than concentrations downstream of conventional WWTP effluent discharge. This research has shown that in addition to regulating water quality and availability, this forest-water system reduced the environmental input of emerging contaminants to a greater extent than a conventional treatment system did. This research can inform the future implementation of water reuse systems and expand emerging contaminant mitigation while providing additional ecosystem benefits.

Forest-water reuse systems should be further explored in order to expand their utility for wastewater reuse. Foremost research objectives should focus on determining the primary exposure pathways for human health risks for water reuse systems such as the forest-water system investigated in this study. If water reuse systems are to be expanded by municipalities and regions throughout the country risk needs to be well-established. Additional work should document the greater extent of emerging contaminant input in this system by evaluating non-regulated contaminants further upstream and downstream of the scope of the current study. Understanding the mechanisms of removal in different compartments of the forest-water reuse system will provide important research as to the mechanisms most critical for larger-scale implementation (i.e. the open-air reservoirs vs soil system, etc.). Finally, in expanding water-reuse systems to edible crop production, terrestrial ecotoxicological and plant uptake studies should be considered. Plants and biota in the forest-water reuse system may experience a greater contaminant burden than plants and biota not irrigated with municipal wastewater and understanding uptake into both plants and biota will speak to the future utility of wastewater land application in edible crop systems. These future research objectives would continue to advance the field towards a greater understanding and greater implementation of sustainable forest-water reuse systems.

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