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Distribution of nonprescription pharmaceuticals in central Indiana streams and effects on sediment microbial activity

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Abstract Since the discovery of trace concentrations of pharmaceuticals in streams and treated drinking water around the world, a call has been made by both the scientific community and the general public to increase understanding of the potential effects these compounds may have on freshwater integrity. We measured abundance and distribution of pharmaceuticals in headwater streams across the Upper White River Watershed of central Indiana. Four nonprescription pharmaceuticals (1,7-dimethylxanthine, caffeine metabolite; acetaminophen; caffeine; cotinine, nicotine metabolite) were found at one or more sites with mean concentrations of 0.038, 0.109, 0.057 and 0.041 μg/l, respectively. Caffeine was measured at trace concentrations at all sites sampled. Higher pharmaceutical concentrations were associated with streams having >90% agricultural land use in the sub watershed, suggesting that nonpoint sources, such as septic tanks, may contribute to stream pharmaceutical contamination. To assess the influence of these pharmaceuticals on stream microbial activity, we measured changes in sediment respiration and nutrient uptake in response to pharmaceuticals using both in vitro and in situ techniques. For in vitro experiments, respiration rates were not significantly different from controls with pharmaceutical exposure. However, net NO_3^--N uptake increased significantly with nicotine concentrations. Net NH_4^+-N uptake was reduced in response to caffeine and nicotine exposure. In situ experiments indicated nicotine exposure increased microbial respiration. Our data show pharmaceuticals are ubiquitous in headwater streams of central Indiana and likely influence stream microbial activity depending on the pharmaceutical compound and history of exposure.

Keywords Pharmaceuticals streams · Freshwater · Land use · Microbial activity

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Introduction

Pharmaceuticals have been found at trace concentrations in streams throughout the United States (Kolpin et al. 2002; Cahill et al. 2004; Glassmeyer et al. 2005; Focazio et al. 2008) and around the world (Lissemore et al. 2006; Roberts and Thomas 2006; Gómez et al. 2007). Pharmaceuticals have also been found in groundwater (Barnes et al. 2008) and treated drinking water (Ternes et al. 2002). Since these discoveries, a call has been made by both the scientific community and the general public to increase understanding of the potential effects these compounds may have on freshwater integrity. If these compounds are influencing the structure or function of aquatic ecosystems, and potentially human health through exposure in drinking water, proactive strategies to reduce environmental contamination are needed.

The primary pathway for pharmaceuticals to enter aquatic ecosystems is through human consumption followed by excretion of un-metabolized pharmaceuticals (Halling-Sørensen et al. 1998; Cain et al. 2004). Only a fraction of pharmaceuticals ingested are metabolized and remaining compounds are excreted in waste either in the parent form or as a metabolite. These compounds then enter the aquatic environment after passing through wastewater treatment facilities, as most current wastewater treatment methods are not designed to remove trace organic pollutants, such as pharmaceuticals (Halling-Sørensen et al.

1998; Ternes 1998; Daughton and Ternes 1999; Roberts and Thomas 2006). Higher pharmaceutical concentrations have been linked to streams receiving effluents from areas draining larger population densities (Buerge et al. 2003). Because of this link between pharmaceutical concentrations in freshwater and human waste, most studies have focused on waterways directly receiving point source pollution from wastewater treatment effluents.

Recent studies have noted pharmaceuticals are not only in wastewater effluent, but also are ubiquitous in streams not receiving effluent (Kolpin et al. 2002; Cahill et al. 2004; Glassmeyer et al. 2005; Lissemore et al. 2006; Roberts and Thomas 2006; Gómez et al. 2007; Focazio et al. 2008). However, data regarding the abundance and distribution of these compounds is still lacking as many studies have limited geographical ranges focused on areas with higher human populations (Kolpin et al. 2002; Glassmeyer et al. 2005; Focazio et al. 2008). Before the potential environmental effects of these contaminants can be assessed, factors that influence the distribution and concentration of pharmaceuticals in the aquatic environment must be further examined.

Pharmaceutical compounds are designed to have a physiological effect on organisms and thus it is likely that they may also alter function of aquatic microbial organisms (Halling-Sørensen et al. 1998). Studies assessing the potential environmental risk of pharmaceuticals in aquatic ecosystems have primarily focused on acute toxicity tests for higher organisms (e.g., invertebrates and fish; Smith and Burgett 2005; Crane et al. 2006; Heckmann et al. 2007) with limited data assessing effects on microbial organisms (Lawrence et al. 2005). Microbial processes (respiration, nutrient uptake) are a holistic measure of ecosystem function as they are essential to nutrient flow and the transformation of organic matter (Hall and Meyer 1998; Hill et al. 2000; Balser et al. 2006; Nweke et al. 2007). Thus, microbial communities are commonly used to examine the influence of contaminants on aquatic ecosystems (Balser et al. 2006) and can provide vital information about the influence of pharmaceuticals to both ecosystems and organisms. Further, processes at lower levels of organization (i.e., microbial respiration, nutrient uptake) may be influenced by contaminants more rapidly than effects on higher organisms (Clements 2000). Therefore, changes in microbial activity may be an early indicator of stress before a compound affects invertebrates or vertebrates.

We examined pharmaceutical abundance and distribution in the Upper White River Watershed (UWRW) of central Indiana. Additionally, the potential influence of nonprescription pharmaceuticals on stream ecosystem function was examined via changes in sediment microbial activity. The objectives were to: (1) quantify the distribution of pharmaceuticals in the UWRW and relate to land use and physiochemical factors; and, (2) assess the influence of four commonly found pharmaceuticals (acetaminophen, caffeine, ibuprofen, nicotine) on microbial respiration and nutrient uptake using both in vitro and in situ experiments.

Materials and methods

Study area

Sampling was conducted in the UWRW of central Indiana (Fig. 1). The UWRW supplies 85% of the surface water needed for human use in Indianapolis and central Indiana (Crawford 1995). The UWRW covers 7040 km^2 across 16 counties with a gradient of urban activities (Upper White River Watershed (UWRW) Alliance 2007). Because of anthropogenic activities, the UWRW is one of the most degraded watersheds in the country. Ten headwater streams within the watershed were selected for sampling to represent a range of urbanization (Fig. 1). Percent land use was calculated using a topographic method with a 1:24,000 USGS topographic map.

Water samples

Water samples were collected from ten sites within 12 h on 23 July 2008 during baseflow conditions. Two modified grab samples were collected from three verticals, at approximately the one-quarter, one-half, and three-quarter points of the transect that extended from the left edge to the right edge of water at each site. The first sample was analyzed for dissolved pharmaceuticals and was collected from the stream into a 1 l baked amber glass bottle. Immediately after sample collection, water was filtered from the glass collection container into another baked amber glass bottle using a Geopump© connected to a syringe filter with an ashed 25 mm Whatman© glass fiber filter (GF/F; 0.7 μm pore size). Samples were packed on ice and shipped overnight to the USGS National Water Quality Laboratory in Denver, CO for analysis of 14 commonly used pharmaceuticals: 1,7-dimethylxanthine, acetaminophen, albuterol, caffeine, carbamazepine, codeine, cotinine, dehydronifedipine, diltiazem, diphenhydramine, sulfamethoxazole, thiabendazole, trimethoprim, and warfarin, using mass spectrometry (USGS schedule 2080). Descriptions of the analytical methods for detection of pharmaceuticals have been detailed elsewhere (Kolpin et al. 2002; Cahill et al. 2004). Water quality parameters were also measured at three points for each site using a Hydrolab© minisonde equipped with an LDO oxygen sensor, turbidity meter, temperature probe, and pH sensor. Mean ($N = 3$) values for each site were used in data analyses.

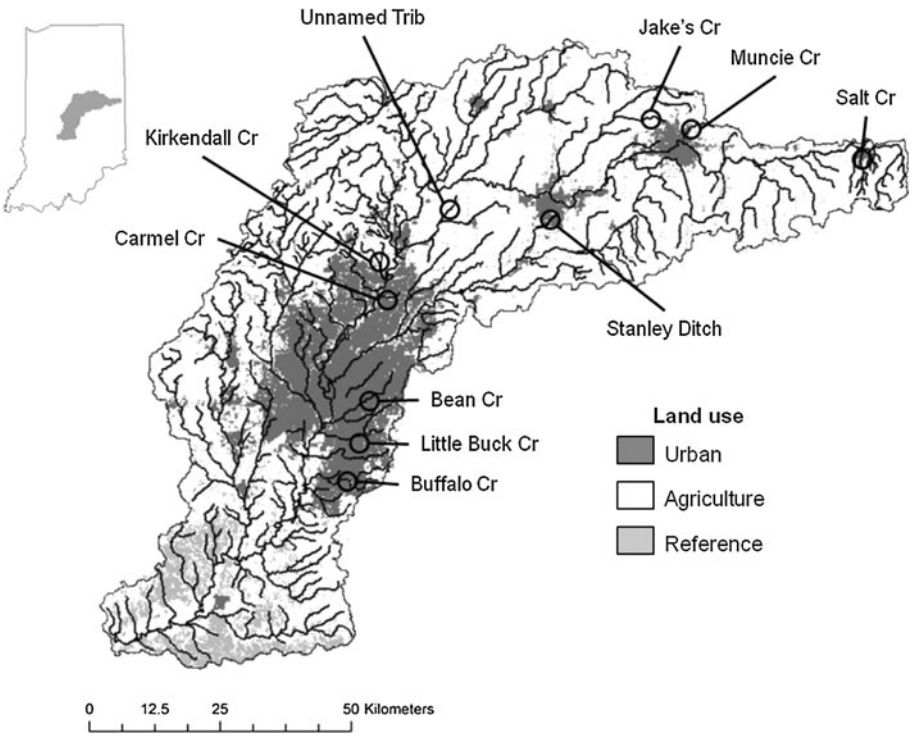

Fig. 1 Sample site locations in the Upper White River Watershed of central Indiana. *Map* depicts dominant land-use types. Reference *land use* refers to forest and wetland

The second water sample was collected into a 1 l acid-washed Nalgene© bottle and placed on ice for return to the laboratory. These samples were filtered in the laboratory within 24 h of collection, using a Geopump© connected to a syringe filter with an ashed 25 mm Whatman© glass fiber filter (GF/F; 0.7 μm pore size), into another acid washed Nalgene© bottle. Samples were then frozen for subsequent analysis of cations (ammonium, calcium, lithium, magnesium, potassium, sodium) and anions (bromide, chloride, nitrate, nitrite, phosphate, sulfate) using ion chromatography (Dionex ICS-3000; Dionex Corporation, Sunnyvale, CA).

Microbial response to pharmaceuticals: in vitro experiments

Sediment and water collection

Stream sediment and water were collected from a headwater stream in Delaware County of east central, IN for two in vitro experiments conducted simultaneously. The stream where collection took place had no point source inputs from wastewater treatment effluents and was a sampling site for measurement of pharmaceuticals as described above. Sediment from the top 5 cm of the stream bottom was collected from the stream thalweg as a natural microbial inoculum (sensu Kemp and Dodds 2002; Inwood et al. 2005). Sediment and water were brought back to the laboratory where sediment was homogenized and invertebrates were removed using a USGS no. 14 sieve; stream water was filtered using a Geopump© connected to a syringe filter with an ashed 25 mm Whatman© glass fiber filter (GF/F; 0.7 μm pore size) as above. Collected sediment and filtered water were stored in acid-washed Nalgene© bottles and refrigerated until experiments began (<24 h). Sediment and water were used for two simultaneous mesocosm experiments assessing microbial response to pharmaceutical treatments: (1) measurement of microbial respiration via dehydrogenase activity (DHA); and, (2) measurement of net nitrate (NO_3^--N) and ammonium (NH_4^+-N) uptake via change in concentrations over time.

Experimental mesocosms

Sieved, homogenized sediment was separated into subsamples by placing 2 ml into acid-washed 12 ml falcon tubes for DHA analyses ($N = 68$) and 30 ml into 120 ml HDPE specimen cups for net nitrogen uptake analyses ($N = 68$). After sediment separation, 2.5 ml of filtered stream water was added to falcon tubes and 90 ml filtered stream water was added to specimen cups. The four treatment pharmaceuticals selected for this study (acetaminophen, ibuprofen, caffeine, nicotine) have been found at trace concentrations throughout US surface waters (Kolpin et al. 2002). These compounds have a range of human uses: acetaminophen is an antipyretic pain reliever; ibuprofen is an anti-inflammatory; caffeine is a stimulant; and, nicotine is a central nervous system stimulant. For both respiration and nutrient uptake experiments,

Table 1 Treatment concentrations for in vitro and in situ pharmaceutical experiments

Pharmaceutical	Treatment concentration (μg/l)			
	Low	Mean	Intermediate	Max
Ibuprofen	0.1	0.2	0.6	1
Acetaminophen	0.055	0.11	5.055	10
Caffeine	0.0405	0.081	3.0405	6
Nicotine	0.012	0.024	0.462	0.9

Treatment concentrations were selected to represent the range of concentrations measured in freshwater (Kolpin et al. 2002)

four concentrations of each pharmaceutical compound (Table 1) and a control (no pharmaceutical addition) were tested (17 treatments total) with four replicate mesocosms for each treatment. Treatment concentrations for each pharmaceutical were low, mean, intermediate, and maximum concentrations based on concentrations observed in U.S. surface waters (Kolpin et al. 2002).

Microbial respiration: dehydrogenase activity assay (DHA)

Dehydrogenases are oxidoreductase enzymes that oxidize organic compounds by transferring electron pairs during microbial respiration (Smith and McFeters 1997). The use of tetrazolium salts as an electron acceptor is a widely accepted method for measuring microbial respiration, as they form a deep red colored formazan when reduced and can be measured using spectrophotometry (sensu Hill et al. 2000). Pharmaceutical treatments (Table 1) were added to prepared mesocosms (2 ml sieved sediment and 2.5 ml filtered water) and vortexed for 1 min to ensure complete mixing. Next, 1 ml of 0.75% iodonitroetrazolium (INT) chloride was added to each tube and tubes were capped and vortexed followed by incubation for 3 h at 27°C. The reaction was then stopped with addition of 8 ml of methanol and again vortexed. Samples were subsequently centrifuged for 10 min and the supernatant collected for spectrophotometric analyses. INT-formazan absorbance was measured using a Schimadzu dual-beam spectrophotometer (UV-1700 Phermaspec) at 428 nm. Background absorbance was subtracted from samples based on mean absorbance of four samples with no INT-chloride added. Formazan concentrations were calculated using a standard curve prepared with INT-formazan and then converted to mg of dissolved oxygen (O_2) consumed. Remaining sediment in falcon tubes was dried at 350°C and sediment grams dry mass (gdm) was measured for each replicate. Sediment microbial respiration was expressed as mg O_2/gdm/h.

Net nitrate and ammonium uptake

Net nutrient uptake was measured as change in nutrient concentration over time in laboratory mesocosms (sensu Kemp and Dodds 2002). Initial nutrient concentrations were determined by removing 10 ml of filtered water from each replicate mesocosm. Pharmaceutical amendments were then added accordingly (Table 1). All mesocosms were then incubated for 48 h at 27°C followed by collection of 10 ml filtered water for measurement of final nutrient concentrations. Nitrate was analyzed using ion chromatography as above followed by calculation of concentrations based on a standard curve. Ammonium was analyzed using colorimetric phenol hypochlorite technique (Aminot et al. 1997) and concentrations were calculated based on a standard curve. After collection of water for nutrient analyses, remaining water was decanted from each replicate mesocosm and sediment was dried and weighed as above. Initial stream nutrient concentrations were 2.04 mg NO_3^--N/l and 90.5 μg NH_4^+-N/l. Nutrient uptake is expressed as change in mg NO_3^--N/gdm/h or change in μg NH_4^+-N/gdm/h. For replicates where final concentration was greater than initial concentration (net mineralization; $N = 30$ for NO_3^--N; $N = 9$ for NH_4^+-N), net uptake rate was estimated as zero.

Microbial response to pharmaceuticals: in situ experiment

Nutrient diffusing substrata (NDS)

NDS is a method to assess microbial colonization and respiration using cellulose sponge substrates over nutrient and pharmaceutical enriched agar. NDS procedures were modified from Tank et al. (2006). Nine agar treatments were prepared including a control (no pharmaceuticals) and low and high treatment concentrations for acetaminophen, ibuprofen, caffeine, and nicotine (Table 1). The treatment concentrations were the mean and maximum concentrations used in the in vitro experiment (Table 1). Agar was autoclaved to 50°C and allowed to cool before pharmaceutical treatments were added. Each treatment had eight replicate NDS prepared. Pharmaceutical-amended agar was poured into 90 ml polycon plastic cups with holes drilled into the hinged lids. After agar solidified, pre-weighed cellulose sponges were placed on top of agar-filled cups. Cellulose sponges were used to select for bacterial colonization once placed into the stream; algae prefer a hard inorganic surface so any changes in weight were attributed to bacterial growth. Agar treatments were randomly attached to L-bars using cable ties with one of each treatment per L-bar. NDS were placed into Muncie Creek in Muncie, IN. This stream was one of the ten sites sampled for pharmaceutical concentrations (Muncie Creek; Fig. 1).

At the time of incubation, stream temperature was 22°C, dissolved oxygen in the water column was 9.2 mg O_2/l, NO_3-N and NH_4-N concentrations were 3.2 and 0.12 mg N/l, respectively, and pH was 8.4. Conditions were comparable to experimental conditions during in vitro experiments. Plastic stakes were used to secure L-bars to the stream bottom and placed into a stream riffle at base-flow conditions. NDS were oriented so ~10 cm of stream water was flowing over sponges at ~0.5 m^3/s during incubation. To protect from animal destruction or tampering, chicken wire was used to enclose NDS during stream incubation. After a 2 week incubation period, L-bars and attached NDS were removed from the stream. In the laboratory, cellulose sponges were separated from the NDS, rinsed lightly with deionized water to remove unattached particles and placed in 50 ml falcon tubes with 10 ml of deionized water. Microbial respiration was analyzed using DHA (as described above). Sponges were then dried in a drying oven (2 days at 100°C) and weighed for measurement of change in grams dry mass (gdm).

Statistical analysis

Pearson's correlation analyses, with Bonferonni correction for multiple comparisons, were used to evaluate the relationship between pharmaceutical concentrations and stream physiochemical parameters or watershed land use. For in vitro experiments, dose response equations were developed using linear and non-linear regression analyses to model respiration and net nutrient uptake rates in response to pharmaceutical concentrations. Models were developed using SigmaPlot v. 10.0 dynamic fit wizard. Models were not forced through the origin as respiration or net nutrient uptake may occur in the absence of pharmaceuticals. For in situ experiments, a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc pair wise comparisons was used to evaluate differences in respiration rates and microbial colonization among treatments with pharmaceutical treatment as the main effect ($N = 9$ treatments each with nine replicates). To normalize data, oxygen consumed was log transformed prior to statistical analyses. ANOVA and correlation statistical analyses were conducted using Minitab 15 (Minitab Inc. 2006). Data are presented as arithmetic means and standard error.

Results

Pharmaceutical concentrations

Of the 14 pharmaceuticals analyzed, four nonprescription pharmaceuticals (1,7-dimethylxanthine, caffeine metabolite; acetaminophen; caffeine; cotinine, nicotine metabolite) were found at one or more sites with mean concentrations of 0.038, 0.109, 0.057 and 0.041 μg/l, respectively (Table 2). Caffeine was ubiquitous with trace concentrations measured at all sites. Concentrations of 1,7-dimethylxanthine (caffeine metabolite), acetaminophen, caffeine, and cotinine (nicotine metabolite) ranged from 0.015 to 0.078, 0.025 to 0.318, 0.019 to 0.176, and 0.037 to 0.045 μg/l, respectively (Table 2). Other pharmaceuticals analyzed but not found in concentrations above detection limits (0.01 μg/l) in the UWRW were dehydronifedipine, diltiazem, diphenhydramine, sulfamethoxazole, thiabendazole, trimethoprim, and warfarin.

Table 2 Pharmaceutical concentrations quantified in study sites in the Upper White River Watershed of central, Indiana USA

Site	Pharmaceutical concentrations (μg/l)			
	1,7-Dimethylxanthine	Acetaminophen	Caffeine	Cotinine
Salt Cr	0.078	0.088	0.176	0.045
Muncie Cr	0.023	0.318	0.056	<DL
Jake's Cr	<DL	0.058	0.057	<DL
Stanley Ditch	0.015	0.056	0.039	<DL
Unnamed trib	<DL	<DL	0.019	<DL
Kirkendall Cr	<DL	<DL	0.047	<DL
Carmel Cr	<DL	<DL	0.043	<DL
Bean Cr	<DL	<DL	0.030	<DL
Little Buck Cr	<DL	<DL	0.021	<DL
Buffalo Cr	<DL	0.025	0.085	0.037

DL (detection limit) = 0.01 μg/l 1,7-dimethylxanthine; 0.02 μg/l acetaminophen; 0.02 μg/l caffeine; 0.02 μg/l cotinine

Physiochemical factors related to pharmaceutical concentrations

Physiochemical parameters varied among streams sampled (Table 3) indicating a range of different freshwater ecosystems were selected. Based on standard deviation, chloride (mean = 40.33 mg/l ± 22.44), sulfate (mean = 30.15 mg/l ± 50.08), and specific conductivity (mean = 775 μs/cm ± 267) varied the most across sites while pH (mean = 7.82 ± 0.46), nitrite (mean = 0.01 mg N/l ± 0.014), bromide (mean = 0.02 mg/l ± 0.007), ammonium (mean = 0.09 mg N/l ± 0.05) and potassium (mean = 1.95 mg/l ± 0.41) varied the least across sites. Water column dissolved oxygen ranged from 5.61 to 8.84 mg O_2/l (mean = 6.72 ± 0.96). Total pharmaceutical concentration (sum of all compounds measured) was correlated with lithium concentrations ($p < 0.01$, $r = 0.84$). As lithium was

Table 3 Physiochemical parameters measured at sites in the Upper White River Watershed of Central IN, USA

	Salt Cr	Muncie Cr	Jake's Cr	Stanley Ditch	Unnamed trib	Kirkendall Cr	Carmel Cr	Bean Cr	Little Buck Cr	Buffalo Cr	Mean ± SD	
Water column characteristics												
Temperature (°C)	21.7	22.2	22.4	17.6	15.2	21.1	20.6	20.0	22.3	22.0	20.5	±2.2
pH	8.6	8.4	8.4	7.5	7.3	7.7	8.0	7.5	7.5	7.3	7.8	±0.5
Specific conductivity (μS/cm)	829	1019	680	1019	1274	634	838	300	568	591	775	±266
Total dissolved solids (mg/l)	0.53	0.65	0.44	0.66	0.82	0.41	0.54	0.01	0.36	0.38	0.48	±0.21
Dissolved oxygen (mg/l)	8.84	6.31	5.88	5.94	8.00	6.35	6.89	6.90	6.43	5.61	6.72	±0.96
Salinity (mg/l)	0.43	0.53	0.35	0.53	0.67	0.32	0.44	0.30	0.29	0.30	0.42	±0.12
Turbidity	10.4	36.9	63.0	0.2	1.7	14.7	9.0	1.6	13.7	4.9	15.6	±18.8
Anions (mg/l)												
Chloride	40.3	36.4	33.0	72.3	95.6	35.9	90.5	58.2	44.3	63.2	40.3	±22.4
Nitrite	0.01	0.02	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	±0.01
Bromide	0.02	0.04	0.02	0.02	0.03	0.04	0.04	0.03	0.03	0.03	0.02	±0.01
Nitrate	1.96	1.13	0.39	1.20	0.23	0.89	0.40	1.35	0.88	0.79	1.96	±0.58
Phosphate	0.08	0.02	0.06	0.01	0.01	0.01	0.00	0.05	0.03	0.03	0.08	±0.03
Sulfate	30.2	181.8	27.8	52.4	119.4	42.0	47.7	45.3	20.1	18.	30.2	±50.1
Cations (mg/l)												
Lithium	0.002	0.004	0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	±0.001
Sodium	16.2	13.5	13.2	27.9	31.1	12.9	35.4	28.2	19.7	27.1	22.5	±7.9
Ammonium	0.11	0.11	0.21	0.08	0.11	0.06	0.03	0.04	0.04	0.11	0.09	±0.05
Potassium	2.12	2.17	1.30	1.41	2.52	2.44	1.90	2.25	1.89	1.48	1.95	±0.41
Magnesium	26.6	35.6	18.2	24.3	29.5	17.0	17.3	17.83	12.4	11.3	21.0	±7.4
Calcium	33.0	52.8	31.7	37.7	52.6	32.1	32.3	35.2	26.5	19.1	35.3	±9.9

Mean and standard deviation (SD) of site characteristics across study streams provided

only present in streams with measurable pharmaceuticals, this pattern indicates potential co-occurrence of these compounds. Concentrations of individual pharmaceutical compound were variably correlated with stream physiochemical parameters (Table 4). Specifically, nitrate concentrations were correlated with 1,7-dimethylxanthine ($p < 0.05$) and caffeine ($p < 0.05$). Acetaminophen concentrations were correlated with sulfate ($p < 0.05$) and magnesium ($p < 0.05$). Caffeine was correlated with phosphate concentrations ($p < 0.05$).

Relationship of land use to pharmaceutical concentrations

Land use in the surrounding sub-watershed ranged from 2 to 99% urban across the ten study sites (Fig. 2). Agriculture land use was ≥90% of the sub-watershed at two sites, Muncie and Salt Creek. Urban land use correlated negatively with total pharmaceutical concentrations in streams ($p < 0.05$, $r = -0.65$), while agricultural land use correlated positively with total pharmaceutical concentrations ($p = 0.05$, $r = 0.64$) (Fig. 2). This correlation was driven by two sites (Muncie and Salt Creek) indicating that streams with large amounts of agricultural land use (>90%) are associated with higher pharmaceutical concentrations, where streams with moderate agricultural land use may be buffered from pharmaceutical inputs via some unknown mechanisms (potentially sewage treatment processes). Forest and wetland land-cover ("reference" land use) accounted for <10% of land in the sub-watershed for all streams and was not correlated with pharmaceutical concentrations. Concentrations of individual pharmaceutical compounds were not correlated with any measure of land use in the sub-watershed ($p > 0.05$).

In vitro microbial response

Pharmaceutical treatments did influence microbial respiration rates in vitro with all treatment compounds eliciting a concentration-dependent response (Fig. 3, Table 5). Microbial respiration increased at low concentrations of ibuprofen, caffeine, and acetaminophen but increased rates became saturated with higher concentrations of ibuprofen and caffeine and inhibited with higher concentrations of acetaminophen. In contrast, increasing concentrations of nicotine yielded an exponential decline in respiration rates. Nicotine yielded a stimulatory effect on net nitrate uptake at lower concentrations but the effect was saturated at

Table 4 Pearson correlation statistics assessing the relationship between stream physiochemical parameters and pharmaceutical concentrations in the Upper White River Watershed of central Indiana

	1,7-Dimethylxanthine		Acetaminophen		Cotinine		Caffeine	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Temperature (°C)	0.17	0.64	0.30	0.41	0.30	0.41	0.39	0.27
pH	**0.63**	**0.05**	0.61	0.06	0.20	0.58	0.59	0.07
Specific conductivity	0.22	0.55	0.35	0.33	−0.10	0.78	−0.01	0.97
TDS	0.22	0.54	0.34	0.34	−0.04	0.92	0.05	0.90
Dissolved oxygen	**0.65**	**0.04**	−0.07	0.85	0.35	0.33	0.47	0.18
Salinity	0.19	0.60	0.33	0.35	−0.18	0.61	−0.10	0.79
Turbidity	−0.03	0.93	0.46	0.19	−0.20	0.57	0.05	0.89
Chloride	−0.30	0.40	−0.41	0.24	−0.14	0.69	−0.35	0.32
Nitrite	0.15	0.68	0.45	0.19	−0.04	0.91	0.22	0.54
Bromide	−0.50	0.14	0.16	0.66	−0.33	0.36	-0.39	0.27
Nitrate	**0.78**	**0.01**	0.31	0.39	0.51	0.13	**0.64**	**0.04**
Phosphate	0.59	0.08	0.04	0.90	0.53	0.11	**0.67**	**0.03**
Sulfate	0.05	0.90	**0.73**	**0.02**	−0.34	0.34	−0.22	0.54
Lithium	0.58	0.08	**0.84**	**<0.01**	0.17	0.63	0.48	0.17
Sodium	−0.33	0.35	−0.48	0.16	−0.09	0.81	−0.34	0.34
Ammonium	0.16	0.65	0.30	0.39	0.20	0.59	0.30	0.40
Potassium	0.11	0.76	0.02	0.96	−0.14	0.69	−0.09	0.81
Magnesium	0.48	0.17	**0.72**	**0.02**	−0.09	0.81	0.15	0.68
Calcium	0.11	0.76	0.53	0.12	−0.43	0.22	−0.24	0.50
1,7-Dimethylxanthine	*	*	0.40	0.25	**0.66**	**0.04**	**0.88**	**<0.01**
Acetaminophen	*	*	*	*	0.03	0.94	0.24	0.51
Cotinine	*	*	*	*	*	*	**0.89**	**<0.01**

Bold values denote significant correlations ($p \leq 0.05$; $N = 10$)

* Indicates duplicate comparison in correlation matrix

higher concentrations (Fig. 4, Table 5). Acetaminophen also stimulated net nitrate uptake at lower treatment concentrations whereas caffeine inhibited net nitrate uptake in an exponential manner. Net nitrate uptake was not influenced by increasing ibuprofen concentrations (Fig. 4, Table 5). Similarly, net ammonium uptake was not influenced by increasing ibuprofen or increasing acetaminophen concentrations (Fig. 5, Table 5). Net ammonium uptake decreased linearly with caffeine concentrations but nicotine increased net ammonium uptake at lower concentrations (Fig. 5, Table 5). Overall, in vitro microbial activity response to pharmaceutical compounds varied with both the pharmaceutical compound tested and the measure of microbial activity.

In situ microbial response

In situ microbial respiration rates associated with pharmaceutical-amended substrates varied among pharmaceutical treatments ranging from 1.75 mg O_2/gdm/h in response to low acetaminophen treatments to 1.0 mg O_2/gdm/h in low caffeine treatments (Fig. 6). Control substrates (no pharmaceutical amendment) had mean respiration rates of 1.6 mg O_2/gdm/h. In contrast to in vitro experiments (Fig. 3), in situ pharmaceutical treatments found no effect of acetaminophen and ibuprofen on microbial respiration rates (Fig. 6). However, respiration in situ was significantly decreased relative to controls in the low nicotine treatment and all caffeine treatments (Fig. 6). Interestingly, microbial biomass on in situ substrates was significantly higher relative to controls in the nicotine and caffeine treatments indicating lower absolute rates of respiration and not simply decreased colonization by microbes (Fig. 6). In situ microbial colonization of pharmaceutical-amended substrates ranged from 0.052 to 0.105 gdm microbial biomass.

Discussion

Pharmaceutical concentrations in the UWRW are comparable to those found in other studies (Table 6) although acetaminophen and caffeine concentration are on the higher end of the ranges previously documented in US streams (Kolpin et al. 2002; Glassmeyer et al. 2005;

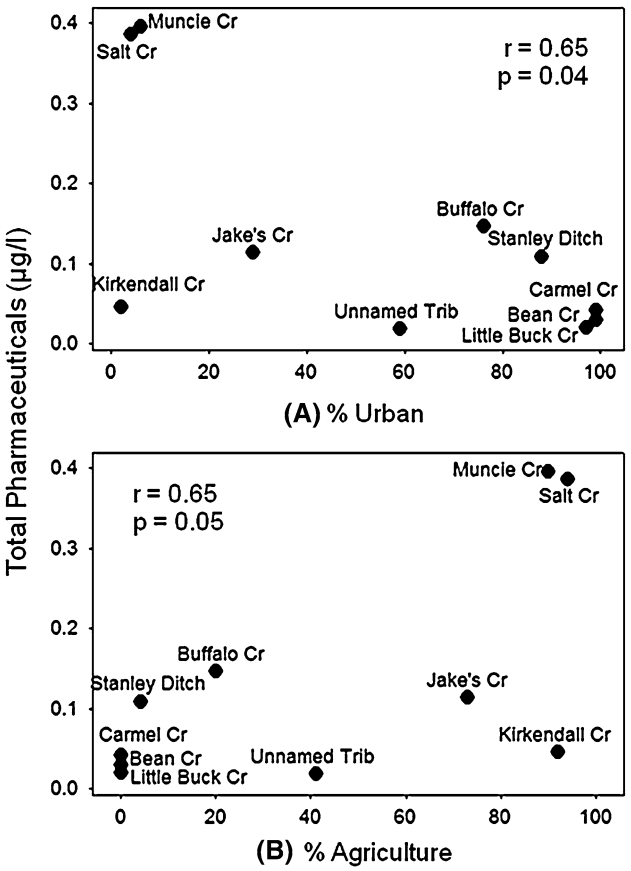

Fig. 2 Relationship between percent **a** urban and **b** agricultural land use in the sub-watershed and total pharmaceutical concentration for ten sites measured in the Upper White River watershed of central Indiana. Pearson correlation statistics noted

Focazio et al. 2008). The frequencies of detection for acetaminophen and caffeine were also higher in the UWRW relative to other studies in the US (Kolpin et al. 2002; Glassmeyer et al. 2005; Focazio et al. 2008). Sites in this study were smaller headwater streams compared to larger streams sampled in other studies. Kolpin et al. (2004) found that at higher flows pharmaceutical concentrations were likely diluted and detected less frequently. Thus, higher concentrations and more frequent detection in our study may be due to less dilution as our streams had lower discharge relative to sites assessed in previous studies.

Pharmaceutical concentrations in streams are thought to be associated with higher population densities and relative sewage input in urban areas (Ellis 2006). In this study, higher pharmaceutical concentrations were found in streams dominated by agricultural land use in the sub-watershed as opposed to more urban areas. Contrary to our hypothesis, a negative relationship between urban land use in the sub-watershed and total pharmaceutical concentrations was observed (Fig. 2). The main pathway for human pharmaceuticals to enter streams, sewage contamination, can be influenced by many factors including age and design of the sewer system (Walsh et al. 2005). Less urbanized areas tend to use more septic tanks and less advanced sewer systems that can leak untreated sewage into streams. This study suggests these nonpoint sources may be contributing to pharmaceutical contamination in freshwaters. Further, the negative correlation with urban land use suggests urban waste treatment processes may be removing pharmaceutical

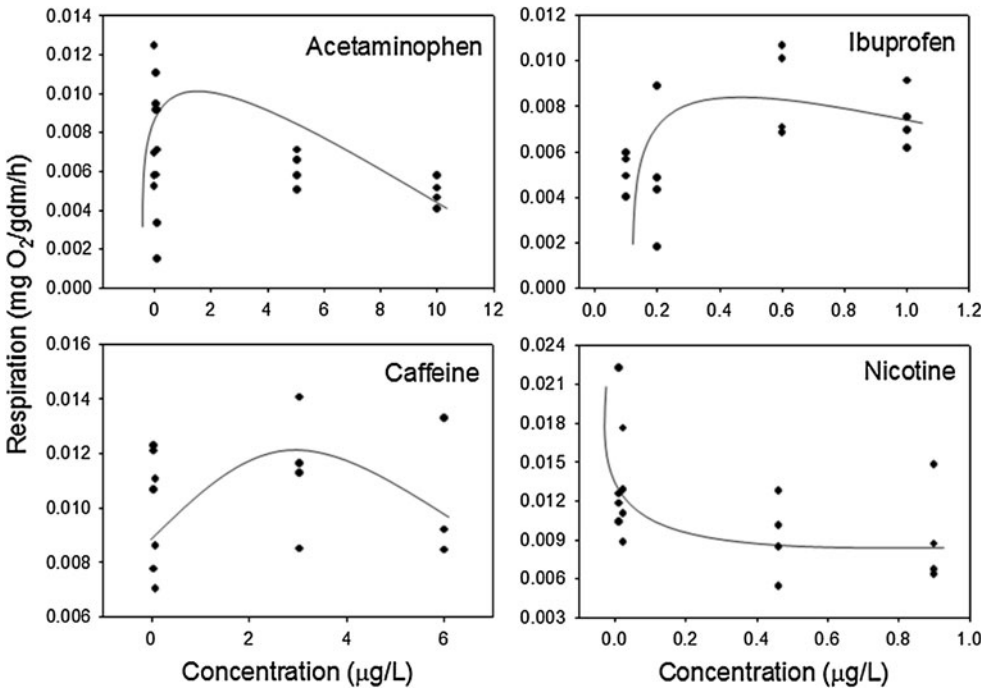

Fig. 3 In vitro sediment respiration response to pharmaceutical treatments. Four replicates shown for each treatment ($N = 4$ treatment concentrations) for each pharmaceutical compound. Significant linear and non-linear response patterns denoted by *lines*. Linear and non-linear equations and statistics provided in Table 5

Table 5 Predictive equations for respiration and nitrogen uptake with increasing pharmaceutical concentrations based on dose–response curves

Treatment	Equation	*p*-value	Type
Acetaminophen			
Respiration	$y = 0.01\ (\exp\ (-0.5\ (x - 2.4/7.3)^2))$	<0.0001	Peak
NO_3^--N uptake	$y = 0.007\ (\exp\ (-0.5\ (x - 0.4/0.1)^2))$	0.0015	Peak
NH_4^+-N uptake	*	*	*
Ibuprofen			
Respiration	$y = 0.0018\ (1 - \exp\ (-12.28x))$	0.007	Rise to max
NO_3^--N uptake	*	*	*
NH_4^+-N uptake	*	*	*
Caffeine			
Respiration	$y = 0.009\ (1 - \exp\ (-675x))$	<0.0001	Rise to max
NO_3^--N uptake	$y = 0.001\ (\exp\ (-0.092x))$	0.0015	Exponential decay
NH_4^+-N uptake	$y = 0.05 - 0.189x$	<0.01	Linear decay
Nicotine			
Respiration	$y = 0.009\ (\exp\ (-0.031x))$	<0.0001	Exponential decay
NO_3^--N uptake	$y = 0.0015\ (1 - \exp\ (-16x))$	<0.0001	Rise to max
NH_4^+-N uptake	$y = 0.17\ (1 - \exp\ (-135x))$	<0.0001	Rise to max

X pharmaceutical concentration (µg/l). For respiration, $y =$ mg O_2/gdm/h; NO_3^--N uptake, $y =$ mgNO_3^--N/gdm/h; NH_4^+-N uptake, $y =$ mg NH_4^+-N/gdm/h. $N = 16$ for each model

* Indicates no significant relationship

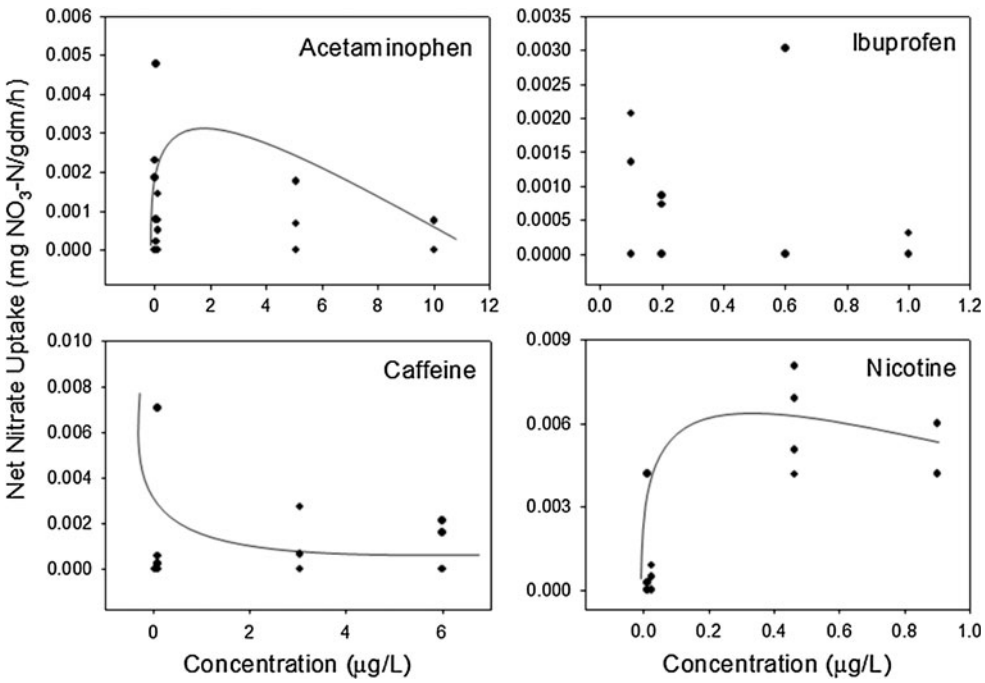

Fig. 4 In vitro sediment net nitrate uptake response to pharmaceutical treatments. Four replicates shown for each treatment ($N = 4$ treatment concentrations) for each pharmaceutical compound. Significant linear and non-linear response patterns denoted by *lines*. Linear and non-linear equations and statistics provided in Table 5

compounds prior to discharge into freshwaters. Urbanized areas tend to have updated sewage systems that carry waste to treatment facilities making contamination in streams not receiving wastewater treatment effluents less apparent, especially at base flow conditions when combined sewer overflows (CSOs) are not contributing to water flow, as in this study. Further, nitrate is normally associated with rural agricultural nonpoint pollution (Ribbe et al. 2008). Our data show concentrations of caffeine and 1,7-dimethylxane are correlated with nitrate concentrations further suggesting these compounds may be linked to rural, agricultural land use activities rather than urban activities in headwater streams. In contrast, urban waste is generally positively correlated with ammonium concentrations (Paul and Meyer

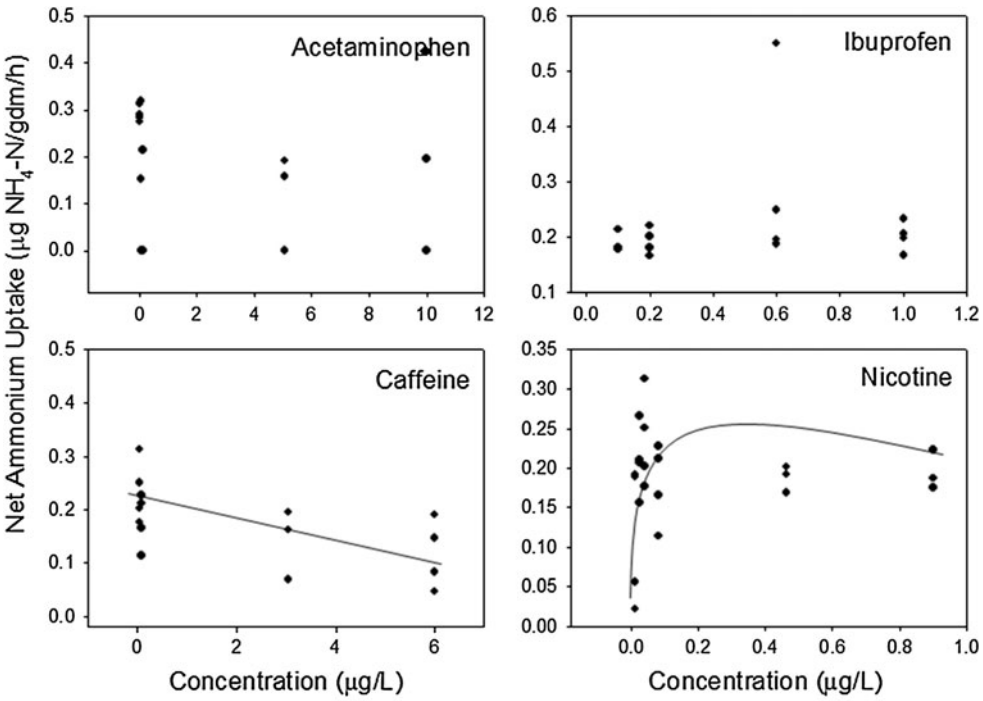

Fig. 5 In vitro sediment net ammonium uptake response to pharmaceutical treatments. Four replicates shown for each treatment ($N = 4$ treatment concentrations) for each pharmaceutical compound. Significant linear and non-linear response patterns denoted by *lines*. Linear and non-linear equations and statistics provided in Table 5

2001) although pharmaceuticals measured in this study were not correlated with NH_4^+-N concentrations either as total pharmaceutical concentration or concentrations of individual pharmaceutical compounds ($p > 0.10$, data not shown). The ubiquity of small nonpoint pollution sources may result in a larger cumulative effect on aquatic ecosystems (Duda et al. 1982), particularly during baseflow, which may be the case for pharmaceuticals in central Indiana.

Higher lithium concentrations were correlated with higher total pharmaceutical concentration across streams indicating pharmaceuticals may co-occur with lithium. Barber et al. (2006) found lithium to be a good indicator of anthropogenic waste inputs into streams, consistent with this study. Lithium is a drug used for the treatment of mood disorders (Price and Heninger 1994). Thus, the pathway lithium takes to enter freshwater is likely similar to other pharmaceutical compounds. Although more studies are needed, these results suggest that examining concentrations of this inorganic compound in streams may be a good indicator of the need for additional testing for organic pharmaceuticals. Lithium concentrations are significantly less expensive and labor intensive to quantify relative to pharmaceutical concentrations (ion chromatography techniques for lithium vs. mass spectrometry techniques for trace organics).

Data from the in vitro experiments suggest nonprescription pharmaceuticals can influence stream sediment respiration and net nitrogen uptake even at the trace concentrations currently found in US freshwaters. Microbes present a unique challenge in understanding the effects of contaminants, as compounds can be assimilated or degraded by microbes, in addition to their potential negative toxic effects. For example, Bradley et al. (2007) found that surface sediment microorganisms can alter the chemical structure of caffeine and nicotine yielding increases in microbial respiration in the presence of these compounds. Our data similarly indicate nicotine stimulated microbial activity as net nitrate and ammonium uptake (Figs. 4, 5) but inhibited overall microbial respiration (Fig. 3). Thus, it is likely that specific microbes can utilize nicotine as a nutritive source though the community overall may be inhibited. Lawrence et al. (2005) found some pharmaceutical compounds (including caffeine and ibuprofen) influenced microbial activity but stimulatory effects, potentially due to use of pharmaceuticals as a nutritive source, and inhibitory effects, potentially due to toxicity, depended on the specific pharmaceutical compound, consistent with our study. Identifying the mechanisms of microbial response in future studies is important to understanding how emerging contaminants could potentially alter nutrient cycling in stream ecosystems and thereby freshwater integrity.

If microbes are using pharmaceutical compounds as a nitrogen source, in place of dissolved NO_3^--N and NH_4^+-N, they may be removing less nitrogen from the water column potentially influencing nitrogen availability for other organisms or decreasing potential for mitigation of nitrogen pollution by microbes. If microbial nitrogen uptake from the water column is decreased, autotrophic production could be stimulated by increased nitrogen availability, potentially

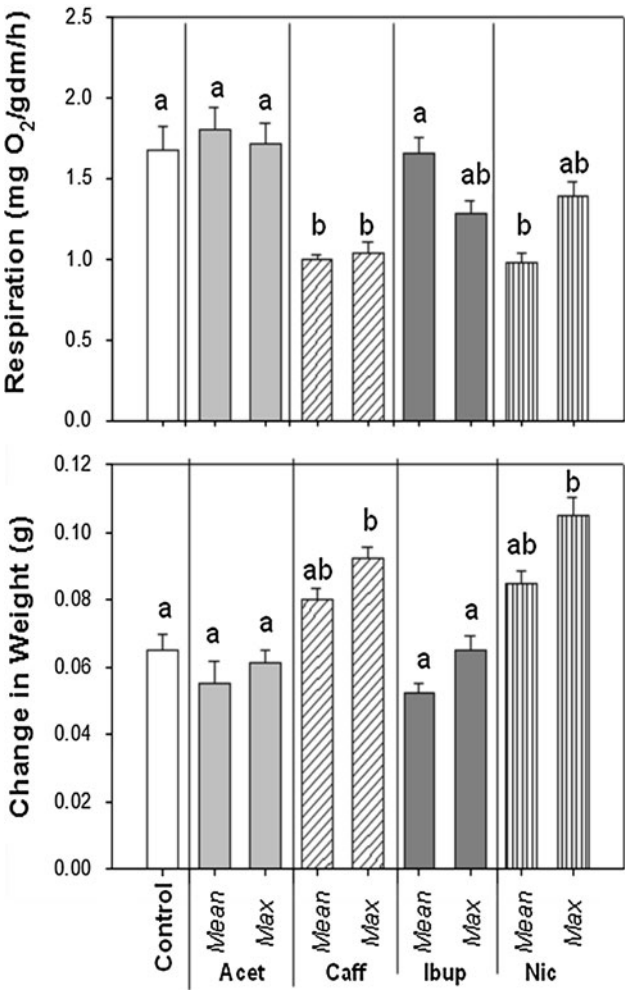

Fig. 6 Mean + SE microbial respiration rates and microbial biomass associated with pharmaceutical-amended nutrient diffusing substrates incubated in situ. *Control* no pharmaceuticals added, *Acet* acetaminophen amended substrate, *Caff* caffeine amended substrate, *Ibup* ibuprofen amended substrates, *Nic* nicotine amended substrates. Substrates were amended with two concentrations of each pharmaceutical compound (mean, max; Table 1). $N = 8$ for each *bar*. *Different letters* above *bars* indicate pair wise differences among treatments (ANOVA $p < 0.01$) when all treatments were considered. *Bars* with one or more of the same letters are not statistically different

resulting in unwanted algal blooms (Dodds and Welch 2000). Both ammonium and pharmaceutical contamination in streams have been linked to waste discharge from urban areas (Paul and Meyer 2001; Glassmeyer et al. 2005). This suggests that high concentrations of both nitrogen and pharmaceuticals are likely to co-occur in some systems. If NH_4^+-N is not removed from the water column because of decreased microbial uptake, due to either decreases in respiration or nutrient uptake, toxic concentrations of NH_4^+-N may occur at higher frequencies in streams affected by sewage. Alternatively, changes in nutrient uptake or respiration may be caused by shifts in the microbial community associated with pharmaceutical exposure. Lawrence et al. (2005) found pharmaceuticals (including caffeine and ibuprofen) alter microbial species in river biofilm communities potentially influencing nitrogen assimilated by the microbial communities. Regardless of the mechanism for changes in microbial activity, inhibited or stimulated microbial activity in response to trace concentrations of pharmaceuticals may alter ecosystem functioning.

In situ NDS experiments were conducted in the headwater stream with the highest concentrations of pharmaceuticals, Muncie Creek (Table 2). Although pharmaceutical sampling was conducted before the NDS experiment, we assume pharmaceutical concentrations detected in Muncie Creek were representative of typical conditions as NDS were incubated at base flow, consistent with timing of pharmaceutical measurements. In situ, caffeine and nicotine exposure significantly decreased microbial respiration (Fig. 6) consistent with the decreases in microbial activity observed in vitro. In contrast to in vitro experiments, there was not a significant microbial response to acetaminophen or ibuprofen exposure in situ. Biomass of microbial biofilms was similarly not influenced by acetaminophen or ibuprofen exposure in situ, though both nicotine and caffeine increased microbial biomass (Fig. 6). Ibuprofen has been shown to decrease biomass, whereas caffeine can increase biomass of aquatic microbial biofilms (Lawrence et al. 2005), consistent with this study. However,

Table 6 Comparison of maximum pharmaceutical concentration and frequency of detection measured in this study relative to previous studies

Reference	Sample type	Pharmaceutical concentration (μg/l)							
		1,7-Dimethylxanthine	%	Acetaminophen	%	Caffeine	%	Cotinine	%
This study	IN streams	0.04	30	0.11	50	0.06	100	0.04	20
Kolpin et al. 2002	US streams	0.11	29	0.11	24	0.08	62	0.02	32
Glassmeyer et al. 2005	US WWTP effluent	<DL	28	0.01	8	0.05	70	0.024	92
	Downstream from effluent	0.02	*	<DL	*	0.05	*	0.024	*
Focazio et al. 2008	US surface & groundwater	0.30	2	0.16	8	0.27	7.5	0.10	35

Frequency of detection is the percentage of samples collected pharmaceutical contamination was detected

% Frequency of detection, *WWTP* waste water treatment plant, *DL* detection limit

*Glassmeyer et al. (2005) averaged frequencies for both sample types

previous studies have not delineated effects of pharmaceuticals on growth or activity. Because microbial biomass increased when exposed to nicotine and caffeine, but respiration rates decreased, absolute microbial activity was altered as opposed to decreased microbial colonization.

Differences in microbial activity in vitro relative to in situ experiments with exposure to acetaminophen and ibuprofen may be because the incubation stream had a history of higher pharmaceutical exposure potentially yielding microbial adaptation and tolerance (Spain and Van Veld 1983; Liebert et al. 1991). Microbial activity in streams contaminated by pharmaceuticals may not be influenced if long-term exposure results in acclimation. An alternative explanation for variable microbial response to pharmaceuticals in the laboratory (in vitro) and field (in situ) experiments is that the microbial response is dependent on multiple factors including pH, organic matter, oxygen, and water chemistry as well as pharmaceutical concentration and history of pharmaceutical contamination in the stream. In vitro experiments were conducted at room temperature (27°C) whereas in situ experiments were conducted at a slightly lower temperature (22°C). Theoretically, the higher incubation temperatures during in vitro experiments would be expected to increase overall microbial respiration rates. However, in situ experiments yielded respiration rates an order of magnitude greater than respiration rates measured in vitro (Figs. 3, 6). Thus, the difference in incubation temperature likely had minimal influence associated with comparisons; rather, continuous supply of nutrients in situ likely was more conducive to higher microbial respiration. Further, water pH, oxygen concentrations, and nutrient concentrations were comparable in water used for in vitro experiments and in situ incubations, allowing for more robust comparisons on the effect of pharmaceuticals. Other factors not measured in this study such as light availability, organic matter, other trace contaminants, and shear stress may have also contributed to differences in observed results in vitro compared to in situ experiments. More comprehensive studies are needed to assess confounding factors on microbial response to pharmaceuticals.

This study demonstrates that pharmaceuticals are ubiquitous in headwater streams of central Indiana and depending on the compound and the concentration these pharmaceuticals may be influencing stream microbial activity. Contrary to our hypothesis, pharmaceutical concentrations were not associated with urban land use. Higher pharmaceutical concentrations were measured in streams with >90% agricultural land use in the sub-watershed and negatively correlated with urban land use in the sub-watershed. Because pharmaceuticals were associated with agricultural land use in streams not receiving direct wastewater treatment effluents, nonpoint sources such as septic tanks are likely contributing to pharmaceutical contamination in these ecosystems. Studies examining the frequency and distribution of pharmaceuticals in streams not receiving wastewater effluents will help in future management of these currently unregulated contaminates.

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References

Aminot A, Kirkwood DS, Kérouel R (1997) Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C 5 questionnaire. Mar Chem 56:59–75

Balser TC, McMahon KD, Bart D, Bronson D, Coyle DR, Craig N, Flores-Mangual ML, Forshay K, Jones SE, Kent AE, Shade AL (2006) Bridging the gap between micro- and macro-scale perspectives on the role of microbial communities in global change ecology. Plant Soil 298:58–70

Barber LB, Murphy SF, Verplanck PL, Sandstorm MW, Taylor HE, Furlong ET (2006) Chemical loading into surface water along a hydrological, biogeochemical, and land use gradient: a holistic watershed approach. Environ Sci Technol 40:475–486

Barnes KK, Kolpin DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB (2008) A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States: I) groundwater. Sci Total Environ 402:192–200

Bradley PM, Barber LB, Kolpin DW, McMahon PB, Chapelle FH (2007) Biotransformation of caffeine, cotinine, and nicotine in stream sediments: implications for use as wastewater indicators. Environ Toxicol Chem 26:1116–1121

Buerge I, Poiger T, Muller MD, Buser H (2003) Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environ Sci Technol 27:691–700

Cahill JD, Furlong ET, Burkhardt MR, Kolpin DW, Anderson LG (2004) Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr 1041:171–180

Cain TG, Kolpin DW, Vargo JD, Wichman MD (2004) Occurrence of antibiotics, pharmaceuticals and sterols at select surface and wastewater sites in Iowa. In proceedings of the 4th international conference on pharmaceuticals and endocrine disrupting chemicals in water, Minneapolis, MN, 13–15 Oct 2004. National Ground Water Association, pp 151–157 (CD-Rom)

Clements WH (2000) Integrating effects of contaminants across levels of biological organization: an overview. J Aquat Ecosyst Stress Recovery 7:113–116

Crane M, Watts C, Boucard T (2006) Chronic aquatic environmental risk from exposure to human pharmaceuticals. Sci Total Environ 367:23–41

Crawford CG (1995) Occurrence of pesticides in the White River, Indiana, 1991–95. U.S. Geological Survey Fact Sheet, pp 233–295

Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change. Environ Health Perspect 107:906–938

Dodds WK, Welch E (2000) Establishing nutrient criteria in streams. J North Am Benthol Soc 19:186–196

Duda AM, Lenet DR, Penrose DL (1982) Water quality in urban streams—what we can expect. J Water Pollut Control Fed 54:1139–1147

Ellis JB (2006) Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environ Pollut 144:184–189

Focazio MJ, Kolpin DW, Barnes KK, Furlong ET, Meyer MT, Zaugg DS, Barber LB, Thurman ME (2008) A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) untreated drinking water sources. Sci Total Environ 402:201–216

Glassmeyer ST, Furlong ET, Kolpin DW, Cahill JD, Zaugg SD, Werner SL, Meyer MT, Kryak DD (2005) Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination. Environ Sci Technol 39:5157–5169

Gómez MJ, Martínez Bueno MJ, Lacorte S, Fernández-Alba AR, Agüera A (2007) Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere 66:933–1002

Hall RO, Meyer JL (1998) The trophic significance of bacteria in a detritus-based stream food web. Ecology 79:1995–2012

Halling-Sørensen B, Nielson SN, Lanzky PF, Ingerslev F, Holten-Lutzhoft J, Jorgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 35:357–393

Heckmann LH, Callaghan A, Hooper HL, Connon R, Hutchinson TH, Maund SL, Sibly RM (2007) Chronic toxicity of ibuprofen to *Daphnia magna*: effects on life history traits and population dynamics. Toxicol Lett 172:137–145

Hill BH, Hall RK, Husby P, Herlihy AT, Dunne M (2000) Interregional comparisons of sediment microbial respiration in streams. Fresh Biol 44:213–222

Inwood SE, Tank JL, Bernot MJ (2005) Patterns of denitrification associated with land use in 9 midwestern headwater streams. J North Am Benthol Soc 24:227–245

Kemp MJ, Dodds WK (2002) The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata. Limnol Oceanogr 47:1380–1393

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–1211

Kolpin DW, Skopec M, Meyer MT, Furlong ET, Zaugg SD (2004) Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Sci Total Environ 328:119–130

Lawrence JR, Swerhone GDW, Wassenarr LI, Neu TR (2005) Effects of selected pharmaceuticals on riverine biofilm communities. Can J Microbiol 51:655–669

Liebert CA, Barkay T, Turner RR (1991) Acclimation of aquatic microbial communities to Hg(II) and CH_3Hg^+ in polluted freshwater ponds. Microb Ecol 21:139–149

Lissemore L, Hao C, Yang P, Sibley PK, Mabury S, Solomon KR (2006) An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. Chemosphere 64:717–729

Minitab, Inc. (2006). Statistical Software, version 16. Minitab, Incorporated, State College, PA

Nweke CO, Alisi CS, Okolo JC, Nwanyanwu CE (2007) Toxicity of zinc to heterotrophic bacteria from a tropical river sediment. Appl Ecol Environ Res 5:123–132

Paul MJ, Meyer JL (2001) Streams in the urban landscape. Annu Rev Ecol Syst 32:333–365

Price LH, Heninger GR (1994) Lithium in the treatment of mood disorders. New Engl J Med 331:591–598

Ribbe L, Delgado P, Salgado E, Flügel WA (2008) Nitrate pollution of surface water induced by agricultural non-point pollution in the Pocochay watershed, Chile. Desalination 226:13–20

Roberts PH, Thomas KV (2006) The occurrence of select pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ 356:143–153

Smith GR, Burgett AA (2005) Effects of three organic wastewater contaminants on American toad, *Bufo americanus*, tadpoles. Ecotoxicology 14:477–482

Smith JJ, McFeters GA (1997) Mechanisms of INT (2-(4 iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride and CTC (5-cyano-2,3,-ditolyl tetrazolium chloride) reduction in *Escherichia coli* k-12. J Microbiol Meth 29:161–175

Spain JC, Van Veld PA (1983) Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure time, inoculum, and chemical structure. Appl Environ Microbiol 45:428–435

Tank JL, Bernot MJ, Rosi-Marshall EJ (2006) Nitrogen limitation, uptake, and turnover in stream ecosystems. In: Hauer FR, Lamberti GA (eds) Methods in stream ecology, 2nd edn. Academic Press, San Diego, CA

Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. Water Res 32:3245–3260

Ternes TA, Meisenheimer M, McDowell D, Sacher F, Brauch H, Haist-Gulde B, Preuss G, Wilme U, Zulei-Seibert N (2002) Removal of pharmaceuticals during drinking water treatment. Environ Sci Technol 36:3855

Upper White River Watershed (UWRW) Alliance (2007) http://www.whiteriveralliance.org. Accessed 4 Oct 2007

Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP (2005) The urban stream syndrome: current knowledge and the search for a cure. J North Am Benthol Soc 24:706–723