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Association between degradation of pharmaceuticals and endocrine-disrupting compounds and microbial communities along a treated wastewater effluent gradient in Lake Mead

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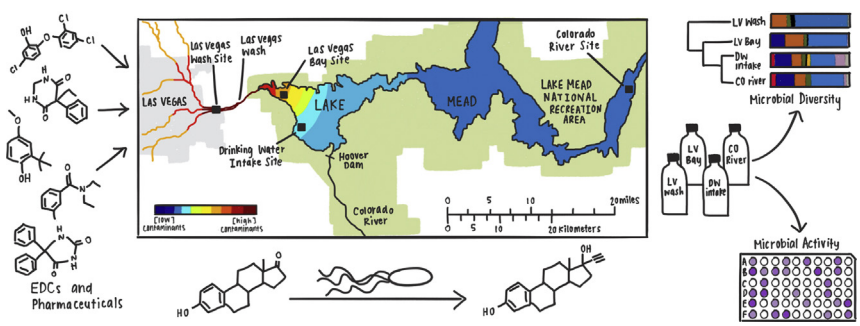
HIGHLIGHTS

- Microbial contaminant degradation was examined in discharged wastewater effluent
- Wastewater effluent was enriched in organics, nutrients, and culturable microbes
- Microbial phylogenetic diversity was higher in wastewater effluent-impacted water
- Microbial metabolic capacity was elevated in wastewater effluent-impacted water
- Contaminant biodegradation corresponded with relative wastewater influence

GRAPHICAL ABSTRACT



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ABSTRACT

The role of microbial communities in the degradation of trace organic contaminants in the environment is little understood. In this study, the biotransformation potential of 27 pharmaceuticals and endocrine-disrupting compounds was examined in parallel with a characterization of the native microbial community in water samples from four sites variously impacted by urban run-off and wastewater discharge in Lake Mead, Nevada and Arizona, USA. Samples included relatively pristine Colorado River water at the upper end of the lake, nearly pure tertiary-treated municipal wastewater entering via the Las Vegas Wash, and waters of mixed influence (Las Vegas Bay and Boulder Basin), which represented a gradient of treated wastewater effluent impact. Microbial diversity analysis based on 16S rRNA gene censuses revealed the community at this site to be distinct from the less urban-impacted locations, although all sites were similar in overall diversity and richness. Similarly, Biolog EcoPlate assays demonstrated that the microbial community at Las Vegas Wash was the most metabolically versatile and

Abbreviations: BHA, butylated hydroxyanisole; CEC, contaminants of emerging concern; DEET, *N,N*-Diethyl-meta-toluamide; EDC, endocrine-disrupting compound; LC-MS/MS, liquid chromatography mass spectrometry; OTU, operational taxonomic unit; PCR, polymerase chain reaction; SPE, solid-phase extraction; TCEP, tris(2-carboxyethyl)phosphine; WWTP, wastewater treatment plant.

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active. Organic contaminants added as a mixture to laboratory microcosms were more rapidly and completely degraded in the most wastewater-impacted sites (Las Vegas Wash and Las Vegas Bay), with the majority exhibiting shorter half-lives than at the other sites or in a bacteriostatic control. Although the reasons for enhanced degradation capacity in the wastewater-impacted sites remain to be established, these data are consistent with the acclimatization of native microorganisms (either through changes in community structure or metabolic regulation) to effluent-derived trace contaminants. This study suggests that in urban, wastewater-impacted watersheds, prior exposure to organic contaminants fundamentally alters the structure and function of microbial communities, which in turn translates into greater potential for the natural attenuation of these compounds compared to more pristine sites.

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1. Introduction

Mechanisms driving the natural attenuation of wastewater-derived organic contaminants in the environment remain relatively understudied, despite their regular detection in surface and groundwaters around the world (Kolpin et al., 2002; Petrovic et al., 2004; Bendz et al., 2005; Bester et al., 2008; Kuster et al., 2008; Kasprzyk-Hordern et al., 2009; Rosen et al., 2010; Alvarez et al., 2012; Rosen et al., 2012a; Meffe and de Bustamante, 2014). Although their ubiquity suggests that, as a class, these compounds are often persistent to varying degrees, the fate and transport of any individual compound is controlled by complex interactions between physiochemical and biological processes, including advection, photolysis, adsorption/desorption, and microbial degradation. As global water resources become increasingly strained by human population growth and climatic factors, wastewater reuse will increase, making water resources more susceptible to contamination (Benotti et al., 2010). Among the broad range of anthropogenic chemicals commonly detected in surface waters and aquifers, contaminants of emerging concern (CECs) (e.g.: pharmaceuticals and endocrine-disrupting compounds [EDCs]) are of special interest due to their recalcitrance and often extremely high potencies (Caldwell et al., 2012). A better understanding of the role of biology, specifically indigenous microorganisms, in the degradation of persistent and harmful organic compounds (Kostitch et al., 2014) is essential.

Much of the available information pertaining to the biodegradability of pharmaceuticals and EDCs stems from investigations of their behavior in wastewater treatment plants (WWTPs, Petrie et al., 2015) and bench-scale experiments (Zwiener et al., 2002; Popa et al., 2014; Cetecioglu et al., 2015). For example, Clara and coworkers showed that an increase in sludge retention time (SRT, >10 days) resulted in almost complete removal of ibuprofen, bezafibrate, bisphenol-A, and naturally occurring estrogens, owing to greater microbial activity, while carbamazepine was shown to be refractory despite longer SRTs (Clara et al., 2005). Similarly, Carballa et al. (2006) found carbamazepine to be resistant to degradation; while diazepam, diclofenac, ethynylestradiol, ibuprofen, and naproxen all showed moderate to high removal in WWTPs. Likewise, trimethoprim (Junker et al., 2006) and fluoxetine (Kwon and Armbrust, 2006; Redshaw et al., 2008) have proven highly resistant to degradation through wastewater treatment. More recently, Tang et al. (2017) showed that biodegradation rates of 12 pharmaceuticals in post-tertiary-treated wastewater effluent increased up to 4-fold with the addition of humic acids.

There is a need to measure degradation rates of pharmaceuticals and EDCs in surface waters, as their behavior in manipulated systems (e.g., WWTPs) may not accurately predict outcomes in natural settings. In a study of pharmaceutical concentrations in wastewater effluent of 50 large WWTPs across the United States (each processing 57–2500 million L of wastewater per day), Kostitch et al. (2014) found detectable concentrations of hydrochlorothiazide, metoprolol, atenolol, and carbamazepine in >90% of WWTPs, and the concentrations of 52 additional pharmaceuticals were variable. Although Kostitch et al. (2014) predicted the potential risks of these pharmaceuticals to aquatic life to be low, their study highlights the need to better understand the accumulation, fate, and transport of these emerging contaminants in surface waters receiving wastewater effluent.

Benotti and Brownawell (2009) calculated first-order degradation rates for low (ng/L) concentrations of pharmaceuticals in a wastewater-impacted estuary based on laboratory experiments. Half-lives of the targeted compounds varied temporally and spatially throughout the estuary, ranging from 0.68 to >100 days. The most labile compounds included nicotine, acetaminophen, fluoxetine, diltiazem, and nifedipine; whereas, the most refractory compounds were sulfamethoxazole, trimethoprim, salbutamol, antipyrine, cotinine, and carbamazepine. In a study conducted by Yamamoto et al. (2009), biodegradation of eight pharmaceuticals in water collected from rivers on two different dates was examined over a period of 120 h. While all eight compounds exhibited a relatively slow biodegradation rate (>24 h), acetaminophen exhibited the quickest removal, although degradation rates appeared to vary depending on the time of sampling. The stark differences in degradation rates between time points in the Yamamoto study suggest that the microbial degradation of pharmaceuticals and EDCs is a dynamic process that varies by time and environment, and may be reflective of the spatial and temporal differences in microbial populations in surface waters.

To date, few studies have combined an examination of the transformation of pharmaceuticals and EDCs in surface waters in parallel with characterization of microbial communities presumed responsible for the degradation (Caracciolo et al., 2015). Lake Mead, USA, provides a unique setting in which to study the microbial transformation of pharmaceuticals and EDCs in surface waters variably impacted by wastewater effluent (Rosen et al., 2010; Rosen et al., 2012b; Patiño et al., 2015). The present study examined microbial community structure, metabolic potential, and microbial degradation kinetics of a diverse suite of pharmaceuticals and EDCs in water collected from four representative locations around the Lake Mead system, comprising a gradient of treated wastewater effluent impact. The objectives of this study were: (i) to identify differences in overall microbial metabolic capacity, diversity, and community composition between the same four sites, (ii) to investigate rates of EDC and pharmaceutical biodegradation at sites along a treated wastewater effluent gradient, and (iii) to determine how differences in microbial community characteristics relate to differences in EDC and pharmaceutical biodegradation.

2. Materials and methods

2.1. Sampling

Water samples were collected from four stations throughout the Lake Mead system (Fig. 1) along a treated wastewater effluent gradient on October 21 and 22, 2008, in coordination with periodic water quality sampling by the Southern Nevada Water Authority (SNWA, Las Vegas, Nevada). Detailed descriptions of Lake Mead are available elsewhere (LaBounty and Burns, 2005; Holdren and Turner, 2010; Rosen et al., 2012b). Briefly, the main source of water supplying Lake Mead is the Colorado River (CO River, 97% of the total average inflow, [36.100°N, −114.116°W]), which enters Lake Mead from the east (Fig. 1). A large secondary input of water to Lake Mead is from the Las Vegas

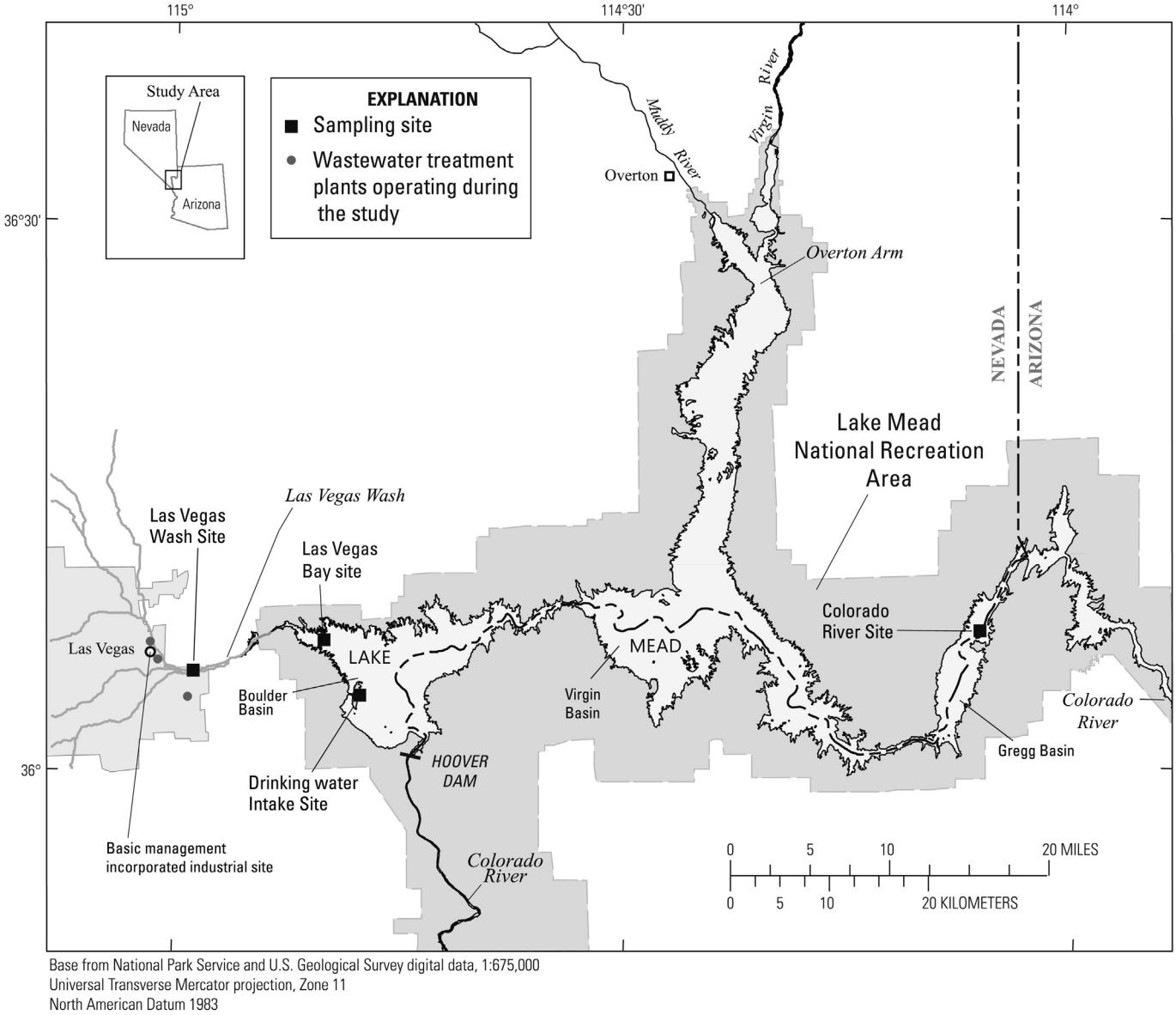

Fig. 1. Map of study site and sampling locations.

metropolitan area's three wastewater treatments plants (>720 million L of tertiary-treated wastewater per day; Turner et al., 2012), which enters from the west via the Las Vegas Wash (LV Wash [36.092°N, −114.969°W]). Water from LV Wash mixes with that of the main lake within Las Vegas Bay (LV Bay [36.106°N, −114.780°W]). The drinking water intake (DW Intake [36.064°N, −114.801°W]) for the city is downstream of the point of wastewater discharge, although at a depth that prevents extensive mixing at current lake levels (Fig. 1).

Individual samples were collected with a peristaltic pump (Masterflex® E/S™ Portable Sampler, Cole-Parmer) and autoclaved platinum-cured silicone tubing (Masterflex® 96420-24, Saint-Gobain Performance Plastics) from shore at the LV Wash site, from a small craft at the Las Vegas Bay (LV Bay) and CO River (CO River) sites, and from a tap connected to the raw water intake of the Alfred Merrit Smith Drinking Water Treatment Facility. All samples were collected from ~1 m below surface, with the exception of the DW Intake, which drew from ~32 m' depth. Physical and chemical parameters, including temperature, dissolved oxygen concentration, pH, and specific conductance were measured at each site with a calibrated multi-parameter sonde (Hydrolab Corporation Model Surveyor). Water quality samples were also collected from flowing sample lines at each site, stored and transported on ice, and analyzed by Weck Laboratories (Monrovia, CA) or SNWA via USEPA protocols within suggested hold times (e.g. Alkalinity, SM 2320B; Ammonia, SM 4500-NH3 C/D; Cations/Metals, EPA 200.7; Nitrate/Nitrite/Anions, EPA 300.0; Total organic carbon, SM 5310C; Dissolved organic carbon, EPA 415.3; and Total phosphorous, EPA 365.3). A sterile 100 μm prefilter was installed in the sample line during water collection to remove larger debris and zooplankton. Samples for microcosm experiments were collected by pumping 19 L of raw lake water into sterile 19 L glass carboys containing a Teflon-coated magnetic stir bar and capped with a sterile rubber stopper fitted with a 0.2 μm air filter. Microbial biomass for DNA analysis was collected from each of the study sites by filtration of a 100 mL subsample of raw water at time zero from each microcosm onto 0.2 μm filters (25 mm, Supor Polyethersulfone, Pall) and stored on dry ice or at −80 °C.

2.2. Microcosms

Microcosm experiments were conducted directly following sample collection. In the laboratory, carboys were fitted with a sterile hose-barbed tap, tubing, and "t" valve assembly to facilitate subsampling and maintained in the dark (to prevent photolysis) at room temperature

with gentle stirring (~100 rpm). A total of five incubations were maintained: the four samples described above, and a bacteriostatic control: LV Bay water containing 1 g/L sodium azide. Each microcosm was spiked with 27 pharmaceuticals and EDCs to an expected initial concentration of 100–500 ng/L, not including the background concentration of these chemicals already present in the water samples (see Tables S2S6 for actual concentrations). A stock spiking solution was made by dissolving each compound in deionized (DI) water and then combining. The low aqueous solubility of some compounds precluded the entire stock from dissolving and explains some of the lower than desired initial concentrations. Some of the higher than expected concentrations were due to already elevated background concentrations in raw water prior to the addition of the spiking solution. Subsamples were collected periodically from the microcosms using the "t" valve assembly for chemical analyses. At each time point, dissolved oxygen (DO) in each sample was determined with an Oxygen CHEMets® Kit (K-7512, Chemetrics, Calverton, VA) to ensure that incubations remained aerobic. Dissolved oxygen concentrations in all microcosms remained stable, with DO values consistently remaining between 6 and 7 mg/L, with the exception of the bacteriostatic control, which ranged from 8 to 9 mg/L. Time points for the chemical analyses were 0, 1, 2, 4, 7, 14, 29, 56, and 120 days.

2.3. Pharmaceutical and EDC methods and data analysis

Prior to pharmaceutical and EDC (steroid hormones) addition, and at each time point, a 500 mL subsample was collected from the microcosms in an amber glass bottle containing 0.5 g sodium azide for analysis of pharmaceuticals and EDCs. Analyses employed cleanup and sample concentration by solid-phase extraction (SPE) and analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Analytical methods were based on published methods for pharmaceuticals (Vanderford and Snyder, 2006) and steroid hormones (Benotti et al., 2009) with the only differences being a modified analyte list (Table S1). Analysis of background pharmaceutical and EDC concentrations in raw water samples were conducted in triplicate. To preserve sample volume in the microcosms throughout the experiment (samples taken at 0, 1, 2, 4, 7, 14, 29, 56, and 120 days post EDC/pharmaceutical spike), replicate samples for pharmaceutical and EDC analysis were not taken.

Attenuation of pharmaceutical and EDC concentrations in the microcosm studies was presumed to follow first-order kinetics. The concentrations at a given time point were normalized to their starting concentrations (concentration at a given time point was divided by the starting concentration) and first-order degradation rate constants were calculated by regression analysis of the natural log of the data (Microsoft Excel, Data Analysis Toolpack). Compounds that exhibited little or no removal over the 120-day experiment, resulting in either the inability to calculate a half-life or in extrapolation to exceedingly long half-lives, were assigned a value of >365 days.

2.4. Microbial community metabolic fingerprinting

Community metabolic potential was assessed with Biolog EcoPlates (Biolog Inc., Hayward, CA), according to the manufacturer's protocol. Briefly, each 96 well microtiter EcoPlate contained three replicates of 31 different carbon substrates, with the remaining three wells containing sterile water as a control. As the various substrates were metabolized, a tetrazolium dye in each well produced a color change, and absorbance at 595 nm was then measured with a BioTek PowerWave 340 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT). To conduct the assay, 120 μL of raw sample water from each of the four sites was added to each EcoPlate well immediately following sample collection and incubated at room temperature in the dark. Absorbance readings were taken after 7 days of incubation. Averages were calculated from the 3 replicate wells and absorbance values above a threshold of 0.3 were considered positive for usage of the specific carbon source for that well. Average values and standard deviations for each compound class for each sample site were calculated from triplicate absorbance values for each compound within each compound class. Statistical analysis of community metabolic diversity (one-way ANOVA, Tukey's Honest Significant Difference post-hoc test, and pairwise *t*-test with Bonferroni correction) was conducted in R using the base package (R Core Team, 2013) to identify significant differences in the capacity for individual microbial communities to metabolize compounds within each of the six compound classes. Only differences with *P*-values <0.01 for both Tukey's honest significant difference test and the pairwise *t*-test with Bonferroni correction were considered significant.

2.5. Microbial community characterization and molecular analysis

DNA was extracted from archived filters using MoBio Ultraclean® Soil DNA kits (MoBio, Solano Beach, CA) according to manufacturer's protocol. 16S rRNA gene amplicons were generated by polymerase chain reaction (PCR) with a Bacteria-specific forward primer (9bF, 5′-GRGTTTGATCCTGGCTCAG-3′) and a universal reverse primer (1512uR, 5′-ACGGHTACCTTGTTACGACTT-3′) (Eder and Ludwig, 1999) on a PXE 0.2 thermal cycler (Thermo Electron Corp., Milford, MA). PCR reactions contained 1.25 U LA Taq® (Takara Bio Inc., Japan), 200 nM of each primer, 0.4 mM of each dNTP, 1 × LA PCR Buffer, 2.5 mM $MgCl_2$, and 2 μL of DNA template. Thermal cycler conditions included an initial denaturation step (5 min at 95 °C); 35 cycles of denaturation (30 s at 95 °C), annealing (60 s at 50 °C), and extension (90 s at 72 °C); and a final elongation step of 20 min at 72 °C. Successful amplification was confirmed on 1% agarose gels stained with 1 mg/mL ethidium bromide. Three replicate 25 μL PCR reactions were pooled for each sampling site, purified (UltraClean™ GelSpin™ DNA Purification Kit, MoBio Laboratories, Inc.), and cloned using TOPO®-TA kits (Invitrogen). Ninety-six clones from each sample site were bidirectionally sequenced (Functional BioSciences, Madison, WI) and contigs were generated from paired forward/reverse reads and manually quality filtered in Sequencher™ 4.8 (Gene Codes). The remaining 16S rRNA sequence analyses were conducted in QIIME v1.9.1 (Caporaso et al., 2010). Chimeric sequences were identified using the uclust algorithm (Edgar, 2010) against the SILVA_119 reference sequence database (Quast et al., 2013) and removed, which resulted in 274 non-chimeric sequences. Sequences were clustered at an operational taxonomic unit (OTU) cutoff of 97% sequence similarity using uclust. Uclust was then used to assign taxonomy, based on the SILVA_119 database, to all OTUs. Diversity and richness indices (OTU richness, Shannon index, Chao 1 estimated richness, and Simspon index) were calculated in QIIME. Evenness was calculated as H/H_{max}, where H is the Shannon diversity estimate and $H_{max} = \log_2(S)$, with S being the total number of corresponding OTUs. Community similarity was determined from UniFrac calculations (Lozupone et al., 2006) determined from 100 rarefactions of 50 sequences per sample.

2.6. Microbial enumeration using flow cytometry and heterotrophic plate counts

Raw sample water was collected from natural samples into sterile 15 mL conical polypropylene centrifuge tubes and preserved with gluteraldehyde (final concentration of 2.5% *v*/v) for cell enumeration with flow cytometry. Total cell counts were determined using a MicroPRO™ flow cytometer ("Total Biomass" assay, Advanced Analytical Technology Inc.) according to the manufacturer's instructions. Sterile bottles for bacterial heterotrophic plate counts (HPCs) were prepared and analyzed using APHA Method 9215. Cell enumeration and HPC analyses were conducted once per sample.

2.7. Nucleotide accession numbers

Near full-length 16S rRNA gene sequences have been deposited in GenBank with the following accession numbers: MF040306-MF040579.

3. Results and discussion

3.1. Water chemistry

Table 1 lists the water quality parameters measured at each site. The strongest indications of wastewater influence corresponded with the LV Wash site (e.g., conductivity, total dissolved solids (TDS), total organic carbon (TOC), nitrate, ammonia, sulfate, and UV_{254} [a proxy for dissolved organic carbon concentration]), reflective of direct wastewater effluent inputs at this location (Bevans et al., 1996; Boyd and Furlong, 2002; Rosen et al., 2006; Rosen et al., 2010). For example, conductivity, TDS, and TOC values in the LV Wash were all roughly twice those of the next highest site (LV Bay) (2433 vs. 1068 μS/cm, 1541 vs. 649.4 mg/L, and 6.0 vs. 3.2 mg/L, respectively). Boron, a common leachate from desert soil, displayed a similar pattern (0.57 mg/L in LV wash, 0.12 in LV Bay, 0.1 in DW Intake and <0.1 in CO River (Table S7)). Nutrients were even more enriched in the LV Wash in comparison to the next most enriched site (LV Bay), with nitrate showing a roughly 16-fold enrichment (14 mg/L vs. 0.88 mg/L) and phosphate showing a > 24-fold enrichment (0.12 mg/L vs. <0.005 mg/L). Background concentrations of most pharmaceuticals and EDCs measured were generally higher in the LV Wash compared to all other sites (Table S1). The concentrations of atenolol (650 ng/L), atorvastatin (6.35 ng/L), carbamazepine (175 ng/L), DEET (108 ng/L), gemfibrozil (115 ng/L), ibuprofen (6.15 ng/L), meprobamate (570 ng/L), naproxen (83.5 ng/L), primidone (145 ng/L), sulfamethoxazole (995 ng/L), TCEP (515 ng/L), and trimethoprim (58.5 ng/L) were 10- to >100-fold higher in the LV Wash than any other site and were consistent with average concentrations measured in wastewater effluent from 50 large wastewater treatment plants in the USA (Kostitch et al., 2014). Conversely, the concentrations of bisphenol-A (19 ng/L) and caffeine (95.5 ng/L) were highest in LV Bay. Pharmaceutical and EDC concentrations measured in DW Intake and CO River were low (<20 ng/L and <10 ng/L, respectively, for each individual compound).

Table 1
Water quality parameters.

	LV Wash	LV Bay	DW Intake	CO River
Temperature (°C)	23.4	21.6	17.8	20.8
pH	7.71	8.27	7.93	8.38
DO (mg/L)	6.57	7.81	5.87	8.57
DO (% saturation)	81	93	65	101
Conductivity (μS/cm)	2433	1068	1030	908
TOC (mg/L)	6.0	3.2	2.7	2.9
Tot. Alkalinity (mg/L)	129	132	143	134
Nitrate (mg/L N)	14	0.88	0.5	0.2
Nitrite (mg/L N)	<0.05	<0.05	<0.05	<0.05
T-Phosphate (mg/L P)	0.12	<0.005	<0.005	0.0055
Ammonia (mg/L N)	0.205	0.024	<0.02	<0.02
Calcium (mg/L)	130	75	81	69
Chloride (mg/L)	340	100	88	80
Iron (mg/L)	<0.1	<0.1	<0.1	<0.1
Magnesium (mg/L)	63	28	27	24
Potassium (mg/L)	32	6.9	5.9	5
Sodium (mg/L)	280	99	96	81
Tot. Hardness (mg/L $CaCO_3$)	590	300	320	270
Alkalinity, CO_3^{2-}	0	0.592	0	2.39
Alkalinity, HCO_3^-	129	131	143	131
Silica (mg/L)	19	6.7	7.3	6.2
Sulfate (mg/L)	570	270	250	220
TDS (mg/L)	1541	649.4	618.4	553.6
Turbidity (NTU)	2.68	0.51	0.33	2.66
UV_{254} (cm^{-1})	0.1000	0.0404	0.0417	0.0511

DO – Dissolved oxygen; TOC – total organic carbon; TDS – total dissolved solids.

The relative degree of wastewater impact at each site can be inferred from these parameters, and ranged from greatest to least impact as follows: LV Wash > LV Bay > DW Intake > CO River. This order is similar to the order of impacted sites in the basin studied by Leiker et al. (2009), Rosen et al. (2010), and Patiño et al. (2015). Dissolved O_2, at 65% of saturation, was lowest in the metalimnetic drinking water intake (~32 m depth) and highest (101%) in surface water from the relatively pristine Colorado River site, upstream from urban influences. At 81% of saturation, dissolved O_2 in LV Wash water was substantially depleted in spite of being highly aerated, likely reflective of strong biochemical oxygen demand imposed by high bulk organic loadings at this site. Additional water quality data are available in Table S7.

3.2. Bacterial counts

Bacterial abundance was measured at the initiation of the microcosm biotransformation experiments, prior to CEC amendment, by both flow cytometry and heterotrophic plate counts (HPCs). These values are generally consistent with the literature, as HPCs have been shown to be 1–4 orders of magnitude lower than those observed for flow cytometry counts of raw surface and drinking waters (Hoefel et al., 2003; Hammes et al., 2008). This is not unusual as it is generally acknowledged that only a small percentage of environmental bacteria are cultivable using routine methodologies (Amann et al., 1995; Staley and Konopka, 1985). The close agreement observed between the HPC and flow cytometry counts for the LV Wash site could be an indication of the ecological stress/opportunity provided by wastewater discharge. It is highly likely that copiotrophic heterotrophic bacteria are more abundant and occur in higher proportions in the LV Wash due to the higher nutrient concentrations at this location.

3.3. Characterization of the microbial community

To examine the microbial communities responsible for biodegradation of pharmaceuticals and EDCs, libraries of near full-length 16S rRNA gene clones were constructed with DNA from each site and representative clones were sequenced (Appendix 1). As we were most concerned with the identities of dominant microorganisms in these samples, the relatively long sequences provided by the amplified library approach and the correspondingly greater capacity for accurate identification to higher levels of specificity justified this approach versus the large number of short sequences that could be obtained by competing technologies, such as Illumina tag sequencing. Microbial community richness, evenness, and diversity estimates for 16S rRNA gene sequences at 97% sequence identity (species level) are shown in Table 3. Diversity indices corresponded to the degree of wastewater effluent impact, with the LV Wash having the highest diversity, and the DW Intake and CO River samples having the lowest diversity. Specifically, the number of individual species detected in the samples (Table 2, OTUs [operational taxonomic units]) was similar and varied from 34 to 46, but the Chao1 richness estimator indicated that the LV Wash and LV Bay communities were more species-rich than the DW Intake or CO River sites. Since the Chao 1 estimate values are much higher than the total number of OTUs, the total coverage of bacterial richness was not achieved. Rarefaction curves performed at the species level (97% sequence identity, Fig. S2) between all four sites indicate no significant difference between them. Rarefaction curves also indicate that sequencing was not exhaustive. Evenness did not vary appreciably between sites at the species level (0.901–0.942). The Shannon diversity index also indicated that the LV Wash community was most diverse, followed by LV Bay, DW Intake, and CO River communities.

To investigate differences in taxonomic composition among the four sample sites, UniFrac distances, a measure of the phylogenetic similarity of microbial communities, were calculated following Lozupone et al. (2006). From this comparison, an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree (Fig. 2) was constructed and

further demonstrated the distinctiveness of the LV Wash community from all other samples. The CO River and DW Intake samples were most phylogenetically similar, reflective of these two samples having lower treated wastewater effluent impact than the remaining samples. Consequently, LV Bay clustered between these two samples and LV Wash, indicative of some degree of treated wastewater influence, which was also observed in the water quality parameters.

Overall, samples showed significant coverage of the recognized bacterial phyla, even though the 16S rRNA gene libraries were relatively small (56–74 full length clones per sample). *Proteobacteria* and *Bacteroidetes* dominated all communities, with relative abundances ranging from 37.0–63.5% and 11.0–25.7%, respectively. This was not unexpected as studies conducted by Arroyo et al. (2010) and Yang et al. (2011) also found microbial communities in WWTP activated sludge to be predominately composed of *Proteobacteria*. *Cyanobacteria* were uniformly distributed across the dataset, although at a somewhat lower proportion in the DW Intake, as might be expected due to the greater depth of this site. The DW site was the only site in which we detected the phyla *Nitrospirae* (5.5%), candidate division OD1 (1.4%), *Gemmatimonadetes* (1.4%), and *Planctomycetes* (1.4%). All sites, with the exception of the LV Wash, contained *Actinobacteria* (21.4–22.5%) and a variable proportion of *Verrucomicrobia* (1.4–12.3%). This is consistent with previous studies reporting that *Verrucomicrobia* are common in environments with low nutrient availability (Sipura et al., 2005) or in oligotrophic surface waters (Urbach et al., 2001). However, the trophic state of Lake Mead is complex and variable due to different water sources entering the lake. In addition, the lake is generally phosphorus-limited and prone to sporadic algal blooms when phosphorus is available (LaBounty and Burns, 2005; Tietjen et al., 2012).

A comparison of bacterial community profiles suggested that the LV Wash community was the most unique and the DW Intake and CO River communities were the most similar of the four sites (Fig. 2, Appendix 1). Of the 46 OTUs detected in the LV Wash community, only three of those OTUs were detected in any other sample (a *Hydrogenophaga* sp., a *Paenibacillus* sp., and an *Arcobacter* sp., detected in LV Bay and absent from DW Intake and CO River samples). The distinctiveness of the LV Wash microbial community is likely due to different environmental stresses imposed by the LV Wash's unique aqueous chemistry profile and its proximity to WWTPs, whose effluents serve as microbial inoculation sources for the LV Wash and greater Lake Mead system.

The LV Wash was dominated by an uncultured *Flavobacterium* species (13.5%) and an uncultured *Sulfuricurvum* species (5.4%, Appendix 1), both of which were undetected in all other samples. Members of the *Flavobacterium* genus have been found to degrade organic pollutants and to be resistant to a variety of antibiotics, including aminoglycoside antibacterial agents that target Gram-negative bacteria (Sun et al., 2011; Nedashkovskaya et al., 2014). *Sulfuricurvum* species are putative chemolithoautotrophic diazotrophs that derive energy from the oxidation of elemental sulfur, sulfide, sulfite, or hydrogen (Handley et al., 2014), physiologies that, while not immediately applicable to the focus of this study, may be consistent with ecophysiological opportunity afforded when organic-laden wastewater effluent is artificially and

Table 2
Diversity and richness estimates from 16S rRNA gene libraries (97% sequence similarity).

	LV Wash	LV Bay	DW Intake	CO River
	$n = 74$	$n = 56$	$n = 73$	$n = 71$
OTU richness[a]	46	35	37	34
Shannon index (H′)[a]	5.19	4.83	4.79	4.58
Chao 1[a]	88.3	85.0	58.0	57.8
Evenness[b]	0.940	0.942	0.919	0.901
Simpson index[a]	0.961	0.955	0.949	0.938

[a] Diversity and richness measurements were determined in QIIME (Caporaso et al., 2010).
[b] Evenness was calculated as $E = H/H_{max}$, where $H_{max} = \log_2(S)$, and S = the total number of phylotypes (OTUs).

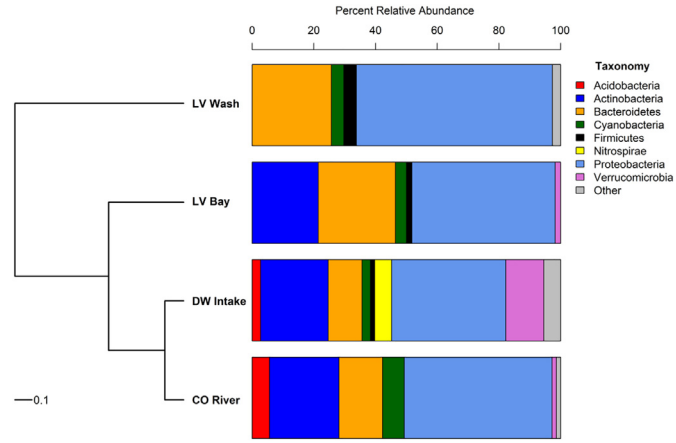

Fig. 2. Comparison of the bacterial community profiles with an UPGMA dendrogram created from abundance-weighted UniFrac distances. Phyla supported by only one clone have been grouped into the 'Other' category.

naturally oxidized by ozonation and released into the environment. The most abundant OTU detected in the LV Bay was an uncultured bacterium in the *Acidimicrobiaceae* family of *Actinobacteria* (10.7%). This OTU was also the most abundant OTU detected in DW Intake and CO River communities (13.7% and 16.9%, respectively). The *Acidimicrobiaceae*, heterotrophic bacteria commonly found in environments impacted by acid mine drainage (Johnson, 2012; Huang et al., 2016), are not commonly reported in waters impacted by treated wastewater effluent, especially in waters whose pH is circumneutral. As such, the role of *Acidimicrobiaceae* in organic contaminant degradation in surface waters remains largely unknown and deserves further investigation.

3.4. Microbial metabolic diversity

To assess the capacity for the four Lake Mead microbial communities to metabolize a range of organic substrates, water samples were tested for activity on 31 substrates with the Biolog EcoPlate system. To simplify and facilitate comparison across sites, the substrates were grouped by their chemical class (Fig. 3, Table S9). Under the conditions tested, metabolic performance of these samples was strikingly different. Most notably, whereas the microbial community in the LV Wash was capable of metabolizing most of the Biolog EcoPlate carbon substrates (29 of 31), the CO River sample was capable of metabolizing 3 of 31 substrates, consistent with differences in microbial community diversity in the wastewater effluent gradient. The amount of substrate utilization per unit time was also significantly higher in the LV Wash site than the DW Intake and CO River sites for all compound classes. This observed higher metabolic diversity and potential in LV Wash could be attributable to a higher density of heterotrophic bacteria and to increased nutrient concentrations (nitrate, ammonia, and phosphate) compared to the remaining three sites. LV Bay, DW Intake, and CO River showed similar utilization patterns for most of the substrates, and there was a consistent trend corresponding with the degree of wastewater effluent impact (LV Bay > DW Intake > CO River, Table S9).

3.5. Transformation of pharmaceuticals and EDCs

Primary degradation for individual compounds in each microcosm varied considerably. First-order rate constants were generally higher in the LV Wash microcosm than in any of the other three (Table S8 and Fig. S1), likely due to higher species diversity and metabolic capacity compared to all other samples. Correspondingly, the majority of the compounds had shorter half-lives at this site than elsewhere (Table 3). The longest half-lives were generally observed in the DW intake and CO River sites, the two sites with the least treated wastewater effluent impact. Many of the more recalcitrant compounds (e.g., DEET, dilantin,

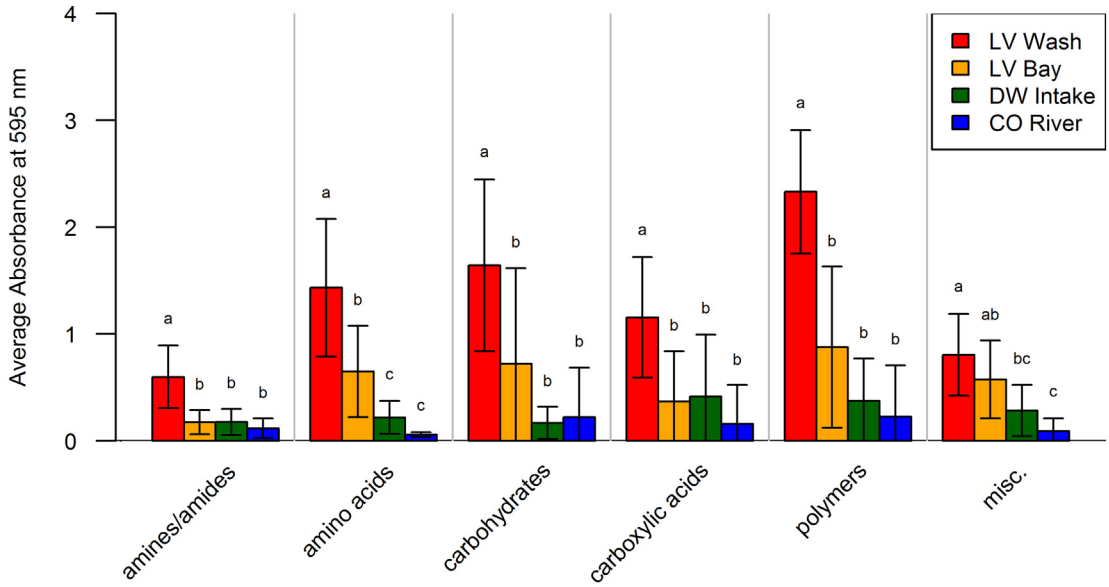

Fig. 3. Capacity of microbial communities to metabolize various classes of organic substrates as determined with Biolog EcoPlates. Bar height represents the mean well color development per compound and whiskers represent the standard deviation. Letters shared between two site means within a compound class indicate no significant differences in mean well color development ($P > 0.01$). *P*-values were obtained by one-way ANOVA followed by Tukey's Honest Significant Difference post-hoc test and pairwise *t*-test with Bonferroni correction. Note: Letters are not comparable between compound classes.

gemfibrozil, meprobamate, and sulfamethoxazole), whose background concentrations were higher in the LV Wash than any other site, had measurable half-lives within the LV Wash microcosm only ($t_{1/2}$ = 53–150 days), but exhibited no measurable removal ($t_{1/2}$ > 365 days) in any of the other microcosms, potentially due to lower microbial diversity, lower metabolic capacity, and a lower capacity to biodegrade these compounds.

Table 3
Half-lives (in days) of pharmaceuticals and EDCs.

	LV Wash	LV Bay	DW Intake	CO River	Bacteriostatic control
Atenolol	**15**[a]	**62**	**250**	**81**	>365[b]
Atorvastatin	**66**	**237**	**90**	**230**	>365
Atrazine	>365	>365	>365	>365	**344**
BHA	**19**	**45**	**27**	**48**	**18**
Bisphenol A	**57**	>365	**73**	>365	>365
Caffeine	**110**	**98**	>365	>365	>365
Carbamazepine	>365	>365	>365	>365	>365
DEET	**57**	>365	>365	>365	>365
Diazepam	>365	>365	**280**	**280**	>365
Diclofenac	**160**	**160**	>365	>365	**223**
Dilantin	**100**	>365	>365	>365	>365
Estradiol	**2.9**	**1.1**	**17**	**1.2**	**83**
Estrone[c]	**27**	**34**	>365	**2.4**	>365
Ethynylestradiol	**150**	>365	**300**	**240**	**315**
Fluoxetine	**310**	**120**	**53**	**120**	>365
Gemfibrozil	**73**	>365	>365	>365	>365
Ibuprofen	**44**	**160**	**43**	**88**	>365
Iopromide	**150**	>365	>365	**260**	>365
Meprobamate	**50**	>365	>365	>365	>365
Naproxen	**69**	>365	**170**	**160**	>365
Primidone	>365	>365	>365	>365	>365
Progesterone	<**0.5**	**5.2**	**1.6**	**20**	**65**
Sulfamethoxazole	**53**	>365	>365	>365	>365
TCEP	>365	>365	**240**	>365	**292**
Testosterone	**0.59**	**0.73**	**1.4**	**0.41**	>365
Triclosan	**30**	**170**	**230**	**150**	>365
Trimethoprim	**54**	>365	>365	**320**	>365

[a] Measurements for which a half-life could be calculated bolded.
[b] For cases which half-lives could not be calculated given the time-scale of the incubations, values are reported as >365 days.
[c] These values include the initial decomposition of spiked estrone and subsequent reappearance from the degradation of estradiol.

The steroid hormones progesterone and testosterone were the most labile within all treatments ($t_{1/2}$ = 0.41–20 days). Conversely, atrazine, carbamazepine, and primidone were the most resistant to degradation, with no measurable removal ($t_{1/2} \geq 365$ days) in any of the treatments. Atrazine (Carboneras et al., 2017), carbamazepine (Clara et al., 2005; Carballa et al., 2006; Benotti and Brownawell, 2009), and primidone (Inyang et al., 2016), all of which contain nitrogenated ring structures, are known to be recalcitrant to biodegradation in WWTPs and bench-scale biodegradation experiments, so this result was not unexpected. Diazepam and TCEP, whose background concentrations were highest in the LV Wash (3.65 ng/L and 515 ng/L, respectively), exhibited no measurable removal in the LV Wash microcosm, but removal was observed in DW Intake (half-lives of 280 and 240 days, respectively) and CO River (diazepam half-life of 280 days, undetectable removal of TCEP). Fernandez-Fontaina et al. (2016) found that biotransformation of diazepam in activated sludge was negligible under nitrification, nitratation, and heterotrophic conditions. Furthermore, Tappin et al. (2014) reported 36% diazepam removal after 21-day microcosm incubations of surface waters receiving treated wastewater effluent, but bacterial degradation was only observed following initial photolysis of diazepam. Liang and Liu (2016) noted that TCEP concentrations increased following biological and physical-chemical treatment during the advanced wastewater treatment process, which suggests that prior exposure to TCEP during wastewater treatment has no effect on the associated microbial community's capacity (e.g.: LV Wash) to degrade this contaminant.

The data for estrone must be regarded carefully, given that it is a biotransformation product of estradiol (Lee and Liu, 2002; Zheng et al., 2007; Combalbert and Hernandez-Raquet, 2010; Blunt et al., 2017). Although data for estrone were treated as undergoing first-order decay, a small amount of estrone production was noted over the course of each of the treatments, and the apparent loss of estrone was actually the difference between the microbial degradation of estrone and its formation due to the microbial oxidation of estradiol. Thus, the half-lives for estrone (in particular that in the DW Intake sample) were likely artifacts of the initial decomposition of spiked estrone and the subsequent reappearance from the degradation of estradiol. Likewise, the slightly slower apparent degradation of estradiol in the LV Wash versus LV Bay microcosms might reflect the transient stoichiometric reduction of estrone to estradiol (Zheng et al., 2012; Prater et al., 2015), an effect which has

been observed for microbial isolates from Las Vegas Wash previously (Blunt et al., 2017).

Although decreases in the concentrations of some compounds were observed in the LV Bay bacteriostatic control microcosm, these losses are likely attributable to abiotic processes such as chemical oxidation, volatilization, or hydrolysis. For example, TCEP is capable of efficiently reducing sulfoxides, sulfonylchlorides, N-oxides, and azides (Faucher and Grand-Maître, 2003), which may have rendered the sodium azide less effective at controlling microbial growth and could have contributed to abiotic degradation of organic contaminants in the bacteriostatic control microcosm. Despite this chemical interaction, most losses in the control were small in comparison to the other microcosms, with half-lives > 365 days for the majority of tested compounds. Only 7 of the 24 compounds tested exhibited any loss within this treatment. Loss due to adsorption was not examined in this study, as it was demonstrated to be insignificant in a previous study of the microbial degradation of pharmaceuticals in water with low particulate loadings (Benotti and Brownawell, 2009).

4. Conclusions

The ecological stress applied by treated wastewater discharge, the structure and characteristics of the microbial community, and the microbial degradation of wastewater-derived contaminants are all coupled. Within Lake Mead, the water quality of discharged, treated wastewater had a clear and measureable effect on the microbial community. Although it is not known if the ecological stress is most associated with one specific water quality parameter (e.g., elevated nutrients, differences in natural organic matter concentration or composition, etc.), this research has demonstrated quantifiable differences between the microbial community associated with the most wastewater effluent-impacted site and other sites along a contamination gradient throughout Lake Mead. In almost all of the metrics used to assess the microbial communities (metabolic diversity, community genetic profiles, and cell counts), the LV Wash site consistently exhibited differences from the other three sites, which is attributable to the effect of wastewater discharge. At the same time, the degradation of pharmaceuticals and EDCs in laboratory studies demonstrated that these compounds were more rapidly removed by the microbial community associated with this site. As wastewater reuse practices increase to offset the effects of population growth and dwindling freshwater resources, the absolute and relative amounts of treated wastewater discharge will increase. Additionally, these results have implications for the environmental fate and transport of wastewater effluent-derived organic contaminants, suggesting that indigenous microbial communities may be altered through community-level adaptation to prolonged wastewater discharge, and thereby altering the microbial transformation of these compounds.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.10.052.

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