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Constructed wetlands for polishing oil and gas produced water releases†

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Produced water (PW) is the largest waste stream associated with oil and gas (O&G) operations and contains petroleum hydrocarbons, heavy metals, salts, naturally occurring radioactive materials and any remaining chemical additives. In some areas in Wyoming, constructed wetlands (CWs) are used to polish PW downstream of National Pollutant Discharge Elimination System (NPDES) PW release points. In recent years, there has been increased interest in finding lower cost options, such as CWs, for PW treatment. The goal of this study was to understand the efficacy of removal and environmental fate of O&G organic chemical additives in CW systems used to treat PW released for agricultural beneficial reuse. To achieve this goal, we analyzed water and sediment samples for organic O&G chemical additives and conducted 16S rRNA gene sequencing for microbial community characterization on three such systems in Wyoming, USA. Three surfactants (polyethylene glycols, polypropylene glycols, and nonylphenol ethoxylates) and one biocide (alkyldimethylammonium chloride) were detected in all three PW discharges and >94% removal of all species from PW was achieved after treatment in two CWs in series. These O&G extraction additives were detected in all sediment samples collected downstream of PW discharges. Chemical and microbial analyses indicated that sorption and biodegradation were the main attenuation mechanisms for these species. Additionally, all three discharges showed a trend of increasingly diverse, but similar, microbial communities with greater distance from NPDES PW discharge points. Results of this study can be used to inform design and management of constructed wetlands for produced water treatment.

Environmental significance

Use of constructed wetlands (CWs) for treatment of oil and gas (O&G) produced water (PW) is expected to increase in the future, due to the relatively low cost associated with this treatment approach. Prior to widespread implementation of this practice, it is important to understand if CWs are effective at PW treatment. In this study we focused on three CW systems downstream of National Pollutant Discharge Elimination System (NPDES) PW discharges in Wyoming, USA and found greater than 94% removal of all detected O&G chemical additives. In contrast, our companion study showed that concentrations of inorganic species were not decreased. Results and recommendations provided in these studies can be used to inform design and management of CWs for PW treatment.

Introduction

Produced water (PW) is one of the largest waste streams associated with oil and gas (O&G), resulting in over three trillion liters of PW generated each year in the United States.[1] Due to conflicts over water scarcity and issues associated with common PW management strategies (*e.g.*, earthquakes caused by high rates of underground PW injection), operators and governments are increasingly interested in finding ways to reuse PW, either in the oilfield or outside.[2–9] One example of PW reuse occurs in the United States where west of the 98th meridian, the federal National Pollutant Discharge Elimination System (NPDES) regulation permits operators to release PW for agricultural beneficial reuse.[2–4,10]

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In most cases, PW must be treated prior to reuse.[11,12] Many approaches for PW treatment have been studied including membrane separation and distillation, forward osmosis, electrocoagulation, advanced oxidation processes, adsorption, and biological treatment.[13–15] Treatment costs range from \$0.25–20 per m^3 depending on treatment type, location, quality of the inlet water and more.[6] Treatment facilities often require skilled staff, high start-up and maintenance costs and external power.[16] These factors can make treatment and reuse financially prohibitive, which may result in temporary or permanent closure of a well.[17]

Constructed wetlands (CWs) have been used for decades to treat domestic, agricultural, and industrial wastewaters. In comparison to other treatment systems, CWs can have relatively low start-up and maintenance costs. Biodegradation, sorption, photo-degradation and plant uptake are the main attenuation mechanisms in these systems and CWs can be designed to enhance one or more removal mechanisms, depending on the contaminant(s) of concern.[18] In some parts of the U.S., natural or constructed wetlands are used to polish PW after treatment with separators and chemical additives.[16,19,20] This practice has been occurring since the 1980s, however, it remains relatively uncommon. PW composition varies throughout the U.S. (*e.g.*, TDS: 100–400 000 mg L^{-1}) and only lower salinity PWs are suitable for treatment *via* CWs.[21,22] Wetlands are currently used for PW polishing in both Wyoming and California, where PW salinity is relatively low (<5000 mg L^{-1}). Even though these CWs have been in operation for decades, minimal analysis has been conducted on the efficacy of PW treatment at these sites.

PW is a complex waste stream containing organic chemicals (both geogenic and anthropogenic), metals, naturally occurring radioactive materials (NORMs), and salts. Previous studies, many of which were conducted in the lab, have shown that CWs are effective at reducing a range of bulk contaminant parameters including chemical oxygen demand (COD), biological oxygen demand (BOD), oil, trace organics, and in some cases, total dissolved solids (TDS).[19,23–25] Additional studies have reported attenuation of metals, including cadmium, copper, nickel and zinc.[23,24] Finally, one study showed decreased acute toxicity in *Daphnia magna* after treatment of PW with reverse osmosis followed by CWs.[23]

This study was conducted at an undisclosed location in Wyoming, USA, where surface flow CW systems are used to polish PW downstream of NPDES PW release points. Previous analysis at one release point showed that volatile organic contaminants (*e.g.*, gasoline range organics (GRO), volatile organic compounds (VOCs)) were removed prior to the first CW. Less volatile species, including diesel range organics (DRO) and organic O&G chemical additives (*e.g.*, surfactants) persisted through this wetland and preliminary results indicated that attenuation of O&G chemical additives increased within the CW. To date, no studies have investigated the fate of organic O&G chemical additives in PW treated *via* CWs. Similarly, no studies have examined the efficacy of CWs for O&G chemical additive removal.

Many O&G chemical additives are biodegradable, can be taken up by plants, and readily sorb to sediment (*e.g.*, nonylphenol ethoxylates) and therefore are ideal candidates for attenuation *via* CWs.[26,27] Some well-maintenance additives are volatile (*e.g.*, toluene) and will be removed from PW *via* volatilization.[3] The environmental fate and impact of other compounds, however, is less certain. For example, biocides are commonly used in the O&G industry to suppress unwanted microbial activity. Biodegradation is a major attenuation mechanism in CWs and thus biocides may negatively impact biodegradation rates in CWs. Additionally, because PW is complex, a chemical analysis alone is insufficient for understanding treatment efficacy and downstream impacts of PW treated in CW systems.[2] Due to the sensitivity of microbial communities to changes in the environment, the characterization of microbial communities may offer additional insights into treatment efficacy, especially in complex water samples such as PW, where the exact chemical composition is unknown.[2,3,28] Microbial community analyses may also provide insight into contaminant removal mechanisms. The composition of PW is unique to other wastewaters that have been managed by CWs. Thus, further investigation into the viability of CWs for PW management is warranted.

The goals of this study are to (1) determine the efficacy of CWs downstream of NPDES releases for O&G chemical additive removal, (2) explore the key mechanisms controlling the fate of organic O&G chemical additives in CWs, and (3) analyze the microbial communities in water and sediment to identify major metabolic processes involved in contaminant fate. To achieve these goals, this study will focus on three surface flow CW systems in Wyoming used to polish PW downstream of three different NPDES PW release points where salt concentrations are relatively low (TDS: 1000–3500 mg L^{-1}). Fate of both organic and inorganic species must be considered to fully understand treatment efficacy and thus a companion study, which focused on radium and TDS components, was also conducted at this site.[4] By determining the environmental fate of organic O&G chemical additives in these systems, this study will provide valuable information for the design and management of additional CWs for PW treatment.

Materials and methods

Site description

This study was conducted at an undisclosed field site in Wyoming, USA, where over 10 NPDES PW discharges are located and has been studied in detail previously.[2–4,9,10] At this site, O&G operations occur in a relatively remote location and there are few other sources of contamination. Analysis focused on three NPDES PW discharges, the wetland(s) used to polish the PW downstream (Fig. 1) and a control site wetland. For the remainder of this study, the NPDES discharges will be referred to as discharge A (DA), discharge B (DB), and discharge C (DC). Treatment of PW prior to release at the NPDES point is relatively similar at all three sites. Multiple wells (10+) are in operation at each site. After extraction from the wells, the oil–gas–PW mixture is combined and sent to the on-site treatment system. Treatment includes a three-phase separator (oil, gas, water) which uses heat, gravity, and emulsion-breaking chemicals.

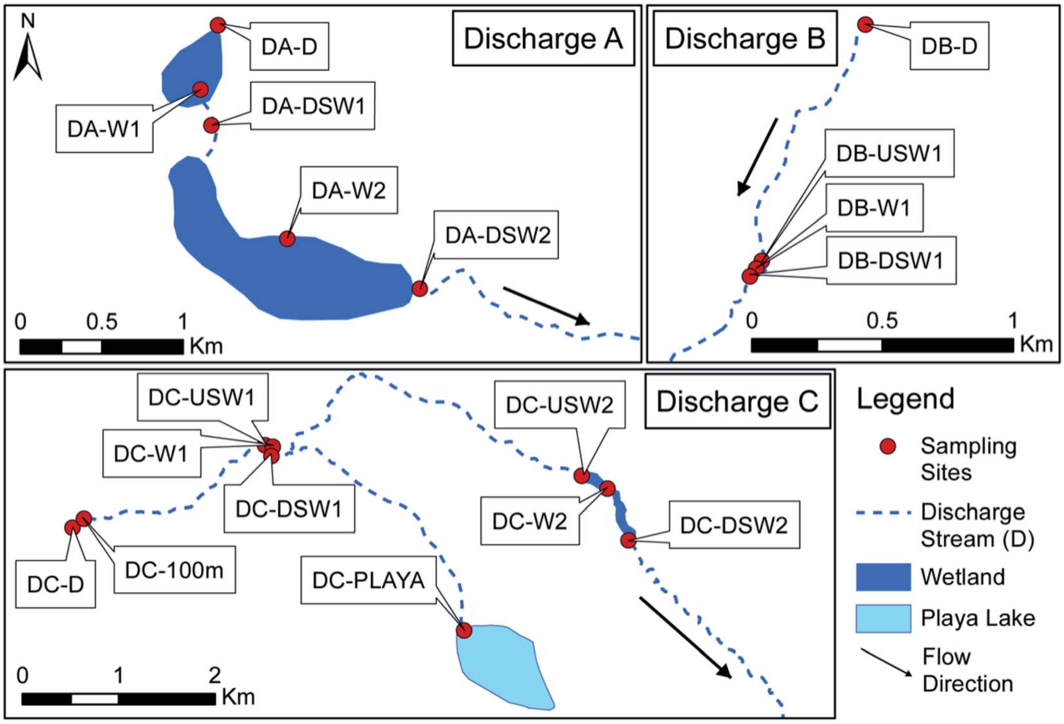

Fig. 1 Map of sampling locations at three undisclosed NPDES PW discharges in Wyoming, discharge A (DA), discharge B (DB) and discharge C (DC). Surface water and sediment grab samples were collected in November 2018. Sites DA-D, DB-D and DC-D were collected directly from the discharge culvert. All other sites were collected upstream (US), downstream (DS) or within the wetlands (W). The first wetland on each discharge is indicated by W1 and the second by W2. When large enough, wetlands are indicated on the map in dark blue. In some instances (DB-W1 and DC-W1) the wetlands are smaller and hidden beneath the sampling site indicators. Site DC-100 m was collected 100 m downstream of DC-D.

Once separated, a portion of the PW is reinjected underground either for enhanced oil recovery or for disposal in cases where TDS exceeds effluent limits. Permits for DB provided detailed information on well maintenance chemicals used onsite including scale inhibitors, corrosion inhibitors, and a water clarifier. The permit stated that hydraulic fracturing occurs every other year at this site. Details regarding well maintenance chemicals and stimulation schedule were not available for the other two discharges.

On average, 1.5 million liters of PW are released each day at DA. At DB and DC, an average of 4.0 and 4.5 million liters are released per day, respectively. At the time of sampling, multiple wells at DB were shut-in due to low oil prices. This resulted in a lower-than-average discharge rate at this site. At all sites, minimally treated PW is polished by wetlands after release. Downstream of DA and DB, the wetlands are natural depressions that are now filled with PW and vegetation. At DC, wetlands were constructed for the purpose of PW treatment. At all three sites, cattle, birds, horses, and other wildlife use the streams and wetlands as a drinking water source. There is little precipitation in the region (average 230 mm per year)[29] and no additional tributaries to the wetlands and streams discussed at these sites. As a result, the wetlands and streams downstream of all three discharges are composed entirely of O&G PW unless there has been a recent precipitation event. Additional site description, along with details on permit effluent limits, are provided in the ESI (Table S1†).

Site sampling

Surface water and sediment grab samples were collected at all three field sites in November 2018. Samples DA-D, DB-D and DC-D were collected directly from the NPDES discharge point (D), immediately before the water entered the streams. All other sampling sites were located immediately upstream, downstream or within a wetland. The naming conventions for these sites indicates their location. For example, DC-USW1 is located upstream (US) of the first wetland (W1). DC-W1 is located within the first wetland (W1) and DC-DSW1 is located downstream (DS) of the first wetland (W1). DC-100 m, which is located 100 m downstream of DC-D, is one exception to this naming and sampling convention. In addition, a control site wetland (CSW) that was unimpacted by PW releases was also sampled. A complete list of site names, site descriptions and distances from the discharge are provided in Table 1. Our companion study on radium and TDS components sampled the same locations and used the same site names.[4]

Water samples were collected in the center of the streams and as close to the center of the wetlands as possible. Water samples for microbial analysis were collected using Sterivex filters (0.22 μm, polyethersulfone, Millipore). Between 40 and 1000 mL of fluid was passed through these filters to collect planktonic biomass for DNA extraction. Sediment samples in the streams were collected near the shore typically in an area on the inner shore of a meander. In the wetlands, sediment

Table 1 Sampling site names, descriptions, distance from discharge point and field parameters

Site name	Distance from discharge (km)	Site description	Temperature (°C)	pH	Conductivity (μS cm^{-1})	Dissolved oxygen (mg L^{-1})
CSW	—	Control site wetland	4.9	8.80	900	1.6
Discharge A (DA)						
DA-D	0.00	NPDES discharge point	35.4	7.07	6400	0.4
DA-W1	0.33	Wetland 1	1.9	8.41	5790	1.7
DA-DSW1	0.53	Downstream of wetland 1	3.6	8.05	6330	2.3
DA-W2	1.41	Wetland 2	2.1	8.30	7830	3.7
DA-DSW2	2.06	Downstream of wetland 2	2.4	8.56	11 400	3.5
Discharge B (DB)						
DB-D	0.00	NPDES discharge point	10.6	7.40	5080	1.2
DB-USW1	0.79	Upstream of wetland 1				
DB-W1	0.82	Wetland 1	1.0	7.62	6420	3.2
DB-DSW1	0.84	Downstream of wetland 1	1.9	7.77	6430	4.5
Discharge C (DC)						
DC-D	0.00	NPDES discharge point	40.4	7.90	2270	0.5
DC-100 m	0.10	100 m downstream of discharge	41.1	8.11	2290	0.6
DC-USW1	1.79	Upstream of wetland 1	29.8	8.40	2090	1.7
DC-W1	1.85	Wetland 1	27.1	8.50	2080	2.0
DC-DSW1	1.90	Downstream of wetland 1	24.4	8.36	2010	1.9
DC-USW2	5.24	Upstream of wetland 2	16.5	7.73	2000	1.6
DC-W2	5.40	Wetland 2	15.7	7.76	1910	1.4
DC-DSW2	6.00	Downstream of wetland 2	2.4	7.81	1990	3.2

samples were collected as close to the water sample as possible, also in an area of sediment accumulation. Except for water samples collected for NPOC analysis, samples for organic analysis were collected in glass bottles with Teflon-lined caps. Water samples for NPOC analysis were collected in plastic bottles and acidified in the field. Prior to collection, all glassware was cleaned with Milli-Q water and methanol and baked in a muffle furnace for 6 hours at 450 °C. Sediment samples for microbial analysis were collected in sterile plastic bags. Field and lab blanks were also collected and processed alongside each analysis. At each site, a Hanna HI98194 probe was used to measure temperature, pH, conductivity, and dissolved oxygen of the water.

Volatile organic compounds, carbon and nitrogen analyses

Water samples were analyzed for non-purgeable organic carbon (NPOC), total nitrogen (TN), and volatile organic compounds (VOCs). NPOC and TN of water samples was analyzed using a Shimadzu TOC-L equipped with a platinum catalyst. For VOC analysis, water samples were prepared following EPA Method 5021A using a Tekmar 7000 Headspace Autosampler and analyzed for volatile organics following EPA Method 8015 using an Agilent 6890N Network Gas Chromatography (GC) system with a Flame Ionization Detector (FID). Additional details on water sample collection and analysis can be found in the ESI.†

Sediment samples were analyzed for total carbon, total nitrogen, and inorganic carbon. Total carbon and total nitrogen of sediments were analyzed using a LECO TruSpec CN. Inorganic carbon (*i.e.*, carbonate) content of sediments was analyzed using a calcimeter, pressure transducer and voltage meter following methods in Sherrod, 2002.[30] Additional details on sediment sample collection and analysis can be found in the ESI.†

Non-volatile organic compounds analyses

Water and sediment samples were analyzed for non-volatile organic compounds (NVOCs). Water samples were collected without headspace, stored on ice in the field and stored at 4 °C in the lab until analysis. Sediment samples were stored on ice in the field and at −20 °C in the lab. Water sample extracts were prepared following methods in McLaughlin *et al.*, 2020a. Sediment extracts were prepared following methods described in Lara-Martín *et al.*, 2011.[31] Details for both methods are provided in the ESI.†

Water and sediment methanol extracts were analyzed for NVOCs using a Quadrupole Time-of-Flight mass spectrometer (Q-ToF-MS). Details of this method are provided in the ESI.† An exact concentration of each surfactant series could not be determined due to a lack of commercial standards with known ethoxymer distribution. Instead, an estimated concentration was determined at the discharge using polyethylene glycol 400, polypropylene glycol (Alfa Aesar, Haverhill, MA), and 4-nonylphenol-polyethylene glycol (Sigma Aldrich, Saint Louis, MO) standards. For alkyldimethylbenzylammonium chloride (ADBAC), three different alkyl lengths (C10, C12, C14) were detected and a dodecyldimethyl-*n*-benzylammonium chloride (Alfa Aesar, Haverhill, MA) standard was used to estimate concentration. Relative concentrations (C/C_0) were determined for samples downstream since all samples were stored in the same manner and extracted and analyzed at the same time. Relative concentration was calculated by dividing the estimated concentration at each sampling location by the estimated concentration at the effluent.

Microbial analysis (16S rRNA gene sequence)

Sterivex filters and sediments for microbial analysis were stored in sterile plastic bags on dry ice in the field and at −70 °C in the lab until analysis. Total nucleic acids were extracted from 0.4 g of sediment using the DNeasy PowerSoil Kit (Qiagen) and eluted with 10 μL of elution buffer, then stored at −20 °C. Extracted DNA purity and quantity were measured on a Qubit Fluorometer (Thermofisher Scientific). DNA was sequenced at the Colorado State University next-generation sequencing facility. Bacterial 16S rRNA gene libraries were prepared according to the two-step PCR workflow in the Illumina 16S Metagenomic Sequencing Library Preparation Protocol (Part 15044223 Revision B). HiFi HotStart ReadyMix (Roche Ltd.) was used to amplify libraries. Individual libraries were pooled at approximately equimolar ratios and library QC included visualization on with Tapestation HS D1000 reagents (Agilent, Inc.) and qPCR using Library Quantification Master Mix and Standards (Roche Ltd.). The pooled libraries were sequenced at 10 pM on a MiSeq instrument (Illumina Inc.) using the 500 cycle (2 × 250 base pair) V2 Reagent Kit with 15% PhiX spike-in to increase base-call heterogeneity during the run. All raw reads for 16S rRNA gene sequencing were submitted to NCBI under BioProject PRJNA722032.

Data processing was conducted with QIIME2 following the protocol of Borton *et al.* 2017.[32] Samples with less than 5000 reads were discarded due to low data quality. Statistical analysis was performed primarily using the R statistical package "vegan" (v2.5.7). Alpha diversity was calculated with the diversity function to investigate species richness, Shannon's diversity and Simpson evenness. Beta diversity was calculated by analyzing Bray–Curtis dissimilarities of the resulting 16S rRNA amplicon features by first converting feature counts to relative abundances, and then plotting these values with nonmetric multidimensional scaling (NMDS) ordinations in R. Both a multi response permutation procedure and mean dissimilarity matrix (MRPP) function and an analysis of similarities (ANOSIM) function were calculated to determine the significance of differences between sample groups. Finally, linear discriminant analysis effect size (LEfSe) was performed on water and sediment samples to determine significantly discriminate microbial features between these two groups.[33]

Results & discussion

Field parameters

Field parameters for the sampling sites are shown in Table 1. For all three discharges, temperature was highest at the discharge point and decreased with distance downstream. At discharge A and discharge B, pH was lowest at the discharge (DA-D: 7.07; DB-D: 7.40), trended upward in the wetlands and with distance downstream. At discharge C, pH was 7.90 at the discharge, increased slightly through the first wetland and then decreased through the second wetland. Previous studies have observed increases in pH downstream of both O&G and coalbed methane (CBM) PW discharges in Wyoming and attributed the increases to evaporation and carbonate precipitation.[10,34] All sampling sites were within the range of the pH permitted at the effluent (pH 6.5–9).

At both discharge A and discharge B, conductivity was lowest at the discharge (DA-D: 6400 μS cm; DB-D: 5080 μS cm^{-1}) and increased with distance downstream. Previous studies conducted in the area showed that increases in conductivity downstream of O&G PW discharges were due to evaporation and oxidation of sulfide to sulfate (SO_4).[10] In discharge A, conductivity was above the permit effluent limits (7500 μS cm^{-1}) at site DA-W2 (7830 μS cm^{-1}) and DA-DSW2 (11 400 μS cm^{-1}). Conductivity at discharge C was lower than observed at discharge A and discharge B and remained relatively steady at all sampling sites, ranging between 1910 and 2290 μS cm^{-1}. At all three discharges, dissolved oxygen was lowest at the discharge and generally increased with distance downstream. In the control site wetland (CSW), temperature was 4.9 °C, pH was slightly higher than in the impacted wetlands (8.80), and conductivity was substantially lower than in the impacted wetlands (900 μS cm^{-1}). Dissolved oxygen in the CSW was 1.6 mg L^{-1}, which is comparable to dissolved oxygen in the two wetlands at discharge C (DC-W1, DC-W2).

Chemical composition of water samples

The chemical composition at all three discharges reflected that of PW previously reported in the area, with relatively low salt concentrations as compared to PWs in other regions of the U.S. (TDS: 1000–4000 mg L^{-1}).[2–4,10] The PW discharge samples also contained O&G chemical additives, including some that were reported as well-maintenance chemicals in the discharge B NPDES permit (Table 2). At discharge B and discharge C, non-purgeable organic carbon (NPOC) was highest at the discharge and generally decreased with distance downstream. At discharge A, NPOC was lowest at the discharge and increased downstream, more than doubling by the last sample (DA-DSW2, ∼2 km downstream) (Fig. S1†). At all three discharges, total nitrogen in water samples generally decreased with distance (Fig. S1†).

VOC analysis

Gas chromatography analysis revealed that volatile organic compounds (VOCs) were present at all three discharges (Table 2). VOCs detected at the discharges included benzene, toluene, ethylbenzene, xylenes (BTEX) and acetone. Except for acetone, these compounds are naturally present in PW.[35,36] All five chemicals are commonly used O&G chemical additives and three of these VOCs (toluene, ethylbenzene, and xylene) were reported as well-maintenance chemicals in the discharge B NPDES permit. In addition, acetone is a known by-product of polypropylene glycol (PPG) biodegradation, another O&G chemical additive detected in all three discharges.[37]

VOC concentrations were highest at DB-D, with benzene and toluene being the most prominent VOCs in the sample (Table 2). Total BTEX released at this site was 2640 μg L^{-1}.

Table 2 Concentrations of BTEX and four chemical additives polyethylene glycols (PEGs); polypropylene glycols (PPGs); nonylphenol ethoxylates (NPEOs); and alkyl dimethyl benzyl alkonium chloride (ADBAC) at the three NPDES PW discharges

	Discharge				Chemical type		
	DA-D	DB-D	DC-D	Surface water acute tox.[a]	DB-D well-maintenance[b]	O&G chemical additive[c]	Geogenic component of PW[c]
Benzene (μg L^{-1})	308	848	22	2300	N	Y	Y
Toluene (μg L^{-1})	252	1067	10	120	Y	Y	Y
Ethylbenzene (μg L^{-1})	88	210	10	130	Y	Y	Y
Xylenes (μg L^{-1})	232	513	27	230	Y	Y	Y
Acetone (μg L^{-1})	96	560	99	28 000	N	Y	N
PEGs (μg L^{-1})[d]	4.4	7.0	2.5	[e]	N[f]	Y	N
PPGs (μg L^{-1})[d]	2.1	4.2	6.4	[e]	N[f]	Y	N
NPEOs (μg L^{-1})[d]	2.7	1.7	3.8	[e]	N[f]	Y	N
ADBAC (μg L^{-1})[d]	347	62	0.1	[e]	Y	Y	N

[a] Data from NOAA screening quick reference tables unless noted. If multiple values available, lowest value was selected.[41] [b] Well-maintenance chemical listed in the discharge B NPDES permit. Chemicals may also be used for other purposes on-site, such as hydraulic fracturing. Chemical additives were not reported in the NPDES permits for discharge A and discharge C. [c] Elliot, 2016.[42] [d] Estimated concentrations. See Methods section. [e] Acute toxicity values not available. [f] Monomer is listed in discharge NPDES permit.

Concentrations of toluene, ethylbenzene and xylenes at this site exceeded the surface water acute toxicity values for aquatic species (Table 2)[38] and this discharge reported failed acute toxicity (*Daphnia magna* and *Pimephales promelas*) tests in 2019. Concentrations of BTEX were lower at DA-D (BTEX: 880 μg L^{-1}) and were lowest at DC-D by another order of magnitude (BTEX: 70.0 μg L^{-1}). Toluene was detected downstream of the discharge point at discharge A (DA-DSW1, DA-DSW2) and discharge B (DB-W1, DB-DSW1) but was below the limit of quantification (LOQ). All other BTEX chemicals were below the detection limit in downstream samples. Previous studies have shown that BTEX removal in both wetlands and streams is dominated by volatilization, as evidenced by the Henry's constants ($5.2–6.6 \times 10^{-3}$ atm-m^3 mol^{-1}).[3,39] Additionally, at all three discharges, produced water is released from a pipe and falls 1–2 m before entering the stream. Thus, these species are expected to partition quickly from water to the air and are generally removed prior to reaching the wetlands. All BTEX chemicals were below detection limit in the CSW.

Acetone concentrations were highest at DB-D (560 μg L^{-1}) and relatively similar at DA-D and DC-D (96–99 μg L^{-1}). Acetone was below detection limit downstream of DA-D and DB-B. Downstream of DC-D, where acetone was the most prominent VOC, this species was detected at DC-UPW1 (60 μg L^{-1}) and DC-DSW1 (55 μg L^{-1}). PPG concentrations are highest at DC-D and degradation of PPG was observed in the first wetland (see section on NVOCs analysis) and thus PPG biodegradation may contribute to acetone concentrations at these sites.[37] Acetone also has natural sources including animals, plants, and trees.[3,40] Concentrations of acetone were below detection limit in the CSW.

NVOCs analysis

Water samples were analyzed for non-volatile organic compounds (NVOCs) using liquid chromatography. Polyethylene glycols (PEGs), polypropylene glycols (PPGs), nonylphenol ethoxylates (NPEOs), and alkyldimethylbenzylammonium chlorides (ADBACs) were present in all three discharges (Table 2 and Fig. 2). PEGs, PPGs and NPEOs are non-ionic surfactants commonly used by the O&G industry as emulsifiers, wetting agents and corrosion inhibitors.[37] Ethylene glycol and propylene glycol, the monomer of PEGs and PPGs, respectively, are both reported in the DB-D NPDES permit, although the polymers are not (Table 2). ADBACs, a cationic surfactant and quaternary ammonium compound mixture commonly used as a biocide in the O&G industry, are also reported in the discharge B NPDES permit.[43]

ADBACs were the most prominent chemical additive detected at both DA-D (347 μg L^{-1}) and DB-D (62 μg L^{-1}), however, concentrations were much lower in DC-D (0.1 μg L^{-1}). At both discharge A and discharge B, ADBAC concentrations exceeded the LC50 values to fish and aquatic invertebrates (280 μg L^{-1} and 5.9 μg L^{-1}, respectively), and thus ADBAC may also have contributed to the failed acute toxicity tests reported at discharge A.[44] Concentrations of PEGs, PPGs, and NPEOs were relatively steady between the discharges, ranging between 1.7 and 7.0 μg L^{-1}. These concentrations are lower than concentrations reported in PW collected from a wellhead in Colorado (PEGs and PPGs ~1000 μg L^{-1}), which was expected since the Colorado wells were recently hydraulically fractured and PW at our site is partially treated prior to release at the NPDES point.[45] To our knowledge, ADBACs have not previously been quantified in PW.

PEGs, PPGs, NPEOs and ADBACs were detected downstream of both DA-D and DC-D. At discharge B, all NVOCs were below LOQ at sampling sites downstream of the discharge. This is likely due to the lower-than-average discharge rate at discharge B, resulting in generally stagnant water and increased hydraulic retention times in both the stream and wetland. Downstream of DA-D and DC-D, concentrations of ADBACs, PEGs, PPGs and NPEOs decreased with distance from the discharge (Fig. 2). In the second sample downstream of DA-D (DA-DSW1), ADBACs were below LOQ. Downstream of the second and final wetland

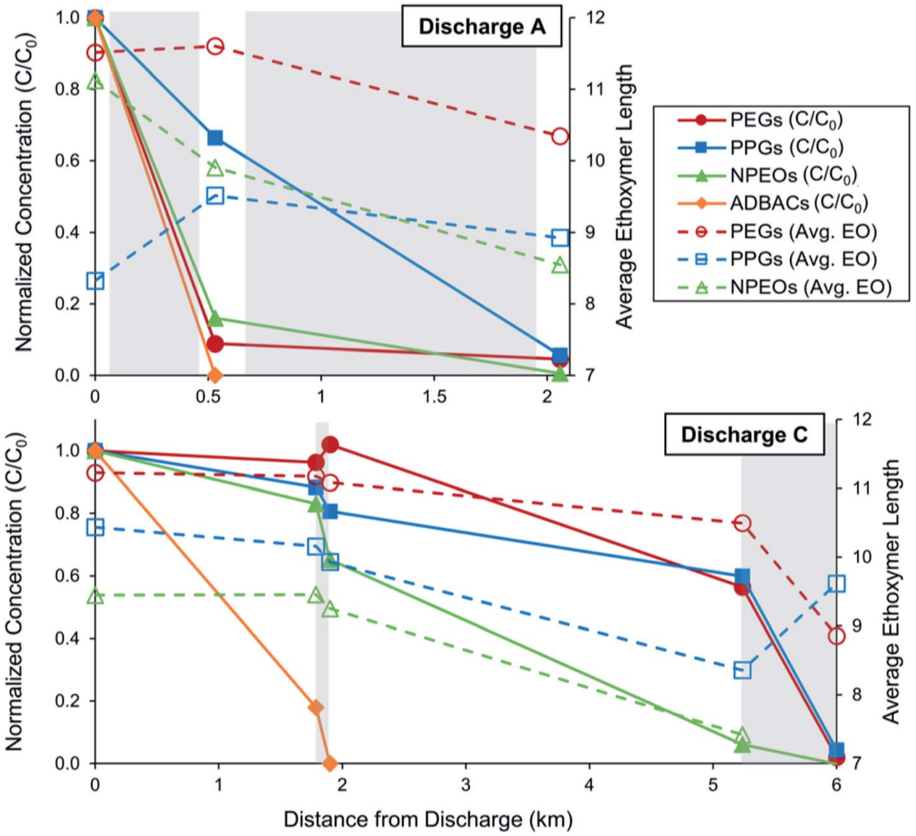

Fig. 2 Relative concentration of polyethylene glycols (PEGs), polypropylene glycols (PPGs), nonylphenol ethoxylates (NPEOs), and alkyl dimethyl benzyl ammonium chlorides (ADBACs) and average ethoxymer (EO) length for PEGs, PPGs, and NPEOs *versus* distance from the NPDES discharge (km) at discharge A (top) and discharge C (bottom). Grey boxes indicate locations of wetlands. Initial concentrations of these species are shown in Table 2.

(DA-DSW2), a 95% reduction in PEGs, 94% reduction in PPGs, and a 99% reduction in NPEOs was observed, as compared to the discharge. This corresponds to estimated concentrations of 0.20 $\mu g\ L^{-1}$ (PEGs), 0.12 $\mu g\ L^{-1}$ (PPGs), and 0.02 $\mu g\ L^{-1}$ (NPEOs). At discharge C, ADBACs were below detection downstream of the first wetland (Fig. 2). Both PEGs and NPEOs were below detection downstream of the second wetland (DC-DSW2), while a 96% reduction in PPGs was observed at this site (0.27 $\mu g\ L^{-1}$), as compared to the discharge. Concentrations of all surfactant classes were below detection limit in water samples collected from the CSW.

ADBACs were the first chemical species removed from the water column downstream of DA-D and DC-D. Aqueous biodegradation of ADBACs have been reported in some instances; however, due to their high soil sorption coefficient ($\log K_{\mathrm{oc}} = 5.5$–7), previous studies have shown that sorption is the dominant removal mechanism for these species.[43,44,46] ADBACs were detected in sediments downstream of all three discharges, as discussed further in the sediments section, providing further evidence to show that this species was removed *via* sorption. Previous studies have shown that biodegradation and sorption are the two main attenuation mechanisms for PEGs, PPGs and NPEOs.[26,37,46–48] These species are known to biodegrade *via* sequential ethoxylate chain shortening, which leads to changes (increase or decrease) in homolog distribution depending on microbial preferences in ethoxymer chain length.[37,47] NPEOs also potentially degrade *via* central cleavage.[49] Decreases in average ethoxymer length can also be an indication of sorption since larger ethoxymers are more hydrophobic and therefore more likely to sorb to sediments.[46,48] Changes in average ethoxymer length were observed for all three non-ionic surfactant species with distance downstream at both discharge A and discharge C (dashed lines, Fig. 2) indicating that changes in concentration were due to transformation and not dilution. Downstream of DC-D, the average ethoxymer length of the surfactants generally decreased with distance, providing evidence for both biodegradation and sorption. In DA-W2, decreases in PEGs, PPGs and NPEOs concentrations were similarly accompanied by decreases in ethoxymer length. The increase in PPGs average ethoxymer length within DA-W1 and DC-W2 may be due to preferential biodegradation of shorter ethoxymer lengths, which has been observed in some studies for PEGs.[47] A combination of removal processes (*e.g.*, biodegradation and sorption) with preferences for different EO lengths could be the reason that the average PEGs EO chain length remains relatively steady in DA-W1, while the relative concentration decreases by nearly 90%.

While concentrations of surfactants generally decreased with distance downstream, a slight increase in PEGs was observed in the first wetland downstream of discharge C. Initially, the concentration of PEGs decreased with distance and 96% remained upstream of the first wetland. Within the first wetland, however, relative concentration of PEGs increased to 102%. This is mostly likely due to variability in the discharge composition; however, the increase may also be due to other mechanisms such as NPEO biodegradation. Previous studies have provided evidence for a central cleavage mechanism for NPEO degradation, which would generate PEGs and nonylphenol.[49]

In addition to biodegradation and sorption, evaporation and photodegradation have the potential to impact the fate of the O&G chemical additives in these systems. Our previous study had documented substantial evaporation at this site, causing increases in concentrations of inorganic solutes along the flow path.[10] Likewise, this process would lead to an increase in concentration of non-volatile organic chemicals, underestimating the removal rates calculated below. Photodegradation may also be occurring at the site, however, studies show that photodegradation of PEGs, NPEOs, and ADBACs is minimal in the absence of a catalyst.[44,50,51]

Removal of PEGs, PPGs and NPEOs downstream of discharge C

Downstream of DC-D, water samples were collected immediately upstream and downstream of both wetlands, allowing for a comparison between the stream and wetlands sections. In contrast to discharge A and discharge B, where the wetlands are ephemeral wetlands that have been filled in with PW, the wetlands downstream of DC-D were constructed for the purpose of treating PW. Thus, this site is more similar to PW CWs that may be built downstream of other NPDES PW discharges.

The amount of mass removed in each of the two stream and wetlands "segments" was calculated for ADBACs, PEGs, PPGs, and NPEOs. These calculations were conducted using the average flow rate of the discharge stream (0.036 cm; Fig. S8†) and assumptions for average depth and width of the stream segments, based on what was observed at the site (Tables S3 and S4†). These results are presented in Table 3 and show the estimated mass per unit width of stream, the removal of mass in each segment and the percent removal in each segment. For both ADBACs and NPEOs, the majority of removal occurred in one of the stream segments. The vast majority (82%) of ADBACs were removed in the first stream segment while the majority of NPEOs (59%) were removed in the second stream segment. In contrast, the majority of PEGs and PPGs removal did not occur in a stream segment but instead within the second wetland segment (56% and 58% of removal, respectively). For all species, when change in mass is normalized over distance, the greatest change in mass occurs in one of the wetlands segments. For ADBACs and NPEOs, this is observed in the first wetland (W1), while for PEGs and PPGs, this is observed in the second wetland (W2). These results show that both wetlands and streams are effective at removing these well-maintenance NVOCs from PW. The wetlands, however, are more effective than streams when normalized by distance.

In addition to these calculations, pseudo-first order rates of removal were calculated for PEGs, PPGs and NPEOs. While removal processes in these systems may follow different kinetic orders and the exact and possibly mixed order is unknown, previous studies have shown that most removal processes that occur in CWs can be estimated with first order kinetics.[52] Results of the kinetic analysis are shown in Fig. 3. This calculation was not conducted for ABDACs since only two data points were available. It should also be noted that interpretation of these results is limited due to the few data points and the flow rate assumptions. PPGs were the only species detected downstream of the second wetland and removal of this species generally follows the pseudo-first order rate of removal throughout the stream and wetland system. When combined with the results in Table 3, this indicates that the increased removal of PPGs within the second wetland is most likely due to the increased retention time in the wetland and not because the rates of major removal mechanisms (*i.e.*, biodegradation, sorption) are enhanced in the wetlands *versus* the stream segments. Studies have shown that an increase in hydraulic retention time (HRT) results in increased removal efficiency in CWs.[53] Larger HRTs generally require larger volume systems, however, and thus enhancing removal rates within CWs is desirable to reduce the space required for CWs.[18,53]

Observations that can be made about PEGs and NPEOs are limited due to the fact that these species are below detection limit downstream of the second wetland. Linear trendlines were added to Fig. 3 to indicate when removal is faster or slower than average. It is clear from these trendlines that the rate of removal for PPGs and NPEOs was slower within the first wetland as compared to the average removal rate of these species throughout the system (dotted black line). The removal rate of PEGs and NPEOs was also slower within the first stream segment, as compared to average. These observations indicate that there may be an initial lag period in removal mechanisms (*e.g.*, biodegradation and sorption) for these two species. Many factors could be contributing to this lag phase. In the case of PEGs, previous studies have shown that biodegradation is faster under aerobic conditions and that biocides, when present at high enough concentration, inhibit PEG degradation.[37,47] Dissolved oxygen increased and ADBAC concentrations decreased with distance downstream and thus could result in increased PEG biodegradation with distance. In the case of NPEOs, changes in sediment composition may result in increased sorption downstream. A study on NPEO absorption partition coefficients (K_d) found that the average K_d value for a NPEO mixture increases with increasing organic carbon (OC) content in sediment.[48] The OC content at DS-USW2, which is the last sampling point shown for NPEOs in Fig. 3, is approximately twice that of upstream samples (3.6% *vs.* 0.37–1.96%) (Fig. S9†). If OC values increase throughout this segment, it would result in increased sorption. Finally, a slight increase in PEG concentration was observed within the first wetland, as noted in the previous section.

Table 3 Estimated removal of ADBAC, PEGs, PPGs and NPEOs mass in each stream and wetland segment downstream of DC-D. Values in bold indicate segments in which the greatest % removal or greatest removal/distance occurred for each species

Segment	Distance (km)	Hydraulic retention time (days)	Estimated mass (μg) per unit width of stream	Removal of mass in each zone (Δ μg)	% removal (of mass present at discharge)	Removal/distance (Δ μg km^{-1})
ADBAC						
Discharge[a]	0.0		0.03			
Stream 1	1.8	0.19	0.01	0.02	**82%**	0.013
Wetland 1	1.9	0.44	0.00	0.01	18%	**0.047**
Stream 2	5.2	0.36	0.00	0.00	0%	0.000
Wetland 2	6.0	4.1	0.00	0.00	0%	0.000
PEGs						
Discharge[a]	0.0		0.84			
Stream 1	1.8	0.19	0.81	0.03	4%	0.018
Wetland 1	1.9	0.44	0.85	−0.05[b]	−6%[b]	−0.427[b]
Stream 2	5.2	0.36	0.47	0.38	46%	0.114
Wetland 2	6.0	4.1	0.00	0.47	**56%**	**0.619**
PPGs						
Discharge[a]	0.0		2.13			
Stream 1	1.8	0.19	1.88	0.25	12%	0.139
Wetland 1	1.9	0.44	1.71	0.16	8%	1.448
Stream 2	5.2	0.36	1.27	0.44	22%	0.132
Wetland 2	6.0	4.1	0.09	1.18	**58%**	**1.549**
NPEOs						
Discharge[a]	0.0		1.25			
Stream 1	1.8	0.19	1.04	0.21	17%	0.119
Wetland 1	1.9	0.44	0.82	0.22	18%	**1.984**
Stream 2	5.2	0.36	0.08	0.74	**59%**	0.222
Wetland 2	6.0	4.1	0.00	0.08	6%	0.100

[a] Discharge is not a segment. Provided to show mass present at NPDES discharge point. [b] Negative values indicate an increase in PEGs concentration which is either due to central cleavage of NPEOs or variability in the discharge composition.

Chemical composition of sediments

Sediment sample extracts were analyzed for non-volatile organic compounds (NVOCs) using liquid chromatography. PEGs, PPGs, NPEOs and ADBACs were detected at all sites downstream of the three discharges (Table 4). PEGs and PPGs were also detected in the control site wetland (CSW: PEGs: 6.9 μg kg^{-1}; PPGs: 2.0 μg kg^{-1}) at concentrations lower than in the PW impacted samples. NPEOs and ADBACs were below detection limit in the control site wetland (CSW).

In samples collected downstream of the NPDES PW discharges, concentrations of PEGs ranged between 14.0 and 202 μg kg^{-1}. Concentrations of NPEOs spanned a similar range (11.0 and 194 μg kg^{-1}) while the range in PPGs was greater, spanning four orders of magnitude (3.4–1150 μg kg^{-1}). The range in ADBACs sediment concentration was greatest, spanning seven orders of magnitude (0.7–455 000 μg kg^{-1}). Previous studies have shown that sediments are sinks for surfactants and that concentrations of cationic surfactants in sediments are generally greater than concentrations of non-ionic surfactants as a result of the greater sorption coefficients for cationic species.[26,46]

In sediments collected from all three discharges, ADBAC concentrations were highest near the discharge, corresponding with the sharp decrease in aqueous ADBAC concentrations at these sites. ADBACs are commonly used in the United States as pesticides and disinfectants and have been widely observed in sediments downstream of wastewater treatment plants (WWTP), as well as other areas.[54] Sorption of ADBAC to sediment occurs because the positive charge on this species sorbs strongly to negatively charged sediments (*i.e.*, clay minerals). Sorption is further enhanced because of the large hydrophobic moieties in ADBAC and their affinity to soil organic matter.[43,44,55] Three different alkyl lengths of ADBAC were detected in water samples including decyl, dodecyl and tetradecyl. At DA-D and DC-D, sediments were enriched in dodecyl- and tetradecyl-ADBACs, as compared to the water samples collected from the discharge, indicating that preferential sorption of the longer ADBACs occurred due to increasing hydrophobic interactions with increasing chain length.[55]

The elevated concentration of ADBACs is most striking at site DA-W1 where the sediment concentration was 455 mg kg^{-1}. Similar concentrations have been observed in sediments downstream of WWTPs.[46,56] The environmental impact of such high ADBAC concentrations in sediments is unknown. Microbial analysis, which is discussed further in the next section, revealed that the alpha diversity metrics at this site (*i.e.*, richness, Shannon's H) were insignificantly lower than in most other samples. No other notable differences between this sample and others were observed in the microbial community analysis. Previous studies have shown that ADBACs can retain

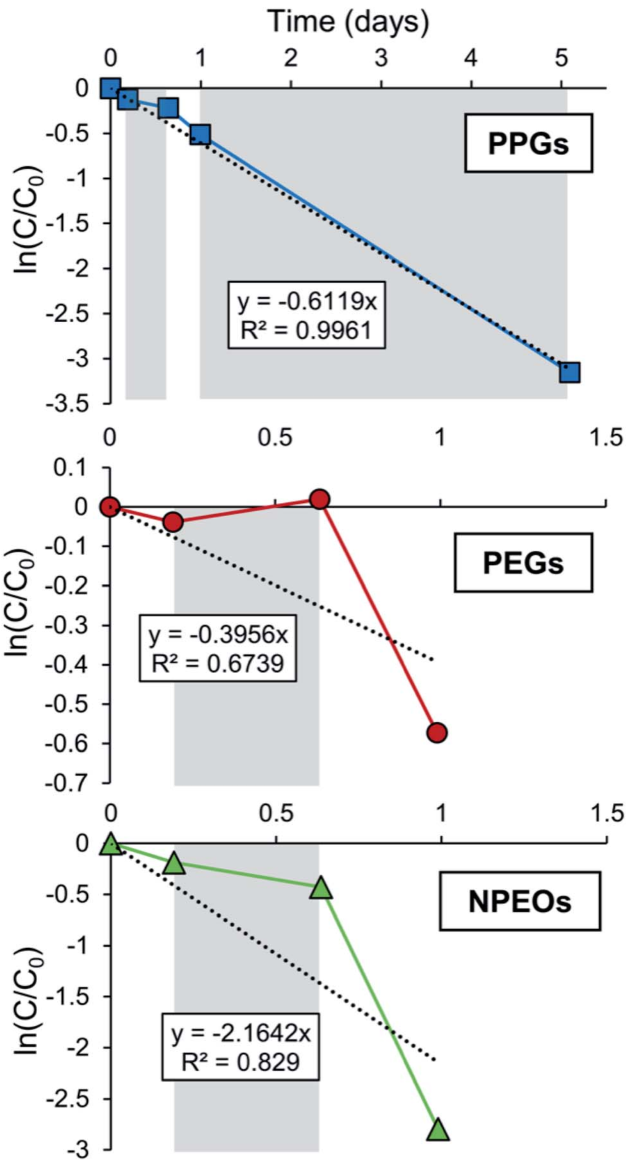

Fig. 3 Pseudo-first order removal rates of PPGs, PEGs and NPEOs downstream of discharge C. PEGs and NPEOs were below detection limit in sample DC-DSW2 and thus only four data points are presented. Grey boxes indicate locations of wetlands. The dotted black line is a trendline, indicating average rate of removal.

their biocidal properties on surfaces and can also sorb beyond their cation exchange capacity, leading to clay aggregation and decreased sorption capacity.[43,55,57] If occurring within the wetland, these outcomes have the potential to negatively impact major attenuation mechanisms (*i.e.*, biodegradation and sorption). Concentrations of PEGs, PPGs and NPEOs were also elevated at this site (DA-W1) relative to other sediment samples collected from this discharge. Biodegradation is a dominant removal mechanism for all four NVOCs in sediment and thus removal rates would be influenced by a decrease in microbial activity.[58] In samples collected downstream of site DA-W1, ADBAC concentrations decreased substantially and concentrations of all non-ionic surfactants trended downward as well.

Table 4 Concentration of polyethylene glycols (PEGs), polypropylene glycols (PPGs), nonylphenol ethoxylates (NPEOs) and alkyldimethylbenzylammonium chlorides (ADBACs) in sediment grab samples collected from the three discharges and the control site wetland (CSW)

Site name	PEGs (μg kg^{-1})	PPGs (μg kg^{-1})	NPEOs (μg kg^{-1})	ADBACs (μg kg^{-1})
CSW	6.9	2.0	Below LOD	Below LOD
Discharge A				
DA-D	15.5	19.2	30.9	1430
DA-W1	196	22.8	70.0	455 000
DA-DSW1	202	10.2	29.2	154
DA-W2	85.1	7.8	43.1	45.0
DA-DSW2	15.7	5.4	23.6	5.7
Discharge B				
DB-D	66.8	1150	132	6110
DB-USW1	33.9	16.1	11.6	1.4
DB-W1	24.8	28.3	44.5	1.8
DB-DSW1	50.0	38.0	28.0	2.8
Discharge C				
DC-D	59.3	76.5	122	83.5
DC-100 m	44.7	38.7	11.6	11.3
DC-USW1	25.8	35.7	138	6.4
DC-W1	18.9	11.8	23.3	3.5
DC-DSW1	14.0	7.3	14.6	1.4
DC-USW2	47.8	184	167	3.7
DC-W2	44.4	382	194	0.7
DC-DSW2	28.6	3.4	11.0	3.9
DC-PLAYA	17.7	76.9	136	1.9

For PEGs, PPGs and NPEOs, there were no clear trends in sediment concentrations *versus* distance. NPEOs have previously been detected in lake sediments impacted by a WWTP treating O&G PW. PEGs and PPGs were not detected in this sediment.[26] The exact composition of the WWTP PW influent was not reported, however, based on an analysis of O&G chemical additives conducted in the area, it is likely that PEGs and PPGs were presented in the water influent.[59] This provides evidence for preferential sorption of NPEOs to sediments, as compared to PEGs and PPGs. PEGs are the most hydrophilic of the surfactants detected at these sites and therefore the least likely to sorb to sediments. Concentrations of PEGs in sediment may be due to sorption or could be the result of *in situ* degradation of other non-ionic surfactants.[46]

Results show that all four surfactant classes are accumulating in sediments downstream of the NPDES PW discharges, even though concentrations are below detection limit in some of the water samples. Degradation of all four surfactants has been observed in sediments and thus additional research is needed to determine if sediment concentrations are changing with time.[46] Finally, it should be noted that a principal component analysis (PCA) was conducted on the sediment samples and revealed no correlation between soil properties (organic carbon, inorganic carbon and total nitrogen) and surfactant concentrations. This finding was not unexpected because sediment surfactant concentrations are influenced by multiple factors including sediment composition, composition of the influent, and distance from the discharge.

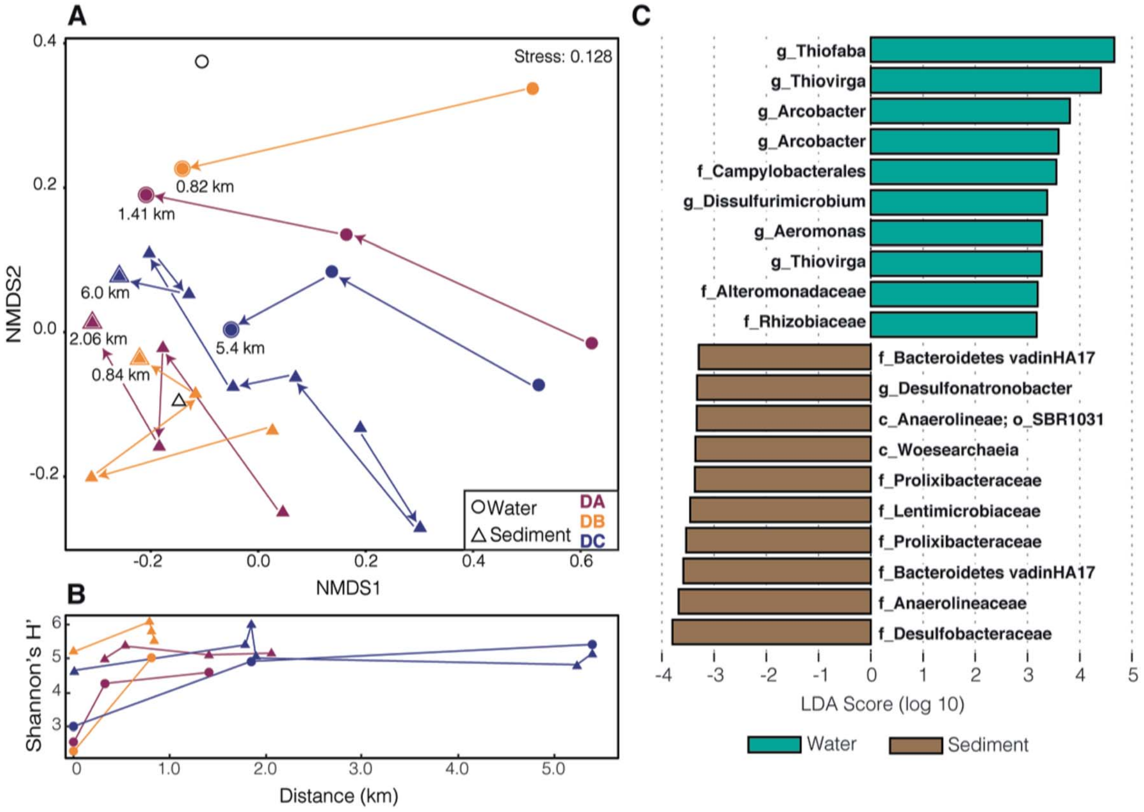

Fig. 4 Microbial community dynamics across wetland samples. In parts A and B, samples from discharge A (DA) are shown in red, samples from discharge B (DB) are shown in yellow and samples from discharge C (DC) are shown in blue. Additionally, water samples are shown as circles and sediment samples are shown as triangles. (A) Multivariate ordination showing spatial dynamics of microbiome profiles. Arrows indicate direction of flow in the CW systems, and the final sample is indicated with a larger data point and a distance (*e.g.*, 0.82 km for discharge B water). The clustering of water and sediment samples with increasing distance from discharge points indicates a diminishing impact of produced water on community composition. (B) Alpha diversity (Shannon's H′) in water samples increases with increasing distance downstream from discharge point. (C) Microbial taxa generating the largest Linear Discriminant Analysis (LDA) scores that are discriminant features between water and sediment samples.

Microbial (16S rRNA gene analysis)

The composition of microbial communities in sediment and water samples was profiled using 16S rRNA gene analyses to identify major metabolic processes involved in contaminant fate and to further understand changes in water and sediment composition with distance downstream (Fig. S9 and S10†). Samples with less than 5000 reads (DA-D and DC-DSW1 sediment samples) are not presented in the results. A series of alpha diversity metrics highlighted greater microbial richness and diversity in sediments, relative to water samples (Fig. S13†). While this observation is likely driven by greater habitat heterogeneity in sediments, the influence of produced water chemistry (*e.g.*, elevated salinity) on constraining microbial diversity may also play a role.[60] In general, communities displayed greater dissimilarity nearer discharge points, and began to converge with increasing distance downstream (Fig. 4A). This effect was observed in both water and sediment samples and suggests a weakening of the environmental factors (*e.g.*, water temperature, inorganic and organic chemistry) that constrain community composition with distance. Further highlighting the role of environmental factors in influencing microbial community assembly, we observed a positive correlation between distance from the discharge point and microbial community richness in water samples (Fig. 4B). Together, these results suggest a trend of increasingly diverse, but similar, microbial communities with greater distance from produced water discharge points.

16S rRNA gene sequencing data revealed strong evidence for sulfur cycling in both sediments and the overlying water impacted by water discharge. In general, water samples were dominated by putative sulfur oxidizing chemolithoautotrophic microorganisms (*i.e.*, Thiovirga[61] and Thiofaba[62]) that were also likely able to tolerate elevated salinity. These enrichments were typically greatest at the discharge points, with a single Thiofaba OTU accounting for 38% and 57% of the community at DC-D and DA-D, respectively and a sulfurospirillum OTU accounting for ~50% of the microbial community at DB-D. Highlighting the dominance of these taxa in the majority of water samples, OTUs associated with Thiovirga and Thiofaba were discriminant features for water samples (Fig. 4C). Previous studies have shown that halotolerant microbial communities such as these are capable of degrading commonly used oil and gas surfactants, including PEGs and PPGs, as well as some biocides.[28,63,64]

Water samples also contained putative signatures of microorganisms that may have derived from produced water itself. Arcobacter is commonly detected in produced water from hydraulic fracturing operations[65,66] and two OTUs affiliated with this genus were discriminant features for water samples. Given that these heterotrophic microorganisms are thought to utilize organic substrates within the well environment, they may also play a role in surfactant biodegradation within the wetland discharge system.

Putative sulfate reducing bacteria affiliated with the order desulfobacterales were frequently some of the most abundant OTUs in sediment samples collected downstream of the PW discharges (*e.g.*, DC-W1, DA-W2, DA-W1, and DC-USW2) and were discriminant features between water and sediment sample groups (Fig. 4C). A previous study observed that bacteria within this group (*e.g.*, desulfovibrio) were enriched in sediments from anaerobic microcosms during degradation of O&G organic chemical additives.[67] However, degradation of PEGs, PPGs and NPEOs is slower under anaerobic conditions and thus, these chemical species may be accumulating in sediments due to the reducing/anaerobic conditions.[37] At the control site wetland (*i.e.*, CSW) and sites less impacted by produced water (*i.e.*, DC-DSW2, DB-USW1, and DB-W1), abundant OTUs affiliated with *Thiobacillus* were also detected in the sediments. Given that *Thiobacillus* microorganisms are frequently implicated in sulfur oxidation,[68] these data suggest that oxidative and reductive portions of the sulfur cycle may be tightly coupled in some near-surface sediments and that the oxidative conditions are generally observed at sites less impacted by PW.

OTUs affiliated with the family *Anaerolineae* were also abundant (1–11% relative abundance in sediments) and strong discriminant features between water and sediment samples (Fig. 4C). Although little is known of metabolisms within this family, the few studies published to date have implicated these microorganisms in processes including hydrocarbon degradation[69] and primary fermentation.[70,71] Therefore, microorganisms affiliated with this family could potentially play key roles in degrading complex polymers, such as surfactants, and hydrocarbons within wetland sediments. Finally, two OTUs affiliated with the *Bacteroidetes* VadinHA17 group were discriminant features between sediment and water samples (Fig. 4C). These OTUs have previously been identified in sulfidic bioreactors where they were implicated in the degradation of complex organic matter.[72]

In two locations (DC-D, DC-100 m), surface waters were found to be over 40 °C and contained elevated concentrations of H_2S (>20 ppm observed on personal H_2S meters). Furthermore, filamentous algal mats were clearly visible associated with near surface sediments. Here, OTUs affiliated with a green filamentous anoxygenic phototrophic (FAP) bacterium, *Candidatus Chlorothrix*, accounted for 18% and 35% of all OTUs in sediment samples at DC-D and DC-100 m, respectively. Given prior laboratory studies indicating that these microorganisms perform sulfide dependent anoxygenic photosynthesis, the specific environmental conditions at these locations likely selected for enrichment of this microorganism.[73,74]

Conclusions

In some arid locations in the western U.S., CWs are used to treat and polish PW downstream of NPDES PW release points. Because of the relatively high costs associated with PW treatment and the relatively low costs associated with CWs for treatment, we predict that PW treatment with CWs will become increasingly popular in the future. Results of this study showed that CW systems at the study site were effective at removing organic O&G chemical additives from PW. Chemical and microbial analyses provided evidence to indicate that biodegradation and sorption are major removal mechanisms of non-volatile O&G organic chemical additives at these sites. Kinetic calculations, however, showed that the rate of removal did not increase within the CWs as compared to the stream segments. This indicates that the rate of removal for the major removal mechanisms was not optimized within the CWs, as compared to the stream segments, and there is likely potential for increased attenuation in these systems.

Treatment optimization within the CWs at these sites can occur by increasing aeration, which could increase biodegradation rates of all three surfactants.[37] As noted in our companion study, a small baffle, which would allow for aeration through a waterfall, would achieve this goal, as would additional cattail vegetation maintaining oxic redox conditions and thereby increasing degradation of these species within the sediments. Aeration of these systems would also enhance removal of any remaining VOCs in these systems. While the VOCs at this site were removed relatively quickly, some VOCs (*e.g.*, benzene) are highly toxic to microbes, livestock and humans, and thus removal of these species is highly important to CW design and performance. To enhance adsorption of ADBAC, clay minerals could be added to the stream segment prior to the wetland. Removing the biocide prior to the first wetland has the potential to increase biodegradation rates of other species within that wetland.[47] Because biocides are one of the most commonly used O&G chemical additives, removal of biocides, based on the physiochemical parameters, should be one of the first considerations in CW design for PW treatment. NPEO sorption could be enhanced by adding additional organic soils to the sediments in the wetlands.[48] Finally, a pilot scale study showed that more shallow water depths (15 cm *vs.* 56 cm) resulted in increased removal rates for organic PW chemicals. The opposite trend was observed for inorganic species. Thus, shallower water in one or more of the CWs would increase removal of the organic chemicals; however, sequential wetlands, with varying depths (and likely other design parameters) are needed to fully treat this complex waste stream.[24]

Sediment analyses showed that despite the relatively low concentrations of O&G chemical additives in the NPDES PW discharge, all four NVOC O&G chemical additives had accumulated in the sediment. This is especially true for ADBACs, which were found at concentrations of 455 mg kg^{-1} in one sample. The environmental implications of such high concentrations of ADBACs, however, remain unknown. Because ADBACs are a component of hand-sanitizers, production, use and release of these compounds has increased substantially in the past year due to the SARS-CoV-2 pandemic.[56] As a result, there is increased attention from the scientific community towards understanding the environmental implications of these commonly used biocides. The concentrations of PEGs, PPGs and NPEOs in all sediments were relatively low, as compared to concentrations previously reported in sediments impacted by WWTP discharges, and thus are not as high priority for additional research as ADBACs.[46,75]

In conclusion, this study shows that CW systems are effective at removing O&G chemical additives from PW. However, the systems at this site could be further optimized to increase removal rates. Additional research is needed to understand the potential impact of cationic surfactants and biocides, such as ADBACs, on benthic organisms, sediment sorption capacity, plant uptake, and the implications for contaminant removal and CW management.[76] Finally, in addition to chemical analysis, analysis of both chronic and acute toxicity is necessary at these sites to fully understand treatment efficacy and determine if the treated water can be safely reused for agriculture.[2,11,77] Results of this study and future studies can be used to inform design and management of CWs for PW treatment.

Conflicts of interest

There are no conflicts to declare.

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