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ORIGINAL ARTICLE

Targeted 16S rRNA amplicon analysis reveals the diversity of bacterial communities in carwash effluents

Timothy Sibanda[1,2] · Ramganesh Selvarajan[1] · Memory Tekere[1]

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Abstract

This study aimed to analyze the bacterial diversity in carwash effluents and to determine their potential for use in microbial degradation of environmental contaminants. Nine carwash effluent samples were collected for physicochemical and bacterial community diversity analysis using multi-digital probes and 16S rRNA gene amplicon sequencing respectively. The pH of all effluent samples was neutral to slightly alkaline. Oil and grease concentrations ranged from 15.3 to 49.7 mg/L. 16S gene amplicon sequencing of the nine samples produced 45,934-sequence reads, which translated to 13 bacterial phyla, 26 classes, and 43 genera. The most dominant phyla were *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Fusobacteria*. Canonical correspondence analysis (CCA) showed that the distribution of the phyla *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Verrucomicrobia* was influenced by the presence of oil and grease, total petroleum hydrocarbons-gasoline range organics (GRO-TPH), and metals species (Pb, Cu, and Zn). The dominant bacterial genera found in the present study were previously proven to biodegrade hydrocarbons, and their presence in carwash effluents could bode well for in situ natural bioremediation of these contaminated sites.

Keywords Carwash · Bacteria · Diversity · Pyrosequencing · Canonical correspondence analysis

Introduction

Vehicle washing produces effluent contaminated with a range of pollutants such as heavy metals, polyaromatic hydrocarbons (PAHs), total petroleum hydrocarbons-gasoline range organics (TPH-GRO), and in some instances, phosphate-based detergents (Aikins and Boakye 2015; Tekere et al. 2016). These pollutants have potential to exert selective pressure on microbial communities found therein. Some studies suggest that environmental bacterial community composition is influenced by geochemical parameters such as pH, total organic carbon (TOC), nutrient availability, and presence (and concentration) of gases and metals (Lu et al. 2006). However, Wu et al. (2017) found bacterial diversity to be strongly related to soil pH, with higher diversity in neutral samples and lower diversity in acidic samples and concluded that pH was the primary determinant of bacterial community composition. Contrastingly, Lozupone and Knight (2007) analyzed the environmental distribution of bacterial gene sequences using 16S rRNA sequence analysis and found salinity to be the major determinant of environmental microbial community composition. Whichever geochemical parameter is the primary determinant of microbial community composition, the fact remains that the presence of contaminants in the environment may translate either to reduced microbial species abundance (Xie et al. 2016), increased species diversity (Siam and Ghobrial 2000), or increased tolerance of the microbes to the pollutants. This can result in either an increase or decrease in species diversity (Wakelin et al. 2014), depending on the nature of the pollutants and their impacts on the resident microbes.

Sequencing of the 16S rRNA gene is used to study the diversity of some bacterial communities in different environments. These include oil-contaminated soils (Peng et al.

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✉ Timothy Sibanda
timsibanda@gmail.com

[1] Department of Environmental Sciences, UNISA Florida Campus, Florida 1710, Republic of South Africa

[2] Department of Biological Sciences, University of Namibia, Private Bag 13301, 340 Mandume Ndemufayo Ave, Pionierspark, Windhoek, Namibia

2015), mine tailings (Liu et al. 2014), coral reefs (Zhang et al. 2015), residential kitchens and the indoor environment (Flores et al. 2013; Stanish et al. 2015), and in the food processing industry (Reynisson et al. 2010). However, there is dearth of information about the diversity of microbial communities found within carwash effluents—microbial niches characterized by an array of potentially toxic chemical substances with the potential to impact environmental water sources. Identifying dominant bacterial taxa at such contaminated sites may provide the first insight into the potential for in situ microbial degradation of contaminants, which in the case of carwashes may include total petroleum hydrocarbons (TPH), benzene, toluene, ethylbenzene and xylene (BTEX), and metals. With further research, such knowledge can be used to examine the microbial populations in terms of their ecological relevance in order to understand the underlying conditions that are conducive to degradative processes. In addition, actual car washing frequencies, washing chemicals/detergents, and effluent flow rates all vary among carwashes. Because of this variation, it is expected that different microbial taxa are found in effluents of different carwashes. While carwashes differ in design and size and thus making their flow rates and hydraulic retention times way different, a carwash holding tank which is 0.6 m deep, 0.5 m wide, and 1.8 m long has an estimated flow rate of 38 L min^{-1} and a hydraulic retention time of 15 min (Fall 2007). A pertinent question that then arises would be, "Are the microbial communities found in carwashes transient or autochthonous?" While attempting to answer this question was beyond the scope of this study, it is much more likely that the microbial communities in carwashes consist of both transient and autochthonous communities, based on the model of the microbiology of spring water as described by Savio et al. (2018). Transient communities are likely introduced in the event of inflows, as when a vehicle is washed, while autochthonous communities are likely adhered to the walls of holding tanks (biofilm mode), becoming suspended when flow rates increase. The study, therefore, was done with an assumption that the diversity of bacterial communities found in carwash effluents is a mixture of transient and autochthonous communities, and this feature was assumed standard across all carwashes studied. High-throughput targeted 16S rRNA amplicon sequencing was used to analyze the bacterial community diversity in the nine carwash effluent samples. To our knowledge, this is the first study to determine the microbial community composition of carwashes.

Materials and methods

Sample collection

Grab carwash effluent samples were obtained from the effluent tanks of nine carwash stations. Selection of carwashes ensured that the sampling regimen gave an approximate representation of Johannesburg Metropolitan. The following carwashes were therefore selected for sample collection: BPSmith, CALTJ, and Sasol (26° 12′ 14.76″ S, 28° 2′ 50.28″ E; Johannesburg Central); Imperial and MRC (26° 6′ 27.36″ S, 28° 3′ 24.12″ E; Sandton); Springchem and Ziggy (26° 19′ 20.64″ S, 28° 7′ 26.4″ E; Alberton); and CAWC and TCW (26° 8′ 37.68″ S, 27° 59′ 42.72″ E; Randburg). Samples were collected into sterile 1-L glass bottles, immediately stored in cooler boxes containing ice, and transported to the laboratory at the University of South Africa (UNISA) Florida Campus for analysis. Sampling was done on the same day in June 2016.

Physicochemical analysis

Physicochemical parameters including pH, electrical conductivity (EC), total dissolved solids (TDS), and dissolved oxygen (DO) were measured and recorded in situ during sampling using a multi-parameter meter (Hanna Instruments PTY LTD, Johannesburg, RSA) while turbidity was determined using a turbidity meter (Hanna Instruments PTY LTD, Johannesburg, RSA). Sulfate ion (SO_4^{2-}) concentrations were determined colorimetrically following earlier descriptions (Dickman and Bray 1940; Pappenhagen 1958), while metals (Pb, Cu, and Zn), oil and grease, and GRO-TPH were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer Optima 5300 DV) at WaterLab (Pretoria, RSA). BOD_5 was determined in the laboratory at the University of South Africa (UNISA, Florida, RSA) by taking the difference between DO_1 and DO_5.

DNA extraction and sequencing

Sample bottles were shaken to homogenize the samples followed by sample filtration through 1.2-μm pore-sized filter membranes to remove coarse particles from the collected water. Each sample filtrate was then passed through a 0.2-μm pore size membrane filter to entrap the microbial cells followed by cutting the membranes into pieces and running them on a Disruptor Genie (VWR, Pennsylvania, USA) to lyse the cells following the manufacturer's protocol. Following this, total DNA extraction was done using a Quick-gDNA™ MiniPrep Kit (ZYMO RESEARCH, Irvin, USA) according to the manufacturer's protocol. Amplification of the 16S rRNA gene was carried out with primer sets 27F (5′-AGAG TTTGATCMTGGC-3′) and 518R (5′-ATTACCGCGGCTGC TGG-3′) as described by García-Moyano et al. (2015). Each primer included a 5′ 26 base pair (bp) adaptor (A or B), a 4 bp library key and a 10-bp multiplex identifier (MID), which were used in conjunction with the 16S rRNA gene primer sets to identify the samples. PCR reactions constituted of 25 μL of one *Taq* 2X Master Mix, 1.5 μL each of the forward and

reverse primers at a concentration of 0.2 μM, 2 μL of extracted DNA (50–100 ng μL^{-1}), and 22 μL of nuclease-free water. The thermal profile consisted of an initial denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min, followed by cooling to 4 °C. PCR products were purified using a DNA Clean & Concentrator Kit (ZYMO RESEARCH, Irvin, USA). Following the purification step, Inqaba Biotechnology (Pretoria, South Africa) sequenced the pooled PCR products on the GS-FLX-Titanium series 454/Roche pyrosequencer.

Sequence analysis

All sequence reads were processed by the next-generation sequencing (NGS) analysis pipeline of the SILVA rRNA gene database project (SILVAngs 1.3) (Quast et al. 2013). Each read was aligned using the SILVA Incremental Aligner (SINA SINA v1.2.10 for ARB SVN) (revision 21,008) (Pruesse et al. 2012) against the SILVA SSU rRNA SEED, and quality controlled (Quast et al. 2013). Reads shorter than 50 aligned nucleotides, more than 2% of ambiguities, or 2% of homopolymers, respectively, were excluded from further processing. Putative contaminations and artifacts, reads with a low alignment quality (50 alignment identity, 40 alignment score reported by SINA), were identified and excluded from downstream analysis. After these initial quality control steps, identical reads were dereplicated while the unique reads were clustered into OTUs on a per sample basis, and the reference read of each OTU classified. Dereplication and clustering was done using cd-hit-est (version 3.1.2; http://www.bioinformatics.org/cd-hit) (Li and Godzik 2006) running in accurate mode, ignoring overhangs, and applying identity criteria of 1.00 and 0.97, respectively. The classification was performed by a local nucleotide BLAST search against the non-redundant version of the SILVA SSU Ref dataset (release v_128; http://www.arb-silva.de) using blastn (version 2.2.30+; http://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard settings (Camacho et al. 2009). The classification of each OTU reference read was mapped onto all reads that were assigned to the respective OTU. Reads without any BLAST hits or reads with weak BLAST hits where the function \(% sequence identity + % alignment coverage)/2″ did not exceed the value of 93 remained unclassified. Community richness indicators (Chao-1) and diversity indexes (Simpson-H) of the samples were calculated using PAST (University of Oslo, USA) while the heat map was generated using the omics tool of XLSTAT (Addinsoft, New York, USA). Sequence reads were deposited into the GenBank-SRA (Sequence Reads Archive) under the accession number SRP117897.

Results and discussion

The physicochemical profiles of the carwash effluents from the nine carwash stations are detailed in Table 1.

The pH of all effluents was neutral to slightly alkaline while SO_4^{2-}, TDS, turbidity, and BOD levels were high and closely resembled levels recorded in a previous study by O'Sullivan et al. (2011). Oil and grease values ranged between 15.3 ± 3.1 and 49.7 ± 5.0 mg/L across the ten sampling sites, which were lower than the average concentration of 1100 mg/L reported by Fall (2007) in carwash wastewater in Mexico City, Mexico. The surveyed carwashes used oil separators for oil and grease removal, and these have an estimated oil and grease removal efficiency of between 73 and 98% (Nadzirah et al. 2015). However, if the oil and grease concentrations observed in this study represent the percentage that remains

Table 1 Physicochemical profiles of the carwash effluents

	BPSmith	CALTJ	CAWC	IMPERIAL	MRC	SASOL	SPRINGCHEM	TWC	ZIGGY
pH	8.4 ± 0.2	7.2 ± 0.2	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.6 ± 0.1	7.4 ± 0.1	7.5 ± 0.2	7.3 ± 0.1
EC (μS/cm)	124.7 ± 3.1	85.7 ± 2.5	30.0 ± 2.0	45.0 ± 4.1	49.7 ± 5.0	54.7 ± 2.5	60.3 ± 5.9	55.2 ± 4.3	51.7 ± 3.2
TDS (mg/L)	684 ± 5.3	488.3 ± 31.0	189.3 ± 8.1	368.3 ± 6.0	453.7 ± 3.2	534 ± 27.1	502 ± 18.3	477.3 ± 13.6	482 ± 6
SO_4 (mg/L)	184 ± 0.0	8.0 ± 2.0	11.3 ± 0.6	36.0 ± 2.0	133.7 ± 3.2	147 ± 6.1	152.3 ± 2.5	145.7 ± 3.1	163 ± 3.0
BOD (mg/L)	354.3 ± 2.5	650 ± 0.0	33.3 ± 5.5	190 ± 5.3	209.3 ± 6.4	204 ± 0.0	224 ± 4.0	117 ± 2.6	325 ± 4.4
Turbidity (NTU)	388.3 ± 6.0	427.7 ± 35.7	137.3 ± 30.1	249 ± 3.6	253.3 ± 3.5	377.3 ± 5.0	234 ± 4.0	474.3 ± 17.5	263 ± 3.0
Oil and grease (mg/L)	15.3 ± 3.1	49.7 ± 5.0	48 ± 5.6	25.7 ± 3.2	34.3 ± 1.5	36.7 ± 1.5	37.0 ± 1.0	30.3 ± 1.5	44.7 ± 1.5
GRO-TPH (mg/L)	2.0 ± 0.0	1.0 ± 1.0	1.3 ± 0.6	1.3 ± 0.6	2.7 ± 0.6	3.0 ± 1.0	1.7 ± 1.2	3.0 ± 1.0	2.0 ± 1.0
Cu (mg/L)	3.3 ± 0.6	13.0 ± 1.0	2.3 ± 0.6	3.3 ± 0.6	2.3 ± 0.6	3.0 ± 1.0	2.0 ± 0.0	3.7 ± 0.6	2.3 ± 0.6
Pb (mg/L)	0.7 ± 0.6	6.0 ± 1.0	1.3 ± 0.6	1.7 ± 0.6	1.3 ± 0.0.6	3.7 ± 0.6	2.0 ± 1.0	1.3 ± 0.6	1.7 ± 1.2
Zn (mg/L)	2.3 ± 0.6	21.0 ± 1.0	3.7 ± 0.6	2.3 ± 0.6	2.0 ± 1.0	2.3 ± 0.6	2.3 ± 0.6	3.0 ± 1.0	2.7 ± 0.6

after removal, environmentalists can still argue that these concentrations are still too high and remain a cause of concern since such pollutants are environmentally persistent, especially considering the cumulative effect of effluent discharges from all carwashes in a unit area. Besides lowering the diffusion of oxygen into aquatic systems, oil and grease are known to contain toxic substances which are carcinogenic to humans (Nadzirah et al. 2015). Concentrations of Cu, Pb, and Zn ranged between 1 and 3 mg/L in nine of the ten sampling sites, the outlier sample being CALTJ whose concentrations of these metals were 13.0 ± 1.0, 6.0 ± 1.0, and 21.0 ± 1.0 mg/L, respectively; which were significantly higher ($p < 0.05$) compared to those of other sites. However, the metal levels detected in this study were still far lower than the levels (Cu = 180.8 μg/L, Zn = 308.5 μg/L, and Pb = 46.4 μg/L) reported by O'Sullivan et al. (2011) in a carwash in Christchurch City, New Zealand. Heavy metal concentrations have been reported to reduce microbial activity and also lead to the inhibition of chlorophyll synthesis (Nadzirah et al. 2015), which may reduce ecosystem productivity.

Statistically, the sites BPSmith, CALTJ, Sasol, and Ziggy had almost the same physicochemical profiles with respect to total dissolved solids, sulfate (SO_4^{2-}), turbidity, biochemical oxygen demand (BOD), electrical conductivity, and the metals zinc (Zn) and copper (Cu) (PCA1 (Fig. S1)). The other five sites had different physicochemical profiles influenced by the distribution of oil and grease and gasoline range organics (GRO-TPH). However, PCA2 (explaining 24.32% of the data) further separated the physicochemical profiles of the carwashes CALTJ and Ziggy from those of Sasol and BPSmith based on the concentrations of turbidity, EC, TDS, and SO_4^{2-} which were higher in the latter two sites compared to the first two. There is evidence that metals and hydrocarbons, and other factors which include water availability, salinity, oxic/anoxic conditions, temperature, pH, pressure, chemical pollution, pesticides, and antibiotics, affect environmental microbial diversity and species richness (Fakruddin and Mannan 2013).

From an environmental and socioeconomic perspective, carwash businesses have become a necessary inclusion into the economic activities of urban areas where the majority of people have busy schedules and cannot afford to wash their cars at home. Even if they had time to do so, the collective damage done to the environment by carwash wastewater would be immeasurable over time. Carwashes close that gap from both a socioeconomic and environmental perspective. However, in so doing, questions arise as to what effect the resultant effluents have on the diversity, abundance, and distribution of microbiota.

Targeted 16S rRNA amplicon sequencing produced 54,934 sequence reads, with a range of 2966 reads in CAWC Carwash sample to 8120 reads in Ziggy Carwash sample and a mean sequence reads of 5104. After performing a sequence quality check, a total of 14,990 OTUs were recovered from all the nine carwash samples, with a range of 622 OTUs in SASOL Carwash sample to 3186 OTUs in Imperial Carwash sample. In the absence of sequence data from a carwash environment with which to compare the results of the present study, the reads data from this present study was compared with data obtained from other, nearly similar environments ranging from thermal springs, saltpans, municipal wastewater effluents, to polluted environments. The average number of reads obtained per sample in the present study was higher than the mean sequence reads of 3459 obtained by Selvarajan et al. (2017) from the analysis of halophilic bacteria diversity in a South African saltpan. It was, however, lower than the mean sequence reads (7901) obtained by Keshri et al. (2015) from the analysis of bacterial communities in South African gold mine-water samples, and also lower than the average number of reads (9755) obtained from the analysis of bacterial community composition of wastewater treatment outfalls reported by Wang et al. (2014).

The percentage contribution of each carwash sample to the total number of recovered OTUs, species richness (Chao-1 index), and species diversity index (Shannon-H index) is presented in Table 2.

Table 2 Number of sequences, OTUs, Chao-1, and Shannon-H indices data for the nine selected carwashes in Johannesburg

Sample name	Total number of sequences	Total number of non-chimeric reads	Number of OTUs	Chao-1	Shannon-H
BPSMITH	3868	1336	968	555.5	2.98
CALTJ	3488	1482	1049	632.5	2.92
CAWC	2966	1294	997	200.4	2.56
IMPERIAL	7583	4246	3186	1047	3.31
MRC	6944	4255	2836	468.3	2.56
SASOL	3255	1746	622	141.6	3.11
SPRINGCHEM	6595	2804	1971	727.8	2.53
TCW	3115	1394	1093	190.8	3.03
ZIGGY	8120	3144	2268	547.2	3.19

*Mean length of the sequence reads for all samples were 244 nts

Bacterial species richness of the carwash samples ranged from 141 in SASOL Carwash samples to 1047 in Imperial Carwash samples. The Shannon index obtained in the present study was lower than richness indicators of 3.72 to 5.09 and 6.26 to 7.34 reported by Wang et al. (2014) and Lu and Lu (2014) in wastewater treatment outfalls and sediments receiving wastewater treatment effluents, respectively, implying that the carwash effluents were characterized by low bacterial species richness. A possible explanation to this observation is that while many bacteria taxa have the potential of assimilating and degrading polyaromatic hydrocarbons, others fail to proliferate in environments contaminated with PAHs (Wu et al. 2017). Further evidence of the detrimental effects of some pollutants is provided by Drury et al. (2013) who analyzed the impacts of wastewater on bacterial community composition by comparing the microbial community composition of two urban rivers' upstream and downstream sites of wastewater discharge points and demonstrated that wastewater effluents can significantly reduce both the bacterial species diversity and richness. Taking the present study as the benchmark for bacterial community studies in carwash environments, further studies of this kind may allow for the quantification of possible detrimental effects of car washing activities on particular taxonomic groups as suggested by Gołębiewski et al. (2014).

A further analysis of the sequence data to a phylum level phylogenetic fingerprint showed the presence of 13 phyla across all carwash samples. Of these, the four most dominant phyla were *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Fusobacteria*. Sequences corresponding to the bacterial domain but which could not be classified at phylum level were the third most abundant in almost all carwash sites, representing a potentially large pool of previously uncultured bacteria. Figure 1 presents the detailed distribution of the bacterial phyla across the nine carwashes.

Despite the differences that were observed from one carwash to the next, and considering only OTUs with at least 1000 sequence reads, the phylum level taxonomic structure of bacterial communities in carwash effluents observed in this study was as follows: *Proteobacteria* > *Bacteroidetes* > uncultured bacteria > *Firmicutes* > *Fusobacteria* > *Lentisphaerae* > *Actinobacteria* > *Acidobacteria* > *Verrucomicrobia*. This picture compares relatively well with the bacterial community composition reported by Sutton et al. (2013) from soils impacted by long-term diesel contamination which were dominated by *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi*. Canonical correspondence analysis showed that the distribution of *Bacteroidetes*, *Lentisphaerae*, *Firmucutes*, *Deinococcus-Thermus*, *Cyanobacteria*, *Planctomycetes*, *Verrucomicrobia*, and *Spirochaetae* was higher in the sites BPSmith, Springchem, Sasol, and CAWC than in the other sites. Similarly, *Proteobacteria*, *Acidobacteria*, *Fusobacteria*, *Actinobacteria*, and *Chloroflexi* were more abundant in the effluents from the sites TCW, Imperial, MRC, CALTJ, and Ziggy (Fig. 2).

CCA Axis 2 (32.67%) showed that the distribution of the phyla *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Verrucomicrobia* was influenced by the presence of oil and grease, GRO-TPH, Pb, Cu, and Zn while the distribution of *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, *Deinococcus-Thermus*, *Firmicutes*, *Fusobacteria*, *Lentisphaerae*,

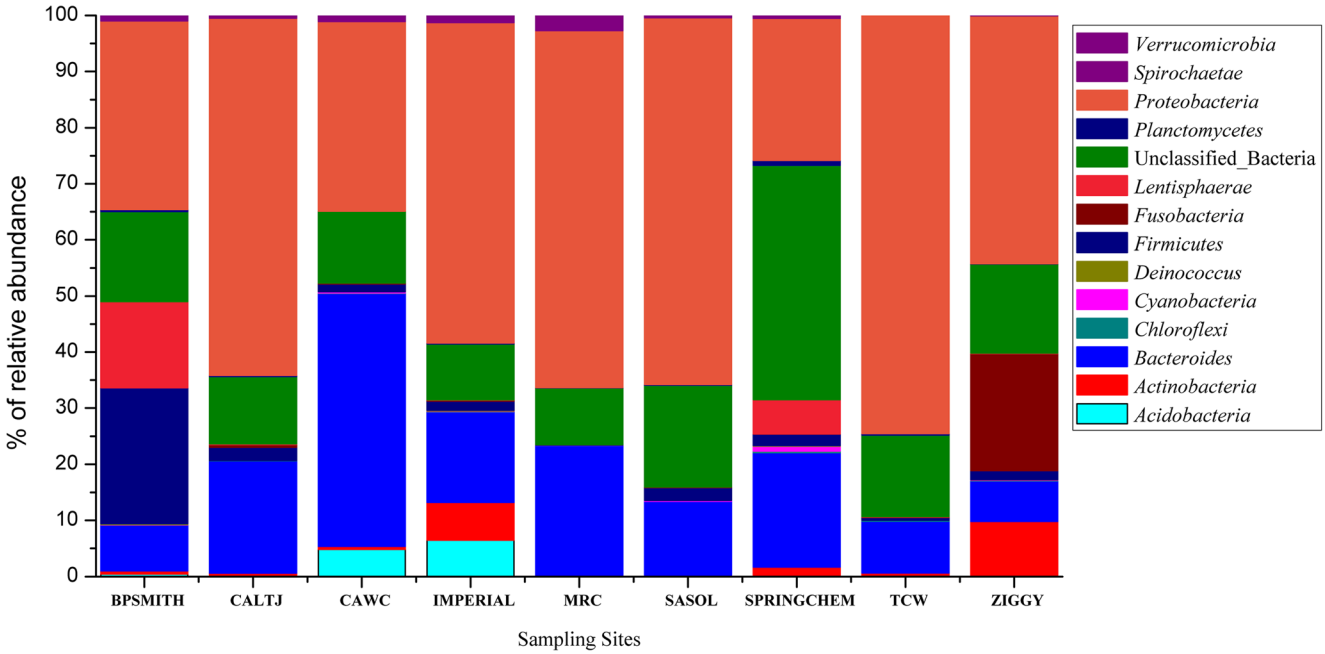

Fig. 1 Phylum level phylogenetic fingerprint of the nine carwash samples

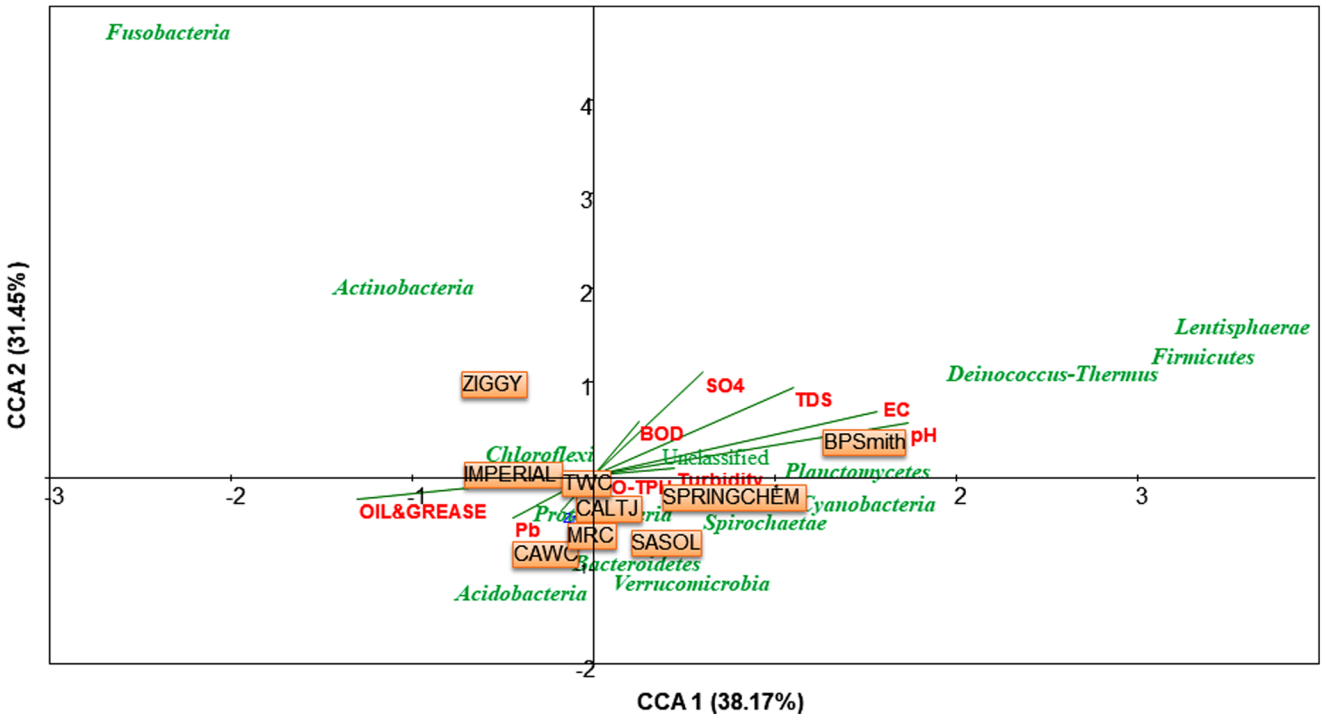

Fig. 2 Canonical correspondence analysis (CCA) showing the distribution and interrelationships of the bacterial phyla and physicochemical parameters in carwash effluents

Planctomycetes, and *Spirochaetae* was influenced more by pH, turbidity, BOD, SO_4^{2-}, TDS, and EC.

From the 13 reported phyla, 26 dominant bacterial classes were observed across the nine carwash samples, with an average of eight bacterial classes in each carwash sample. Of these classes, 31% were classified under the phyla *Proteobacteria* and *Bacteroidetes*, with five (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria*) belonging to the phylum *Proteobacteria* and three (*Bacteroida*, *Flavobacterium* and *Sphingobacterium*) to the phylum *Bacteroidetes*. As in this study, the abundance and overall dominance of the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria* and *Deltaproteobacteria* over other bacterial classes was also noted in a study of bacterial communities inhabiting South African mine-water samples using an Illumina next-generation sequencing platform by Keshri et al. (2015). Class level representation for the remaining phyla was as follows: *Firmicutes* (*Clostridia* and *Bacilli*), *Fusobacteria* (*Fusobacteria*), *Lentisphaerae* (*Lentisphaeria* and *Oligospaeria*), *Acidobacteria* and *Holophagae* (*Holophagae*), *Actinobacteria* (*Actinobacteria*), and *Verrucomicrobia* (*Verrucomicrobia*). Figure 3 comprehensively details the distribution and relative abundance of bacterial classes across the nine carwashes.

A genus level phylogenetic heat map generated using XLSTAT (USA) (Fig. 4) shows 43 dominant bacterial genera across all carwash samples. Ziggy, Springchem, MRC, and Imperial carwashes jointly had the highest number of dominant bacterial genera (with a relative abundance of 0.78 to > 1). BPSmith Carwash sample had the next highest number of dominant genera followed by CALTJ and TCW carwashes, each of which had six dominant bacterial genera.

The 43 bacterial genera all belonged to seven of the described phyla as follows: *Proteobacteria* (55%), *Bacteroidetes* (15%), *Firmicutes* (9%), *Actinobacteria* (6%), *Lentisphaerae* (6%), *Acidobacteria* (6%), and *Fusobacteria* (1%). *Proteobacteria* represents a ubiquitous and metabolically diverse group of Gram-negative bacteria while *Bacteroidetes* are Gram-negative heterotrophic bacteria, both of which are known to degrade high-molecular-weight organic compounds, including petroleum hydrocarbons (Drury et al. 2013). The abundance of bacteria from these two phyla over others could have been as a result of their ability to metabolize complex organic compounds in the effluents, including petroleum hydrocarbons. Canonical correspondence analysis (CCA) (Fig. 2) showed the distribution of these bacterial phyla to be skewed towards samples with higher concentrations of metals, oil and grease, and GRO-TPH, which seemed to confirm the finding by Drury et al. (2013). Polynuclear aromatic hydrocarbons comprise of a family of priority environmental contaminants which are often difficult to biodegrade owing to an imbalance in the carbon:nitrogen:phosphorous (CNP) ratio (caused by high carbon and low nitrogen concentrations) obtaining in hydrocarbon contaminated environments

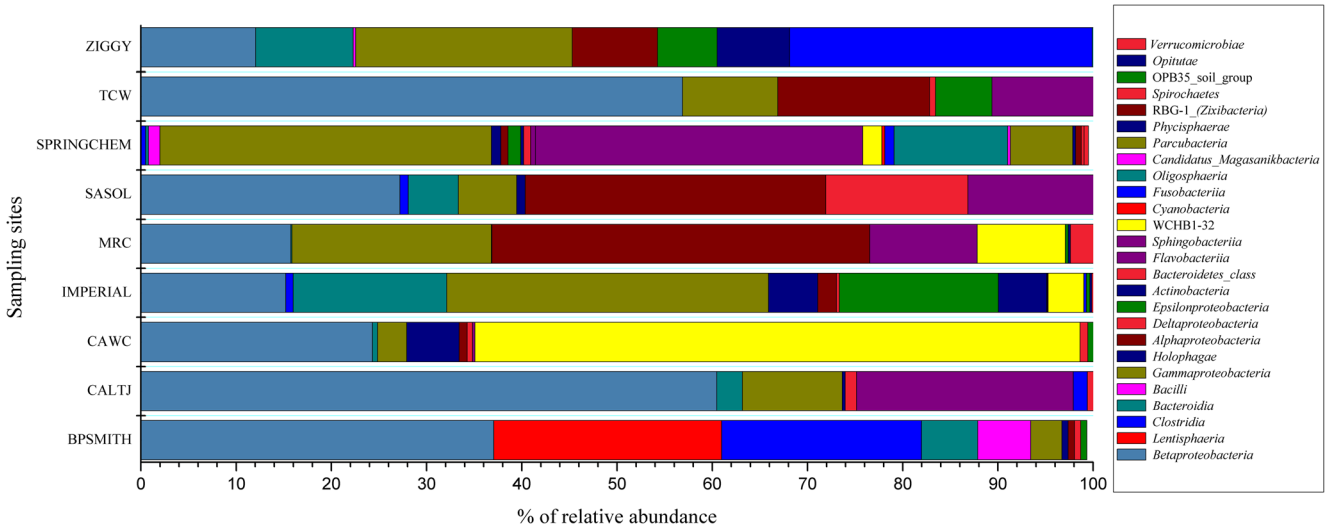

Fig. 3 Class level phylogenetic fingerprint of the nine carwash samples

(Nwinyi et al. 2016). However, *Pseudomonas* spp. are known to exhibit not only an extraordinary capability for degrading xenobiotics but are also equipped with broad metabolic potential for degrading naturally occurring aromatics, largely as a result of enzymes coded for by genes carried by plasmids, chiefly plasmid F1 (Palleroni et al. 2010). Based on these, and

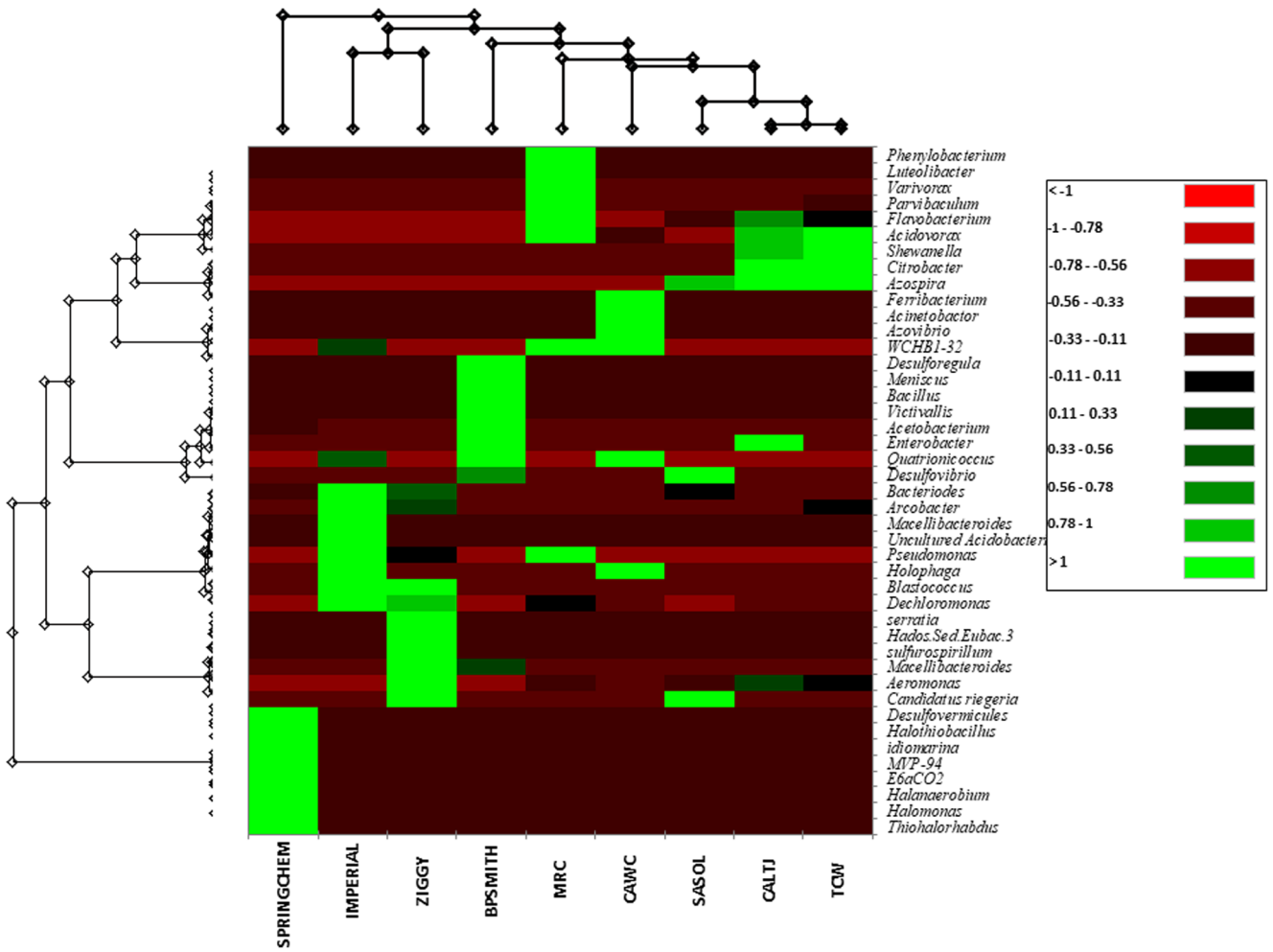

Fig. 4 Genus level phylogenetic fingerprint of the nine carwash samples

other Das and Chandran (2011) findings, it can be assumed that *Pseudomonas* sp. found in oil-rich carwash effluents could play a pivotal role in the bioremediation of oil and grease contaminated environmental milieus.

Previous findings (Costa et al. 2015) have also proven that metal-contaminated freshwater sediments exhibit an extremely complex and well-adapted community dominated by *Proteobacteria*, especially *Betaproteobacteria* and *Bacteroidetes*. In this study, *Betaproteobacteria* had a relative abundance of between 10 and 50% across the nine carwash sites, commensurate with the findings in other settings (Costa et al. 2015). The metabolic versatility of *Proteobacteria* is demonstrated by its recorded dominance in various other environments like acid mine drainage (Keshri et al. 2015), rhizosphere of para grass growing under saline conditions (Mukhtar et al. 2016), diesel contaminated soils (Sutton et al. 2013), and even pristine environments (Basak et al. 2016).

Further, CCA Axis 1 showed that the distribution of two other phyla, *Acidobacteria* and *Verrucomicrobia*, was biased towards samples from Imperial and MRC carwashes, which had higher concentrations of metals (Pb, Zn, and Cu) and oil and grease respectively. CCA Axis 2 also showed that there were more sequences corresponding to the phyla *Fusobacteria* and *Actinobacteria* in Ziggy Carwash samples than in other carwash samples. Similarly, sequences corresponding to *Firmicutes* and *Lentisphaerae* were more abundant in BPSmith and Springchem samples, which were also characterized by higher concentrations of SO_4^{2-}, TDS, EC, turbidity, and BOD. Most of these bacterial genera have known bioremediation potential (Yergeau et al. 2012), and their presence in contaminated sites such as carwashes bodes well for environmental conservation since *on site* bioremediation of contaminants is the most feasible clean up solution of such sites.

As we draw conclusions from this study, a number of analysis limitations need to be taken into account. Firstly, the 1.2-μm pore-sized filters used for straining the samples could have excluded bacteria that are > 1.2 μm from the analysis. Previous studies involving size analysis of soil bacteria indicated that > 60% of soil bacterial cells were found to be smaller than 1.2 μm in diameter, although this percentage was likely to be higher in nutrient-poor habitats (Portillo et al. 2013). Hypothetically, it could be that the present analysis could have excluded as much as 30% of bacterial cells from the analysis. The second limitation concerned the potential inhibition of the polymerase chain reaction amplification of the extracted DNA, plus the varying number of ribosomal operons in different bacteria, which could have lessened the accuracy of results obtained by PCR. Together, these limitations could mean that the results obtained in this study were less than 100% representative of the bacterial community diversity in the surveyed carwash effluents.

In conclusion, results of this study show that carwash effluents harbor diverse bacterial communities dominated by *Proteobacteria* and *Bacteroidetes* whose distribution is influenced by the presence and concentration of petroleum hydrocarbons, metal contaminants, and sulfate. Sequences corresponding to uncultured bacteria also represent a significant proportion of bacterial communities in carwash effluents, and this being the first study of its type, further studies of bacterial community composition in this type of environment are encouraged along with functional predictions, especially with a view of establishing the presence of bacterial families with potential for application in bioremediation initiatives.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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