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GeoChip-Based Analysis of Microbial Functional Gene Diversity in a Landfill Leachate-Contaminated Aquifer

Zhenmei Lu,[†,‡] Zhili He,[‡,∥] Victoria A. Parisi,[§,∥] Sanghoon Kang,[‡] Ye Deng,[‡] Joy D. Van Nostrand,[‡] Jason R. Masoner,[⊥] Isabelle M. Cozzarelli,[#] Joseph M. Suflita,[§,∥] and Jizhong Zhou[‡,∥,▽,◊,*]

[†]College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China;

[‡]Institute for Environmental Genomics;[§]Institute for Energy and the Environment;[∥]Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019, United States;

[⊥]U.S. Geological Survey, Oklahoma City, Oklahoma 73116, United States;

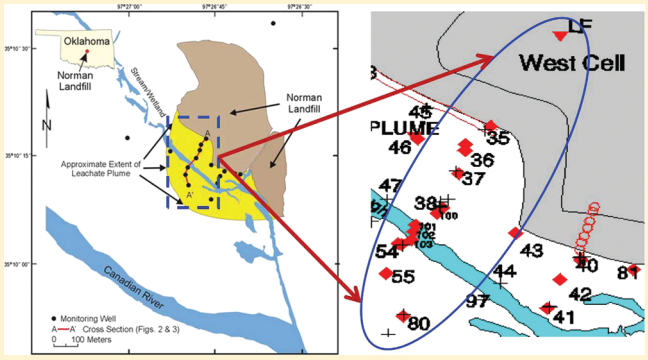
[#]U.S. Geological Survey, 431 National Center, Reston, Virginia 20192, United States;

[▽]Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States;

[◊]State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, People's Republic of China

Supporting Information

ABSTRACT: The functional gene diversity and structure of microbial communities in a shallow landfill leachate-contaminated aquifer were assessed using a comprehensive functional gene array (GeoChip 3.0). Water samples were obtained from eight wells at the same aquifer depth immediately below a municipal landfill or along the predominant downgradient groundwater flowpath. Functional gene richness and diversity immediately below the landfill and the closest well were considerably lower than those in downgradient wells. Mantel tests and canonical correspondence analysis (CCA) suggested that various geochemical parameters had a significant impact on the subsurface microbial community structure. That is, leachate from the unlined landfill impacted the diversity, composition, structure, and functional potential of groundwater microbial communities as a function of groundwater pH, and concentrations of sulfate, ammonia, and dissolved organic carbon (DOC). Historical geochemical records indicate that all sampled wells chronically received leachate, and the increase in microbial diversity as a function of distance from the landfill is consistent with mitigation of the impact of leachate on the groundwater system by natural attenuation mechanisms.



1. INTRODUCTION

The disposal of municipal solid wastes (MSW) is a global problem as nations develop and world populations increase.[1] Despite continued advances in recycling and incineration as treatment options, landfills remain the primary mechanism for MSW management.[2] The U.S. Environmental Protection Agency reported that about 250 million tons of MSW were generated in the U.S. in 2008 and about 135 million tons (~54%) were discarded in landfills.[3] Since 1988, over 6000 U.S. MSW landfills closed, forcing greater reliance on the remaining facilities.[4] Since most municipalities, at some time had facilities for MSW disposal, the number of landfills, dumps and other facilities in the U.S. is estimated to be as high as 100 000.[5]

All landfills are predicted to eventually fail and release leachate into recipient environments including aquifers, creating groundwater contaminant plumes.[6−8] As a result, landfills constitute one of the largest pollutant threats to groundwater.[2] Landfill leachate typically contains high concentrations of DOC, dissolved inorganic carbon (DIC), ammonium (NH_4^+), methane (CH_4), inorganic cations and anions, heavy metals, and numerous xenobiotic organic compounds.[7,9] Natural attenuation has been shown to be an alternative for remediating contaminated groundwater of landfill.[7] Indigenous microbial communities are expected to play a preeminent role in the biodegradation of contaminants in response to the prevailing geochemical conditions. Also, the composition of microbial communities is indicative of the potential for intrinsic bioremediation at landfill leachate polluted aquifers.[10] Thus, understanding the microbial functional diversity and the environmental factors shaping the microbial community structure is important not only for assessing the fate of specific components of contaminant

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plumes, but also for evaluating strategies for landfill bioremediation and management efforts.

The Norman Landfill (OK, U.S.) accepted municipal waste for more than 60 years until it was closed in 1985. Dissolved constituents of the leachate derived from dissolution and degradation of the buried waste material have leached into the underlying aquifer. Groundwater near this landfill contains high concentrations of DOC, DIC, NH_4^+, boron (B), chloride (Cl^-), dissolved ferrous iron (Fe^{2+}), and dissolved CH_4, and is depleted in sulfate (SO_4^{2-}).[9,11] Previous studies showed that anaerobic microbial activity was responsible for attenuating volatile organic compounds, derived from the landfill waste, along the predominant groundwater flow path.[9,12] These processes have been linked with the biogeochemical cycling of sulfate and iron and the formation of methane, which in turn affect the geochemistry in the aquifer.[9,11,13−16] However, relatively little is known about the diversity of functional genes, or the linkage between microbial community and ecosystem functioning near landfills.

Many methods have been used to investigate the microbial biodiversity of groundwater.[10,17−20] Although these approaches have provided important insights into the phylogenetic diversity and composition of subsurface microbial communities as well as their relationships with site geochemistry or specific pollutant compounds,[21−23] little is known about the functional diversity and metabolic potential of landfill leachate-contaminated microbial communities. Recently, functional gene array (e.g., GeoChip) technology has been proven to be a generic tool for profiling microbial communities from a variety of habitats,[24−27] such as contaminated groundwater,[28−30] marine sediments,[31] deep-sea hydrothermal vents,[32] and an oil plume from the Deepwater Horizon blowout.[33,34]

In this study, we hypothesize that the diversity of functional genes in the aquifer microbial community was altered by landfill leachate contamination, and such alternation is related to the distance from landfill source point and groundwater properties. To test this hypothesis, groundwater samples were collected from eight wells along a flowpath downgradient of the Norman Landfill, and analyzed by GeoChip 3.0. Our results indicate that the microbial community functional diversity tends to vary within the site as a function of the groundwater geochemistry variables and inherent spatial heterogeneity, and is significantly shaped by the groundwater geochemistry variables.

2. MATERIALS AND METHODS

2.1. Site Description. The Norman Landfill Environmental Research Site is a closed MSW landfill on the Canadian River alluvium in Norman, OK, U.S. The site (http://ok.water.usgs.gov/projects/norlan/) is currently part of the United States Geological Survey (USGS) Toxic Substances Hydrology Program (http://toxics.usgs.gov/) and has operated as an experimental site since the mid-1990s. The landfill is unlined, reflecting past disposal practices and leachate has contaminated a shallow aquifer.[11,35] This site has been geochemically and hydrologically characterized and the subject of many previous investigations (http://ok.water.usgs.gov/projects/norlan/pubs.html).[9,11,13,15,36,37]

2.2. Groundwater Sampling and Analysis. For geochemical and microbiological analyses, groundwater was collected by peristaltic pump at a depth of 5 m below ground surface from eight monitoring wells located along a groundwater flowpath (Figure S1, Supporting Information, SI). Specific conductance (EC), pH, and temperature (T) of groundwater were determined in the field using specific electrodes with thermocouples. Dissolved oxygen (DO), ammonium (NH_4^+), and hydrogen sulfide (H_2S) concentrations were determined in the field by using colorimetric CHEMets kits (CHEMetrics Inc., Calverton, VA). DOC concentrations were analyzed in the laboratory by high temperature combustion using a Shimadzu TOC-Vcsn Analyzer (Shimadzu Corporation). Dissolved methane (CH_4) concentrations in groundwater were measured in the laboratory by headspace analysis and gas chromatography using a molecular sieve capillary column and a 5890 Series II HP Gas Chromatograph with split/splitless inlet FID (flame ionization detector). Anion concentrations (Cl^-, NO_3^-, SO_4^{2-}) were measured in the laboratory by ion-exchange chromatography using a Dionex 120 Ion Chromatograph. Cation and trace element concentrations, including iron (Fe^{2+}), were determined on an inductively coupled plasma optical emission spectrometer and inductively coupled plasma mass spectrometer using a PerkinElmer ICPOES Optima 4300DV and a Perkin-Elmer ICPMS Elan 9000. Additional details on the geochemical methods can be found in the Supporting Information for Cozzarelli et al.[11] For GeoChip analysis, 5 L of groundwater from each well was filtered (0.45 μm; Millipore, Corp., Bedford, MA, U.S.) on the site, where upon the filters were immediately placed on dry ice, transferred to the laboratory, and stored at −80 °C prior to DNA extraction.

2.3. DNA Extraction, Amplification, and Labeling. High molecular weight DNA was extracted from the filters by the freeze-grind method[38] and then quantified with a NanoDrop spectrophotometer (ND-1000, Nanodrop Inc., DE, U.S.) and Quant-It PicoGreen (Invitrogen, Carlsbd, CA). Approximately 100 ng of DNA was amplified using a Templiphi kit (GE Healthcare, Piscataway, NJ, U.S.).[39] Amplified DNA (3.0 μg) from each sample was labeled and then purified for hybridization.

2.4. GeoChip Hybridization and Data Preprocess. GeoChip 3.0 was used for this study. [25] All hybridizations were carried out in triplicate on different modules at 45 °C for 10 h with 50% formamide using a TECAN HS4800 station (US TECAN, Durham, NC, U.S.) following a 45-min prehybridization step as previously described.[28,39] GeoChip scanning, and image processing were also as previously described.[25,31,39] Hybridization data are available at the Web site of the Institute for Environmental Genomics (http://ieg.ou.edu/4download/).

2.5. Statistical Analysis. Multivariate statistical analyses of preprocessed GeoChip data including Mantel test and CCA for linking microbial communities to environmental variables,[40] partial CCA for covariation analysis of well distance and environmental variables (variation partitioning analysis, VPA) were performed. Selection for CCA modeling was conducted by an iterative procedure of eliminating redundant environmental variable based on variance inflation factor (VIF). Mantel tests were performed by the vegan package in R 2.9.1.[41] CCA were performed using Canoco (version 4.5, Biometris-Plant Research International, The Netherlands).[42]

Geochemical factors were Z transformed to convert all measurements to the same scale.[43] The analysis was focused on interspecies distances, and significance was tested using the Monte Carlo permutation (999 times). Hierarchical clustering was performed with CLUSTER 3.0 using uncentered correlations and the complete average linkage for both genes and samples, and trees were visualized in TREEVIEW.[44]

3. RESULTS

3.1. Site Geochemical Properties. Groundwater geochemical parameters are detailed in Tables 1 and S1 of the SI. The entire flowpath was suboxic (DO < 0.30 mg/L) and concentrations of many geochemical constituents varied in the wells. Well LF2B, immediately downgradient from the landfill, had the highest groundwater specific conductance, CH_4, NH_4^+, and DOC levels relative to the other wells. In contrast, the most distal downgradient well MLS80, had the lowest specific conductance, CH_4, NH_4^+, and DOC values while all wells had comparable levels of Cl^- suggesting that dilution alone does not account for losses of some leachate constituents along this flowpath. The relatively high SO_4^{2-} concentrations in the MLS80 well are consistent with previous observations in areas downgradient from the landfill.[11,14] Similarly, CH_4 concentrations in the five wells north of the wetland (referred to as a slough, Figure S1 of the SI) were significantly ($p < 0.05$) higher than those of three further downgradient wells. The concentration of DOC showed a significant negative correlation with distance ($r = -0.86$, $p < 0.01$), suggesting that natural attenuation of organic carbon may occur. Other geochemical variables including EC, concentrations of DO, NH_4^+, NO_3^-, Cl^-, SO_4^{2-}, and H_2S did not show a significant correlation with distance from the landfill although the values of those constituents from the three wells to the south of the slough were notably different from the five wells to the north of the slough. The geochemical results indicate that there were substantial changes in leachate contaminants and other geochemical parameters in the groundwater along the predominant flowpath.

3.2. Functional Gene Diversity. The number of functional genes and their abundance (signal intensity) detected were used to calculate the Shannon–Weiner index (H'), Simpson's index ($1/D$), and Simpson Evenness index (E). The overall microbial functional diversity was significantly ($p < 0.01$) lower in well LF2B, immediately below the landfill, and its closest neighboring well (MLS35) than that in the other wells based on H', $1/D$ and richness (S). However, the evenness of all samples did not show a significant difference as all wells fell within a relatively narrow range, 0.48 to 0.60. The well MLS38, had the greatest percentage of unique genes (28.1%), while LF2B had the fewest (7.9%). The well MLS36 and MLS37, in the vicinity of the slough, had the most overlapped genes (710, 38.8%), while LF2B and MLS54 had the fewest (349, 20.2%) (Table 2). All wells had higher percentages of genes detected in the categories of organic remediation (36.8–42.4%) and metal resistance/reduction (23.1–25.1%) relative to the percentages of these gene probes (31% and 18%, respectively) designed. The relative abundances of the other gene categories detected were similar among all wells although slight differences in the diversity of functional genes among wells were observed (Table S2 of the SI).

Hierarchical clustering analysis of functional genes reflected the patterns of microbial community structure in the wells (Figure 1). The eight samples could be separated into three clusters: (i) two wells (LF2B and MLS35) directly downgradient of the landfill, (ii) five further downgradient wells (MLS36, MLS37, MLS54, MLS55, and MLS80), and (iii) one well (MLS38) closest to the slough (Figure 1a). The results suggest that the response of key functional populations to landfill contaminants may be differentially reflected by those clusters. Also, all detected genes could be clustered into eight

Table 1. Geochemistry Variables Measured in Groundwater from Each Monitoring Well[a]

sample wells	dis[b] (m)	T (°C)	EC[c] μS/cm	pH	DO[d] (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	H_2S (mg/L)	SO_4^{2-} (mg/L)	DOC[e] (mg/L)	CH_4 (mg/L)	Cl^- (mg/L)
LF2B	0.00	23.0	5675	6.73	0.20	146.53	ND	0.16	0.4	114.9	15.07	620.8
MLS35	47.24	16.7	4766	6.68	0.25	90.83	<0.5	0.05	0.3	95.3	11.26	617.7
MLS36	79.97	15.7	4284	6.70	0.17	44.62	<0.5	0.05	0.2	77.8	15.80	466.4
MLS37	101.70	16.3	5286	6.77	0.08	190.21	<0.5	0.05	1.0	101.4	10.15	545.0
MLS38	138.13	ND	4853	6.90	0.10	146.23	<0.5	0.06	1.7	98.1	5.70	649.0
MLS54	188.84	15.3	4440	6.70	0.15	22.48	<0.5	0.03	0.6	76.0	3.63	609.4
MLS55	220.54	15.4	5548	7.00	0.30	40.78	<0.5	0.08	7.7	66.5	0.16	648.1
MLS80	251.98	16.2	3921	6.72	0.03	7.53	0.7	0.04	32.0	59.7	0.12	662.1

[a]ND, no data collected. See Table S1 in the SI for more information about the levels of contaminants in the groundwater wells. [b]Distance from the landfill center. [c]Electric conductivity. [d]Dissolved oxygen. [e]Dissolved organic carbon.

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Table 2. Gene Overlap (Italicized), Uniqueness (Bold) and Diversity Indices of Landfill Samples

well	LF2B	MLS35	MLS36	MLS37	MLS38	MLS54	MLS55	MLS80
LF2B	**36 (7.9%)**	*249 (31.8%)*	*339 (24.6%)*	*363 (26.4%)*	*299 (24.5%)*	*349 (20.2%)*	*319 (25.3%)*	*287 (24.5%)*
MLS35		**70 (12.2%)**	*387 (26.7%)*	*395 (27.1%)*	*311 (23.5%)*	*416 (23.4%)*	*343 (25.4%)*	*324 (25.9%)*
MLS36			**201 (15.9%)**	*710 (38.8%)*	*509 (28.1%)*	*790 (37.7%)*	*582 (32.3%)*	*525 (30.2%)*
MLS37				**235 (18.4%)**	*547 (30.5%)*	*764 (35.8%)*	*582 (32.0%)*	*536 (30.8%)*
MLS38					**298 (28.1%)**	*546 (25.6%)*	*479 (28.2%)*	*413 (25.1%)*
MLS54						**426 (26.3%)**	*623 (29.4%)*	*561 (27.3%)*
MLS55							**265 (23.7%)**	*471 (28.6%)*
MLS80								**229 (22.9%)**
diversity indices								
H^a	5.88	6.12	6.89	6.90	6.66	7.11	6.74	6.62
$1/D^b$	266.26	344.97	710.81	714.72	510.97	884.61	555.82	537.85
E^c	0.58	0.60	0.56	0.56	0.48	0.55	0.50	0.54
richness[d]	458	574	1262	1280	1060	1621	1120	999

[a]Shannon–Weaver Weiner index, higher number represents higher diversity. [b]Reciprocal of Simpson's index, higher number represents higher diversity. [c]Simpson Evenness index. [d]Detected gene number.

major groups (Figure 1b). Functional genes for organic contaminant remediation were detected in all wells but predominantly in group 5. These genes are generally involved in the metabolism of aromatic chemicals, chlorinated solvents, and pesticide-related compounds. The diversity indices and clustering analysis revealed that landfill leachate affected subsurface microbial functional diversity, composition, and structure along the groundwater flowpath, but the influence of the slough may superimpose some conditions that allow the patterns to appreciably deviate. Further analyses are focused on functional genes involved in organic contaminant degradation, and geochemical cycling of C, N, and S.

3.3. Genes Suggesting Organic Contaminant Degradation. Biodegradation of organic contaminants (e.g., aromatics, pesticides, pharmaceuticals) in landfill leachate is one of major processes that attenuate these compounds as leachate moves into the downgradient aquifer. A total of 176 to 535 organic remediation genes involved in the biodegradation of a myriad of contaminants were detected in each well along the flowpath. Aromatic compounds are common landfill constituents[45] and 127 to 476 genes implicated in the metabolism of these substrates were detected in various wells. Since oxygen levels at this site are generally low, examples are focused on genes involved in anaerobic degradation of organic contaminants. That is, the anaerobic metabolism of aromatic acids is implicated by the detection of *bclA* gene (benzoate-coenzyme A ligase) in all wells. The gene *bbs* (β-oxidation of benzylsuccinate) derived from *Thauera aromatic* and *tutFDG* (benzylsuccinate synthase) from *Aeromonas hydrophila* subsp. hydrophila ATCC 7966, *Desulfobacterium cetonicum* and uncultured bacterium for anaerobic BTEX and related aromatics degradation showed high abundance in the wells. Bacteria metabolizing aromatic amino acids are also implicated by the detection of *hmgABC* genes. These genes showed a high abundance in wells MLS36, MLS37, MLS38, MLS54, and MLS55, but a low abundance in wells LF2B, MLS35, and MLS80, a pattern that is similar to the distribution of *mdlABC* involved in the mandelate metabolic pathway. Genes involved in the degradation of polycyclic aromatic hydrocarbons were also detected.

Pesticides/herbicides are relatively common landfill leachate constituents.[46] In a consistent fashion, 172 genes involved in the degradation of pesticide- or herbicide-related compounds showed positive hybridization signals. The gene *tfdA* (α-ketoglutarate-dependent 2,4-D dioxygenase) for the metabolism of phenoxyacetic acids herbicides were detected in all wells. The atrazine catabolic genes (*atzABC*, *trzN*, *trzA*, and *trzE*) and gene *phn* (C−P lyase) responsible for degrading organophosphonates were also detected with a high abundance. The gene *linB* (haloalkane dehalogenase) for the initial transformation of β-hexachlorocyclohexane, one of the most extensively used organochlorine pesticides during the 1940s[47] was detected in wells MLS36, MLS54, and MLS55.

3.4. Genes Suggesting Carbon Cycling. The DOC leaching from the Norman Landfill is a highly heterogeneous mixture of organic components such as lignin, cellulose, and hemicelluloses that are derived from the dissolution and degradation of the buried waste.[9,48] Other major carbon components in the groundwater are CH_4 and DIC. A total of 459 genes implicated in the cycling of these compounds were detected and genes for carbon fixation, methane oxidation, and methane production showed positive hybridization signals. For carbon cycling, the α-amylase (*amyA*) was most closely related

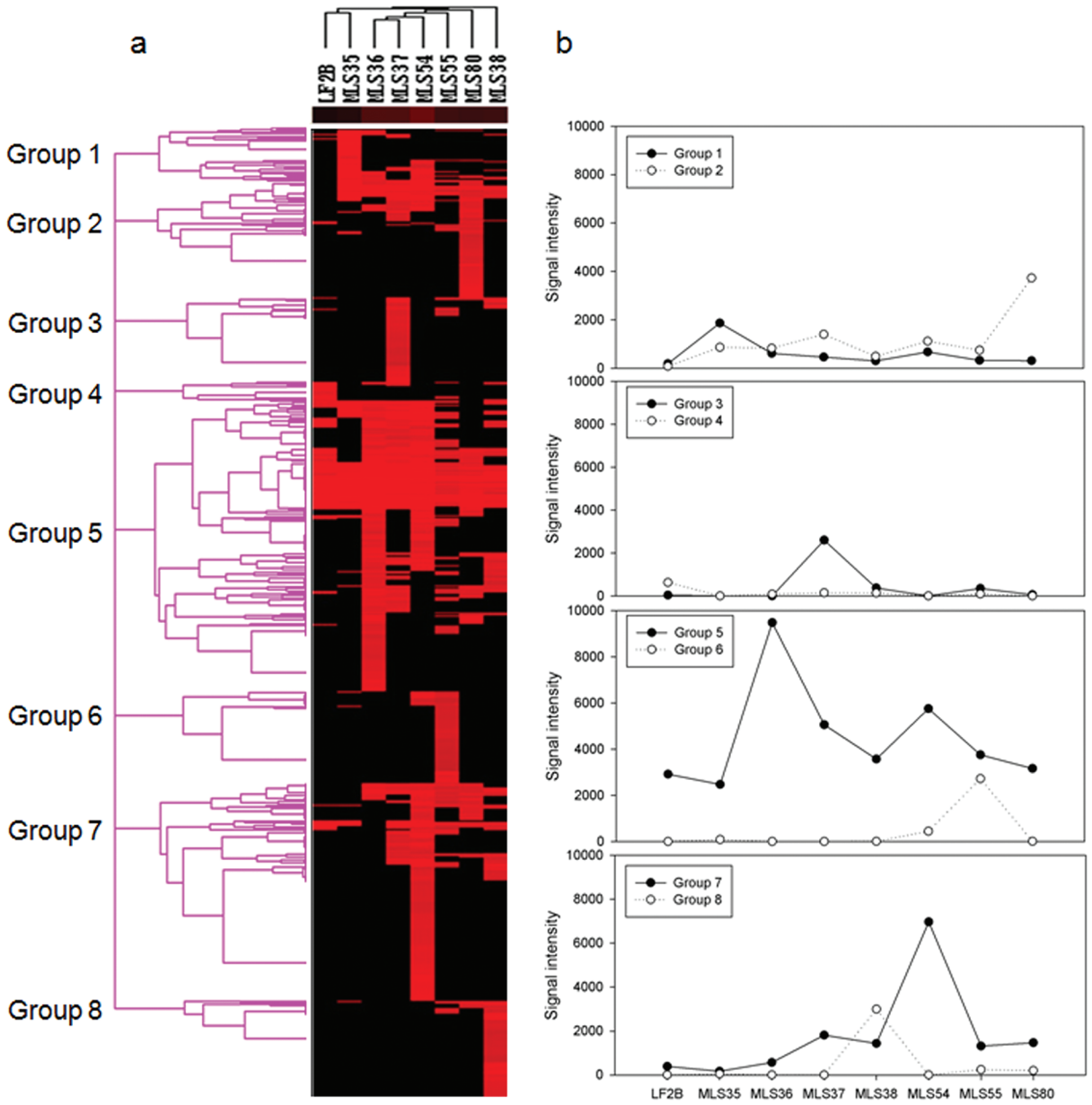

Figure 1. Hierarchical cluster analysis of all genes detected by GeoChip3.0. The figure was generated using CLUSTER and visualized in TREEVIEW. Black indicates signal intensities below the threshold value, while red indicates positive hybridization signals. The color intensities indicate differences in hybridization signal intensity. The well LF2B and MLS35, nearer to the center of the landfill, clustered together, while the wells MLS36, MLS37, MLS54, MLS55, and MLS80, in the direction of the downgradient plume, grouped together, and the well MLS38, nearer to the slough, was not obviously clustered. Eight gene patterns were observed and indicated by numbers in the tree (a), and also illustrated in the graphs (b).

to *Parvularcula bermudensis* HTCC2503. Similarly, other genes including *limC* (dichlorophenolindophenol-dependent carveol dehydrogenase), *mnp* (lignin peroxidase isozyme), and *endochitinase* (Chitinase) were detected in all samples (Figure S2 of the SI).

A total of four methane monooxygenase genes (*pmoA*) were detected with representatives of *Methylosinus trichosporium* and *Methylococcaceae bacterium* IT-4, but most (93.8%) were from uncultured bacteria. Also, six methyl coenzyme-M reductase (*mcrA*) genes for methanogenesis were detected with representatives of *Methanocorpusaclum labreanum* and *Methanoculleus marisnigri* particularly enriched in MLS37 (Figure S3 of the SI).

3.5. Genes Suggesting Sulfur Cycling. A total of 17 to 61 *dsrA/B* genes for sulfite reduction and 7 to 17 *sox* genes for sulfide oxidization were detected in all wells (Figure S5 and S6 of the SI). Specifically, *dsrA* genes derived from *Desulfovibrio aerotolerans*, *Chlorobium phaeobacteroides* DSM 266, and *Pyrobaculum calidifontis* JCM 11548 were detected in all wells, but most (65−88%) of them were from uncultured microorganisms. In a similar manner, *sox* genes derived from *Methylobacterium populi* BJ001, *Methylobacterium* sp. 4−46, and *Pelodictyon phaeoclathratiforme* were detected in all samples and serve as a functional marker for sulfite oxidation.

3.6. Relationships between Microbial Communities and Environmental Parameters. Statistical analyses were performed to determine how major environmental parameters

Table 3. Significant Relationships ($p < 0.1$) of Key Functional Genes to Different Concentration of Environmental Factors[a]

gene category	gene name	coded enzyme	no. of detected sequences	dominated genus	environmental factors	r_M	p
C cycling	*pmoA*	methane monooxygenase	27	uncultured bacteria; *Methylococcaceae*; *Methylosinus*	NH_3N, CH_4, conductivity, DO	0.316	0.094
					NH_3N, CH_4, DO	0.392	**0.034**
					CH_4, DO	0.375	0.061
					DO	0.421	**0.024**
					pH, DO	0.347	0.098
	cellobiase	cellobiase	16	*Bradyrhizobium*; *Opitutaceae*; *Pseudomonas*; *Shewanella*	NH_3N, DOC, DO	0.360	0.056
					NH_3N	0.334	**0.038**
	pectinase	pectinase	9	uncultured bacteria; *Aspergillus*; *Colletotrichum*; *Klebsiella*; *Penicillium*	Cl^-	0.442	0.053
	mnp	manganese peroxidase	7	*Phanerochaete*; *Trametes*; *Ceriporiopsis*	SO_4^{2-}	0.347	**0.049**
	vanA	vanillate demethylase	14	*Sphingomonas*; *Verminephrobacter*; *Chromohalobacter*; *Marinomonas*	Cl^-	0.379	**0.048**
					pH	0.375	0.062
S cycling	*dsrB*	sulfite reductase	43	uncultured bacteria; *Desulfobacter*; *Desulfovibrio*; *Pyrobaculum*	DO	0.384	0.057
					pH, DO	0.476	0.072
					SO_4^{2-}, pH, DO	0.470	0.076
	sox	sulfur oxidation	31	*Bradyrhizobium*; *Paracoccus*; *Rhodopseudomonas*; *Roseobacter*	pH	0.566	**0.014**
N cycling	*nasA*	nitrate reductase	15	uncultured bacteria; *Acinetobacter*; *Janthinobacterium*; *Novosphingobium*	DO	0.399	**0.027**
					pH	0.393	0.077
	nrfA	dissimilatory N reductase	18	*Geobacter*; *Shewanella*; *Actinobacillus*; *Yersinia*	SO_4^{2-}	0.509	0.069
P cycling	*ppk*	polyphosphate kinase	20	uncultured bacteria; *Aeromonas*; *Bordetella*; *Flavobacterium*	pH	0.390	0.060
Organic remediation	*nagI*	gentisate 1,2-dioxygenase	15	*Sphingomonas; Magnaporthe; Aspergillus*	DOC	0.324	0.068
					SO_4^{2-}	0.741	0.089
					NH_3N	0.317	0.074
	narG	salicylate hydroxylase	28	*Aspergillus*; *Burkholderia*; *Pseudomonas*; *Ralstonia*; *Rhodopseudomonas*	SO_4^{2-}	0.548	**0.012**
					DOC	0.365	**0.029**
					CH_4	0.329	0.054
					HCO_3^-	0.382	**0.045**
	ohbAB	halobenzoate dioxygenase	6	*Ralstonia; Pseudomonas; Sphingomonas;*	conductivity	0.350	**0.044**
	chnB	cyclohexanone 1,2-monooxygenase	13	*Mycobacterium; Methylobacterium*; *Burkholderia*; *Parvibaculum*	CH_4, Cl^-, HCO_3^-	0.313	0.068
					Cl^-, HCO_3^-	0.378	**0.049**
					Cl	0.458	**0.022**
					DOC, Cl^-	0.422	**0.030**
	dehH	haloacetate dehalogenase	14	*Roseobacter*; *Ralstonia*; *Streptomyces*	Cl^-	0.496	**0.048**
	PhaB	acetoacetyl-CoA reductase	23	*Bordetella*; *Caulobacter*; *Halorhodospira*; *Nitrobacter*	Cl^-	0.442	**0.038**
					pH	0.314	0.079
	proO	protocatechuate dioxygenase	28	*Azoarcus*; *Burkholderia*; *Chromohalobacter*; *Klebsiella*; *Marinomonas*; *Pseudoalteromonas*	Cl^-	0.593	**0.042**
	todC	toluene dioxygenase	8	uncultured bacteria; *Arthrobacter*; *Thauera*	Cl^-	0.464	**0.017**
	xylF	2-hydroxymuconate semialdehyde hydrolase	10	*Gloeobacter*; *Pseudomonas*; *Synechococcus*; *Ralstonia*	HCO_3^-	0.509	**0.049**
					DO	0.434	**0.036**
					conductivity	0.363	**0.044**
					CH_4	0.249	0.063
					$SO4_2^-$	0.841	**0.003**

[a]The values in this table are the p values determined by Mantel test. Any p value <0.05 is bolded.

influence microbial community and ecosystem functioning. Mantel tests showed that the abundance of many functional genes was significantly correlated with the environmental parameters. For instance, *nagG* was correlated with NH_3–N concentration (r_M = 0.42, $p < 0.05$), *dsrB* with SO_4^{2-} concentration and pH (r_M = 0.47, $p < 0.05$), *sox* with pH (r_M= 0.57, $p < 0.05$), and *pmoA* with CH_4 and DO (r_M = 0.42, $p < 0.05$) (Table 3).

CCA was used to determine the most significant environmental variables to shape the community structure. On the basis of VIFs, six variables including pH, SO_4^{2-}, DO, NH_3–N, DOC, and specific conductance were selected to perform CCA according to the signal intensity of all detected functional genes. The specified CCA model explained 39.6% of the total variance with 2.092 for the sum of all eigenvalues, which was significant (p = 0.022) (Figure 2a). On the basis of the relationship between the environmental variables and the microbial functional structure, many functional genes (e.g., *dsrA/B*, *sox*) detected in this system may depend on sulfate concentrations, which is consistent with previous studies that showed sulfate

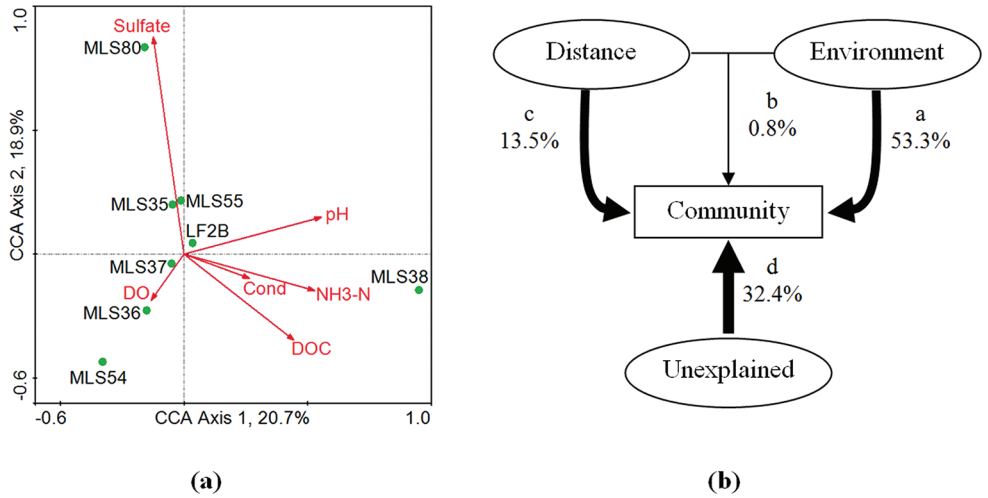

Figure 2. (a) Canonical correspondence analysis (CCA) compares the GeoChip hybridization signal intensities (symbols) and groundwater environmental variables (arrows). Environmental variables were chosen based on significance calculated from individual CCA results and variance inflation factor (VIF) calculated during CCA. The percentage of variation explained by each axis is shown, and the relationship is significant (p = 0.022). (b) Variation partitioning based on CCA for all functional gene signal intensities. A CCA-based VIF was performed to identify distance to the landfill and environmental variables important to the microbial community structure. Environmental variables included pH, SO_4^{2-}, DO, NH_3–N, DOC, and specific conductance.

was one of the most important environmental factors in this system.[14−16,37,49] The microbial community structure of well MLS80 with the highest SO_4^{2-} concentration, the lowest conductivity, and DOC levels was positively correlated with SO_4^{2-} concentration and negatively correlated with conductivity and DOC, while that from wells MLS36 and MLS37 were correlated with DO.

To assess contributions of groundwater environmental properties as a function of position in the transect (i.e., distance from the landfill) to the microbial community structure, VPA was performed (Figure 2b). Environmental variables and distance from the landfill showed a significant correlation (p = 0.034) with the community structure. Distance was able to independently explain 13.5% of the variation observed while environmental variables explained 53.3%. About 32.4% of the community variation based on GeoChip data remained unexplained by the selected variables. These results suggest that environmental conditions and spatial heterogeneity greatly influenced the functional structure of aquifer microbial communities in the Norman Landfill.

4. DISCUSSION

The unlined Norman Landfill is located on a river alluvium and releases leachate to an underlying shallow aquifer. This study comprehensively examined the functional gene diversity and metabolic potential of landfill microbial communities and the linkages between the microbial community structure and predominant environmental variables. Specially, we showed that the functional gene diversity of indigenous aquifer microbial communities changed in response to the chronic leachate exposure and as a function of distance along the predominant groundwater flowpath, and such changes were closely correlated with groundwater pH, and concentrations of sulfate, ammonia and DOC, which generally supports our hypotheses.

The Norman Landfill contains a mixture of contaminants, such as organic compounds (e.g., aromatics, aromatic hydrocarbons) and heavy metals. Also, such landfill waste is generally enriched with carbon (e.g., DOC) and nitrogen (e.g., NH_4^+). Previous studies revealed that some contaminants moved through the aquifer with little attenuation, whereas others were attenuated by various processes including sorption and biogeochemical interactions with aquifer solids. Dynamic hydrologic and chemical conditions at the interfaces between the contaminant plume and overlying recharge water and at a discharge interface in a shallow slough have resulted in substantial geochemical gradients in this system,[9,11,13,15,36,37] which are generally consistent with this study. It is hypothesized that such environmental changes affect aquifer indigenous microbial communities and their functions. To test this, we used community DNA to measure the metabolic potential of microbial communities by the abundance change of key functional genes and their associated populations along the flowpath. Although the functional activity is better examined by microbial community mRNA, due to the limited amount of biomass obtained from each sample, we could not use mRNA for GeoChip hybridization in this study. Also, the detection of functional activity with mRNA from environmental samples currently presents a number of challenges, such as low abundance, rapid turnover, and instability.[24]

Environmental factors may have fundamental impacts on the functional diversity of microbial communities in landfill leachate contaminated aquifers. A previous study demonstrated that environmental factors significantly influenced methanogenesis in this landfill site.[50] Another study showed that the level and type of pollution in the landfill were correlated with *Geobacteraceae* community composition.[51] In this study, the functional gene diversity is lower beneath the landfill and directly downgradient, while no significant differences were observed in other locations. No significant differences were observed in the number of detected functional gene categories across the locations. Functional genes categorized wells into three distinct groupings, and eight gene groups were identified as important in the array, of which a handful of these genes are correlated to specific geochemical analytes. The other multivariate statistics (CCA, VPA) appear to support these general findings. In addition, the slough may have great impact on the hydrologic and chemical conditions at the interfaces between

the plume and overlying recharge water,[9] leading to different patterns of microbial communities for the wells closet to the slough.

This study comprehensively surveys major microbial functional processes that may occur in Norman leachate-contaminated aquifers. The landfill leachate contaminated aquifers have a high concentration of DOC, especially those with highly branched, cyclic aliphatic organic compounds.[48] Previous studies showed that indigenous microorganisms were responsible for the degradation of organic compounds along the flow path.[9,12,14,52] Consistently, a large number of functional genes (e.g., *bclA, tutFDG, bbs, hmgABC, tfd, rd*) involved in the degradation of those contaminants were detected, indicating that indigenous microbial communities are functionally diverse and may play an important role for bioremediation of those contaminants. Sulfate reduction and methanogenesis have shown to be predominant processes in leachate contaminated aquifer systems.[7,9,11,14,16,50] Indeed, a high diversity of *dsrA/B* and *mcrA* genes were detected in this study. Numerous investigators have documented the importance of S cycling in landfill systems.[14−16,37,49] For example, at the boundaries of the contaminant plume, such as the saturated/unsaturated zone interface, oxidation of iron sulfides often occurs.[15,37] Our results are consistent with previous studies, and indicate S cycling and methanogenesis are among most important functional processes of leachate-impacted aquifer microbial communities in the Norman Landfill. Therefore, GeoChip analysis of key functional processes generally supports previous studies, and also extends our understanding of the functional gene diversity, especially for sulfate reduction, methanogenesis, and carbon and organic contaminant degradation, in landfill contaminated aquifer systems.

Due to low levels of oxygen in all wells, biodegradation of leachate contaminants in this system may be largely dependent on the utilization of alternate soluble and insoluble electron acceptors like SO_4^{2-}.[9,11] Among all detected genes and associated microorganisms, many require oxygen as a cosubstrate, raising a question if such microbial microorganisms are really active in such an environment where oxygen is typically low. Several explanations may address this question. First, some microorganisms are facultative and can grow and function at aerobic or anaerobic conditions.[53] Second, some aerobes may survive in low or no oxygen conditions although they may not be active. Third, aerobic or facultative microorganisms were widely detected in groundwater samples, which were assumed to be anaerobic, such as coal-tar waste contaminated groundwater[18] and the leachate plume of Banisveld landfill.[54] In addition, even the same organism could perform both anaerobic and aerobic functions.[55] This study focuses on the functional diversity and potential of landfill leachate contaminated aquifer microbial communities. Hopefully, the results can lead to more hypothesis-driven studies, such as the detection of functional activity using metatranscriptomics or microarray approach,[56−58] and the detection of active microorganisms using stable isotope probing (SIP) method.[59]

■ ASSOCIATED CONTENT

Supporting Information

Additional methods description and figures/tables. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (405) 325-6073; fax: (405) 325-7552; e-mail: jzhou@ou.edu.

Notes

The authors declare no competing financial interest.

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