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Enhanced detection of groundwater contamination from a leaking waste disposal site by microbial community profiles

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[1] Groundwater biogeochemistry is adversely impacted when municipal solid waste leachate, rich in nutrients and anthropogenic compounds, percolates into the subsurface from leaking landfills. Detecting leachate contamination using statistical techniques is challenging because well strategies or analytical techniques may be insufficient for detecting low levels of groundwater contamination. We sampled profiles of the microbial community from monitoring wells surrounding a leaking landfill using terminal restriction fragment length polymorphism (T-RFLP) targeting the 16S rRNA gene. Results show in situ monitoring of bacteria, archaea, and the family Geobacteraceae improves characterization of groundwater quality. Bacterial T-RFLP profiles showed shifts correlated to known gradients of leachate and effectively detected changes along plume fringes that were not detected using hydrochemical data. Experimental sediment microcosms exposed to leachate-contaminated groundwater revealed a shift from a β-Proteobacteria and Actinobacteria dominated community to one dominated by Firmicutes and δ-Proteobacteria. This shift is consistent with the transition from oxic conditions to an anoxic, iron-reducing environment as a result of landfill leachate-derived contaminants and associated redox conditions. We suggest microbial communities are more sensitive than hydrochemistry data for characterizing low levels of groundwater contamination and thus provide a novel source of information for optimizing detection and long-term monitoring strategies at landfill sites.

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1. Introduction

[2] Inadequate waste disposal practices over the past century have resulted in disposal sites cited as a significant source of groundwater contamination in both the United States and Europe [*U.S. Environmental Protection Agency* (*EPA*), 2000; *European Environmental Agency*, 2003]. As recently as the 1980s, landfills designated to accept municipal and commercial solid wastes in the United States could be unlined and sited without consideration to site hydrogeology and were often subject to industrial or hazardous waste dumping. As a result, municipal solid waste landfill (MSWLF) leachates percolating into the subsurface have impacted groundwater resources by discoloration, adverse smell, increased loading of carbon and nitrogen nutrients, elevated anion and cation species, and the leaching of various trace metals or anthropogenic organic compounds that adversely affect public and environmental health (e.g., arsenic, benzene, and trichloroethene) [*Christensen et al.*, 1994; *Kjeldsen et al.*, 2002].

[3] The first line of protection between MSWLFs and the subsurface environment is groundwater monitoring wells placed strategically up-gradient and down-gradient of disposal activities. Regular sampling of the detection well network and statistical methods are used to track changes in physiochemical water quality parameters during the landfill's active life and a 30 year postclosure period [*EPA*, 1993, 2009]. Unfortunately, the variability of leachate composition and its associated water quality impacts [*Gibbons et al.*, 1999; *Kjeldsen et al.*, 2002] necessitate the monitoring of dozens of parameters during this time. In addition, detection efficiency is lower if numbers of sentinel wells or their spatial distribution are insufficient relative to site hydrogeologic characteristics [*Hudak*, 1999, 2005]. As a result, contamination may spread over a larger area prior to its detection or characterization, reducing remediation efficiency and increasing remedial and long-term monitoring costs [*Massmann and Freeze*, 1987].

[4] Methods to improve the detection efficiency of groundwater-monitoring networks have primarily focused

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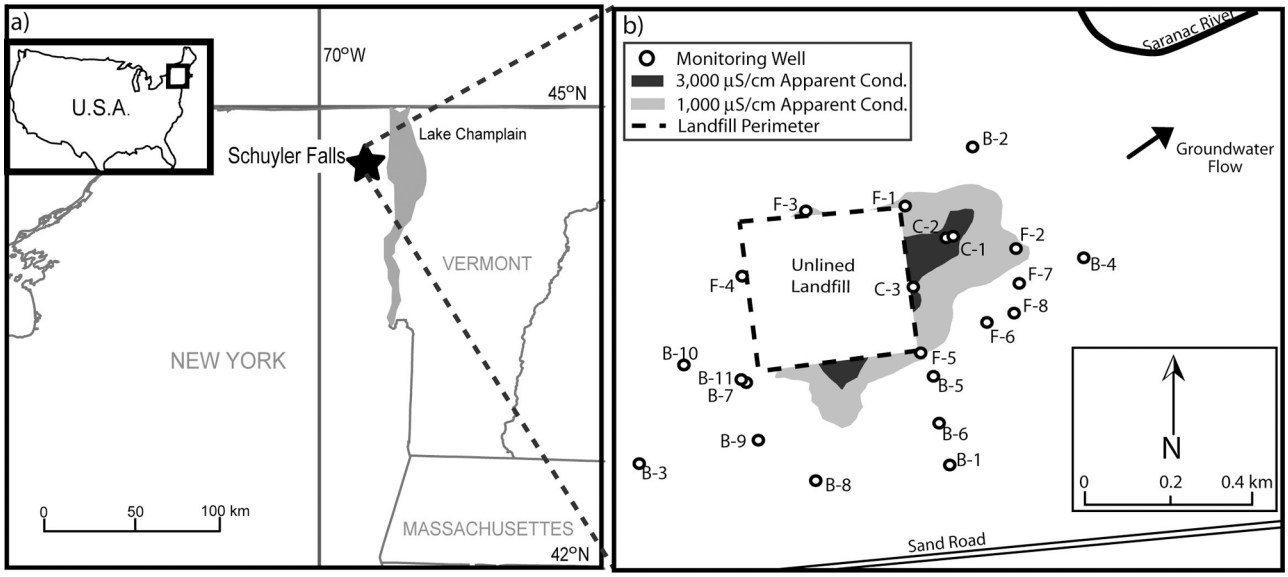

Figure 1. (a) Location of Schuyler Falls in northeastern New York. (b) Plan view of Schuyler Falls landfill indicating the monitoring well locations, the direction of groundwater flow, and the extent of the landfill leachate plume based on electromagnetic surveys. B, background; F, fringe; C, contaminated.

on physical and chemical properties of aquifer systems, such as better characterization of subsurface heterogeneities [*Meyer et al.*, 1994], modifications to traditional statistical methods for water quality trend detection [*Gibbons*, 1996], and optimization of well network design using computational methods [*Loaiciga et al.*, 1992; *American Society of Civil Engineers*, 2003; *Reed and Minsker*, 2004]. One area that is not well researched is the improvement of detection sensitivity in existing monitoring well networks through the incorporation of biological data sources such as microbial community composition.

[5] Groundwater microbial ecology is strongly influenced by subsurface biogeochemical processes [*Anderson and Lovley*, 1997; *Chapelle*, 2000; *Griebler and Lueders*, 2009] and, in particular, the presence and concentration of landfill leachate-derived nutrients [*Beeman and Suflita*, 1987; *Barlaz et al.*, 1989; *Ludvigsen et al.*, 1999; *Cozzarelli et al.*, 2000; *Röling et al.*, 2001]; therefore, the relative distribution or patterns of microorganisms associated with leachate composition should be indicative of its spatiotemporal impact in the subsurface. Molecular genetic techniques now allow rapid description of the microbiological community in subsurface environments [*Madsen*, 2000]. These approaches have been successfully applied to the broad characterization and biodegradation potential of the microbial ecology in shallow contaminated groundwater aquifers [*Watanabe et al.*, 2000; *Lovley*, 2003; *Weiss and Cozzarelli*, 2008; *Mouser et al.*, 2009]. One challenge to using molecular genetic-based microbial information for improving the detection of leachate pollution is the large amount of multivariate data generated from a discrete sample and the incorporation of these data into traditional statistical methodologies.

[6] The objective of this work was to explore the use of molecular genetic data generated by targeting the 16S rRNA gene of two broad prokaryote groups, archaea and bacteria, and the dissimilatory iron-reducing bacterial family Geobacteraceae [*Lovley et al.*, 1993; *Caccavo et al.*, 1994; *Snoeyenbos-West et al.*, 2000; *Holmes et al.*, 2007] for detecting the onset of landfill leachate contamination using multivariate statistical methods. At the field scale, groundwater microbial community profiles provided increased sensitivity for detecting leachate contamination along a known plume fringe over hydrochemical information alone. Experimental sediment microcosms confirmed an association between leachate composition and the increased abundance of certain bacterial community members. Our results suggest that combining molecular genetic data from bacterial communities with a limited set of biogeochemical parameters may be one way to improve detection sensitivity and efficiency of groundwater-monitoring strategies at MSWLF sites.

2. Background and Methods

2.1. Study Area Hydrogeology and Plume Delineation

[7] The Schuyler Falls Sanitary Landfill is an unlined, 30 acre waste disposal site in Clinton County, New York (Figure 1), that received municipal, commercial, and industrial wastes between 1977 and 1996 [*Barton and Loguidice*, *Personal Consultants* 1996]. The landfill is located in deltaic sands and silt that are underlain by Pleistocene age till and outwash soils deposited over dolomite bedrock [*Fisher*, 1968; *Denny*, 1974; *Barton and Loguidice*, *Personal Consultants*, 1996]. Surface soil depths range from 15 to 40 m from west to east. Clinton County receives a mean annual rainfall of about 90 cm. Average rates of advective groundwater transport in the deltaic unit are on the order of 25 m/yr northeast toward the Saranac River (Figure 1b) and eventually to Lake Champlain.

[8] The detection of anthropogenic organic compounds in down-gradient monitoring wells prompted hydrogeologic investigations and a detailed EM-34 electromagnetic survey that revealed leachate contamination in surficial soils and groundwater toward the east and south of

the landfill (Figure 1b) [*Barton and Loguidice*, *Personal Consultants*, 1996]. We used the apparent conductivity data from the EM-34 geophysical survey to characterize the degree of spatial correlation and to estimate the extent of subsurface contaminated by leachate. Experimental semivariograms were produced for 10 and 20 m horizontal dipole (HD) and 10 and 20 m vertical dipole (VD) EM surveys (664 total survey points). Semivariogram models were best fit to the experimental data using the nonlinear model fitting function in JMP version 5.1 (SAS Institute Inc., Cary, North Carolina). Values of apparent conductivity for each of the four surveys were estimated across a 15 m regularly spaced grid of the site using the method of ordinary kriging in MATLAB version 6.1 software (MathWorks Inc., Natick, Massachusetts) [*Journel and Huijbregts*, 1978; *de Marsily*, 1986; *Goovaerts*, 1997]. A conservative parameter field of leachate contamination was produced by selecting the maximum apparent conductivity for each grid cell from the four survey estimates. Although geophysical surveys are generally not conducted at MSWLF sites unless evidence suggests a leak has occurred or for the purpose of characterizing dump perimeters (e.g., when historic boundary data are unavailable), the high-resolution geophysical data available at this site were used to classify groundwater-monitoring locations into three management "zones" of interest (background (labeled B), fringe (labeled F), and contaminated (labeled C) as shown in Figure 1b) and to validate microbial community composition results.

2.2. Groundwater Sampling

[9] Groundwater samples were obtained from the 22 monitoring wells shown in Figure 1b using dedicated bailers or bladder pumps. Samples were purged according to standard practices, and field measurements (temperature, turbidity, oxidation reduction potential (ORP), pH, and conductance) were assessed. Groundwater samples for hydrochemical analysis were placed immediately on ice and transported to Columbia Analytical Services (Rochester, New York) for analysis of Appendix IX constituents [*EPA*, 1993]. Samples for microbial community analysis were collected in 500 mL HDPE bottles, placed on ice, and transported to the University of Vermont where they were pelleted by centrifugation at 20,000 rpm, flash frozen, and stored at −20°C until extraction.

2.3. Experimental Microcosm Construction and Sampling

[10] Microcosms were constructed with deltaic sand and silt soils collected from 8 feet below ground surface and stored on ice [*Denny*, 1974; *Barton and Loguidice*, *Personal Consultants*, 1996]. Within 12 h, 40 g of soil was mixed with 50 mL of groundwater that was collected from a background (B-1) or a leachate-contaminated well (C-1, Figure 1b). Microcosms were developed in duplicate with six groundwater mixtures, 0% (all from B-1), 5% (2.5 mL from C-1 combined with 47.5 mL from B-1 groundwater, etc.), 10%, 25%, 50%, and 100%. After 14 days of incubation at 25°C, microcosms were sampled as described below for biogeochemical parameters and microbial community analysis.

[11] Analysis of redox chemistry was conducted using a mercury drop electrode. Under anaerobic gassing (80% N_2:20% CO_2), 10 mL of groundwater was collected and measured for redox species O_2, Mn^{2+}, Fe^{2+}, and $FeS_{(aq)}$ using methods described previously [*Druschel et al.*, 2008]. Sediments and pore water (approximately 5 g) were sampled for microbial community analysis using presterilized spatulas, flash frozen, and stored at −20°C. Groundwater (25–40 mL) was collected for analysis of cations and nitrogen species (NH_4^+, NO_3^-, and TIN) at the University of Vermont Agricultural and Environmental Testing Center as previously described [*Morales et al.*, 2006].

2.4. Nucleic Acid Extractions, Amplification, and Clone Library Construction

[12] Nucleic acids for T-RFLP analysis and clone library construction were extracted from soils and groundwater using the MoBio Powersoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, California). Polymerase chain reaction (PCR) amplification of the 16S rRNA gene for T-RFLP analysis was done using three primer pairs targeting archaea (46F/907R) [*Lane et al.*, 1985; *Ovreas et al.*, 1997], bacteria (8F/1392R) [*Lane et al.*, 1985], and Geobacteraceae (8F/825R) [*Snoeyenbos-West et al.*, 2000] using PCR reagents described previously [*Anderson et al.*, 2003].

[13] Archaeal targets were amplified using a denaturing step of 4 min at 94°C followed with 35 cycles of denaturation (30 s, 95°C), primer annealing (1 min, 56°C), and the primer extension (1 min, 72°C), followed by a final elongation step of 5 min at 72°C. Geobacteraceae targets were amplified using the same steps as archaea, with the exception of primer annealing temperature and time (20 s, 55°C). For amplification of bacterial targets, a denaturing step of 5 min at 95°C was followed with 28 cycles of denaturation (30 s, 95°C), primer annealing (30 s, 57°C), and primer extension (3 min, 72°C), followed by a final elongation step of 7 min at 72°C. The minimum number of cycles for PCR reactions was determined by visual inspection of the DNA band in gel electrophoresis using PCR gradient analysis. Forward primers were labeled with tetrachloro-6-carboxy (archaea) (TET, Sigma-Genosys, The Woodlands, Texas), 6-carboxy (bacteria) (6-FAM, Sigma-Genosys), and phosphoramidite (Geobacteraceae) (NED, Applied Biosystems, Foster City, California) fluorescein dyes. PCR amplification was verified on a 2% agarose gel, cleaned using a QIAquick PCR purification kit (Qiagen, Valencia, California), and quantified using spectrophotometry (NanoDrop, Wilmington, Delaware).

[14] The 16S rRNA gene libraries were generated using unlabeled primers 8F/1392R as described above and cloned into the TOPO TA vector pCR 2.1 and chemically competent TOP10 cells (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. Inserts from 48 clones for each library were amplified with M13 primer, sequenced, and aligned using the Lasergene software (DNASTAR, Inc., Madison, Wisconsin). Phylogenetic placement for clone library sequences was assigned by BLAST analysis [*Altschul et al.*, 1990]. All genetic analyses were done at the Vermont Cancer Center DNA Analysis Facility.

2.5. T-RFLP Microbial Community Profiling and Data Analysis

[15] Restriction digests (10 μL total volume) were performed with enzyme *Msp*I by mixing fluorescently labeled

and purified PCR product (150 ng, quantified using a NanoDrop spectrophotometer), enzyme buffer, 5 units (U) of enzyme, and water as previously described [*Morales et al.*, 2006]. One microliter of each digest was mixed with 0.4 μL Genscan 500 ROX size standard (Applied Biosystems), brought to 10 μL total volume in deionized formamide, and quantified using capillary electrophoresis (ABI Prism 3100-Avant Genetic Analyzer, Applied Biosystems). Negative controls were run for each primer set. T-RFLP profiles were analyzed using GeneMapper (Applied Biosystems) with parameters set to exclude fragments shorter than 20 base pairs (bp) or larger than 600 bp and those under 50 fluorescence units [*Blackwood et al.*, 2003]. Several levels of duplication were used in this study. Capillary electrophoresis of samples was conducted in triplicate to assess the consistency of base pair size calling determinations, to quantify the variability in fragment peak heights, and to establish the minimum fluorescence intensity of T-RFLP fragments (T-RF) to be included in further statistical analysis [*Osborn et al.*, 2000]. Fragments were binned into T-RFs at a spacing of 1.0 ± 0.2 bp standard deviation [*Dunbar et al.*, 2001]. T-RFs were excluded from the set if they occurred in less than two of the three triplicate runs or were below 50 fluorescence units. Bias associated with T-RF absence (i.e., similarities caused by double zeros) was minimized by requiring T-RFs to be present in a minimum of two field samples and in both duplicate microcosms for inclusion in statistical analysis. Thus, average and standard deviations for T-RF peak fluorescence intensity presented for the six different levels of leachate treatment represent $n = 6$ values across the duplicate microcosms.

2.6. Statistical Methods

[16] Principal component analysis (PCA) on correlation matrices was used to detect correlations within hydrochemical data and T-RFLP microbial community profiles and to transform data into statistically independent variables for classifying contamination levels (e.g., background, fringe, or contaminated). Four separate PCAs were conducted on hydrochemistry, on bacterial community composition, on all three microbial communities (archaea, bacteria, and Geobacteraceae), and between hydrochemistry and the combined microbial community profiles.

[17] For each microcosm treatment, we report the number of T-RFs shared across duplicate microcosms and two different diversity indices: the Shannon-Wiener Index H' and the Jaccard Index J. The Shannon-Wiener Index is calculated as $H' = -\sum \pi \ln \pi$, where π represents the relative abundance of a T-RF in a given fingerprint [*Hill et al.*, 2003]. The Jaccard Index is calculated as $J(a, b) = \frac{a \cup b}{a \cap b}$, where $\cup$ is the number of shared T-RFs (or union) between two fingerprints (a, b) and $\cap$ is the number of total T-RFs, or intersection between fingerprints (a, b) [*Chao et al.*, 2005]. In this application, the Shannon-Wiener Index describes the sample diversity by accounting both for abundance and evenness of T-RF phylotypes, while the Jaccard Index measures phylotype similarity between any two samples on a scale of 0–1, with a value of 1 indicating identical phylotype composition. Jaccard Index values were calculated between the background treatment and all other treatments. Relevant T-RFs were compared to the Ribosomal Database II using Mica and FragSort [*Sciarini and Michel*, 2005] as described previously [*Morales et al.*, 2006]. Significance levels for statistical analyses, including testing for normality, analysis of variance (ANOVA), Tukey-Kramer method, and Spearman's Rho correlation analyses, were conducted at the $\alpha = 0.05$ level in JMP software.

3. Results

3.1. Statistical Analysis of Hydrochemistry and Microbial Community Composition

[18] Leachate-contaminated groundwater-monitoring locations (C-1 through C-3) had significantly elevated levels of 14 parameters compared with background wells, while fringe locations differed from background only by ORP and iron levels (Table 1). Univariate methods were unable to detect significant differences for any of the 25 parameters listed in Table 1 across all three levels of groundwater quality (background, fringe, and contaminated).

[19] Using multivariate statistical methods, PCA revealed that a large proportion of the groundwater hydrochemistry data set variance (~80%) could be explained in four principal components. Consistent with univariate statistical techniques, the first principal component score, PC1, plotted across the x axis in Figure 2a, showed large differences in groundwater quality between the three contaminated locations (C-1 through C-3) when compared with all other fringe and background locations. Differences among fringe and background wells were not well pronounced, as evidenced by the overlap in B and F well designations in Figure 2a. PC1 described about 48% of the variance in the groundwater hydrochemistry data set and was significantly correlated ($r > 0.8$) to general leachate indicators, including TOC, COD, TDS, Mg and Na, specific conductance, chloride, hardness, and alkalinity (see Auxiliary Materials, Table S1).[1] PC2, plotted across the y axis in Figure 2a, described an additional 16% of the data set variance, with significant correlations to BOD, sources of organic and inorganic nitrogen (TON and NH_3), and phenols, along with Ca and K. The third and fourth PCs explained about 16% of the total data set variance and were largely correlated to redox parameters, including pH, ORP, NO_3^-, Fe, Mn, and SO_4^{2-} (Auxiliary Materials, Table S1).

[20] T-RFLP analysis revealed a total of 40, 115, and 54 T-RFs for archaea, bacteria, and Geobacteraceae primers, respectively, across the 22 monitoring locations. When PCA was conducted using bacterial community profiles, 12 PCs were needed to describe the large majority (~80%) of data set variance. However, plots of PC1 and PC2, describing only 11% and 9% of the data set variance, respectively, indicate that despite data set variability, bacterial profiles detected differences among the monitoring wells that were consistent with the monitoring location's position relative to the unlined landfill and leachate plume (Figure 2b). Fringe locations near the up-gradient landfill perimeter (F-3 and F-4) were separated along PC1 (x axis), while contaminated and fringe locations near the leading edge of the plume (C-1 through C-3, F-1, F-2, and F-7) were separated from background locations across PC2.

[21] The addition of archaea and Geobacteraceae T-RFs created a similar pattern of separation to bacterial community

[1] Auxiliary materials are available in the HTML. doi:10.1029/2010WR009459.

Table 1. Summary of Water Quality Parameters for Background, Fringe, and Landfill Leachate–Contaminated Groundwater Monitoring Locations[a]

Parameter	Background Wells (B1–B11)	Fringe Wells (F1–F8)	Contaminated Wells (C1–C3)
Temperature (°C)	8.9 ± 1.2	10 ± 1.1	11.1 ± 2
Oxidation reduction potential (ORP, mV)	48 ± 55 (a)	−16 ± 35 (b)	−38 ± 13 (b)
pH	7.0 ± 0.4	6.6 ± 0.4	6.8 ± 0.3
Specific conductance (μS/cm)	520 ± 340 (a)	1380 ± 630 (a)	7070 ± 1850 (b)
Turbidity (NTUs)	39 ± 72	17 ± 18	50 ± 10
Alkalinity (as $CaCO_3$)	230 ± 160 (a)	550 ± 245 (a)	3440 ± 900 (b)
Hardness (as $CaCO_3$)	302 ± 179 (a)	427 ± 166 (a)	1420 ± 498 (b)
Total dissolved solids (TDS)	367 ± 214 (a)	592 ± 240 (a)	3547 ± 802 (b)
Chloride (Cl^-)	25 ± 37 (a)	49 ± 43 (a)	728 ± 228 (b)
Sulfate (SO_4^{2-})	52 ± 40	47 ± 41	11 ± 14.7
Nitrate (NO_3^-)	1.9 ± 2.0	0.5 ± 0.2	<0.5 ± 0
Ammonia (NH_3)	0.14 ± 0.26 (a)	31 ± 29 (a)	347 ± 177 (b)
Total organic nitrogen (TON)	0.31 ± 0.24	0.5 ± 0.7	3.3 ± 5.8
Total organic carbon (TOC)	3.3 ± 2.2 (a)	17.5 ± 8.4 (a)	328 ± 271 (b)
Chemical oxygen demand (COD)	8.9 ± 4.7 (a)	48 ± 20.8 (a)	1056 ± 850 (b)
Biological oxygen demand (BOD)	2.0 ± 0 (a)	17.8 ± 14.5 (a)	230 ± 340 (b)
Total phenols	0.005 ± 0 (a)	0.007 ± 0.005 (a)	0.4 ± 0.7 (b)
Calcium (Ca)	88 ± 57.5 (a)	91 ± 53 (a)	276 ± 230 (b)
Cadmium (Cd)	0.005 ± 0	0.005 ± 0	0.005 ± 0
Iron (Fe)	1.2 ± 1.6 (a)	48 ± 28 (b)	47 ± 37 (b)
Lead (Pb)	0.005 ± 0	0.005 ± 0.001	0.005 ± 0
Magnesium (Mg)	20.1 ± 13.7 (a)	37 ± 20 (a)	178 ± 35 (b)
Manganese (Mn)	1.4 ± 2.7	2.7 ± 2.0	1.3 ± 1.0
Potassium (K)	4.7 ± 3.1 (a)	28.0 ± 30 (a)	233 ± 161 (b)
Sodium (Na)	18 ± 19 (a)	53 ± 38 (a)	692 ± 220 (b)

[a]Values represent the mean concentration plus standard deviation (in parentheses) for the reported parameters in units of milligrams per liter (mg/L) unless otherwise denoted. Cations are reported as dissolved species. Letters in parentheses indicate significant differences among locations ($P < 0.05$; Tukey-Kramer test for multiple comparisons among means). If no letters are given, no significant differences were observed among the three groups of monitoring wells.

composition used alone (Figure 2c). The combined microbial PCA required 12 PCs to explain more than 80% of the data set variance, with fringe wells separating from others across PC1 (x axis) and contaminated wells separating from background monitoring locations along PC2 (y axis). Monitoring location F-6 fell within the background locations in the bacterial PCA but within the contaminated locations in the combined microbial PCA (Figures 2b and 2c, respectively).

[22] The largest degree of separation between all three groups of monitoring locations (background, fringe, and contaminated) was observed when the microbial community profiles were combined with the hydrochemistry data in a fourth PCA analysis (Figure 2d). Twelve components were again necessary to explain more than 80% of the data set variance. Yet despite the data set variability, contaminated locations separate clearly from others across PC1 (12% of the data set variance), and fringe locations separate from background locations across PC2 (11% of the variance). PC1 loadings remain strongly correlated to leachate indicators, including sources of carbon, nitrogen, and ionic concentrations (Ca, Cl, K, Mg, and Na), in addition to significant loadings from T-RFs belonging to Geobacteraceae, bacteria, and archaea (see Auxiliary Materials, Table S2). The second PC is strongly correlated to ORP, Fe, and NO_3^-, along with a dozen microbial fragments. Parameter loadings on the third PC include pH and various T-RFs (see Auxiliary Materials, Table S2).

3.2. Biogeochemistry in Microcosm Treatments

[23] Microcosms were constructed with site soils and mixtures of groundwater collected from a background well (B-1) or leachate-impacted well (C-1) to replicate the spatiotemporal "fringe" effect (biogeochemical transition between aerobic, nitrate-reducing, and iron-reducing electron accepting processes) observed in the PCA analyses. Microcosms with 5% or less C-1 groundwater exhibited detectable levels of oxygen and nitrate, while little ammonium, dissolved iron, or iron sulfide concentrations had accumulated (Table 2). For microcosms treated with 10%–25% C-1 groundwater, low levels of oxygen and nitrate (near equipment/analytical detection limits) are detectable concurrent with reduced electron acceptors: Fe^{2+}, Mn^{2+}, and $FeS_{(aq)}$. Once C-1 groundwater concentrations surpass 50%, oxygen and nitrate levels are depleted, and reduced concentrations of iron and manganese species begin to accumulate at higher levels.

[24] Using univariate and multivariate statistical analysis of hydrochemistry as a guide for parameters indicative of leachate contamination, we tested what level of known leachate (0%–100%) could be detected for nine different parameters in Table 2. These included iron species (Fe, Fe^{2+}, and FeS), forms of inorganic and organic nitrogen (NH_4^+, TIN, and TON), and cation species (K, Mg, and Na). Only inorganic nitrogen (NH_4^+ and TIN) could be used to detect differences across all six treatments (see footnotes in Table 2). Cationic species, including K and Na, detected four levels across microcosms, while Mg detected only three. Total Fe detected only two levels of microcosm treatments, while reduced iron species (Fe^{2+} and FeS) detected three. No statistical differences were observed in organic nitrogen (TON) (Table 2).

3.3. Bacterial Community Dynamics in Microcosm Treatments

[25] Bacterial community dynamics in response to biogeochemical changes due to leachate were assessed from

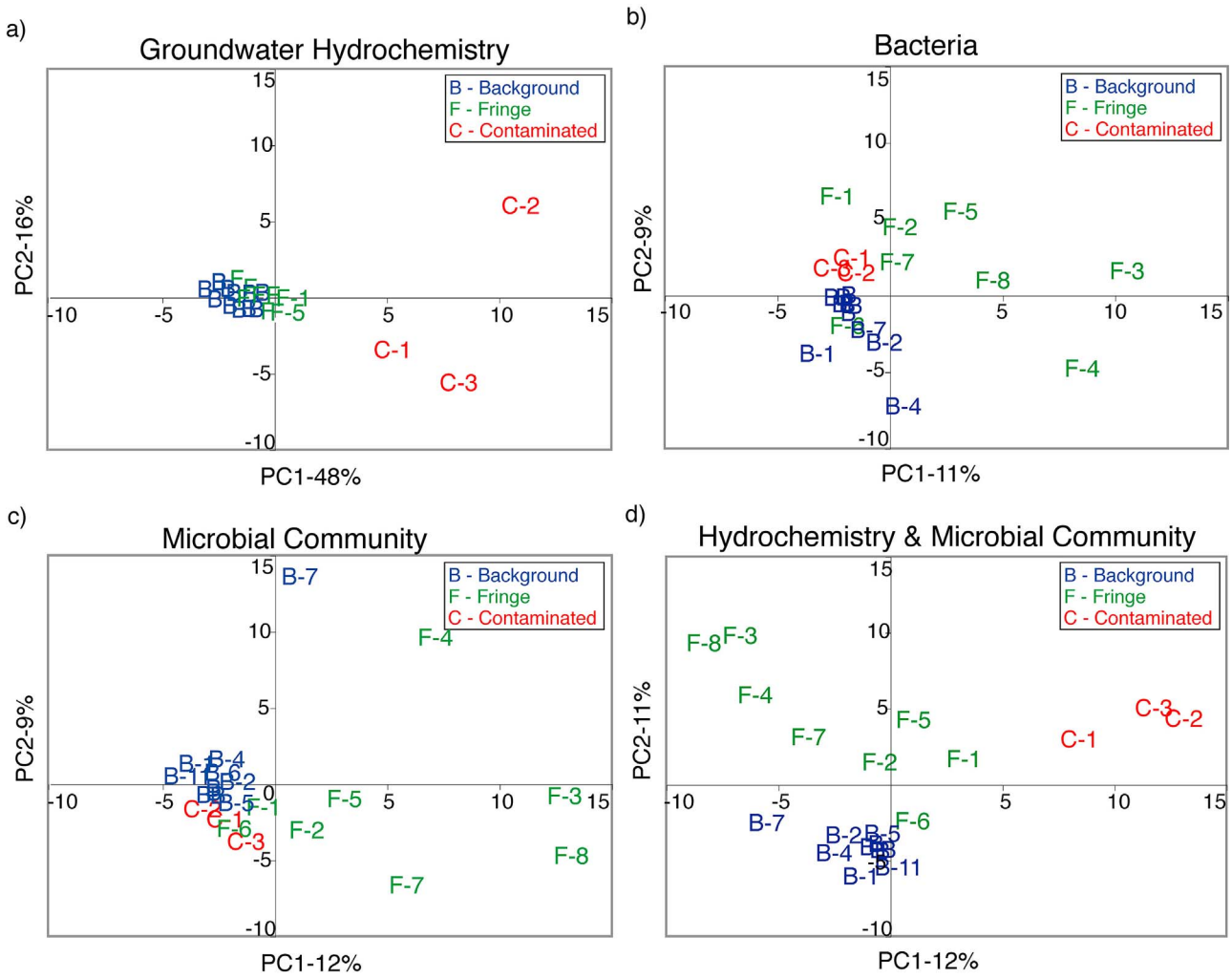

Figure 2. The first two component scores (PC1 and PC2) for principal component analysis conducted on (a) groundwater hydrochemistry, (b) 16S rRNA gene bacterial community composition, (c) microbial community composition (archaea, bacteria, and Geobacteraceae), and (d) combined hydrochemistry and microbial community composition collected from monitoring wells surrounding an unlined landfill.

microcosms by analysis of the 16S rRNA gene. A total of 86 bacterial T-RFs were detected across the gradient of microcosm treatments in this study, with their frequencies shown in Figure S1 in the auxiliary material. At a bacterial ecology level, neither the number of T-RFs (48 ± 8.2) nor the Shannon-Wiener Diversity Index H' (3 ± 0.4) were significantly correlated to the percent leachate (Spearman's Rho > −0.77, $p > 0.07$). In contrast, the Jaccard Index calculated between background (B-1, 0% leachate) microcosms and all others decreased significantly as the percent of leachate increased (Spearman's Rho > −0.99, $p < 0.001$) (Figure 3a).

[26] Analysis of 16S rRNA gene clone libraries indicates a shift in bacterial community composition from clones with high similarity to Actinobacteria, α- and β-Proteobacteria, and Verrucomicrobia under background (B-1, 0% leachate) conditions to one with clones with high similarity to Chloroflexi, Firmicutes, and α- and δ-Proteobacteria under leachate-contaminated conditions (C-1, 100% leachate) (Figure 4). Clone sequences similar to a diversity of α- and β-Proteobacteria (90%–98% similarity) made up the largest percentage of bacteria in background microcosms and included species within the Bradyrhizobiaceae, Caulobacteraceae, Methylobacteriaceae, Comamonadaceae, and Burkholderiaceae families. Background microcosms had a number of Actinobacteria clones with high similarity to Actinomycetales (91%–97%) and clones with high similarity to the *Opitutus* species (91%–95%) within the division Verrucomicrobia.

[27] The microcosms containing leachate (C-1, 100% leachate) were abundant in clones with high similarity to organisms typically involved in the cycling of both simple and complex organics under iron- and sulfate-reducing conditions, such as Chloroflexi, Firmicutes, and α- and δ-Proteobacteria (Figure 4). Within the phylum Firmicutes, this included clones with sequences similar to *Clostridium*, *Sporobacter*, and *Desulfotomaculum* species (84%–97%). Microcosms with C-1 groundwater were abundant in clones highly similar to the Desulfuromonadales, including *Geobacter* and *Pelobacter* species (87%–97%). Clones with

Table 2. Summary of Biogeochemical Parameters Sampled From Groundwater and Sediment Microcosms Incubated Over a 14 Day Period[a]

Parameter	0%	5%	10%	25%	50%	100%
O_2 (mg/L)	2.3 ± 0.6	3.2 ± 0.2	1.7 ± 0.3	2.4 ± 0.4	<1 ± 0.1	<1 ± 0.5
NO_3^- (mg/L)	2.0 ± 1.1	<0.9 ± 0	0.9 ± 0	1.0 ± 0.1	<0.9 ± 0	<0.9 ± 0
Mn (mM)	4.4 ± 0.01	5.1 ± 0.03	5.3 ± 0.31	5.3 ± 0.7	4.9 ± 0.8	5.7 ± 0.2
Mn^{2+} (μM)	280 ± 19	233 ± 2	262 ± 32	174 ± 6	283 ± 13	346 ± 5
Fe (mM)	<0.8 ± 0.2 (a)	<0.8 ± 0.1 (a)	1.7 ± 0.8 (a)	1.1 ± 0 (a)	5 ± 2 (a, b)	7.4 ± 0.7 (b)
Fe^{2+} (μM)	<5 ± 3 (a)	<5 ± 0 (a)	54 ± 32 (a, b)	21 ± 9 (a, b)	187 ± 61 (b, c)	252 ± 31 (c)
FeS (μA)	<5 ± 2 (a)	<5 ± 0 (a)	35 ± 21 (a, b)	13 ± 5 (a, b)	120 ± 39 (b, c)	162 ± 20 (c)
SO_4^{2-} (μM)	199 ± 28	262 ± 13	263 ± 10	243 ± 21	244 ± 31	215 ± 23
NH_4^+ (mM)	0.1 ± 0.02 (a)	0.4 ± 0.03 (b)	0.7 ± 0.02 (c)	1.5 ± 0.04 (d)	2.2 ± 0.03 (e)	6.4 ± 0.04 (f)
TIN (mg/L)	2 ± 0 (a)	6 ± 0 (b)	10 ± 0 (c)	21 ± 1 (d)	31 ± 0 (e)	90 ± 1 (f)
TON (mg/L)	61 ± 16 (a)	23 ± 13 (a)	10 ± 0 (a)	9 ± 3 (a)	20 ± 8 (a)	9 ± 6 (a)
Ca (mg/L)	78 ± 2	104 ± 4	103 ± 6	112 ± 17	104 ± 26	130 ± 1
K (mg/L)	26 ± 2 (a)	32 ± 1 (a, b)	35 ± 1 (a, b)	45 ± 3 (b)	61 ± 4 (c)	110 ± 3 (d)
Mg (mg/L)	12 ± 0.5 (a)	16 ± 0.5 (a, b)	17 ± 0.5 (a, b)	22 ± 2.2 (a, b)	25 ± 4.9 (b)	41 ± 1.4 (c)
Na (mg/L)	33 ± 1.3 (a)	35 ± 0.8 (a)	37 ± 0.1 (a)	48 ± 0.5 (b)	61 ± 0.8 (c)	99 ± 1.9 (d)
B (mg/L)	0.1 ± 0.05	0.1 ± 0.04	0.1 ± 0.04	0.3 ± 0.05	0.5 ± 0.05	1 ± 0.05
Cu (mg/L)	0.1 ± 0.004	0.1 ± 0.01	0.05 ± 0.002	0.1 ± 0.01	0.03 ± 0.01	0.01 ± 0.001
Zn (mg/L)	1 ± 0.01	1 ± 0.04	1 ± 0.11	1 ± 0.13	1 ± 0.09	1 ± 0.09

[a]Values represent the median concentration plus range (in parentheses) for the reported parameters in units of milligrams per liter (mg/L), milimolar (mM), micromolar (uM), or microamp (uA). Percentages shown in the column heads represent the ratio of background groundwater from monitoring location B-1 relative to leachate-contaminated groundwater from monitoring location C-1, with 100% representing all C-1 groundwater. Letters in parentheses denote parameters tested for significant differences ($P < 0.05$; Tukey-Kramer test for multiple comparisons among means). Where differences among treatments were observed, letters vary across experimental treatments.

similarity to several *Dehaloccoides* species (85%) within the Chloroflexi phylum were also more abundant in microcosms containing leachate-contaminated groundwater.

[28] The mean peak height intensities of nine fragments were positively correlated to the percent leachate (i.e., fragment intensity increased with increased leachate (Spearman's Rho > 0.82, $p < 0.05$)), while the mean intensities of 22 other fragments were negatively (inversely) correlated to leachate (fragment intensity decreased when percent leachate increased (Spearman's Rho > −0.82, $p < 0.05$)). A comparison of observed to in silico predicted fragments indicated that shifts in T-RF patterns were consistent with clone library results. T-RFs that increased in abundance with percent leachate matched in silico fragments belonging to Bacteroidetes, Firmicutes, and α- and δ-Proteobacteria, while those that decreased in abundance with percent leachate were related to in silico fragments matching Actinobacteria, Chloroflexi, Firmicutes, and α- and β-Proteobacteria (Table 3).

[29] Specific examples of T-RF shifts in response to increased leachate treatment were plotted for six fragments found to be significantly correlated to leachate content. In Figure 3b, fragment 161 (in silico matches to Firmicutes Bacillales and α-Proteobacteria, Table 3) was detected across all treatments, increasing threefold when 50% or more C-1 groundwater was added to microcosms. T-RFs 471 (in silico match to Firmicutes Clostridia) and T-RF 504 (in silico match to δ-Proteobacteria) were below detection at 25% and 10% leachate, respectively, yet increased in abundance with leachate composition (Figure 3b). The in silico matches to T-RF 504 included *Desulfovibrio* sp. and *Geobacter* sp.

[30] A similar inverse trend was observed for fragments with significant negative correlations to leachate. T-RFs 68, which matched in silico fragments similar to Firmicutes Lactobacillales, decreased by more than threefold with the onset of leachate and was not observed at analytical detection limits when 100% leachate was applied to microcosms. T-RF 136 (in silico match to β-Proteobacteria Burkholderiales) decreased only slightly with 25% or less leachate but was reduced to below detection limits at 50% or higher leachate levels (Table 3 and Figure 3c). Fragments matching Actinobacteridae and β- and γ-Proteobacteria were found as in silico matches to T-RF 143. The peak fluorescence of T-RF 143 decreased by more than fourfold from 0% to 25% leachate, then remained below detection limits with larger percentages of leachate. The in silico matches to T-RF 143 included *Burkholderia* sp. and *Pseudomonas* sp.

4. Discussion

4.1. Statistical Characterization of a Groundwater Fringe

[31] Multivariate analyses have been used to link specific microbial community changes in response to ecosystem changes [*Dollhopf et al.*, 2001; *Fields et al.*, 2006; *Morales et al.*, 2006; *Brielmann et al.*, 2009; *Feris et al.*, 2009]. Specifically, PCA has previously been used for assessing correlations between hydrochemistry and microbial composition in leachate-contaminated groundwater [*Ludvigsen et al.*, 1997; *Röling et al.*, 2001] and to produce independent variables for estimating the spatial correlation of microbial communities in leachate-contaminated groundwater [*Mouser et al.*, 2005]. While the degree of autocorrelation for microbial communities in pristine and contaminated sediments is estimated at less than 1 m [*Mummey and Stahl*, 2003; *Brad et al.*, 2008], the spatiotemporal dynamics of microbial populations in groundwater is not well established and should be considered in monitoring strategies. Multivariate statistical analyses performed here confirm that for one snapshot in time, profiles of bacteria, archaea, and Geobacteraceae more distinctly identified fringe monitoring locations, while hydrochemistry data were more efficient in separating contaminated locations. This is consistent with observations by others [*Fields et al.*, 2006] suggesting that microbial phylogeny and functionality in intermediate

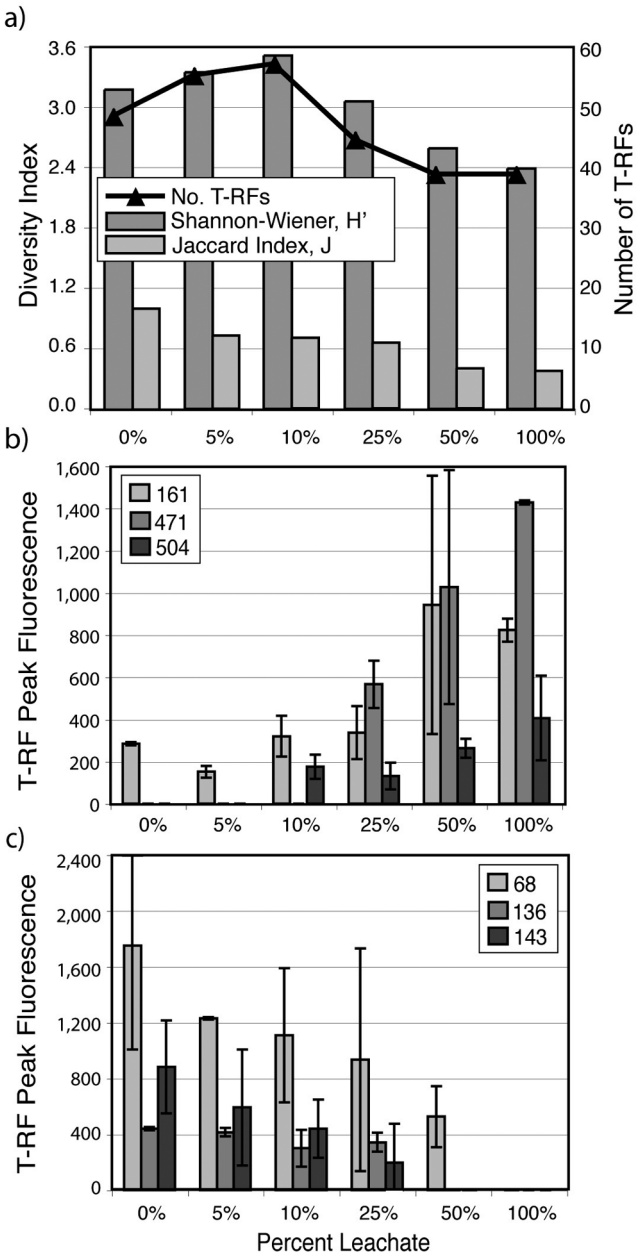

Figure 3. (a) Ecological indices for duplicate microcosms, including the number of T-RFs, Shannon-Wiener Index *H'*, and Jaccard Index *J*. (b) Change in fragment intensity for three T-RFs positively correlated to landfill leachate in microcosm treatments. (c) Change in fragment intensity for three T-RFs inversely correlated to leachate treatment. T-RF peak fluorescence values represent triplicate fragment height readings for duplicate microcosms (n = 6), with means (bars) and standard deviations (whiskers).

monitoring locations are different from background and contaminated locations. An improved characterization of groundwater quality was achieved when microbial community profiles were combined with the hydrochemistry information. The combined PCA separated background, fringe, and contaminated locations across PC1 and PC2 in loading patterns that are related to the primary electron accepting processes and dominant microbial community in the subsurface. Background locations remained clustered together; fringe locations dominated by nitrate and low-level iron reduction were separated across PC2, while other fringe locations and contaminated locations undergoing iron and sulfate reduction were separated across PC1. The multivariate separation of discrete monitoring locations is consistent, in general, with classification of plume zones based on high-resolution data collected using geophysical methods and interpolated using geostatistics and highlights the value of biological information for an improved sensitivity in the monitoring network in the absence of a detailed geophysical survey.

[32] This improved classification is important not only from a detection standpoint but also for long-term monitoring of leachate-contaminated plumes. It allows for a more sensitive measure of groundwater quality impacts from landfills and provides a gauge of natural attenuation processes along a site-specific "subtle" to "gross" contamination spectrum. Gross changes, such as those separating microbial communities present in the plume center of mass (C-1 through C-3) or directly down-gradient of the landfill (F-1 and F-5), would probably be associated with statistically detectable differences in hydrochemistry, including increased ionic content, organic carbon demand, or the presence of anthropogenic compounds not typically observed in groundwater aquifers. Subtle changes, as evidenced in differences observed in fringe wells along the up-gradient landfill perimeter (F-3 and F-4) and down-gradient plume fringe (F-2, F-5, F-7, and F-8), were related to decreased redox potentials, increased available inorganic nitrogen, and increased iron species across groundwater-monitoring wells and in microcosm treatments. Reduced iron is mobile; therefore, a shift from oxic to anoxic conditions would be expected to produce increased levels of iron groundwater-monitoring wells down-gradient from the leachate source. While these biogeochemical trends may be difficult to detect at relatively low leachate content through statistical means, changes were distinctly observable using microbial community profiles and PCA statistical analysis.

4.2. Challenges in Using Hydrochemical Data for Detecting Contamination

[33] Microcosm treatments replicated the spatiotemporal biogeochemical gradient between oxic (aerobic and nitrate reduction) and anoxic (iron and sulfate reduction) electron-accepting processes that have been described in other leachate-contaminated subsurface environments [*Christensen et al.*, 1994]. However, even across a known gradient of leachate, statistically significant differences in hydrochemistry were difficult to detect in microcosms without a larger percentage (what would be considered 50% or greater) of leachate present. Cation species (Fe, K, Mg, and Na) tested in microcosms separated into two to four statistical groups, while redox-sensitive iron species (Fe^{2+} and $FeS_{(aq)}$) and inorganic forms of nitrogen (NH_4^+ and TIN) separated into three and six groups, respectively. Thus, iron species and inorganic nitrogen may be two of the better hydrogeochemical parameters for detecting the onset of leachate in the groundwater.

[34] At the field scale, we detected differences between two distinct groups of water quality for a similar listing of cation and nitrogen species to those noted in microcosm

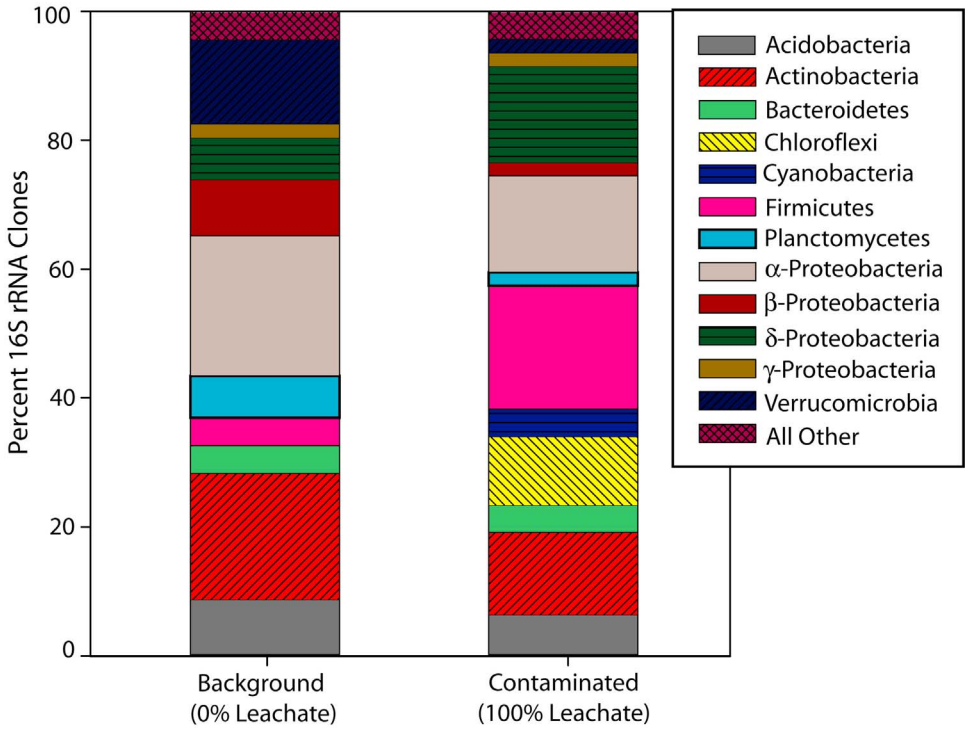

Figure 4. Bacterial community composition based on 16S rRNA clone sequences extracted from microcosms treated with background groundwater (B-1) or leachate-contaminated groundwater (C-1).

treatment; however, none of the 25 hydrochemical parameters analyzed at the site could differentiate fringe locations from either contaminated or background groundwater quality. This demonstrates the difficulty in comparing physiochemical trends using a traditional up-gradient versus down-gradient monitoring strategy at landfills, specifically if background water quality conditions are not characterized prior to the onset of waste disposal activities. Of the 14 parameters listed in Table 1 with statistical differences, ORP levels and iron concentrations in fringe wells were more similar to contaminated locations, while other noted statistical differences grouped fringe wells with background locations. ORP and iron were also two of the key hydrochemical drivers in the separation of fringe from background locations once microbial community profiles were added to hydrochemistry data in the combined PCA. Unfortunately, while ORP and iron-related biogeochemical changes may be related to the onset of landfill leachate in this aquifer example, it may also result from changes in surface hydrology, groundwater recharge, or loading of organic materials not related to landfill leachate [*Chapelle et al.*, 1995; *Anderson and Lovley*, 1997]. Thus, statistical differences in plume biogeochemistry alone are not sufficient for detecting or classifying landfill leachate contamination in this example.

[35] Microbial community profiles provided an alternative measure of groundwater quality information that complimented hydrochemistry data and was related to the contamination source. Overall biodiversity of sediment microcosms was consistent with or slightly lower than numbers observed in other shallow aquifers [*Brielmann et al.*, 2009; *Stein et al.*, 2010]. At a broad level, the Jaccard Index showed a divergence away from the background bacterial community composition as a larger percentage of leachate-contaminated groundwater was added to the microcosms. Significant decreases in 25% of community members (22/86 of detected T-RFs) due to the onset of contaminants is consistent with a reduction in bacterial population complexity and diversity observed in other studies [*Griebler and Lueders*, 2009]. While its possible that shifts in T-RF community composition in the field and at the lab scale are related solely to changes in cation species alone [*Brielmann et al.*, 2009], it is more likely that fringe-observed differences in the microbial community are related to carbon and nitrogen nutrient materials driving redox conditions, in particular, nitrate, iron, or sulfate-reducing TEAPs and associated microbial biota. Community characterization derived through T-RF in silico analysis and the sequencing of the 16S rRNA gene indicate this shift is from a community dominated by β-Proteobacteria and Actinobacteria typical of a pristine aquifer to one with a larger proportion of Firmicutes and δ-Proteobacteria [*Griebler and Lueders*, 2009]. Certain Clostridia, such as clones with high similarity to *Sporobacter termitidis* and *Clostridium* spp. observed here, have been associated with the degradation of complex organic materials derived from landfill leachates [*Van Dyke and McCarthy*, 2002; *Burrell et al.*, 2004] and may be involved in similar decay processes at this site. Dissimilatory metal-reducing bacteria such as clones with high similarity to *Geobacter* spp., *Pelobacter* spp., and *Desulfovibrio* sp. observed here are capable of coupling the oxidation of simple organics to the reduction sediment-derived iron [*Snoeyenbos-West et al.*, 2000; *Anderson et al.*, 2003; *Cummings et al.*, 2003; *Lin et al.*, 2005; *Holmes et al.*, 2007].

Table 3. Comparison of T-RFs Observed in This Study to T-RFs Predicted From the Ribosomal Database II in Silico Digest Analysis[a]

Observed T-RF (bp)	Correlation	Predicted T-RF (bp)	Bacterial Phylum, Order, and Family (Based on in Silico Digest Analysis)
		Positive Correlation	
92	0.82	92	Bacteroidetes Candidatus Cardinium
125	0.83	126	Unidentified/uncultured bacteria
		127	Proteobacteria α-Proteobacteria Rhizobiales
158	0.89	158	Actinobacteria Actinobacteridae Actinomycetales
161	0.89	161	Firmicutes Bacillales Bacillaceae
		161	Proteobacteria α-Proteobacteria Rhizobiales
204	0.89	204	Actinobacteria Actinobacteridae Actinomycetales
269	0.94	268	Fusobacteria Fusobacteriales Fusobacteriaceae
471	0.94	473	Firmicutes Clostridia Clostridiales
504	0.93	505	Proteobacteria δ-Proteobacteria Desulfovibrionales
		506	Proteobacteria δ-Proteobacteria Desulfuromonadales
517	0.93	517	Chloroflexi uncultured bacterium
		Negative (Inverse) Correlation	
61	−0.94	63	Proteobacteria δ-Proteobacteria uncultured bacterium
62	−0.83	63	Proteobacteria δ-Proteobacteria uncultured bacterium
65	−0.94	67	Actinobacteria Actinobacteridae Actinomycetales
68	−0.99	69	Firmicutes Lactobacillales Carnobacteriaceae
72	−0.89	72	Proteobacteria γ-Proteobacteria Thiotrichales
89	−0.89	89	Bacteroidetes uncultured bacterium
91	−0.84	91	Bacteroidetes Flavobacteria Flavobacteriales
110	−0.87	109	Unidentified/uncultured bacteria
115	−0.94	116	Unidentified/uncultured bacteria
117	−0.93	117	Chloroflexi Chloroflexales Chloroflexaceae
127	−0.89	127	Proteobacteria α-Proteobacteria Rhizobiales
136	−0.93	135	Proteobacteria β-Proteobacteria Burkholderiales
		136	Unidentified/uncultured bacteria
143	−0.94	143	Actinobacteria Actinobacteridae Actinomycetales
		143	Proteobacteria β-Proteobacteria Burkholderiales
		143	Proteobacteria γ-Proteobacteria Pseudomonadales
148	−0.83	148	Firmicutes Bacillales Bacillaceae
154	−0.93	154	Proteobacteria α-Proteobacteria Rhizobiales
167	−0.94	167	Firmicutes Bacillales Salinicoccus
175	−0.94	174	Actinobacteria Actinobacteridae Actinomycetales
		175	Proteobacteria α-Proteobacteria environmental samples
178	−0.94	179	Firmicutes Bacillales Bacillaceae
180	−0.87	180	Firmicutes Bacillales Bacillaceae
		180	Firmicutes Clostridia Halanaerobiales
192	−0.99	192	Planctomycetes Planctomycetacia Planctomycetales
		192	Proteobacteria γ-Proteobacteria environmental samples
227	−0.93	227	Proteobacteria γ-Proteobacteria Oceanospirillales
		227	Planctomycetes Planctomycetacia Planctomycetales
267	−0.87	266	Unidentified/uncultured bacteria
		268	Fusobacteria Fusobacteriales Fusobacteriaceae

[a]Fragments reported in Table 3 were significantly correlated (Spearman's Rho > ±0.82, $p < 0.05$) to landfill leachate–contaminated groundwater in microcosm treatments.

Thus, the increased abundance of these δ-Proteobacteria driving the separation of fringe samples and in microcosms containing a larger proportions of leachate is consistent with their described role in other contaminated subsurface environments.

4.3. Implications for Incorporating Microbial Information in Monitoring Strategies

[36] Changes in key T-RFs from microcosm treatments showed that certain bacteria believed to belong to Bacteroidetes, Firmicutes, and α- and δ-Proteobacteria increase in abundance with higher leachate composition. These bacteria could be present in the subsurface prior to contamination or advectively transported via leachate-derived colloids in the subsurface. In the latter case, bacteria may represent an indicator organism or tracer tying the contaminated groundwater to its leachate source, while in both cases, bacteria may represent an increased sensitivity to landfill leachate–related biogeochemical changes when analytical methods are unable to quantify low levels of contaminants. Fecal bacteria and molecular ribotyping methods have been used for determining sources of surface water pollution in watershed systems [*Meays et al.*, 2004]; however, these same tracking methods do not appear as efficient in the subsurface owing to the rates of groundwater transport and source specificity in porous media systems [*Cimenti et al.*, 2005]. Nevertheless, while this study was not designed to link specific T-RFs to leachate contamination, our results suggest that microbial communities may potentially be used for both purposes and that further research is warranted.

[37] This work provides a basis for improving detection methods through the incorporation of molecular-based microbial composition data and multivariate statistical analyses. Tracking changes in the abundance of specific bacterial species in the groundwater may prove to be infeasible for the purpose of detection or long-term monitoring strategies at landfill sites because of the variability of leachate composition and site-specific hydrogeologic characteristics. However, we show here that tracking shifts in T-RF patterns appear to be a sensitive means for detecting low levels of leachate in groundwater from leaking MSWLFs, particularly along plume fringes. It also suggests the possibility of optimizing detection strategies using microbial information and a limited set of hydrochemical parameters to lower long-term monitoring costs at landfills.

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