GeoChip-based Analysis of Groundwater Microbial Diversity in Norman Landfill

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http://ok.water.usgs.gov/projects/norlan/
http://vimss.lbl.gov

ABSTRACT

The Norman Landfill is a closed municipal solid waste landfill located on an alluvium associated with the Canadian River in Norman, Oklahoma. It has operated as a research site since 1994 because it is typical of many closed landfill sites across the U.S. Leachate from the unaﬁlled landfill forms a groundwater plume that extends downward approximately 250 m from the landfill toward the Canadian River. To investigate the impact of the landfill leachate on the diversity and functional structure of microbial communities, groundwater samples were taken from eight monitoring wells at a depth of 5 m, and analyzed using a comprehensive functional gene array covering about 50,000 genes involved in key microbial processes, such as biogeochemical cycling of C, N, P, and S, and bioremediation of organic contaminants and metals. Wells are located within a transect along a presumed ﬂowpath with different distances to the center of the leachate plume. Our analyses showed that microbial communities were obviously impacted by the leachate-component from the ﬁll. The number of genes detected and microbial diversity indices in the center (LF2B) and its closest (MLS35) wells were signiﬁcantly less than those detected in other more downgradient wells, while no signiﬁcant changes were observed in the relative abundance (i.e., percentage of each gene category) for most gene categories. However, the microbial community composition or structure of the landfill groundwater did not clearly show a signiﬁcant correlation with the distance from well LF2B. Burkholderia sp. and Pseudomonas sp. were found to be the dominant microbial populations detected in all wells, while Bradyrhizobium sp. and Ralstonia sp. were dominant populations for seven wells except LF2B. In addition, Mantel test and canonical correspondence analysis (CCA) indicate that pH, sulfate, ammonia nitrogen and dissolved organic carbon (DOC) have signiﬁcant effects on the microbial community structure. The results suggest that the leachate from unaﬁlled landﬁlls signiﬁcantly impact the structures of groundwater microbial communities, and that more distal wells were recovered by natural attenuation.

SITE DESCRIPTION

Norman Landﬁll is a closed municipal solid waste landﬁll sited on the Canadian River alluvial aquifer in Norman, Oklahoma. The landﬁll, which is unaﬁlled and has no leachate collection system, received solid waste for surface disposal beginning in 1922 and disposed in trenches during 1960-80. It has been designated as a U.S. Geological Survey research site under the USEPA Toxic Substances Hydrology Program since 1995 (http://ok.water.usgs.gov/projects/norlan/)

METHODS

* DNA extraction, ampliﬁcation and labeling: Eight wells at the depth of 5m were sampled. LF2B, MLS35, MLS36, MLS37, MLS38, MLS54, MLS55, and MLS80. 5L undisposed water for each well was ﬁltered through 0.45µm pore size membrane on-site and immediately chilled and sent to the laboratory for later analysis. High yield total DNA was extracted by freeze-grinding methods. 100ng DNA was ampliﬁed using a Thermus kit, and the ampliﬁcation products were labeled with Cy-5 using random priming method.

*GeoChip hybridization, scanning and image analysis: A functional gene array (GeoChip 3.0) was used for underground water DNA hybridization. All hybridizations were carried out in triplicate at 42°C for 16 h to 30 h with 50% formamide using a TECAN HS400. The array was scanned by a ScanArray Express Microarray Scanner (Perkin Elmer). The TECAN image version 6.40 was then used for image quantiﬁcation.

*GeoChip data analysis: Functional gene diversity was calculated using Simpson’s U, 1-D, Shannon Weavers’ H and evenness.

*Statistical analysis: Detrended correspondence analysis (DCA) was used to determine the overall functional diversity. Multivariate statistical analyses including ANDiS3, Mantel test, CCA and partial CCA analyses were performed to link microbial communities to environmental variables.

** CHANGES IN FUNCTIONAL GENE DIVERSITY AND RELATIVE ABUNDANCE

Table 1. Gene overlap (italicized), uniqueness (bold) and diversity indices of Landfill samples.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>LF2B</th>
<th>MLS35</th>
<th>MLS36</th>
<th>MLS37</th>
<th>MLS38</th>
<th>MLS54</th>
<th>MLS55</th>
<th>MLS80</th>
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<tbody>
<tr>
<td>Diversity indices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Shannon-Weaver's H'</td>
<td>5.88</td>
<td>6.12</td>
<td>6.89</td>
<td>6.90</td>
<td>6.66</td>
<td>7.11</td>
<td>6.74</td>
<td>6.62</td>
</tr>
<tr>
<td>Simpson's (1/D)</td>
<td>0.26</td>
<td>0.34</td>
<td>0.70</td>
<td>0.74</td>
<td>0.51</td>
<td>0.84</td>
<td>0.86</td>
<td>0.55</td>
</tr>
<tr>
<td>Simpson's</td>
<td>0.58</td>
<td>0.60</td>
<td>0.56</td>
<td>0.56</td>
<td>0.48</td>
<td>0.55</td>
<td>0.50</td>
<td>0.54</td>
</tr>
<tr>
<td>% of detected genes</td>
<td>458</td>
<td>574</td>
<td>1281</td>
<td>1280</td>
<td>1068</td>
<td>1621</td>
<td>1120</td>
<td>999</td>
</tr>
</tbody>
</table>

GENES INVOLVED IN CARBON AND SULFUR CYCLING

A. Recalcitrant carbon degradation

B. Carbon ﬁxation

C. Labile carbon degradation

D. Sulfur cycling

GENES INVOLVED IN NITROGEN CYCLING

E. Nitrogen ﬁxation

F. Nitrogen assimilation

G. Nitrogen mineralization

H. Nitrogen reduction

I. Nitrogen storage

J. Nitrogen utilization

RELATIONSHIPS BETWEEN COMMUNITY STRUCTURE AND ENVIRONMENTAL VARIABLES

The microbial diversity was found to be the lowest in LF2B, while highest in MLS54. All the wells have higher percentages than genes at 36.78%~42.39% and metal resistance genes at 23%~25.09%. The relative abundances of the gene categories were similar.

SUMMARY

The microbial community structure within the leachate plume showed high heterogeneity from each well and clearly impacted by landfill leachate.

The leachate caused a decrease in microbial diversity, but recovery with distance was also noted.

Environmental variables greatly inﬂuenced functional gene composition.

ACKNOWLEDGEMENT

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