Microbial diversity patterns in relation to hydrocarbon seepage

I. Microbial mat pushcores
II. Sediment gravity cores

*The MC118 subsurface microbial community - microbial players within and outside of subsurface fluid advection paths*

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How does hydrocarbon seepage affect microbial diversity in sediments?

How many different microbial phyla are present, and which ones?

How many different microbial “species” (operationally defined by gene sequences) are present?

Which biogeochemical controls affect microbial diversity in seep sites?

Two sample types: pushcores (25 cm) from small-scale Beggiatoa mats gravity cores (ca. 2 m) from diverse MC118 sediments

High seepage activity

Low seepage activity
How does hydrocarbon seepage affect microbial diversity in sediments?

Assessment of microbial “species” diversity with V6-tag pyrosequencing: yields ca. 30000+ sequences/sample, 100-1000 x classical clone libraries

Survey of 8 different hydrocarbon seep samples

<table>
<thead>
<tr>
<th>Geographic Location</th>
<th>Mat?</th>
<th>Depth</th>
<th>Methane(mM)</th>
<th>Sulfate(mM)</th>
<th>T(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Oak River</td>
<td>N</td>
<td>24 cmbsf</td>
<td>0.18</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td>Gulf of Mexico (MC118)</td>
<td>Y</td>
<td>12 cmbsf</td>
<td>2.0</td>
<td>0.9</td>
<td>5</td>
</tr>
<tr>
<td>Gulf of Mexico (MC118)</td>
<td>N</td>
<td>12 cmbsf</td>
<td>0.04</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>Gulf of Mexico (MC118)</td>
<td>n/a</td>
<td>2.12 mbsf</td>
<td>2.0</td>
<td>0.25</td>
<td>6</td>
</tr>
<tr>
<td>Storegga seep enrichment</td>
<td>Y</td>
<td>0.1 cmbsf</td>
<td>2.0</td>
<td>28</td>
<td>n/a</td>
</tr>
<tr>
<td>Black Sea carbonate reef</td>
<td>Y</td>
<td>0.1 cmbsf</td>
<td>2.0</td>
<td>16</td>
<td>n/a</td>
</tr>
<tr>
<td>Guaymas Basin</td>
<td>Y</td>
<td>1 cmbsf</td>
<td>2.1</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Guaymas Basin</td>
<td>Y</td>
<td>5 cmbsf</td>
<td>2.7</td>
<td>4</td>
<td>50</td>
</tr>
</tbody>
</table>
How does hydrocarbon seepage affect microbial diversity in sediments?

Geochemical regime: sulfate-reducing and methanogenic underneath the mat

Reduced microbial diversity for bacteria and archaea underneath the mat compared to bare sediments (Lloyd et al., PLoS One, in revision)

Microbial mat pushcores

![Image of microbial mat pushcore](image)
How does hydrocarbon seepage affect microbial diversity in sediments?

Archaea: High-throughput V6 tag sequencing shows dominance of two anaerobic methane-oxidizing Archaeal groups (ANME-1, ANME-2) under the mat, typical for very active methane seeps (Guaymas Basin, Black Sea); a more diversified archaeal community occurs in sediments outside of the mat.
How does hydrocarbon seepage affect microbial diversity in sediments?

**Bacteria:** High-throughput V6 tag sequencing shows dominance of Deltaproteobacteria (sulfate- and sulfur-reducing anaerobes) under the mat; a more diversified bacterial community occurs in sediments outside of the mat.

Jennifer Biddle, Bay Paul Center at MBL
How does hydrocarbon seepage affect microbial diversity in sediments?

Results from pushcore *Beggiatoa* mat study:

1) Active methane and hydrocarbon seepage skews microbial diversity towards an anaerobic, methane-oxidizing archaeal community, and towards a deltaproteobacterial, sulfate-or sulfur reducing bacterial community.

2) Electron acceptor depletion in seep fluids limits the range of microbial metabolisms, and reduces the number of microbial taxa. Oxidized sediments with different electron acceptors are more microbially diverse.

3) Sharp transition from seepage-controlled to benthic sediment microbial communities

4) Prediction (Valentine, Nature Microbiology, 2007): Energy limitation, including electron acceptor limitation, favors diversity and biomass of archaea – metabolically adapted to extremely reducing conditions and electron acceptor depletion – relative to bacteria. Quantitative checks by qPCR or rRNA blotting

5) Substantiate and explore further with new pushcore sample set from mat-covered seep areas in Rudyville, July 2009
How does hydrocarbon seepage affect microbial diversity in sediments?

**Sediment gravity cores**
29 Gravity cores in May and October 2005 (Lapham et al. 2008)
16+ Gravity cores in April 2008
5 Gravity cores in July 2009

Lapham, Chanton, Martens, Sleper, Woolsey 2008
How does hydrocarbon seepage affect microbial diversity in sediments?

Selected cores representing 3 geochemical categories of high, low, and intermediate seep activity were analyzed with 16S rRNA clone libraries for microbial community structure and diversity.
16S rRNA clone libraries show highest proportion of anaerobic methane oxidizers (ANME-1, ANME-2) in high-activity core No. 26, and in deep layers of intermediate core 30.
How does hydrocarbon seepage affect microbial diversity in sediments?

1. Chao1 diversity estimates show lowest archaeal diversity (Nos. of 16S rRNA phylotypes) in the high-seepage, high-methane gravity core.

2. The highest archaeal diversity is found in the oxidized surface layers for all cores.
Conclusions

• Hydrocarbon seepage reduces microbial diversity for bacteria and archaea, measured with different techniques (16S rRNA clone libraries, V6-tag sequencing)
• Paucity of electron acceptors prevents or limits oxidation of potentially energy-rich substrates
• Microbial community shifts towards specially adapted methane- and sulfur-cycling microbial community
• In less active sediments, the archaeal community shifts from ANME to cosmopolitan benthic subsurface archaea (MCG, SAGMEG, MBG-B, MBG-D)
• Work in progress →
Work in progress:

- mcrA gene sequencing, diagnostic for methanogens and methane oxidizers, for the high/medium/low activity gravity cores (Charlie Martin)
- Flesh out the high seepage core dataset by reanalysis of core 31 from 2005
- 16S rRNA clone libraries of high seepage core No. 6 from April 2008 (Sarah Underwood)
- Separate datasets by proximity to faults and their specific geochemistry
- Work up the 2009 *Beggiatoa* pushcores from Rudyville
- Do meta-analysis and comparison of *Beggiatoa* mat pushcore and gravity core clone libraries and V6-tag datasets, to identify microbial community signatures of strongest seep activity

![Graphs showing microbial community](image)

- 56 archaeal clones, 11-13 cm depth
- 76 bacterial clones, 20 cm depth
Acknowledgements

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Jennifer Biddle (UNC), Susan Huse, Mitch Sogin (MBL)
16S and functional gene cloning and sequencing:
Charlie Martin, Sarah Underwood (UNC)
Sequence data crunching (Genbank, ARB):
Jennifer Biddle, Karen Lloyd, Katrina Twing, Charlie Martin,
Sarah Underwood (UNC)
2008/9 core sampling: Karen Lloyd, Jennifer Biddle,
crews of RV Pelican, RV Brooks McCall
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How does hydrocarbon seepage affect microbial diversity in sediments?

Appendix: 2005 Gravity core samples cloned and sequenced to-date; Dataset with asterisk remains to be worked up

<table>
<thead>
<tr>
<th>Activity of core, as described by Lapham et al. 2008</th>
<th>High</th>
<th>Intermediate</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core number</td>
<td>31*</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>Depth 1 (cmbsf)</td>
<td>5-8</td>
<td>10-14</td>
<td>6-8</td>
</tr>
<tr>
<td>Depth 2 (cmbsf)</td>
<td>24-27</td>
<td>21-24</td>
<td>28-31</td>
</tr>
<tr>
<td>Depth 3 (cmbsf)</td>
<td>72-76</td>
<td>42-44</td>
<td>75-78</td>
</tr>
<tr>
<td>Depth 4 (cmbsf)</td>
<td>106-10</td>
<td>94-97</td>
<td>114-17</td>
</tr>
</tbody>
</table>

Dataset with asterisk remains to be worked up