Nitrogen Biogeochemistry of the Deep Underground

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Nitrogen Biogeochemistry in the Deep Underground

- Nitrogen is an essential element for all forms of life
  Proteins, Nucleic acids, Cell wall amino-sugars

- What is predominant source of nitrogen for microbes in the deep subsurface?

- What can predominant nitrogen-cycle processes tell us about subsurface community metabolism?
Nitrogen Sources for a Deep Subsurface Microbe

- **N₂**
  - Fixation
  - Dissimilation
  - Degradation

- **Fixed N**
  - Assimilation
  - Excretion
  - Mineralization

- **Atmosphere**
- **Rock**
<table>
<thead>
<tr>
<th>Rock type</th>
<th>N, mg kg(^{-1})</th>
<th>(\delta^{15})N, ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Igneous</td>
<td>0-3350</td>
<td>-11.2 to +14.9</td>
</tr>
<tr>
<td>Metasedimentary</td>
<td>0-2140</td>
<td>1.0 to 17.0</td>
</tr>
<tr>
<td>Sedimentary</td>
<td>17-20,300</td>
<td>-2.68 to +8.48</td>
</tr>
<tr>
<td>Ore and Hydrothermal</td>
<td>0-230,000</td>
<td>-0.9 to +23.7</td>
</tr>
</tbody>
</table>

Source: Holloway & Dahlgren, 2002, Global Biogeochem Cycles
Nitrogen Dissimilatory Processes

N Valence

Reduction

+5

Oxidation

+3

+1

0

-3

Dissim. Nitrate Reduction

Dissim. Nitrate Reduction

Nitrite and nitrous oxide are important; even if only trace amounts.

Dissolved gases are important.

Processes fractionate N and O isotopes.

Nitrification

Anammox

Denitrification

Nitrite and nitrous oxide are important; even if only trace amounts.

Dissolved gases are important.

Processes fractionate N and O isotopes.
Denitrification in Ground Water
An Example

WATER TABLE

Groundwater Flow

Physical

Biological

Geochemical

Controlling Factors

Denitrification in Ground Water

An Example

NO$_3^-$ $\rightarrow$ N$_2$

Cells that contain nirK
Cells that contain nirS

Micro-colony on sand grain

OXYGEN

WATER TABLE

NITRATE

DOC

GROUND WATER FLOW
Nitrogen Biogeochemistry at HUSEP

3 Phase Approach

1. Nitrogen Geochemistry Characterization.
3. Nitrogen Cycle Process Assessment
Nitrogen Biogeochemistry at HUSEP

1. Nitrogen Geochemistry.

- What are the chemical forms and quantities of N present?
  - Solid vs. dissolved
  - Bulk vs. trace
  - Ionic vs. gaseous
  - Organic vs. inorganic

- What is the isotopic signature of the N species?
  - Evidence for biological fractionation?

- What is potential for N enrichment via construction process?
  - Possible tracers?

Approach
Collect water and rock samples and assay for N species and N isotopes. Correlate with local and regional geology.
## Nitrogen in Henderson Water Samples

<table>
<thead>
<tr>
<th>Site</th>
<th>Nitrate µM</th>
<th>Nitrite µM</th>
<th>Nitrous oxide µM</th>
<th>Ammonium µM</th>
<th>Total DIN µM</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>17</td>
<td>26</td>
<td>6</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>D4</td>
<td>47</td>
<td>46</td>
<td>17</td>
<td>112</td>
<td>222</td>
</tr>
</tbody>
</table>

Samples collected March 23, 06 with packer to maintain redox
2. Nitrogen Cycle Functional Gene Assessment

- What are the predominant N cycle functional genes present in subsurface biomass?
  - Nitrogen fixation: \textit{nifH}, \textit{nifD}
  - Denitrification: \textit{nirS}, \textit{nirK}, and \textit{nosZ}
  - Nitrification: \textit{amoA}

**Approach**

Collect biomass samples; extract DNA; perform quantitative PCR analysis for selected functional genes.
Correlate with other studies on microbial community analysis and enrichment culture.
3. Nitrogen Cycle Process Assessment

- What N cycle processes are active in the deep subsurface? Are the *in situ* rates or even potential rates quantifiable?

- Processes that might be examined:
  - Nitrogen fixation: acetylene reduction
  - Denitrification: acetylene block; $^{15}$N tracer tests with pathway intermediates
  - Nitrification: $^{15}$NH$_4^+$ tracer tests

**Approach**

A. Conduct long-term, controlled activity assays in lab.

B. Conduct tracer tests in the field.