Denitrification in a Shallow Aquifer Underlying a Dairy Farm: New Approaches to Characterization and Modeling: UCRL-PRES-207404

Bradley Esser, Harry Beller, Steven F. Carle, G. Bryant Hudson, Staci Kane, Roald Leif, Tracy Letain, Jean E. Moran, Walt W. McNab, and Andrew F.B. Tompson

Lawrence Livermore National Laboratory
We must understand denitrification to simulate nitrate transport

- Denitrification requires
  - Denitrifying bacteria
  - Low oxygen conditions (< 0.6 mg/L)
  - An electron donor

- Heterotrophic denitrification
  \[ 4\text{NO}_3^- + 5\text{CH}_2\text{O} + 4\text{H}^+ \rightarrow 2\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O} \]

- Autotrophic denitrification
  \[ 14\text{NO}_3^- + 5\text{FeS}_2 + 4\text{H}^+ \rightarrow 7\text{N}_2 + 10\text{SO}_4^{2-} + 5\text{Fe}^{2+} + 2\text{H}_2\text{O} \]
The Goal is to predict the future concentration of nitrate at a receptor (drinking water well)

Rate of nitrate change at a given receptor

\[
\frac{\partial c_{NO_3^-}}{\partial t} = \text{Dispersion: mechanical dispersion, molecular diffusion} + \text{Advection: bulk groundwater flow} + \text{Source/sinks: mass loading, reactions}
\]

\[
\frac{\partial (D \frac{\partial c_{NO_3^-}}{\partial x})}{\partial x} - \frac{\partial (\nu c_{NO_3^-})}{\partial x} + \sum_i S_{NO_3^-} + R_{NO_3^-}
\]

Local groundwater velocity:
(Darcy’s Law; age gradient)

Source loading:
nitrate migration to the water table

Denitrification rate expression:
microbial kinetics

Parameters that may vary in space and hence will be modeled stochastically
Quantifying denitrification requires a multi-disciplinary approach

- **Characterization of groundwater flow**
  - Historical and current WLs, pump tests
  - Tritium-helium age dating
  - Stable isotopes of the water molecule
  - Vadose zone instrumentation

- **Characterization of nitrate biogeochemistry & source**
  - Real-time quantitative PCR
  - Microbial kinetics
  - Excess nitrogen
  - Stable isotopes of nitrate
    - $\delta^{15}$N and $\delta^{18}$O of NO$_3$
  - Co-contaminants as source tracers

- **Modeling groundwater flow and chemistry**
  - Stochastic models (for spatial heterogeneity)
  - Streamline & gridded flow & transport models
  - Reactive transport
We are using a molecular biology approach to measuring denitrification rates in the field.

Population-normalized denitrification rate
(µmol nitrate/time/cell, determined in the laboratory)

Aquifer denitrification rate
(µM nitrate/time, input for transport model)

Denitrifier population
(# cells/volume from qPCR analysis of field sample)

X

Population (# gene copies)* x Specific rate (rate/cell)** = Potential Denitrification Rate

# mRNA copies* x Rate/mRNA copy** = Actual denitrification rate

Nitrite reductase (NirS, NirK)

DNA → mRNA → protein

NO₂⁻ → NO

Quantitative real-time PCR is a rapid, sensitive, and highly specific method that can be used to quantify denitrifying bacterial populations based on a diagnostic, functional gene.
Key Aspects of Method Development: An assay for denitrifier population & population-normalized denitrification rate constants

- **Quantitative real-time PCR:**
  - Determining denitrifier populations
  - Determined sequence for an autotrophic denitrification gene (*nirS*).
  - High homology to heterotrophic *nirS* genes.
  - We are now designing a functional test for denitrifier cell populations using qPCR.

- **Microbial kinetics:** **Biomass-normalized ("specific") denitrification rate**

  Zero-Order Denitrification Rates (normalized to cell population)

  The physical state of the electron acceptor (aqueous or solid) is major control on rate.

  Denitrification rates are zero-order with respect to nitrate concentration.
Excess nitrogen measurements distinguish denitrification from dilution

- The end product of denitrification is molecular nitrogen ($N_2$, g)
  - Groundwater contains air above equilibrium solubility levels
  - “Excess nitrogen” is the non-atmospheric $N_2$ component due to denitrification
  - The atmospheric component is determined from the dissolved Ar concentration

- Excess $N_2$ allows quantification nitrate transformation
  - $F = 1 - \text{residual/initial nitrate}$
  - $\text{Initial} = \text{residual} + \text{excess } N_2$
  - With age information, a bulk denitrification rate can be determined
The excess nitrogen approach

Membrane inlet mass spectrometry
- Measures nitrogen, argon, oxygen, carbon dioxide, and methane
- Allows determination of excess nitrogen
- Fast, portable and inexpensive
Early results indicated that denitrification was taking place.
Several constituents were analyzed in the field during direct-push sampling

- **Field methods**
  - Nitrate “sticks”
  - Horiba water quality meter
    - DO, Conductivity, Temp, pH, ORP
  - MIMS: Excess N2, CO2, CH4, excess air

- **Samples**
  - Water samples: SS bailer (~500 mL)
    - 3 VOA vials for MIMS
    - 1 filtered 5 ml for IC
    - 125-mL or 1-L plastic for nitrate isotopics
  - Soil samples: 12-inch sections
    - RNA: Dry ice/ethanol → dry ice (15-ml tube)
    - DNA: Dry ice or ice (plastic bag)

- **Shallow water = irrigation?**
  - High nitrate; no excess N2; high DO
  - High excess air; high dissolved CO2

- **Deeper water = mixed canal/irrigation?**
  - Low or no nitrate; variable excess N2; low DO
  - Very low excess air; low dissolved CO2
Results of Excess Nitrogen Analysis:

DP1

<table>
<thead>
<tr>
<th>Depth (ft)</th>
<th>NO3 (or equivalent) ppm</th>
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<tbody>
<tr>
<td>26</td>
<td></td>
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<tr>
<td>31</td>
<td>residual nitrate</td>
</tr>
<tr>
<td>36</td>
<td>Xs N₂ as NO₃</td>
</tr>
<tr>
<td>41</td>
<td></td>
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<tr>
<td>46</td>
<td></td>
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</tbody>
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Water sample
The same pattern occurs all across the dairy site.
CPT-5
(upgradient site; NW corner)

CPT-6
(downgradient site; E edge)

Denitrification zones are not correlated to lithology

Newly installed MWs
We measured ORP, DO, pH, and other constituents. Field-measured ORP, nitrite, ammonium, and pH follow predictable trends.
Analytical results provide input for geochemical models

Dissolved CO₂ is negatively correlated with pH

Dissolved inorganic carbon decreases with depth
Nitrogen and Oxygen Isotopes of Nitrate indicate nitrate source and denitrification

Isotope signatures of nitrate sources may overlap

Changes in isotopic composition along a flowpath can indicate denitrification
Using nitrate co-contaminants as tracers

Herbicides, pesticides, and fecal sterols are likely co-contaminants

- **Method Development**
  - Solid Phase Extraction suitable for selected target analytes
  - GC-MS
  - GC-ECD Dual column
  - LC-MS/MS

- **Field Studies**
  - Lagoon source characterization for fecal sterols
  - Identified triazine herbicides
  - Identified norflurazon and desmethyl norflurazon

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**Herbicide-Chlorophyll/Carotenoid Pigment Inhibitor**

Conclusions

- Excess nitrogen is a fast, accurate method for identifying and quantifying denitrification in groundwater
- Denitrification should be considered in CAFO regulations
- 15 new shallow monitoring wells installed in September 2004
  - More accurate geochemical and isotopic data
  - Mixing of irrigation and canal water (age dating)
- Development of flow and transport models
  - Canal and irrigation recharge to perched aquifer
  - Use of CPT data as conditioning
  - Integration of kinetic models
  - Calibration to groundwater ages and measurements of DO, ORP, CO₂, excess air and N₂
  - Reactive transport modeling using analytical results