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

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We must understand denitrification to simulate nitrate transport

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

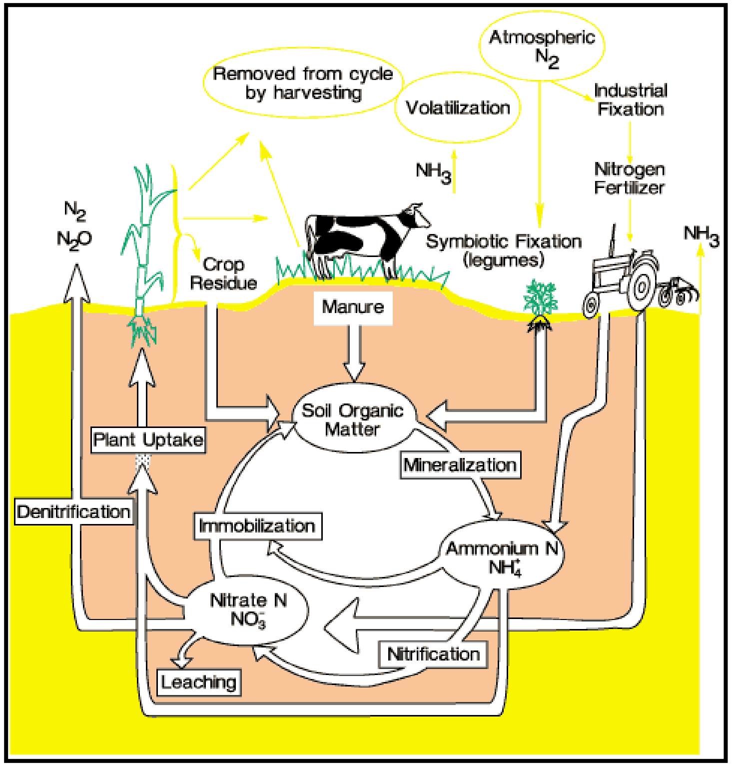
$NO_3^- \rightarrow$	$NO_2^- \rightarrow$	$NO \rightarrow$	$N_2O \rightarrow$	N_2
Nitrate (+5)	Nitrite (+3)	Nitric Oxide (+2)	Nitrous Oxide (+1)	Nitrogen (0)

- **Heterotrophic denitrification**

$$4NO_3^- + 5CH_2O + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$$

- **Autotrophic denitrification**

$$14NO_3^- + 5\,FeS_2 + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O$$



The Goal is to predict the future concentration of nitrate at a receptor (drinking water well)

Rate of nitrate change at a given receptor = **Dispersion**: mechanical dispersion, molecular diffusion + **Advection**: bulk groundwater flow + **Source/sinks**: mass loading, reactions

$$\frac{\partial c_{NO_3^-}}{\partial t} = \frac{\partial\left(D \frac{\partial c_{NO_3^-}}{\partial x}\right)}{\partial x} - \frac{\partial(\upsilon c_{NO_3^-})}{\partial x} + \sum_i S_{NO_3^-} + R_{NO_3^-}$$

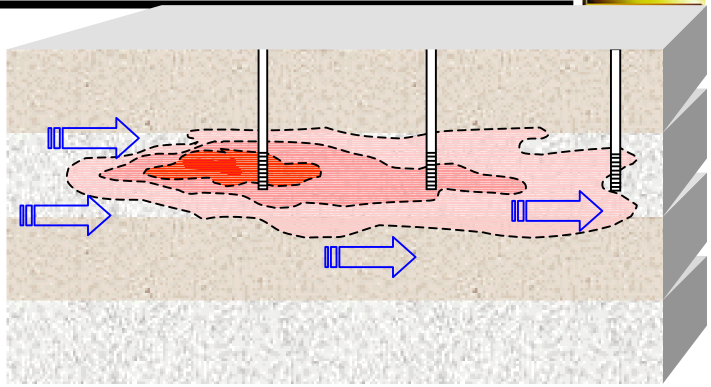
Local groundwater velocity: (Darcy's Law; age gradient)

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

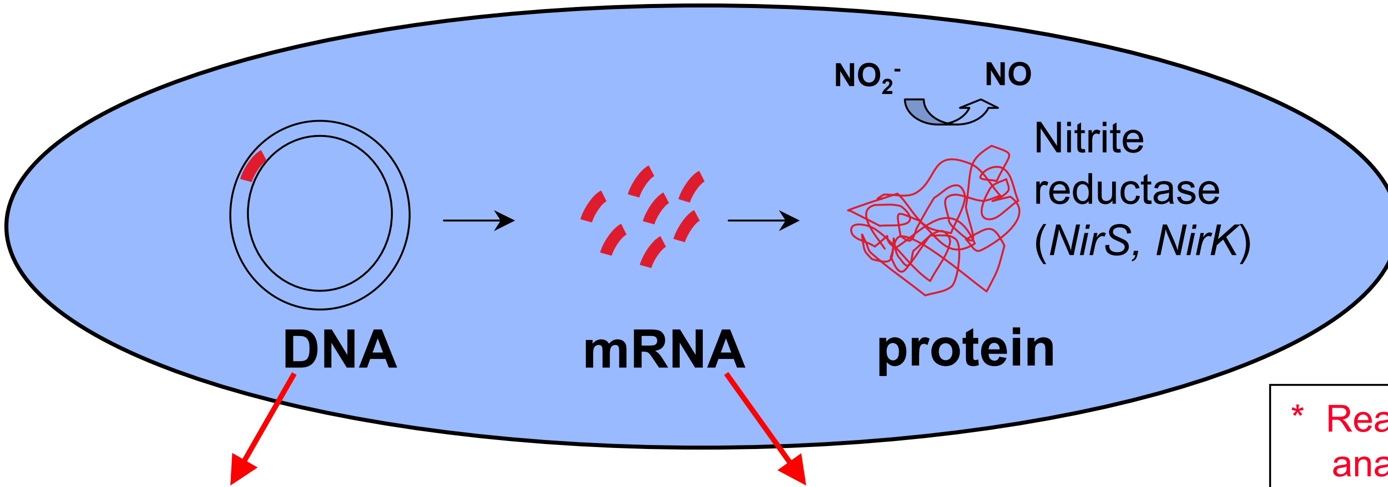
Denitrifier population
(# cells/volume from qPCR analysis of field sample)

X

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

=

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



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

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

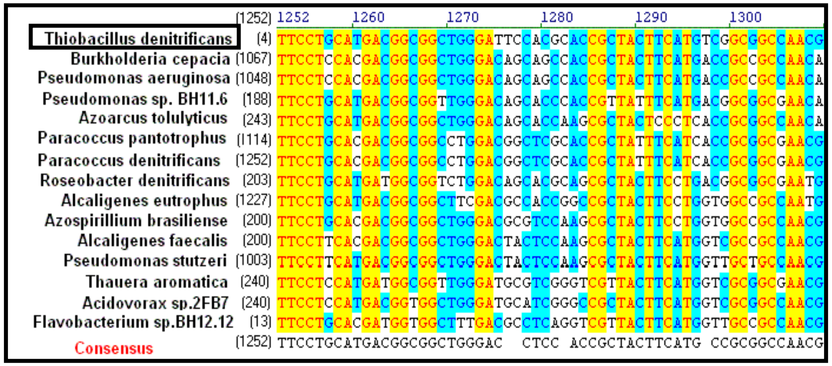
* Real-time PCR analysis of aquifer sample
** Lab-determined denitrification rate

- **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

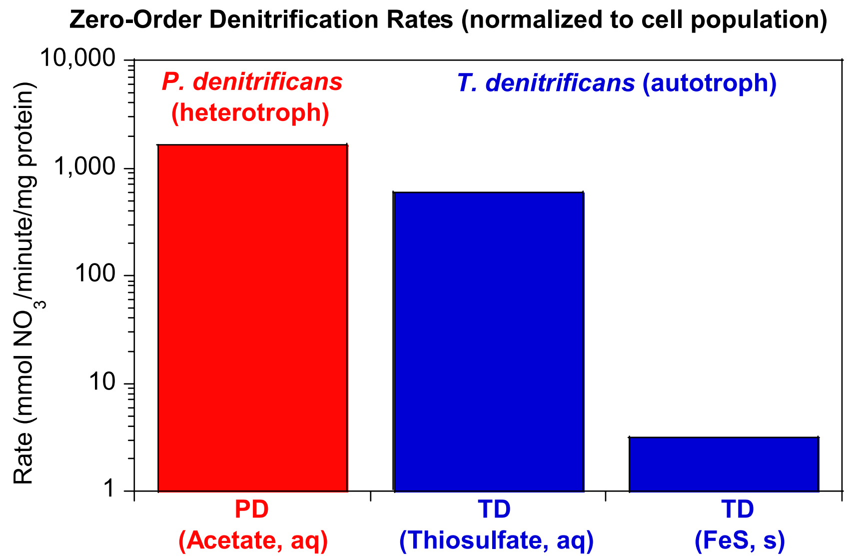
Quantitative real-time PCR:
Determining denitrifier populations

- 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.

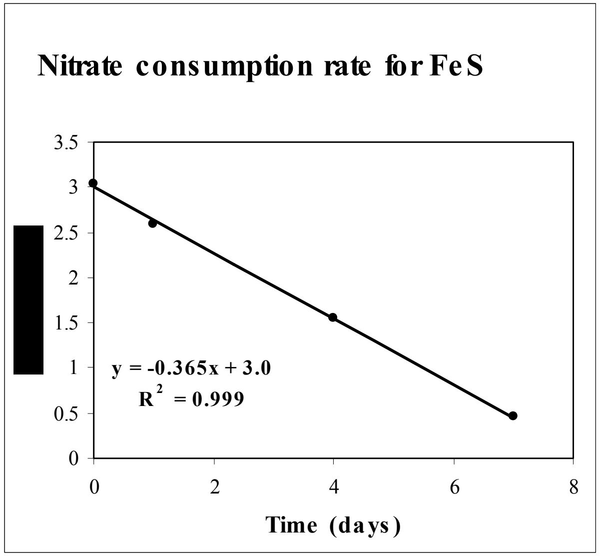


Gene sequences for denitrification gene

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



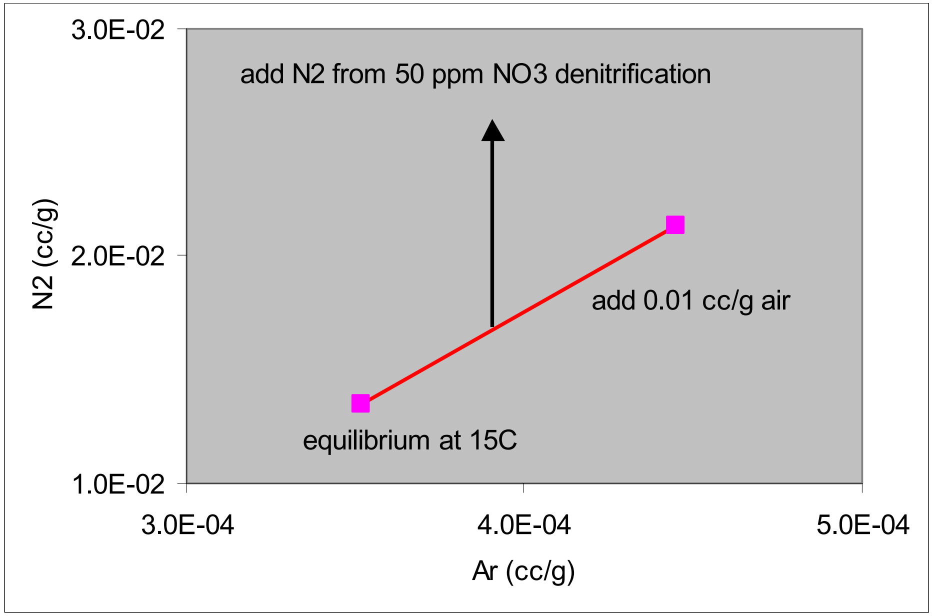
The physical state of the electron acceptor (aqueous or solid) is major control on rate.



Denitrification rates are zero-order with respect to nitrate concentration.

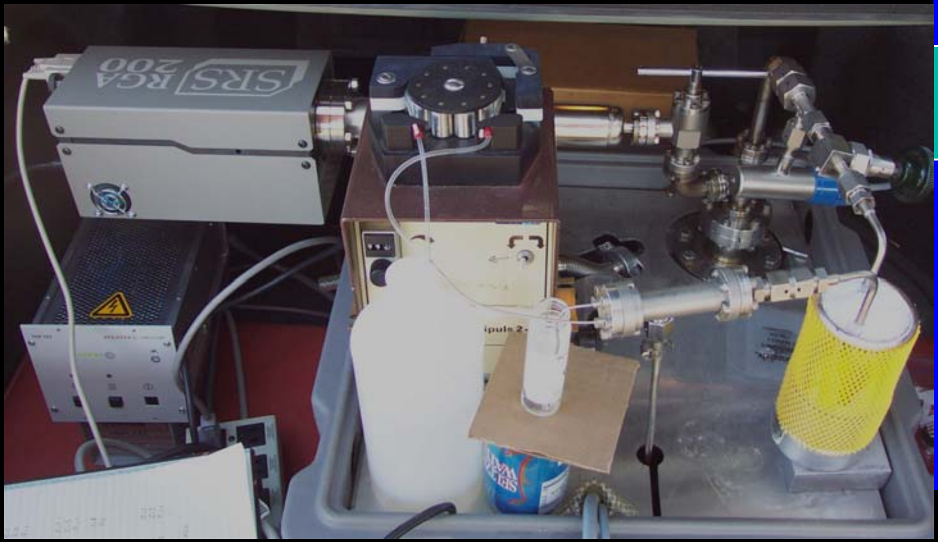
Excess nitrogen measurements distinguish denitrification from dilution

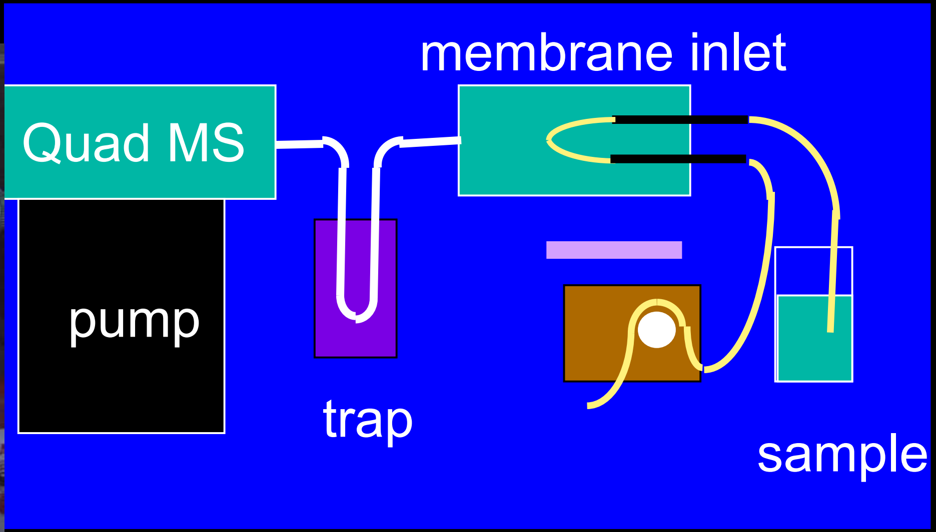
NO_3^- →	NO_2^- →	NO →	N_2O →	N_2
Nitrate (+5)	Nitrite (+3)	Nitric Oxide (+2)	Nitrous Oxide (+1)	Nitrogen (0)



- **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 - residual/initial nitrate**
    Initial = residual + excess N_2
  - **With age information, a bulk denitrification rate can be determined**

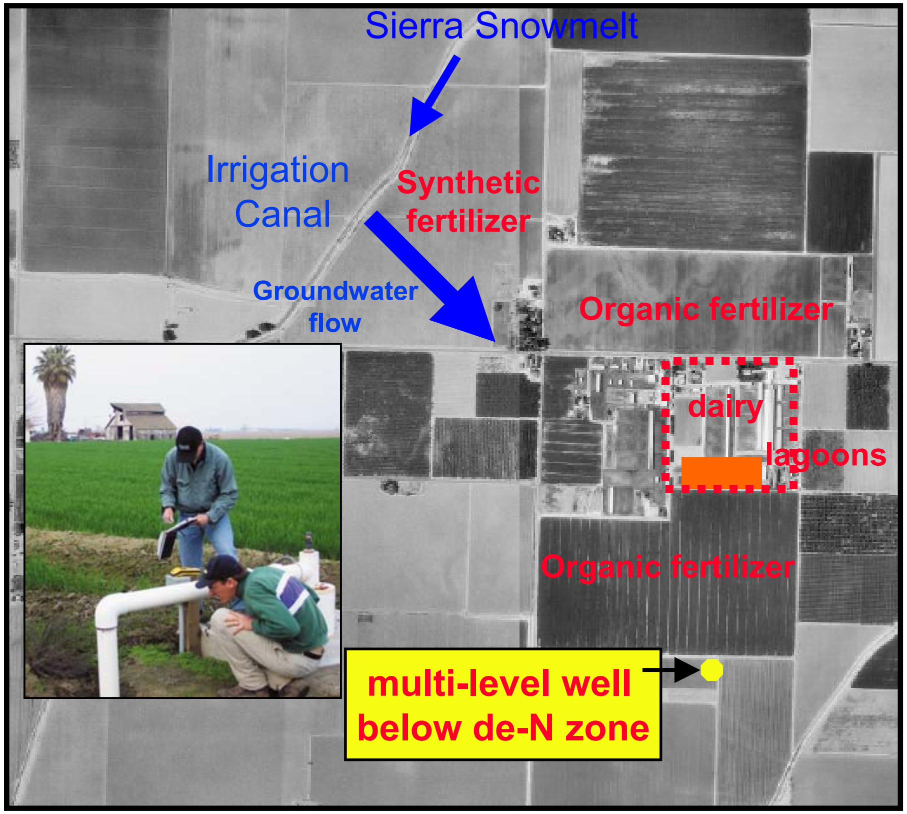
The excess nitrogen approach

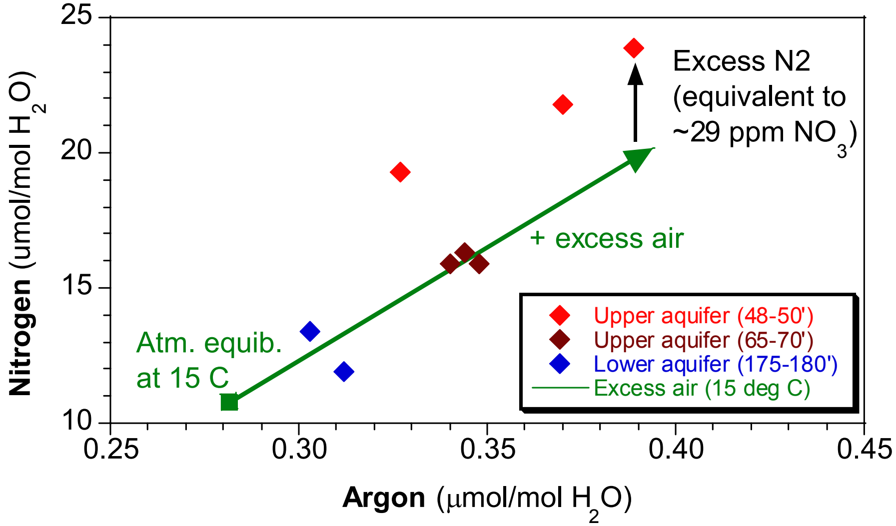




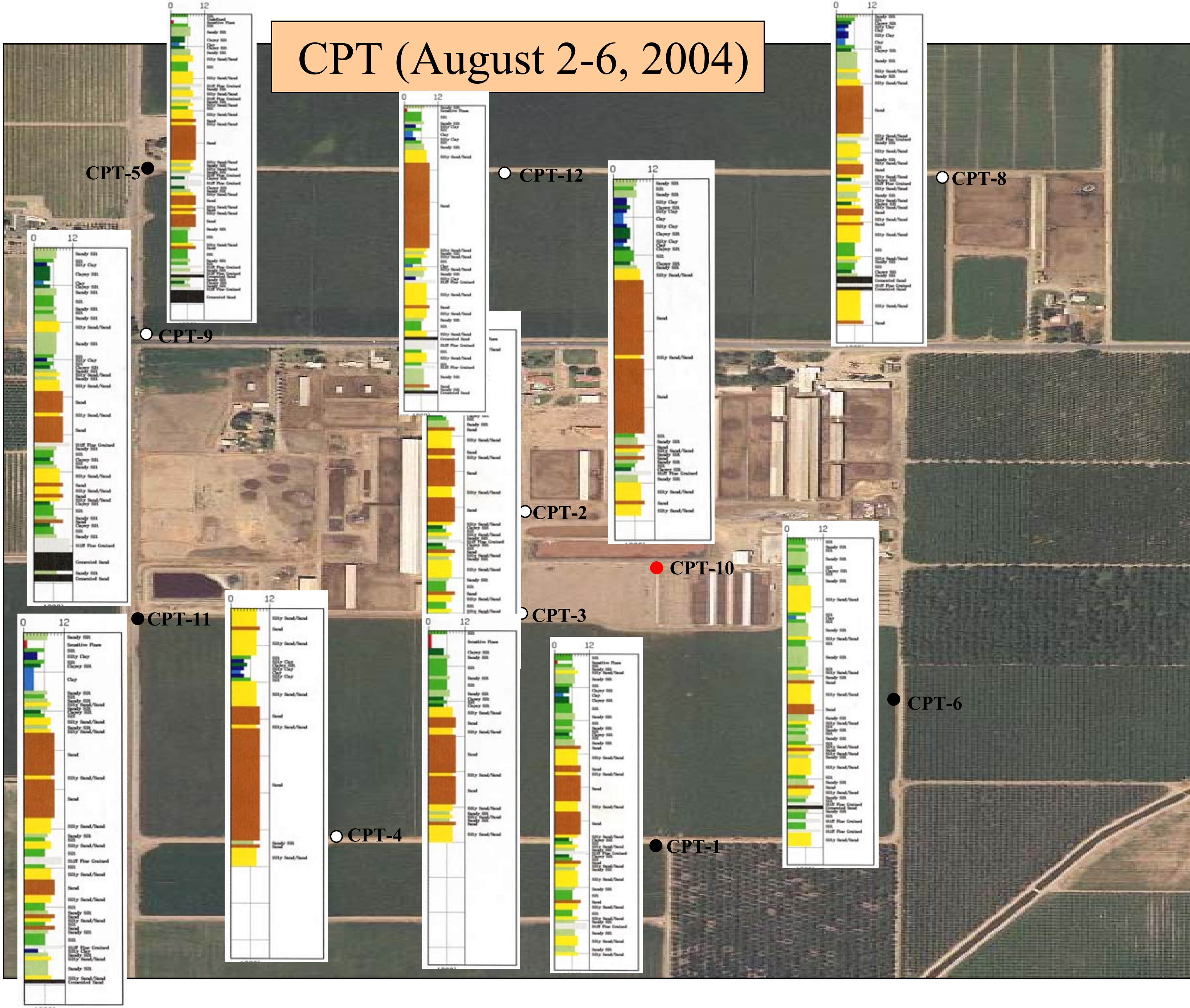
- **Membrane inlet mass spectrometry**
  - **Measures nitrogen, argon, oxygen, carbon dioxide, and methane**
  - **Allows determination of excess nitrogen**
  - **Fast, portable and inexpensive**

Early results indicated that denitrification was taking place





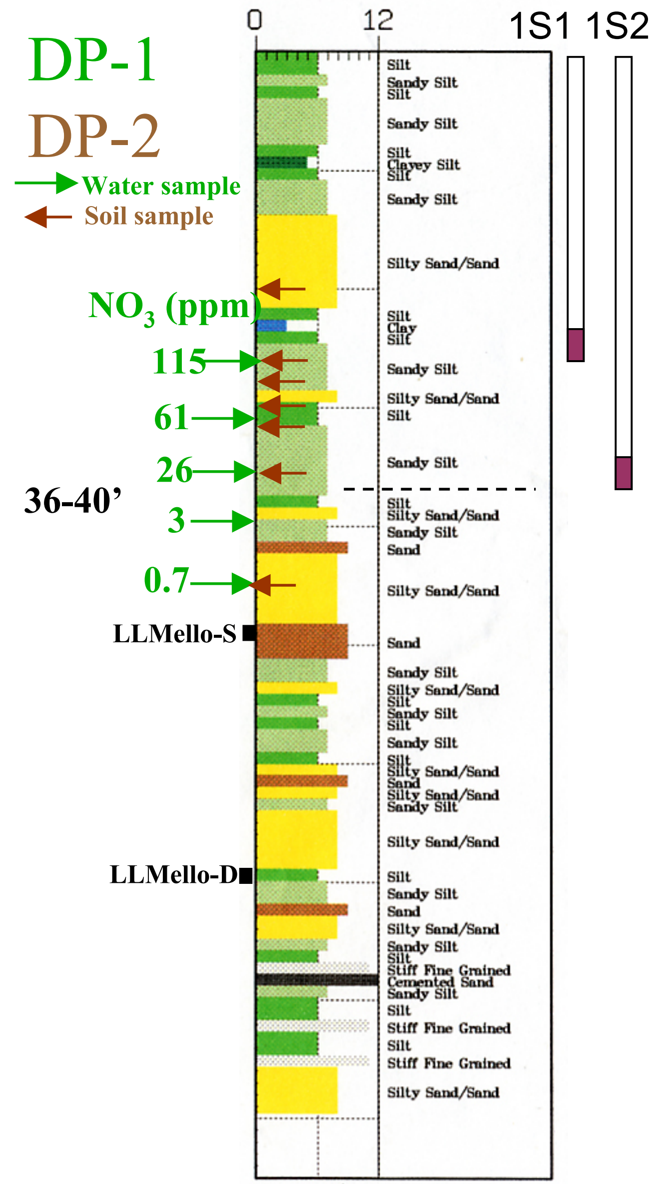
CPT (August 2-6, 2004)



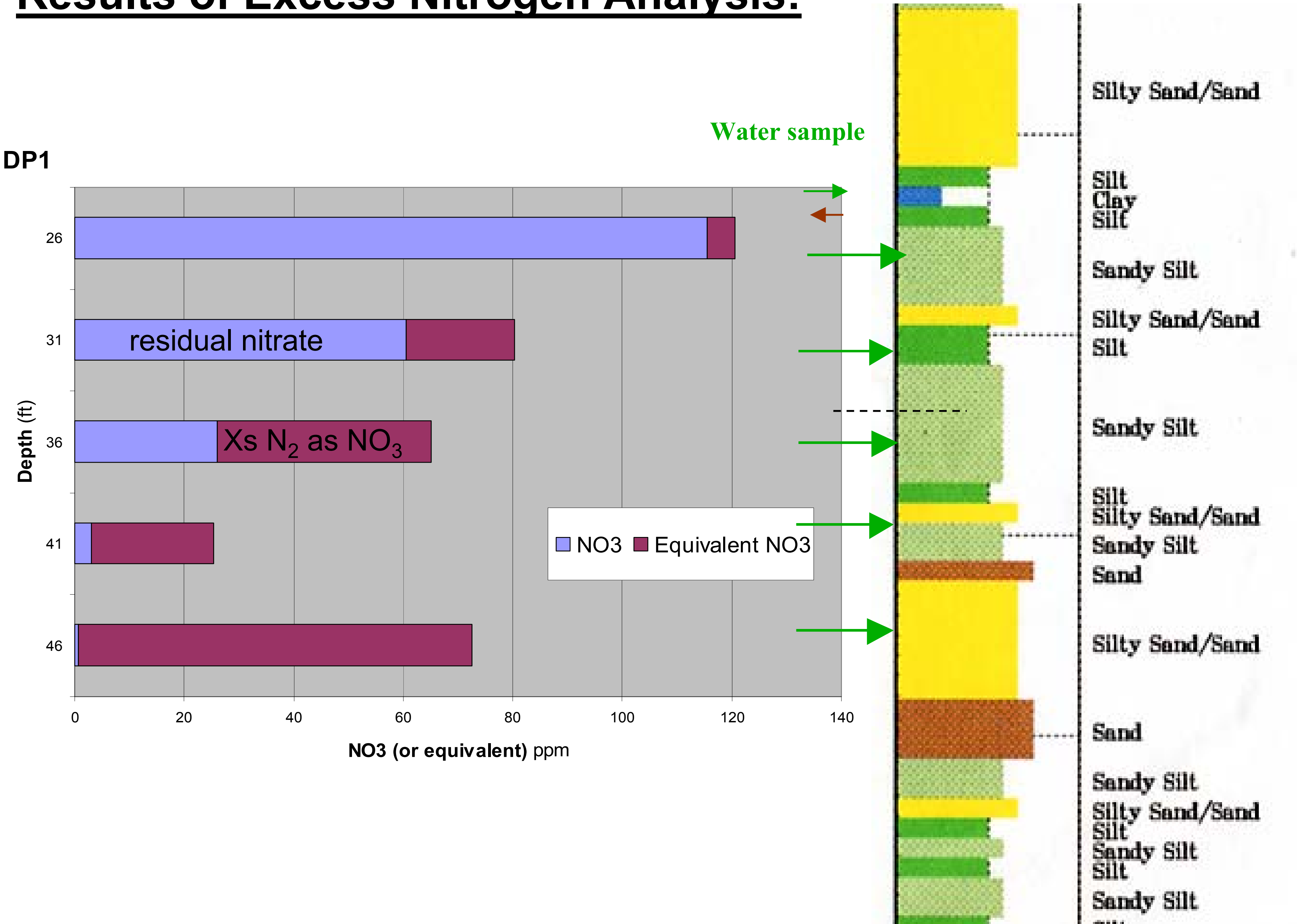
Several constituents were analyzed in the field during direct-push sampling

CPT-1

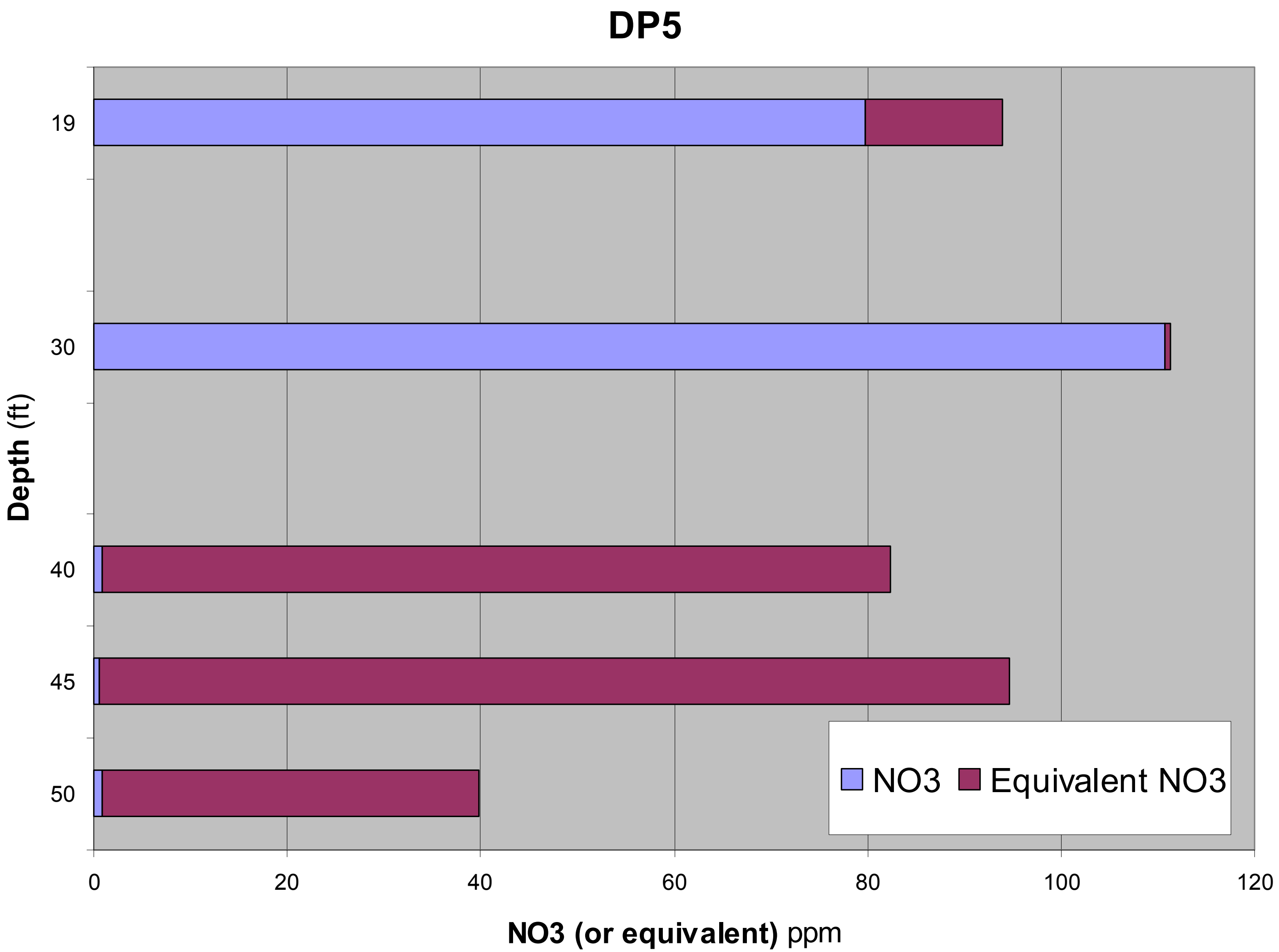
- **Field methods**
  - **Nitrate "sticks"**
  - **Horiba water quality meter**
    - **DO, Conductivity, Temp, pH, ORP**
  - MIMS: **Excess N2, CO2, CH4, excess air**
- **Samples**
  - **Water samples: SS bailer (~500 mL)**
    - **3 VOA vials for MIMS**
    - **1 filtered 5 ml for IC**
    - **125-mL 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**

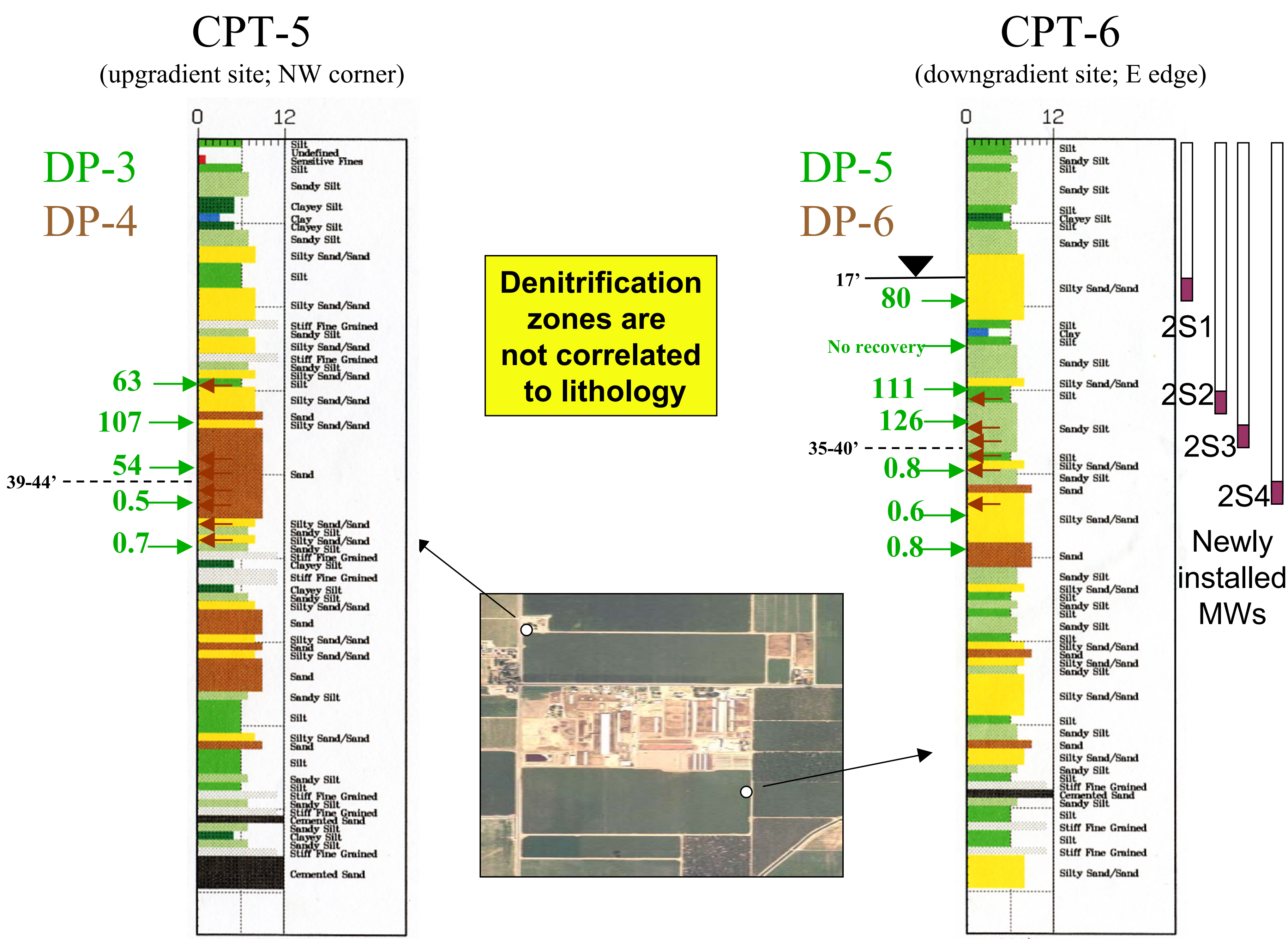


Results of Excess Nitrogen Analysis:



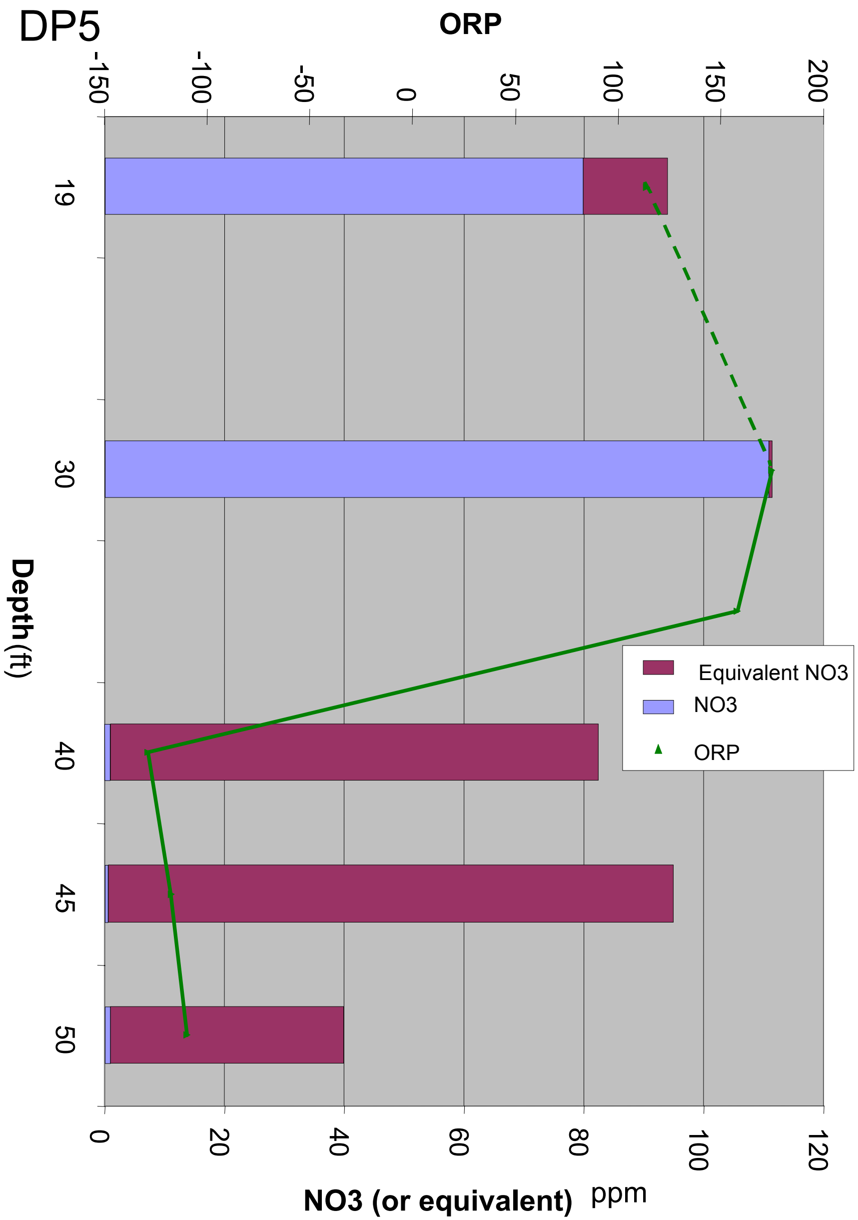
The same pattern occurs all across the dairy site.

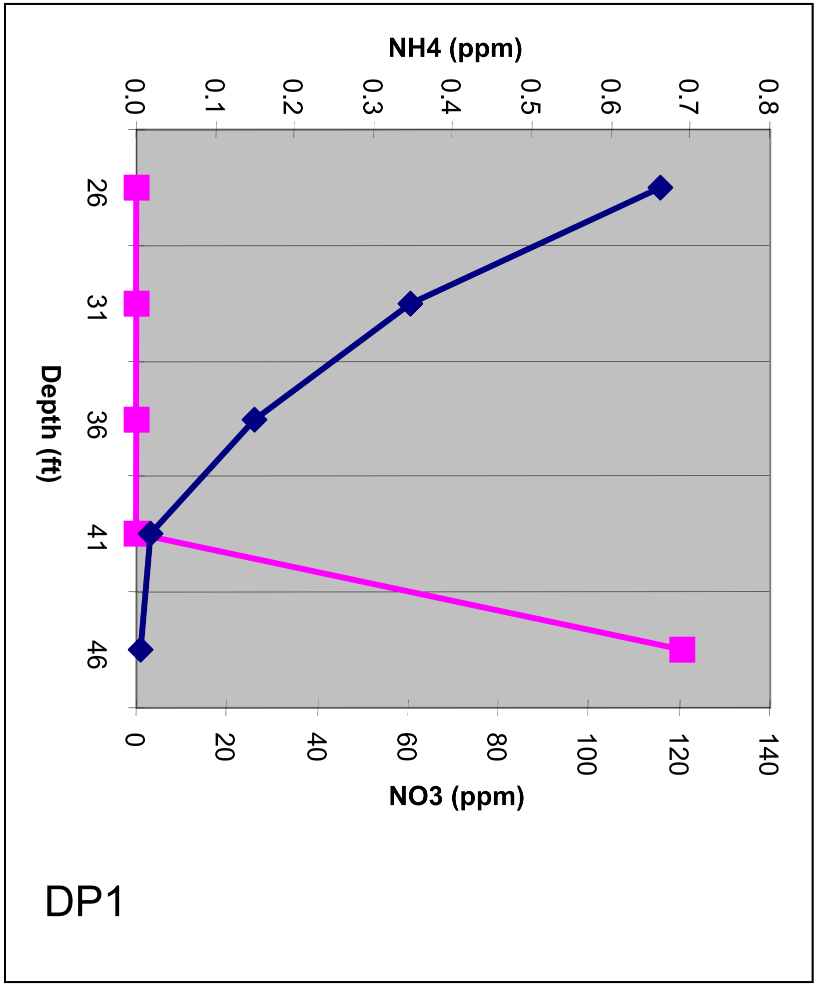



CPT-5
(upgradient site; NW corner)
CPT-6
(downgradient site; E edge)
DP-3
DP-4
DP-5
DP-6
Denitrification zones are not correlated to lithology
0
12
63
107
54
39-44'
0.5
0.7
17'
80
No recovery
111
126
35-40'
0.8
0.6
0.8
2S1
2S2
2S3
2S4
Newly installed MWs
Silt
Undefined
Sensitive Fines
Sandy Silt
Clayey Silt
Clay
Silty Sand/Sand
Stiff Fine Grained
Sand
Cemented Sand

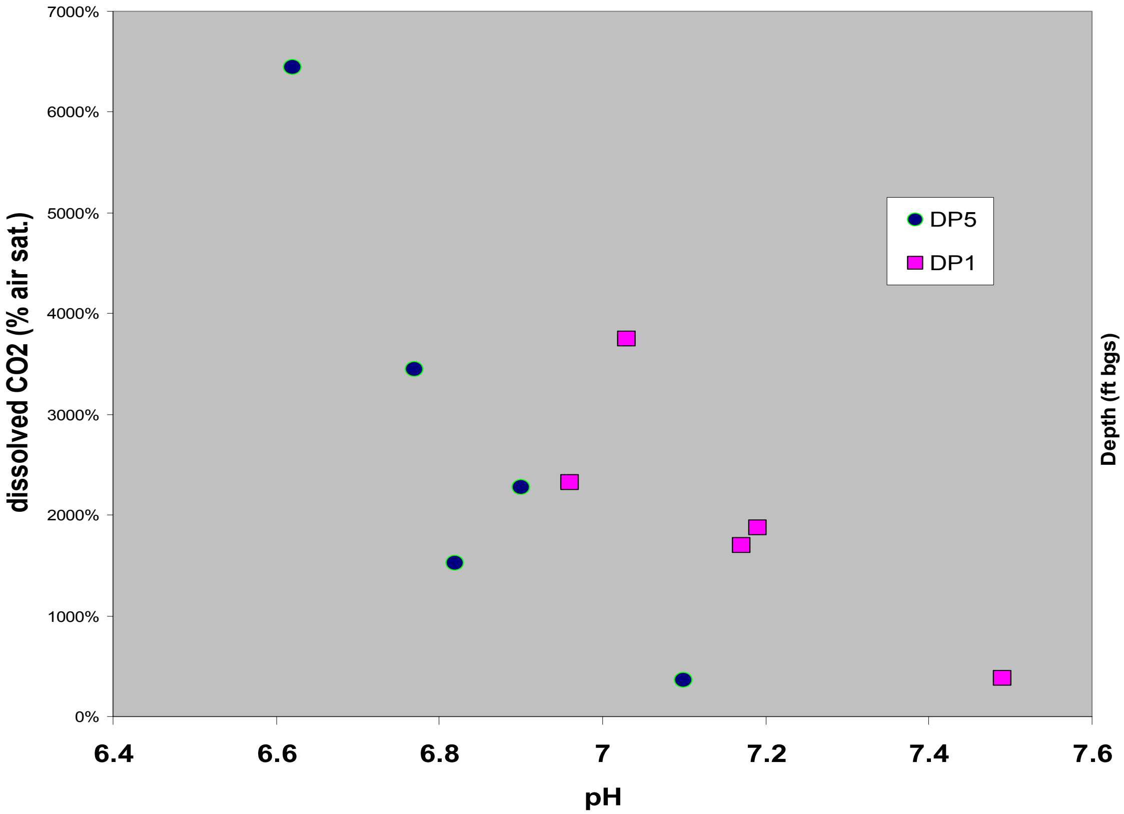
We measured ORP, DO, pH, and other constituents

Field-measured ORP, nitrite, ammonium, and pH follow predictable trends

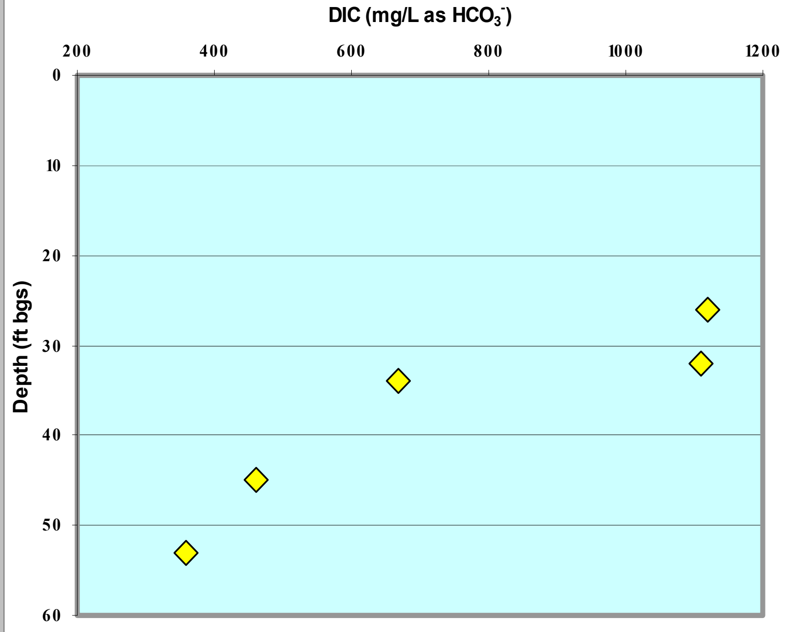




Analytical results provide input for geochemical models

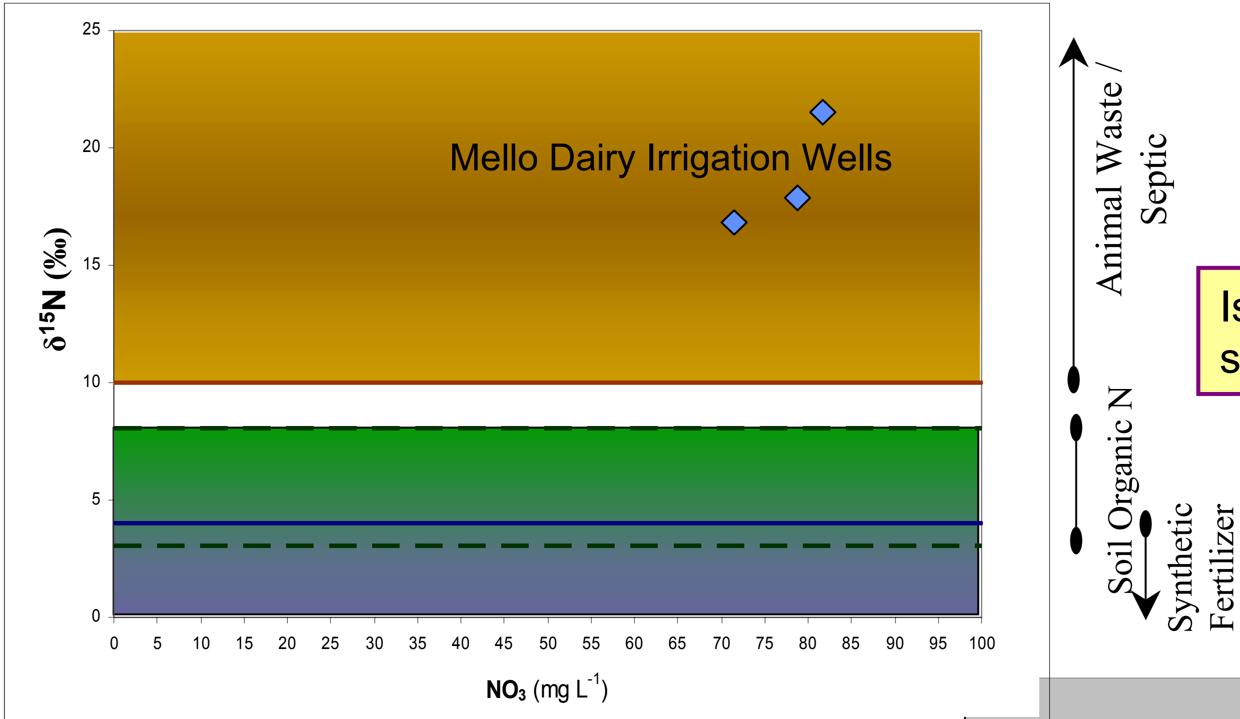


Dissolved CO_2 is negatively correlated with pH



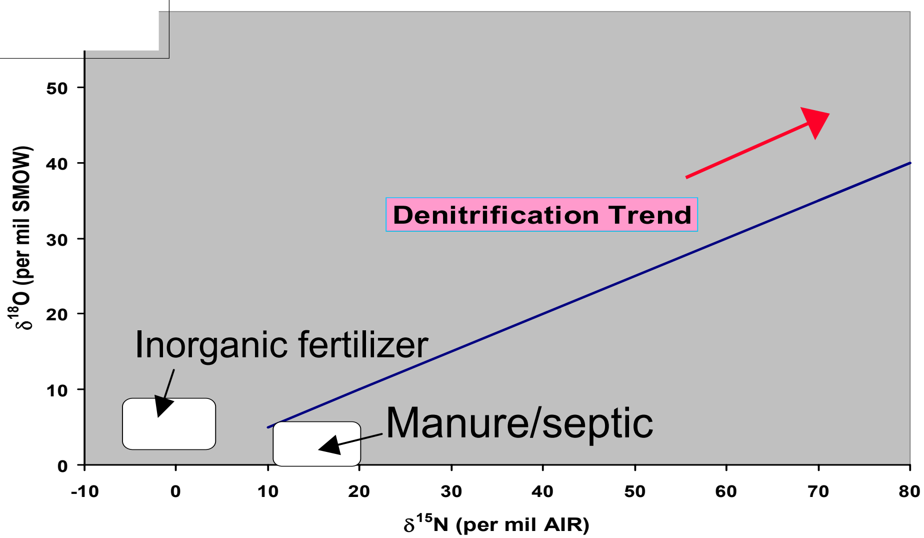
Dissolved inorganic carbon decreases with depth

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

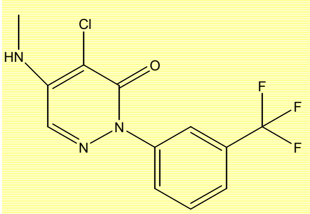


Using nitrate co-contaminants as tracers

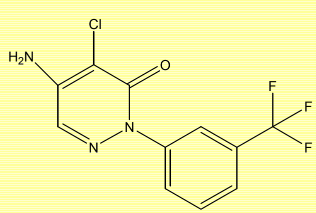
Herbicides, pesticides, and fecal sterols are likely co-contaminants

- **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 desmethylnorflurazon

Herbicide-Chlorophyll/Carotenoid Pigment Inhibitor

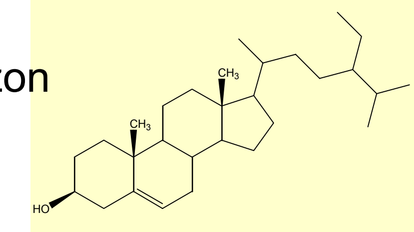


Norflurazon [27314-13-2]

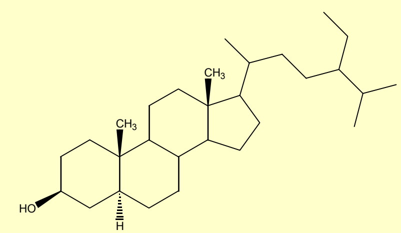


Desmethyl norflurazon [112748-69-3]

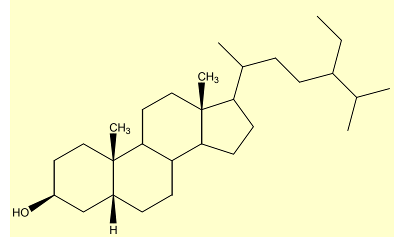
C_{29} Sterols



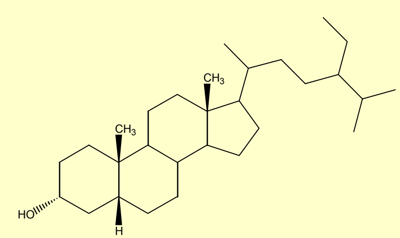
24-Ethylcholest-5-en-3β-ol (24-Ethylcholesterol or β-Sitosterol)



24α-Ethyl-5α-cholestan-3β-ol (24-Ethylcholestanol or Stigmastanol)



24-Ethyl-5β-cholestan-3β-ol (24-Ethylcoprostanol)



24-Ethyl-5β-cholestan-3α-ol (24-Ethyl-epicoprostanol)

Conclusions

- **Excess nitrogen is a fast, accurate method for identifying and quanitfying 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, CO_2, excess air and N_2**
  - **Reactive transport modeling using analytical results**