H31B-03: Trends of potential denitrification below the root zone in an agricultural setting, San Joaquin Valley, California, USA.

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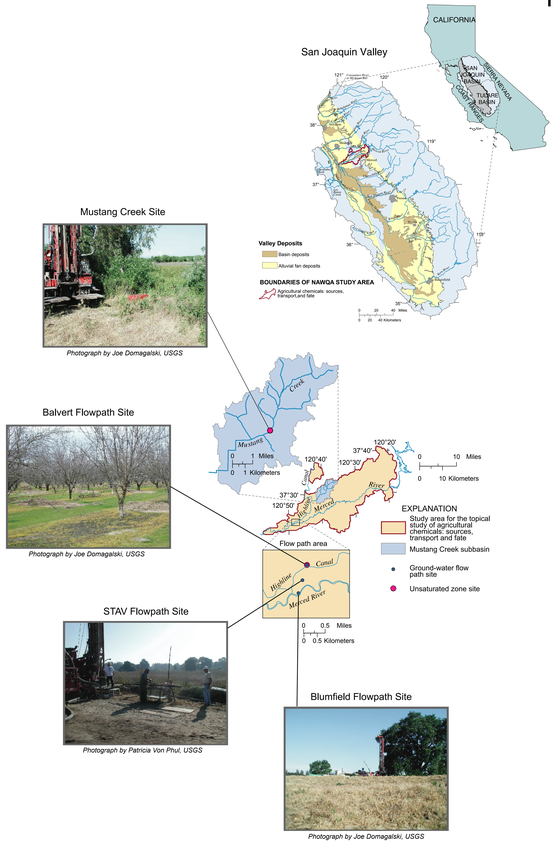


Figure 1. Maps and photographs of Mustang Creek and Flowpath sites.

Map figures adapted from Gronberg et al., 2004

1. Introduction

- We investigated correlations between potential denitrification and physical and chemical properties in the subsurface.

- Samples were collected above and below the water table from 4 sites in the San Joaquin Valley, California (Fig. 1). One site was located in the Mustang Creek sub-watershed. 20 km to the southwest, the Balvert, STAV, and Blumfield sites were located along a 1 km flowpath near the Merced River.

- Subsamples were analyzed for organic matter, nitrate, sulfate, orthophosphate, and grain size distributions.

- Denitrification enzyme assays (DEA) were completed on subsamples.

- N_2O production was modeled to estimate microbial growth and denitrification parameters.

- Correlations were measured for microbial, chemical, and physical parameters for the entire data set and for the Flowpath and Mustang Creek sites individually.

2. Methods

2a. Sample Collection and Handling

At the flowpath sites, an initial round of unsaturated samples was collected by drill rig and hand auger between 5/12/03 and 5/19/03. Hand auger samples were collected using a bucket auger, and were stored in plastic bags on ice, or in a refrigerator until DEA analysis. Drill rig bore holes were advanced using a blown-air technique and samples were collected with a driven 2 ft core barrel with plastic sleeve liner. All samples were stored on ice or in a refrigerator until DEA analysis. DEA analyses were run between 5/27/03 and 5/30/03, and a set of control analyses was run between 6/5/03 and 6/9/03.

At the Mustang Creek site, samples were collected during the initial round of drilling from 5/19/03 to 5/22/03. These included unsaturated sediments and a single sample of sediment from the capillary fringe. DEA analyses were run between 6/9/03 and 6/12/03, with control trials run between 7/5/0

Saturated samples were collected at the flowpath sites between 10/22/03 and 10/31/03. All samples were collected by drill rig using hollow stem augers with drilling mud. The drilling mud was a bentonite slurry amended with KBr to provide a means of determining the extent to which the drilling mud had entered sediment samples. Cores were collected in a driven 5 ft core barrel with plastic sleeve liners and were stored on ice until DEA analyses began on 11/6/03.

2b. Organic Matter

Organic matter content was estimated using loss on ignition (L.O.I.). Samples were placed in a muffle furnace at 430 degrees C for 24 hours (Navarro et al., 1993). Percent organic matter was calculated by dividing the mass loss after ignition by the dry weight of the sample.

2c. Nutrients

Nitrate, Orthophosphate and Sulfate concentrations were analyzed by ion chromatography following procedures described in McMahon et al., 2003.

2d. Grain Size Distribution

Random sub-samples were obtained by splitting samples in a Gilson Co. spinning riffler. Sub-samples were analyzed for grain size distribution using a Coulter LS-230 Particle-Size Analyzer which uses laser beam scatter to determine volumetric proportions of ranges of grain sizes (Gee and Or, 2002).

2e. DEA

Triplicate subsamples were run for each sample. DEA analyses were completed using the following procedure:

1) placed 5 gram subsamples in 50 mL flasks.
2) added 20 mL of filtered groundwater amended with 200 mg/L KNO_3 and 300 mg/L glucose.
3) sparged headspace and liquid in the flasks with helium for 10-15 minutes
4) injected 3 cc of acetylene into the sealed headspace of each flask
5) incubated at 22.5 degrees Celsius.
6) Periodically extracted 100uL or less of headspace from each flask and analyzed for N_2O content using a Shimadzu gas chromatograph.

2e. Kinetic Parameter Estimation

- A mathematical model of exponential growth with lag time was used to estimate microbial kinetics parameters by calibrating model predictions to measured values.

- Equations were solved with explicit finite differences.

- The model was calibrated to measured data by minimizing the sum of the squared log errors using a conjugate gradient optimization routine.

$$\frac{\P N}{\P t} = Mk_c$$

$$M = \begin{cases} M_0 & \text{for } t < t_{lag} \\ M_0 \exp(k_m t) & \text{for } t > t_{lag} \end{cases}$$

t : time
t_{lag} : the time before microbial growth commences
N : mass of NO_3 consumed
M : microbial biomass
k_c : the kinetic rate constant for degradation of NO_3
M_0 : initial biomass
k_m : maximum growth rate

3. Results

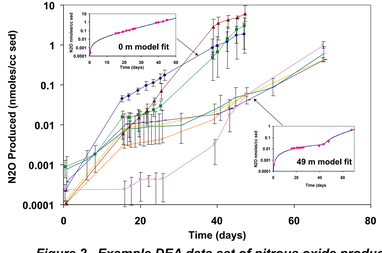
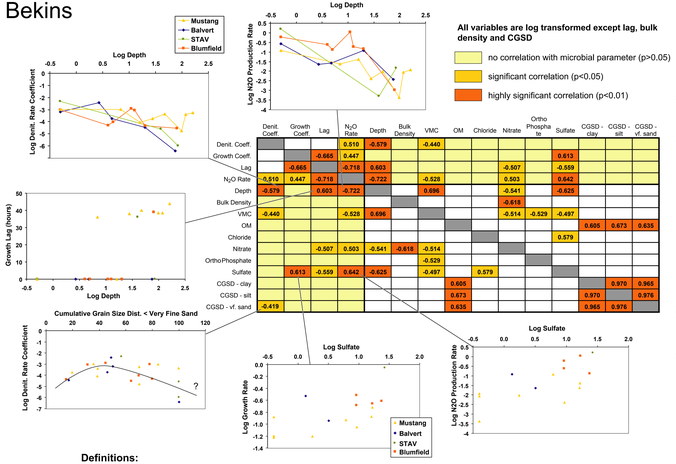


Figure 2. Example DEA data set of nitrous oxide production. Inset graphs show kinetic model fits to DEA data.



Definitions:

Denit. Coeff. - Denitrification coefficient (M_0*k_c from kinetic equations)
Growth Coeff. - Microbial growth coefficient (k_m from kinetic equations)
Lag - Microbial growth lag time (t_{lag} from kinetic equations)
N_2O rate - Amount of N_2O produced in first 24 hours of incubation
VMC - Volumetric moisture content
OM - Organic matter
CGSD - Cumulative grain size distribution (% volume) below the specified USDA category: clay, silt, or v.f. (very fine) sand.

Figure 3a. Correlation Matrix and scatterplots showing significant correlations for full dataset.

4. Discussion

- DEA datasets showed distinct differences in nitrous oxide production rates between samples (Fig. 2).

- The kinetic model was able to closely match measured nitrous oxide production for most samples (Fig. 2). Growth coefficients ranged over an order of magnitude. Denitrification coefficients ranged over 4 orders of magnitude (scatterplots in Fig. 3a), which might result from large variations in the initial microbial population term (M_0) in the denitrification coefficient (see kinetic equations).

- Significant correlations existed between physical, chemical and microbial parameters for the full data set and for the Flowpath and Mustang Creek sites (Fig 3a, b, c).

- For the full data set, the denitrification coefficient correlated most strongly with depth (probably reflecting decreasing population with depth) and the growth coefficient correlated most strongly with sulfate (Fig 3a).

- Grain size was correlated with the denitrification rate coefficient for the full data set and for the Flowpath sites. Scatterplots show a possible non-linear trend with maximum coefficients occuring at CGSD-silt = 10% (not shown) and CGSD-v.f. sand = 45%, probably reflecting larger populations for this sediment texture (Fig 3a).

- At the Mustang Creek site, there were no significant correlations with denitrification rate. There was a weak (p<0.1) correlation between sulfate and growth rate (Fig 3c).

- Organic matter content does not correlate well with microbial parameters, possibly due to interference in L.O.I. measurements by structural water in clay minerals, predominance of non-labile forms of organic matter, or other factors (Fig 3a, b, c).

	Denit. Coeff.	Growth Coeff.	Lag	N_2O Rate	Depth	Bulk Density	VMC	OM	Chloride	Nitrate	Ortho Phosphate	Sulfate	CGSD - clay	CGSD - silt	CGSD - vf. sand
Denit. Coeff.				0.614	-0.778		-0.657						-0.757	-0.559	
Growth Coeff.									0.668						
Lag				-0.736											
N_2O Rate	0.614		-0.736		-0.704		-0.674						-0.564		
Depth	-0.778			-0.704			0.838								
Bulk Density								-0.751			-0.719				
VMC	-0.657			-0.674	0.838							-0.793			
OM						-0.751				0.743	0.805			0.617	0.601
Chloride		0.668										0.834			
Nitrate									0.743						
Ortho Phosphate						-0.719		0.805						0.765	
Sulfate							-0.793		0.834						
CGSD - clay	-0.757			-0.564										0.882	0.874
CGSD - silt	-0.559							0.617			0.765		0.882		0.937
CGSD - vf. sand								0.601					0.874	0.937	

Figure 3b. Flowpath correlation matrix

	Denit. Coeff.	Growth Coeff.	Lag	N_2O Rate	Depth	Bulk Density	VMC	OM	Chloride	Nitrate	Ortho Phosphate	Sulfate	CGSD - clay	CGSD - silt	CGSD - vf. sand
Denit. Coeff.				0.733				0.541*							
Growth Coeff.														0.633*	
Lag					0.745					-0.713					
N_2O Rate	0.733									0.638*					
Depth			0.745									-0.695*			
Bulk Density															
VMC															
OM	0.541*													0.809	
Chloride															
Nitrate			-0.713	0.638*											
Ortho Phosphate															
Sulfate		0.633*			-0.695*										
CGSD - clay														0.711	0.746
CGSD - silt								0.809					0.711		0.911
CGSD - vf. sand													0.746	0.911	

* most significant correlations with microbial parameters (Sulfate and Nitrate p=0.09, OM p=0.17)

Figure 3c. Mustang Creek correlation matrix

5. Conclusions

- Calibration of the kinetic model of potential denitrification separates effects of microbial population, denitrification rate, and microbial growth parameters in DEA.

- Statistical analyses show correlations between microbial, physical, and chemical parameters and suggest potential relationships between sulfate and growth rate and between grain size distributions and denitrification rate.

- Correlations are consistent with a conceptual model of potential denitrification in which the overall rate is controlled by *in-situ* microbial abundance which tends to decrease with depth. However other parameters such as texture and nutrient concentrations may affect microbial kinetics.

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