

# Denitrification and Molecular Detection in Riparian Buffer Soils

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## Abstract

In North Carolina, riparian buffers are an important best management practice (BMP) in regulating the transport of nitrate in groundwater flow from uplands to surface water. Denitrification, the bacterially mediated conversion of nitrate into gaseous forms of nitrogen, is a major biogeochemical process contributing significantly to the groundwater purification. Denitrification has received much attention because it also produces nitrous oxide (N<sub>2</sub>O), a greenhouse gas that can promote ozone depletion. Extensive work has examined the denitrification rate in different riparian buffers and detected the putatively responsible denitrifying genes. However, the relationship between chemical denitrification rate and microbial activity is poorly understood. We measured the nitrate loss rate and N<sub>2</sub>O production rate as a function of organic carbon concentration given carbon types by continuous column experiments. Citric acid promoted denitrification while humic acid and alginate acid did not contribute to denitrification. From the biological perspective, we conducted real-time PCR to examine the responsible gene copy number in the soil. The results will help to link biological and chemical measurements of denitrification.

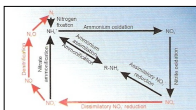
## Objectives

- To assess the rate of denitrification as a function of carbon type and concentration in riparian buffer soils through the column experiments.
- To quantify the gene copy number of the enzyme responsible for denitrification with method of real-time PCR.

## Introduction

### Denitrification

- Primary type of dissimilatory nitrate reduction in soil
- Affected by organic carbon, hydrology, vegetation, pH and etc.



### Responsible Genes

*nirK* (nitrite reductase K)

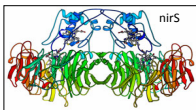
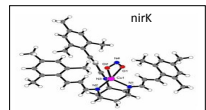
*nirS* (nitrite reductase S)

ATG\_GCC GAA CAG ATG.....

ATG CCA TTT GGC AAG.....

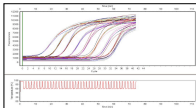
GCA CCA TCT GGC ACG TAA

CAG CAC GAC GTG TAC TGA



### Real-time PCR

- Amplification linked to fluorescence
- Rapid and quantitative assessment of the abundances of specific genes
- Not limited by cultivability



## Methods

### Continuous Column Experiments

- Soil collected from the R4W buffer at Center for Environmental Farming Systems (CEFS) which is the subject of a long term monitoring study
- Soil columns treated with 3 carbon types and 4 concentrations
- System purged with Argon gas to promote anaerobic conditions
- pH, electrode potential, and flow rate measured on daily base for 6 days
- Nitrate loss and nitrous oxides production measured with Automated Ion Analyzer and Gas Chromatograph, respectively

### Genes Quantification

- Genomic DNA extraction  
Performed with Mo Bio® Mega PowerSoil DNA Kit (Mo Bio, USA)  
Two extractions from each column  
DNA concentration measured spectrophotometrically with Nanodrop
- Primers Design

Target gene	Primer
16s rDNA	341F: 5'- CCT ACG GGA GGC AGC AG 515R: 5'- ATT CCG CGG CTG GCA
<i>nirK</i>	nirK876 5'- ATY GGC GGV AYV GCG A nirK1040 5'- GCC TCG ATC AGR TTR TGG TT
<i>nirS</i>	nirSCd3aF 5'- AAC GYS AAG GAR ACS GG nirSR3cd 5'- GAS TTR GGR TGS GTC TTS AVG AA

- Real-time PCR Assay  
QuantFast™ SYBR® Green PCR Kit (Qiagen, USA)  
Carried out in Mastercycler® ep realplex (Eppendorf, USA)  
Standards genome: *nirS* (ATCC33942-D); *nirK* and 16s(ATCC19718-D)  
Triplicate for each DNA extraction  
Real-time PCR conditions: 300s at 95°C for activation, 10s at 95°C for denaturation and 30s at 60°C for combined annealing and extension  
Fluorescence measured at the end of each data acquisition step  
Melting curve analysis performed

	Citric Acid	Alginate Acid	Humic Acid
0ppm	Control		
4ppm	CA 4	AA 4	HA 4
8ppm	CA 8	AA 8	HA 8
16ppm	CA 16	AA 16	HA 16



## Results

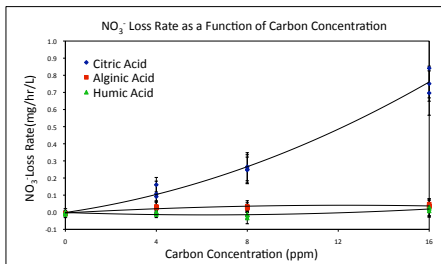


Figure 1\*. Nitrate loss rate as a function of carbon concentration given different carbon types: citric acid, alginate acid and humic acid.

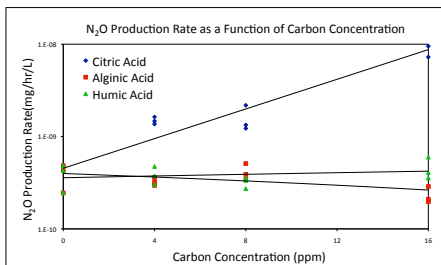


Figure 2\*. Nitrous oxide production rate as a function of carbon concentration given different carbon types: citric acid, alginate acid and humic acid.

\* Lines do not represent a model fit.

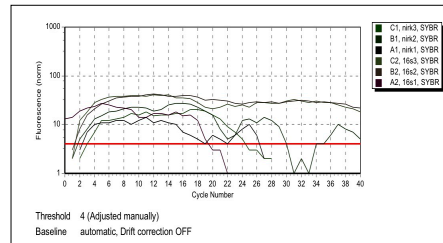


Figure 3. Amplification plot from the real-time PCR assay.

## Conclusions.

- The nitrate loss rate increased with increasing concentration of citric acid. The rate of nitrate loss was below the detection limit for all alginate acid and humic acid treatments.
- Nitrous oxide production trends were similar to those for nitrate loss, which was significantly increased with raising citric acid concentration.
- Real-time PCR held great promise for directly detect the gene responsible for the denitrification.

## References

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