# Aerobic Denitrification: Implications for Nitrogen Fate Modeling in the Missouri-Ohio-Mississippi (MOM) River Basin

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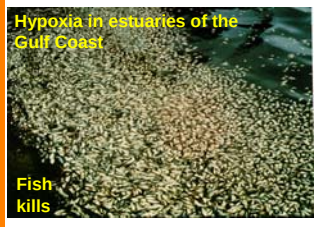
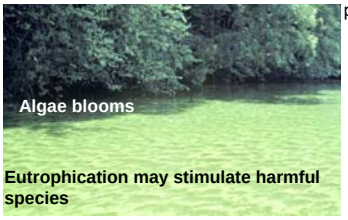
## Introduction

- N-compunds are the cause in ~50% of impaired water bodies.
- 1.6 million metric tons of N compounds discharged annually from the MOM Basin.
- $NO_3^-$ is dominant N species; understanding its behavior is essential for N-fate modeling.
- $NO_3^-$ has risen 3 – 10 fold since the early 1900s in major US rivers.
- High $NO_3^-$ renders groundwater impotable, rivers degraded & coastal productivity impacted.
- Denitrification is an important process for removing $NO_3^-$ from ecosystem because inert $N_2$ is the final product; however denitrification is a step-wise biogeochemical reduction of $NO_3^-$:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

- Moreover, convention holds that denitrification is a *strictly* anaerobic process.

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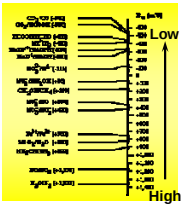
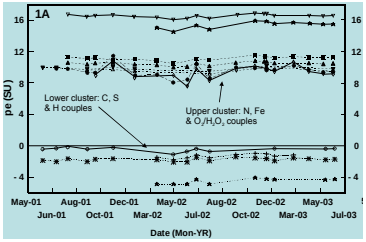
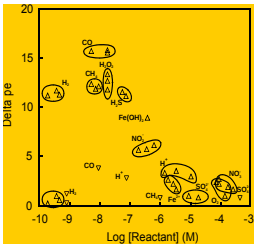
- The propensity for reduction reactions to proceed is often predicted by the oxidation state of a system.
- TEAP – widely accepted model for characterizing oxidation state.
  - Microbes reduce oxidants ***sequentially*** from high to low energy.
- Thus, under the TEAP model denitrification does not proceed until all of the oxygen is reduced or removed from the system.
- However, with thermodynamic analyses of field data, Washington et al. (2005) found dissolved oxygen and $C_{org}$ draw other couples toward potentials imposed by them, generating a bimodal clustering.




- While the redox clustering indicates ***thermodynamic*** control, the two potential clusters, instead of one, suggests ***kinetic*** constraints as well.
- Moreover, the rate of reaction is determined more by concentration than relative potential with reactants < $10^{-6}$M being slow to react & divergent from the clusters, whereas reactants >$10^{-6}$M are near equilibrium & in a cluster

## Hypothesis

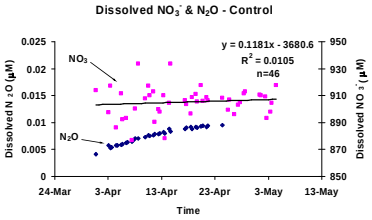
- These observations suggest that reactions of higher-potential oxidants with lower-potential reductants commonly proceed ***simultaneously, regardless of the presence of other potential reactants***.
- Thus, redox reactions can proceed in parallel as opposed to sequentially as often conceived in the TEAP conceptual model.
- Conventional thought long has held that nitrate reduction, or denitrification, does not take place when oxygen is present.
- However, based on the thermodynamic modeling, dissolved $O_2$ and $NO_3^-$ were reduced to nearly equal redox potentials in every sample we analyzed, suggesting simultaneous reduction of $O_2$ and $NO_3^-$.

*We propose that denitrification is **NOT** a strictly anaerobic process*

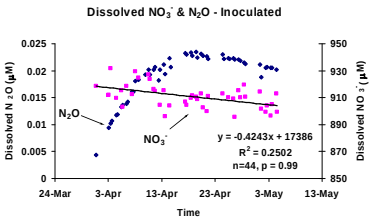
## Methods

- Four microcosm reactors were constructed.
- 800 mL of filtered (0.2μm) river water added to each jar and mixed with a magnetic stir bar.
- Added Ca-nitrate to 10 mg/L as N final concentration
- 1 jar as control; 3 jars inoculated with 3 mL of agricultural wetland sediment slurry (0.01 mg solids dry wt).
- Reactors were sealed with atmospheric-gas headspace, which was recirculated through the solution.
- Headspace gas and the solution were sampled twice daily for $N_2O$ and $NO_3^-$, respectively
- Dissolved $O_2$ measured by Fiber Optic Oxygen (FOXY) Electrodes

## Results




Control jar only modest increase in $N_2O$ to slightly above atmospheric values and no significant decrease in $NO_3^-$, while the inoculated jar produced $N_2O$ almost 3 times greater than atmosphere accompanied by significant reduction in $NO_3^-$ at p = 0.99. Dissolved oxygen was ~6.4 mg/L in both jars for the duration of the experiment.




These results have been corroborated with aerobic experiments on synthetic solutions we have inoculated and several environmental samples, including: spring, wetland, and river waters; GA wetland sediment, TN soil, SC pond sediment, and SC estuarine sediment suspensions. In every environmental medium we have tested, without exception, we have observed significant $N_2O$ ingrowth under aerobic conditions, supporting that aerobic denitrification is a common environmental process.

## Conclusions

- Contrary to TEAP theory, $NO_3^-$ can be reduced in oxic settings (e.g., the Mississippi River)
- Aerobic denitrification is expected to have a high impact on N-fate modeling as most existing models call for denitrification to take place in anoxic settings, yet most impacted surface waters are aerobic.
  - For example, within the denitrification module of the EPA's Water Quality Analysis Simulation Program (WASP), the kinetic expression for denitrification contains a first order rate constant, a temperature correction term, and a DO correction term, which calculates the decline in denitrification rate as DO levels rise above 0 using a user specified half-saturation constant $K_{NO_3}$ ($K_{NO_3}$ = DO level where denitrification rate is reduced by half). Currently, the default value is effectively zero ($10^{-20}$), preventing this reaction at all non-zero DO levels.
- While our experiments have qualified aerobic denitrification, our future work will be towards quantifying denitrification rates at varying DO concentration and temperature. Plans are to collaborate with WASP developers to incorporate aerobic denitrification into the model.

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