Quantifying Nitrate Loss Rates and Mechanisms in the Upper Floridan Aquifer Using Push-Pull Tracer Tests and Microbial DNA

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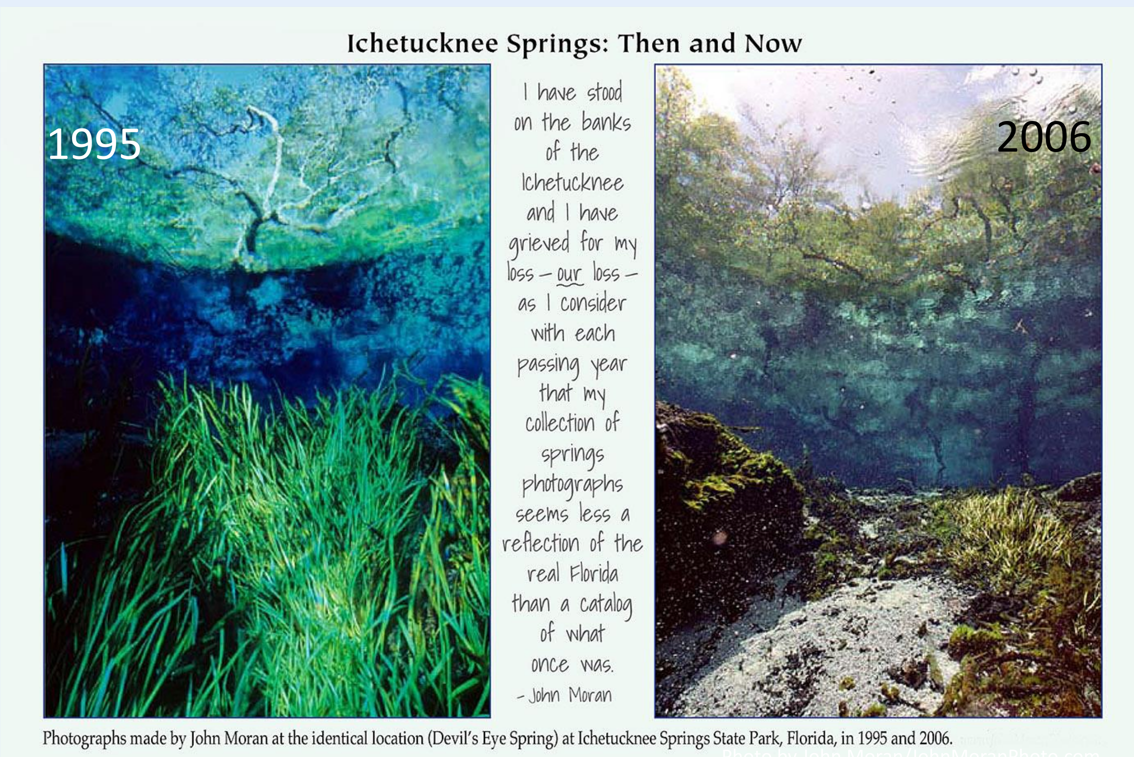
INTRODUCTION

Spring Ecology is Shifting



Reduced Plant Diversity/Species Richness

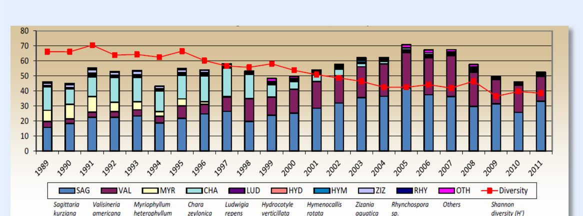


Fig. 1 Historic decreases in species diversity and richness richness for 10 surveyed plant species in the Ichetucknee River (Florida Springs Institute 2012)

Nitrate Concentrations Increasing

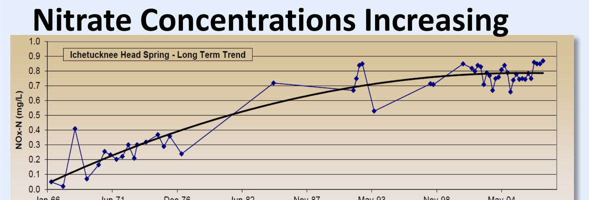


Fig. 2 NOx concentrations since 1966 in the Ichetucknee Head Spring showing long term increases (Florida Springs Institute 2012)

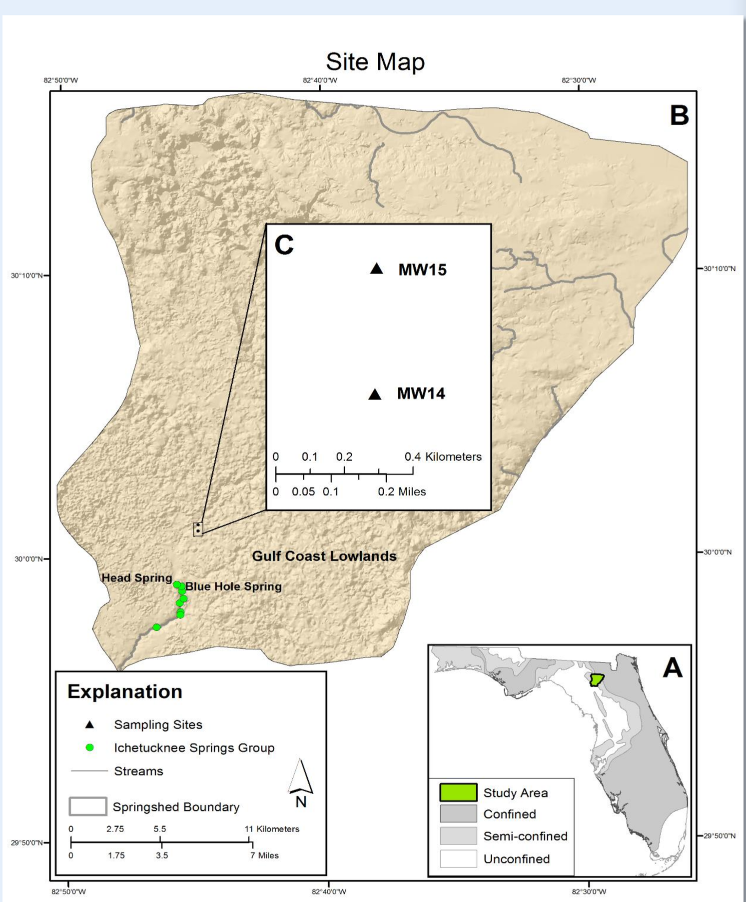


Fig.3 (a) Map of the confined, semi-confined, and unconfined portions of the Floridan Aquifer in north Florida (FDEP, 2006; modified). **(b)** Topographic map of the Ichetucknee springshed **(c)** Site map showing location of wells used for push-pull tests.

- Changes in spring vegetation have been correlated with increased nitrate concentrations.
- The fate of nitrogen in the Upper Floridan Aquifer is poorly understood.
    - Denitrification has been documented
    - Dissimilatory Reduction of Nitrate to Ammonium (DNRA) is assumed to be negligible
- In-situ transformation rates have not been measured.

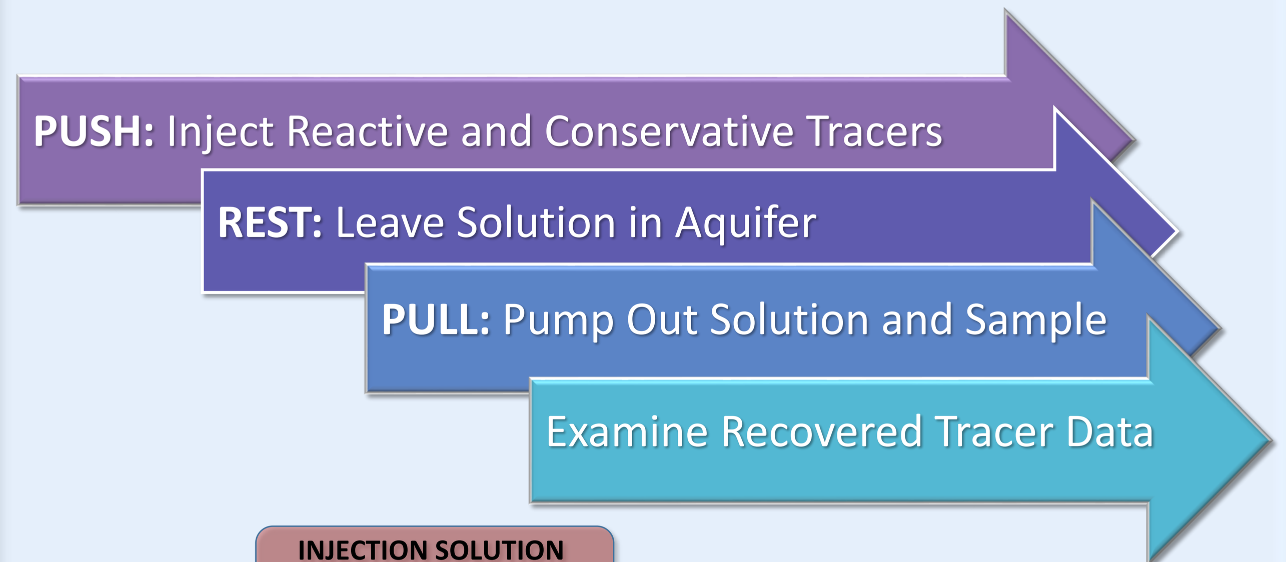
Research Questions

How do nitrate transformations vary across a redox gradient in the Upper Floridan Aquifer?

What is the effect of dissolved organic carbon (DOC) on observed nitrate transformation rates?

METHODS

Push-pull tracer tests and microbial DNA/cDNA examine in-situ nitrate transformations



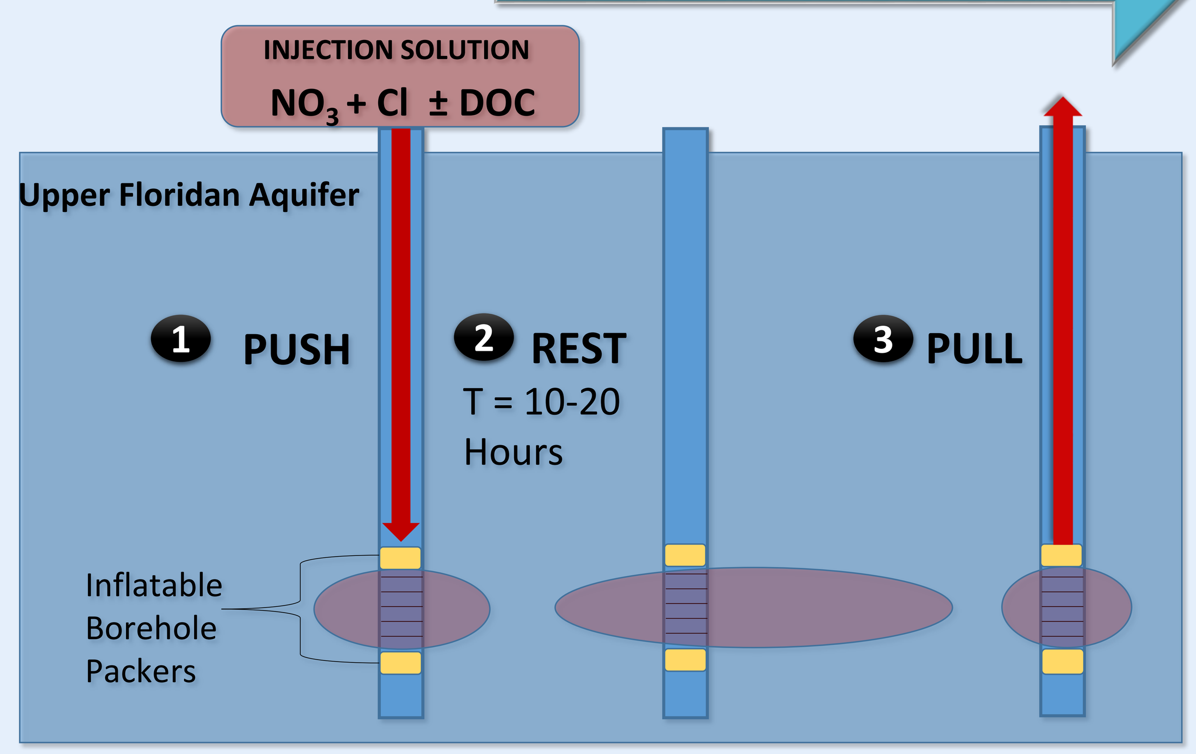


Fig. 4 Diagram showing push-pull method for determining in situ nitrate transformations.

Derive zero[th] order nitrogen loss rates from analysis of conservative and reactive tracer break through curve

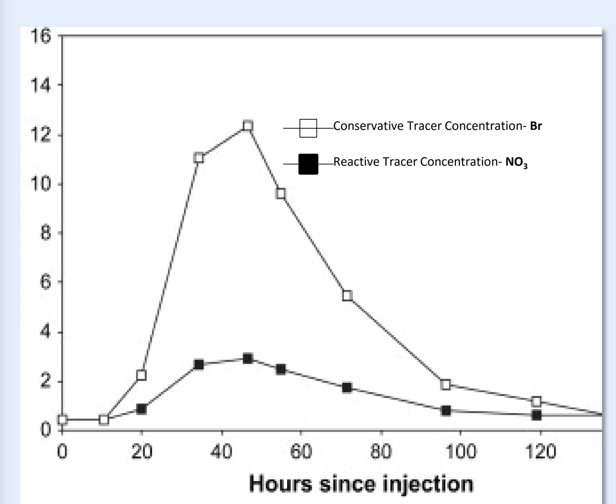


Fig. 5 Hypothetical break through curve for push-pull tracer test.

From Istok et al. (1997)

$$r = \frac{M_{inj} - \frac{M_{ext}}{R_{tracer}}}{V_{inj} * t^*}$$

Where:
r = Zero Order Reaction Rate
M_{inj} = mass injected
M_{ext} = mass extracted
R_{tracer} = recovered conservative tracer fraction
V_{inj} = volume injected
t^* = Time since tracer injection

Use microbial DNA and cDNA data to uncover nitrate transformation mechanisms.

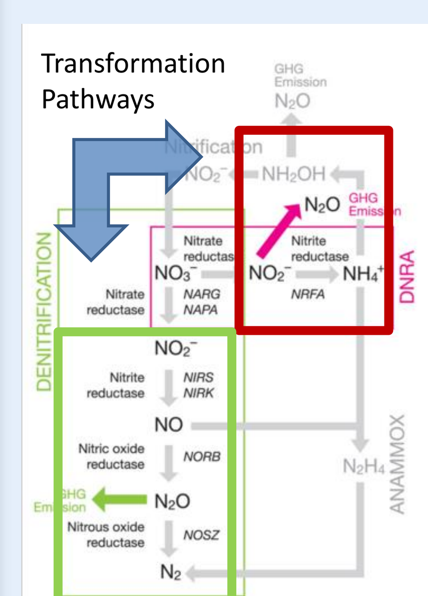


Fig. 6 Nitrate transformation pathways

Two Major Pathways:

DNRA: Nitrate conversion to Ammonium
Nitrogen is not removed from system.
Expected to occur in anoxic aquifer with high C:N ratio

NrfA- DNRA gene

DENITRIFICATION: Nitrate conversion to N_2 gas
Reactive nitrogen is removed from system.
Expected to occur only in anoxic aquifer with carbon

NirS- Denitrification gene
NirK- Denitrification gene

RESULTS

Push-pull Tracer Tests

OXIC SITE

Nitrate loss occurred

No significant NH_4

Zero order loss rate: 80 µmol*L^{-1}hour^{-1}

Nitrate loss occurred

Some NH_4 with carbon addition

Zero order loss rate: 50 µmol*L^{-1}hour^{-1}

ANOXIC SITE

Nitrate loss occurred

Increase in NH_4 with lack of carbon suggests chemolithotrophic DNRA

Zero order loss rate: 40 µmol*L^{-1}hour^{-1}

Maximum Nitrate Loss

No significant increase in NH_4 with carbon addition

Zero order loss rate: 130 µmol*L^{-1}hour^{-1}

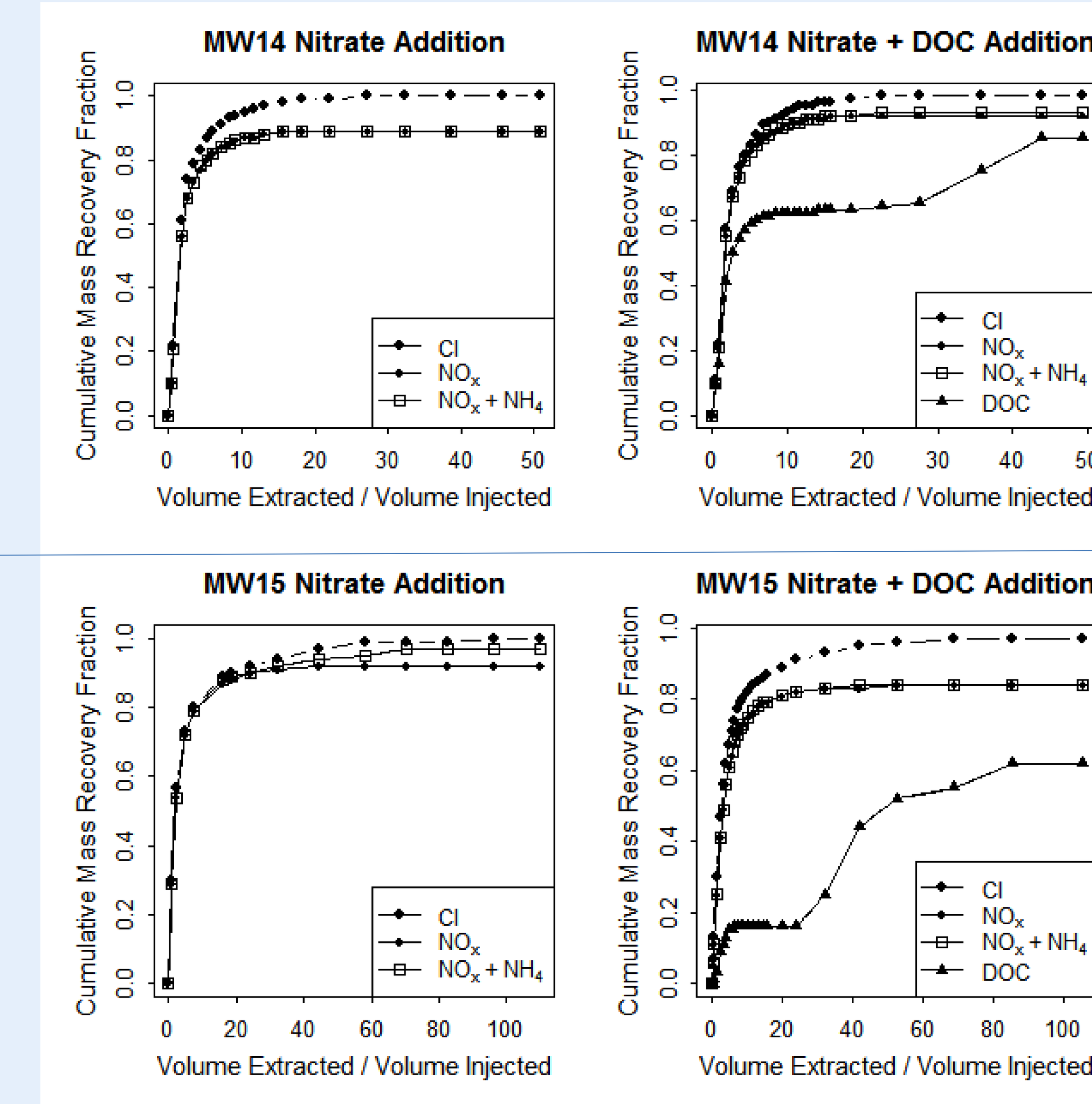


Fig. 7 Push-pull tracer test results

Microbial Genetic Data

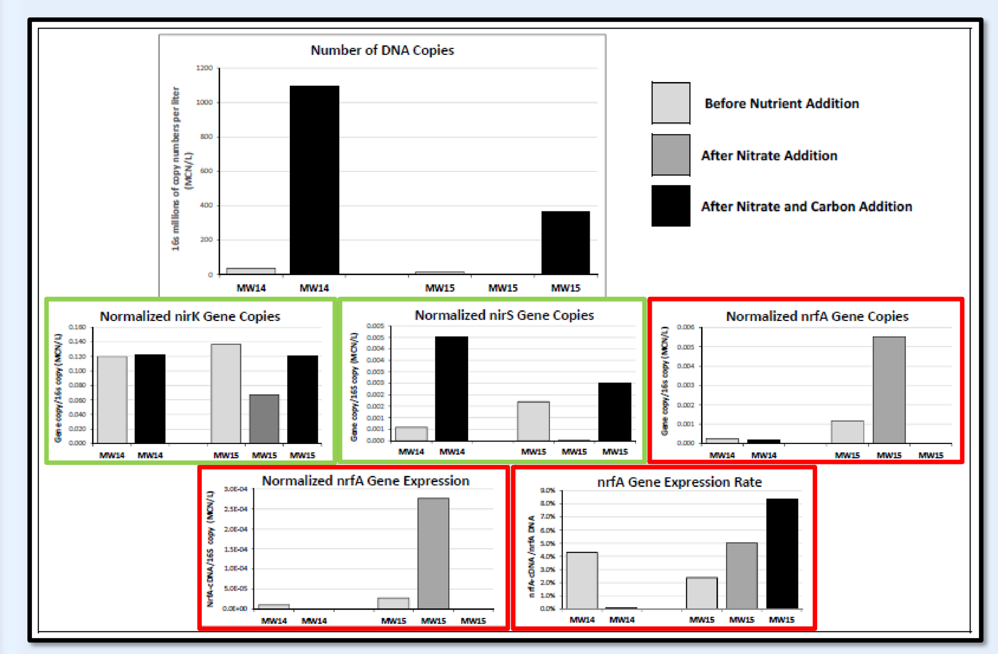


Fig. 8 Microbial genetic data

Addition of carbon stimulated increase in population.

DNRA (Red):
- **Anoxic site increase in NrfA gene copies, expression and expression rate.**

Suggests DNRA is significant nitrogen transformation mechanism

Denitrification (Green):
- **NirS gene increased with carbon addition, NirK no significant change.**
- **No denitrification gene expression at either site.**

Suggests bulk aquifer denitrification is not occurring

SUMMARY

Question 1 Insights:

- Loss rates were not driven solely by redox state; nitrate losses were observed in anoxic and oxic aquifers with and without carbon addition.
- It was unexpected that DNRA was a significant process at the anoxic site with nitrate-only addition.
    - 65% of dissimilatory nitrate reduction was conversion to ammonium.
- Nitrate losses were associated with assimilation and DNRA; however, these do not account for all observed dissimilatory nitrate reduction.
- Denitrification may have occurred in microbial biofilms that were not sampled using the biological sampling of recovered tracer.

Question 2 Insights:

- DOC addition greatly increased rates of nitrate losses in the anoxic aquifer but did not influence rates in oxic aquifer, suggesting oxygen inhibition of denitrification in the bulk aquifer.
- With no carbon addition, microbial conversion to ammonia (DNRA) and subsequent uptake or adsorption, was favored in the anoxic aquifer.