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Nitrogen Compound Metabolism

Insights Gained by a Holistic Testing Regime

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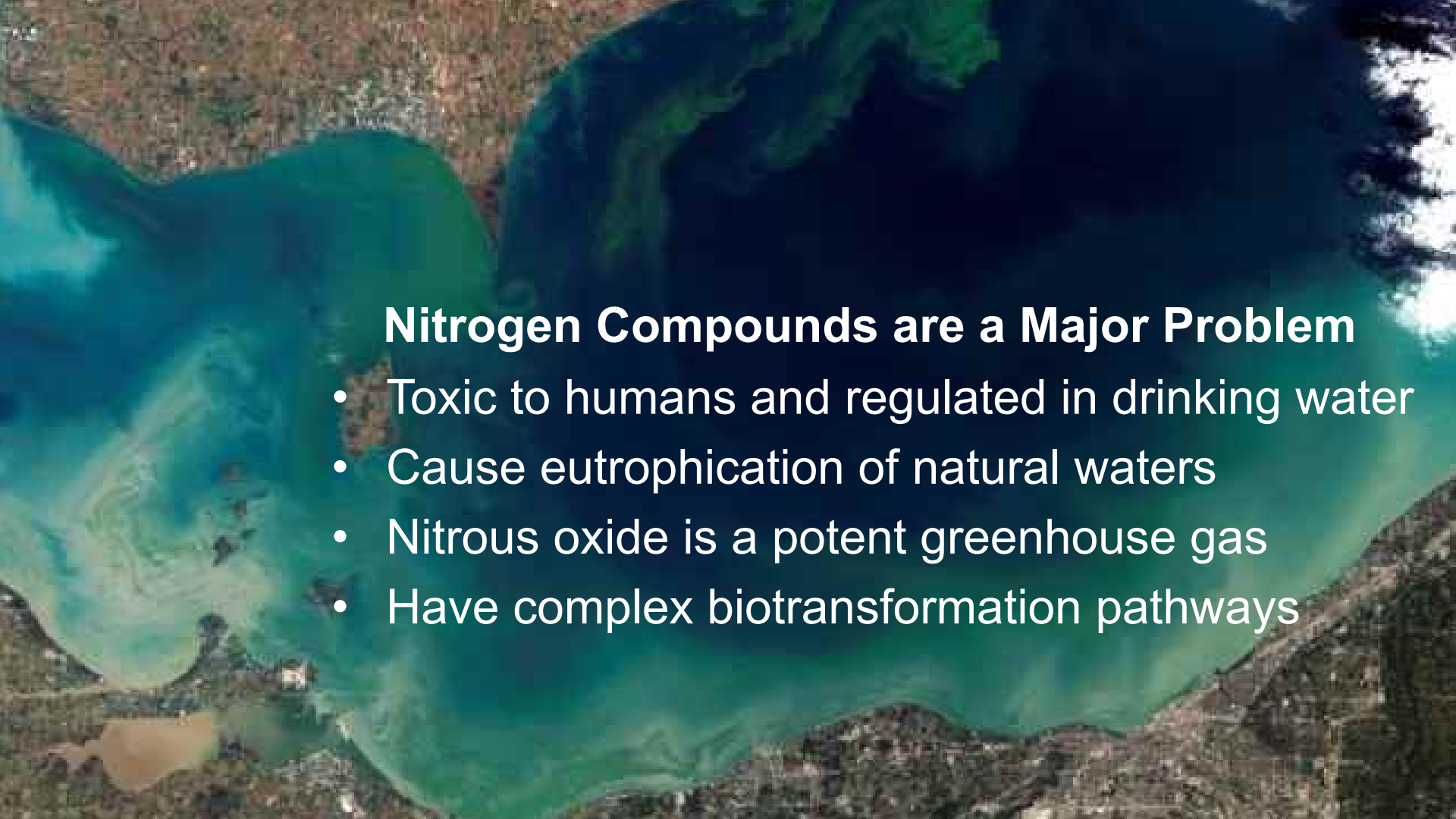
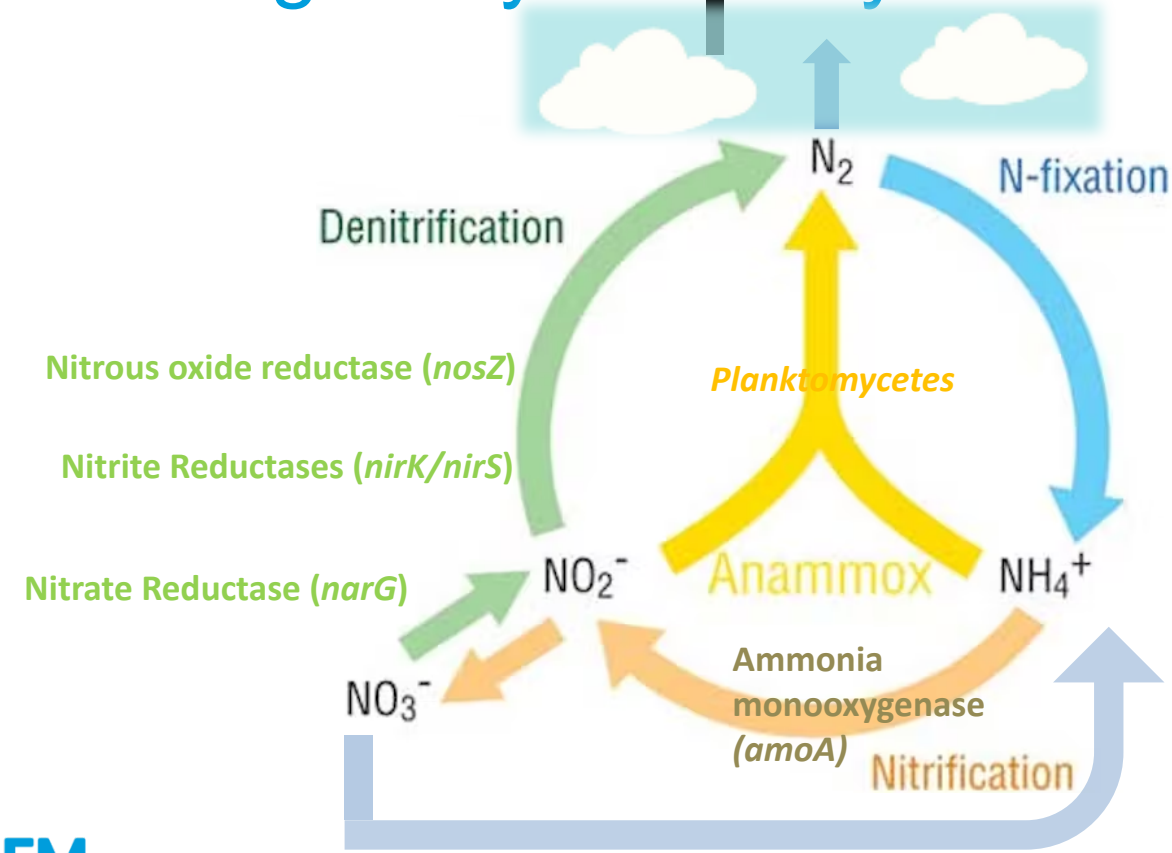


Nitrogen Compounds are a Major Problem

- Toxic to humans and regulated in drinking water
- Cause eutrophication of natural waters
- Nitrous oxide is a potent greenhouse gas
- Have complex biotransformation pathways

Nitrogen Cycle & Key Functional Genes



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Understanding Nitrogen Metabolism

- What form are nitrogen compounds in?
- Do you have suitable geochemistry to transform?
- Are sufficient nutrients available (electron donors)?
- Are nitrogen metabolizing microbes present?
- What are the likely end products N_2? NH_4? NO_3? N_2O?
- Are concentrations declining over time?

A combination of analytical, molecular biological and isotopic tests can answer the above questions

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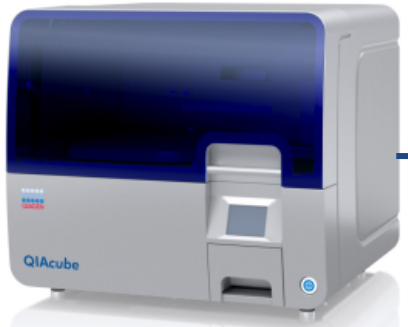
NitroGen™ ANALYTICAL SUITE

Molecular Biological Tools (MBTs)

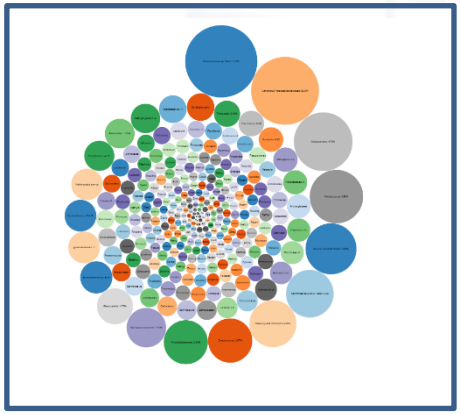
Samples

DNA Extraction



qPCR

Next Gen Sequencing



Microbial Community Profiles

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Certificate of Analysis: Gene-Trac® NitroGen™
Ammonia Monooxygenase A Assay

Customer: Savannah Volkoff, Geosyntec Consultants

SiREM Reference: S-8258
Report Date: 4-Oct-21
Data Files: QS3A-amoA-QPCR-0102

Table 1d: Test Results

Sample ID	Ammonia Monooxygenase A *amoA* (archaeal)		Ammonia Monooxygenase A *amoA* (bacterial)	
	Percent [2]	Gene Copies/Liter	Percent [2]	Gene Copies/Liter
MW-2-20210803	0.01 - 0.03 %	3×10^5	NA	1×10^4 U
MW-1-20210803	0.006 - 0.02 %	5×10^4	NA	1×10^4 U
INJ1-20210803	0.002 - 0.007 %	1×10^5	NA	1×10^4 U

See final page for notes.

Quantify Specific Gene targets

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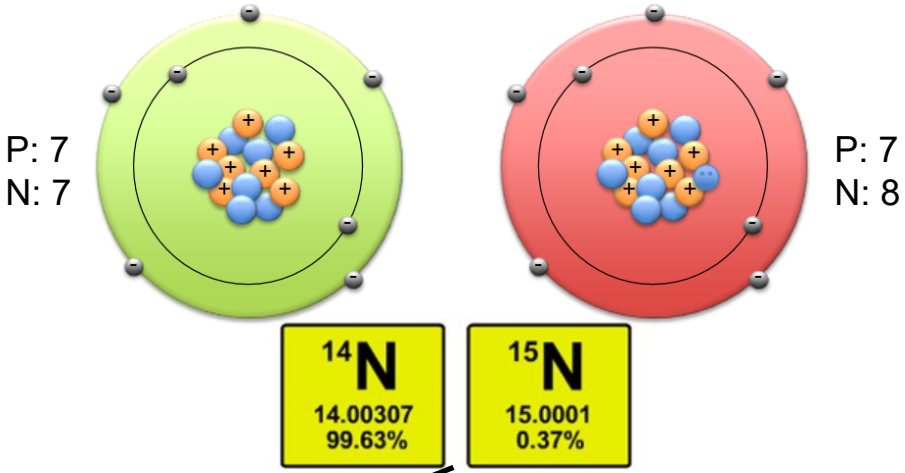
$\delta^{15}N$ Isotopes

Stable Nitrogen Isotopes



Delta-Notation for $^{15}N/^{14}N$

$$\delta^{15}N = \left[\frac{\left(\frac{^{15}N}{^{14}N}\right)_{sample}}{\left(\frac{^{15}N}{^{14}N}\right)_{AIR}} - 1 \right]$$

Increasing $\delta^{15}N$ = greater biotransformation e.g., nitrification

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www.isodetect.de

Advantages of MBTs & Isotopes to Assess N-Transformation

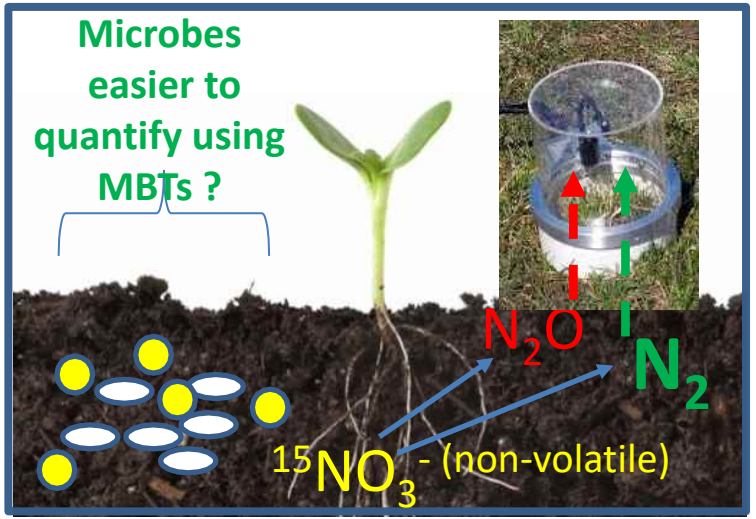
- **Determining soil and water N-flux is challenging**

Gas flux difficult to quantify

- Flux events highly variable
- End products (N_2O/N_2) gaseous and ubiquitous (N_2)

- **Advantages of Isotopic methods**
- Integrate long term transformation via enriched $\delta^{15}N$ ^{18}O
- $\delta^{15}N$ ^{18}O quantified in non-volatile NO_3 and NH_3

- **Advantages of MBTs**
- Microbes are non-volatile, don't dissipate like gases
- MBTs are very sensitive
- MBTs detect potential functions, even if not active
- Tracking populations over time is informative



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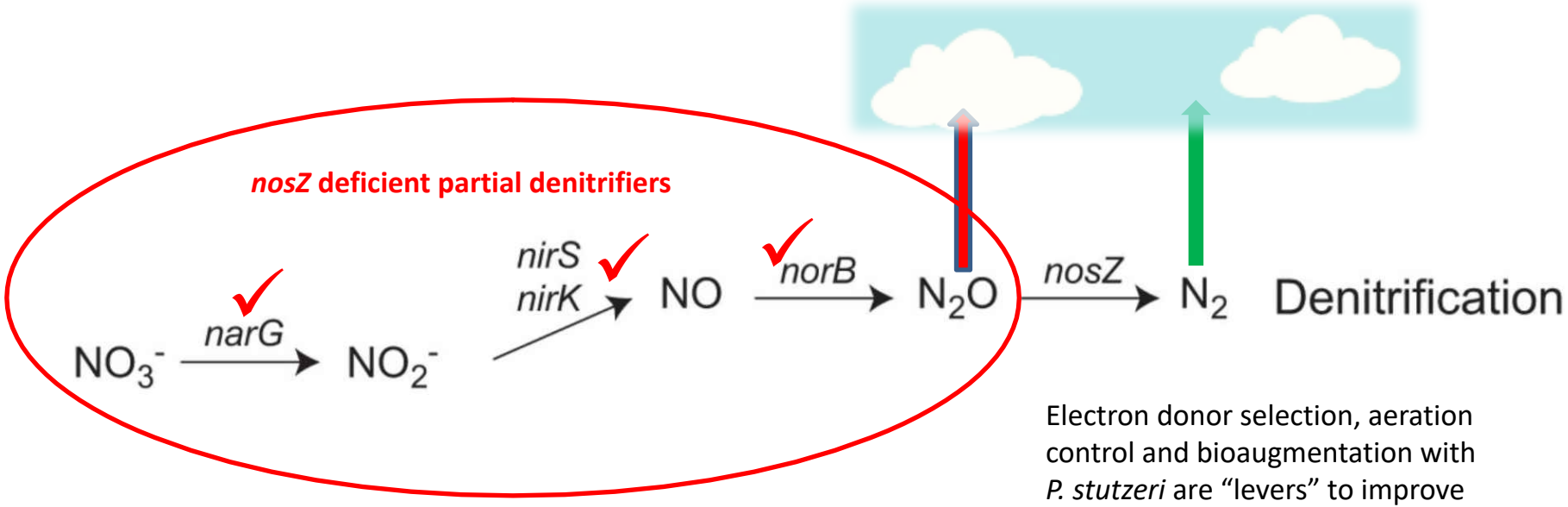
Incomplete Denitrification–Greenhouse Gas Impacts



Electron donor selection, aeration control and bioaugmentation with *P. stutzeri* are "levers" to improve complete denitrification

In denitrification want full gene set, particularly *nosZ, to* prevent N_2O emissions

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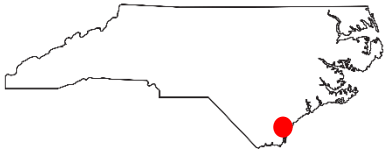
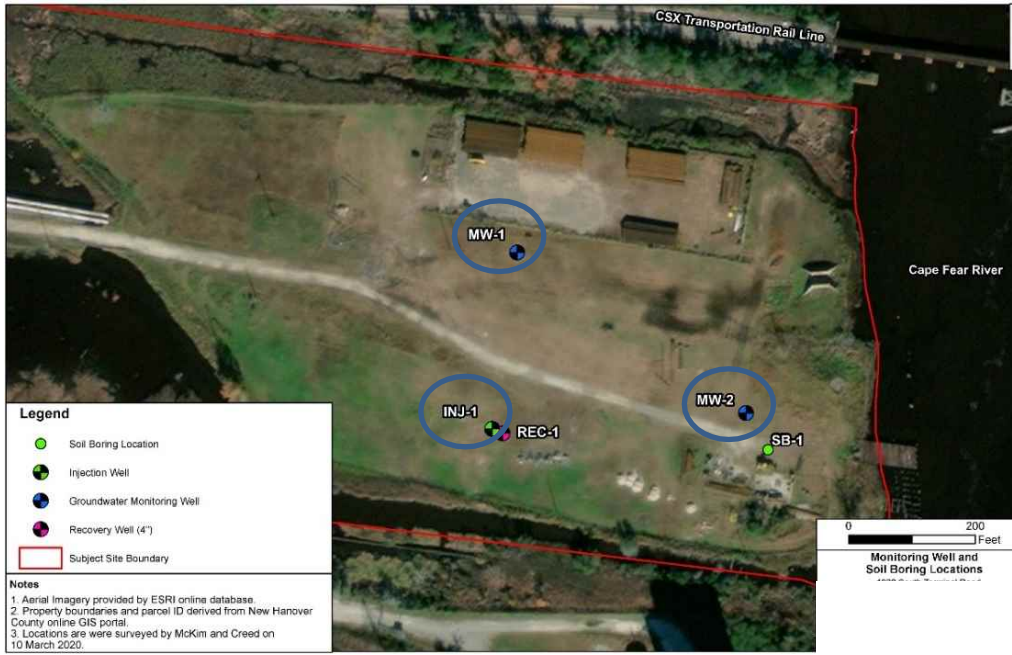
Former Fertilizer Plant (Wilmington, NC)



- Fertilizer plant 1930s-1982
- Fertilizer & petroleum contamination of Groundwater (GW)
- 2018 GW ammonium ~ 83 mg/L
  NC NH_4^+ GW standard 1.5 mg/L
- 2021 ammonium Results:
  - Max 31 mg/L
  - Min 9 mg/L
- No active remediation MNA



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Key Question is Ammonium Being Transformed or Transported?

Data Summary
NC Site

NitroGen™ ANALYTICAL SUITE Results Summary

Parameter	Sample		
Analytical	**MW-2-20210803**	**MW-1-20210803**	**INJ1-20210803**
Nitrate mg/L	<0.09	<0.09	<0.09
Nitrite mg/L	14	9.7	9.7
Ammonia mg/L	24	30.2	10.4
Total Kjeldahl Nitrogen (TKN)	22.4	27.2	9.85
Sum N-Compounds mg/L	38	39.9	20.1
Phosphate	7.5	1.9	ND
Sulfate	1.8	ND	ND
Isotopes			
δ^{15}N Ammonia	15.33	13.905	3.81
δ^{15}N δ^{18}O Nitrate	ND	ND	ND
qPCR			
Anammox			
Anammox Bacteria	1.00E+04	1.E+06	2.00E+03
Denitrification			
narG	1.E+08	3.E+07	8.E+07
nirS	2.E+08	4.E+06	2.E+07
Nitrification			
amoA (Archaea)	3.00E+05	5.00E+04	1.00E+05
amoA (Bacterial)	ND	ND	ND
Nitrobacter	2.00E+04	1.00E+04	3.00E+04
Electron Donors			
TOC mg/L	27.5	18.4	17.6
Total VFA mg/L	91.8	83.75	79.62
Field Parameters			
ORP mV	-125	-26.1	-126.4
DO mg/L	0.05	0.52	0.1
pH	6.41	6.25	6.69
Groundwater Temperature °C	21.5	24.8	24.4
Summary			
Comments	Denitrification predominant/ ammonium most biodegraded in this sample	Mixture of denitrification and anammox at this location /ammonium degradation intermediate in this location and may be driven by anammox	Denitrification predominant/least evidence of ammonium degradation in this sample

Forms of N

Isotopes

Microbes

Electron Donors

Field Parameters

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NC Site Nitrogen Pathways Molecular Biological Tools Summary

MBTs Indicated

- 3 potential modes of N-metabolism
- Denitrification gene copies highest
- Anammox highest at MW-1
- Nitrification highest at MW-2

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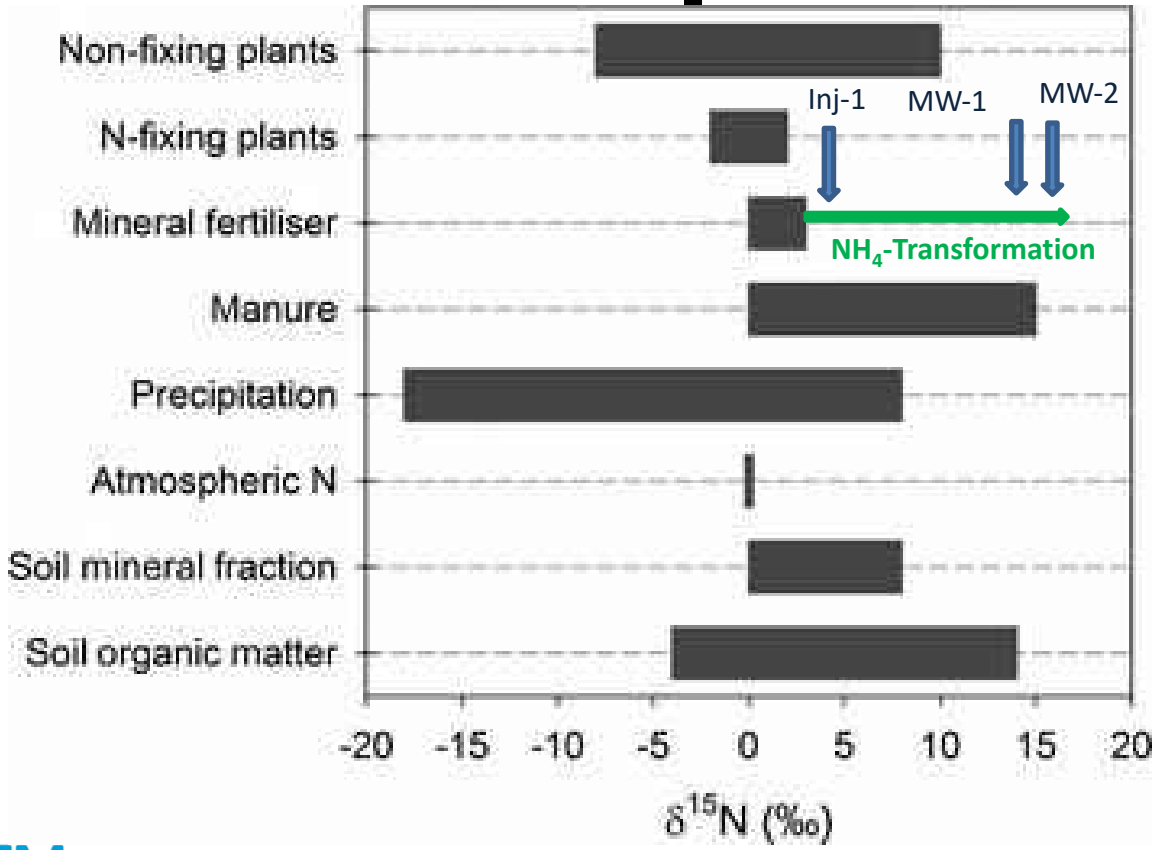
$\delta^{15}N$ Isotopes NC Site



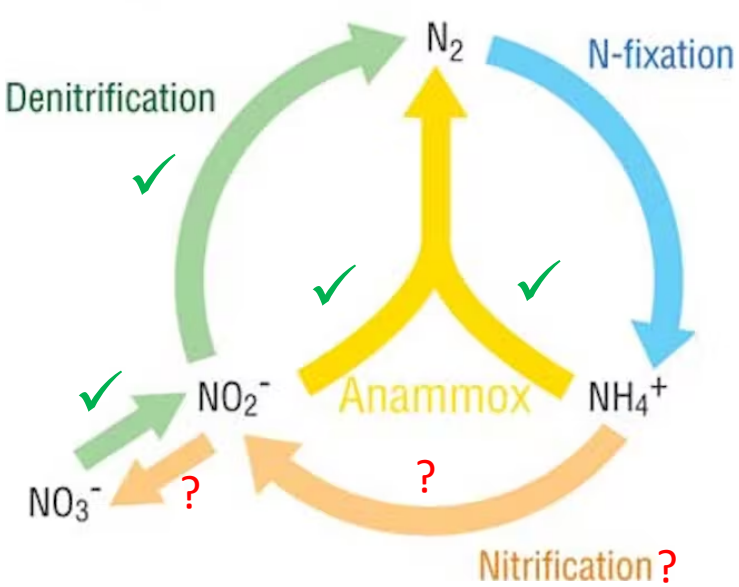
Isotopes Indicate

Ammonium transformation highest in downgradient monitoring wells

Fiorentino et al, 2014

Conclusions Former Fertilizer Plant NC

Ammonium declining 2018-2021. Dilution? Biotransformation? What pathways?



- Strong isotopic evidence for NH_4 transformation in downgradient MW wells

- **Three potential N-metabolism pathways**
  - **Denitrification** using VFAs as electron donors -likely
  - **Anammox** Co-transformation ammonium and nitrite-likely
  - **Nitrification** potential source of nitrate/nitrite site -redox not supportive?

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Conclusions

- Nitrogen compounds are widespread and have significant human heath and environmental impacts.
- Holistic approach to N-compound analytical including MBTs and isotopes leads to a better understanding of whether N-transformation is occurring and how.
- Better knowledge can lead to increased ability to manage and optimize N-compound remediation outcomes.

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Questions? Comments!

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