Application of Stable Isotopic and Omics Methods for Assessment of RDX Natural Attenuation in Groundwater

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PROJECT TEAM

- **Dr. Paul G. Koster van Groos**, Aptim
  Stable isotope data analysis
  Rate estimate calculations

- **Dr. Kate Kucharzyk**, Battelle Memorial Institute
  Metagenomics and proteomics

- **Dr. Neil Sturchio**, University of Delaware
  Compound specific stable isotope analysis

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Outline

- Background
- Sampling and Analysis
- Results
  - GW Chemistry
  - Stable Isotope Analysis
  - Metagenomics
  - Proteomics
  - RDX Biodegradation Potential
- Conclusions

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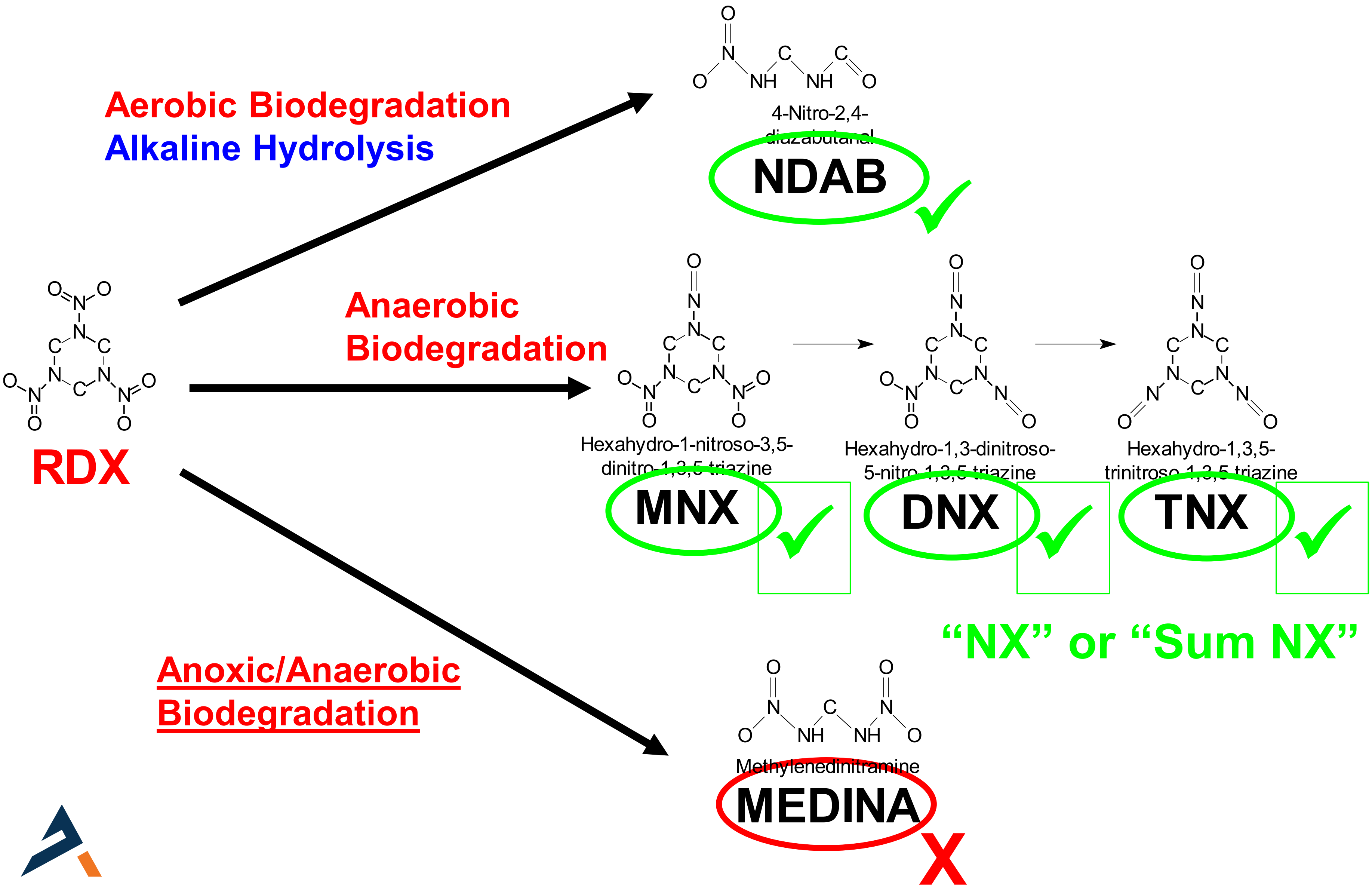
RDX Degradation



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PROJECT OBJECTIVE

Assess the potential of natural attenuation of RDX in a perched aquifer using multiple lines of evidence, including:

- Groundwater chemistry.
- Concentrations of RDX and RDX breakdown products
- Evidence of stable isotopic fractionation of nitrogen in RDX
- Metagenomic and proteomic for biomarkers of RDX degrading bacterial strains
- RDX biodegradation potential of the indigenous microbial community

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Hydrogeological Setting and Contamination History

- Perched aquifer overlying a deep regional aquifer
- Generalized groundwater flow to the southeast
- Groundwater flow velocities variable in direction and magnitude

- RDX and other explosives released from 1950's to 1980's
  - Untreated RDX wastewater flowed into a ditch to an open pond
  - Seepage from the pond connected to the perched aquifer

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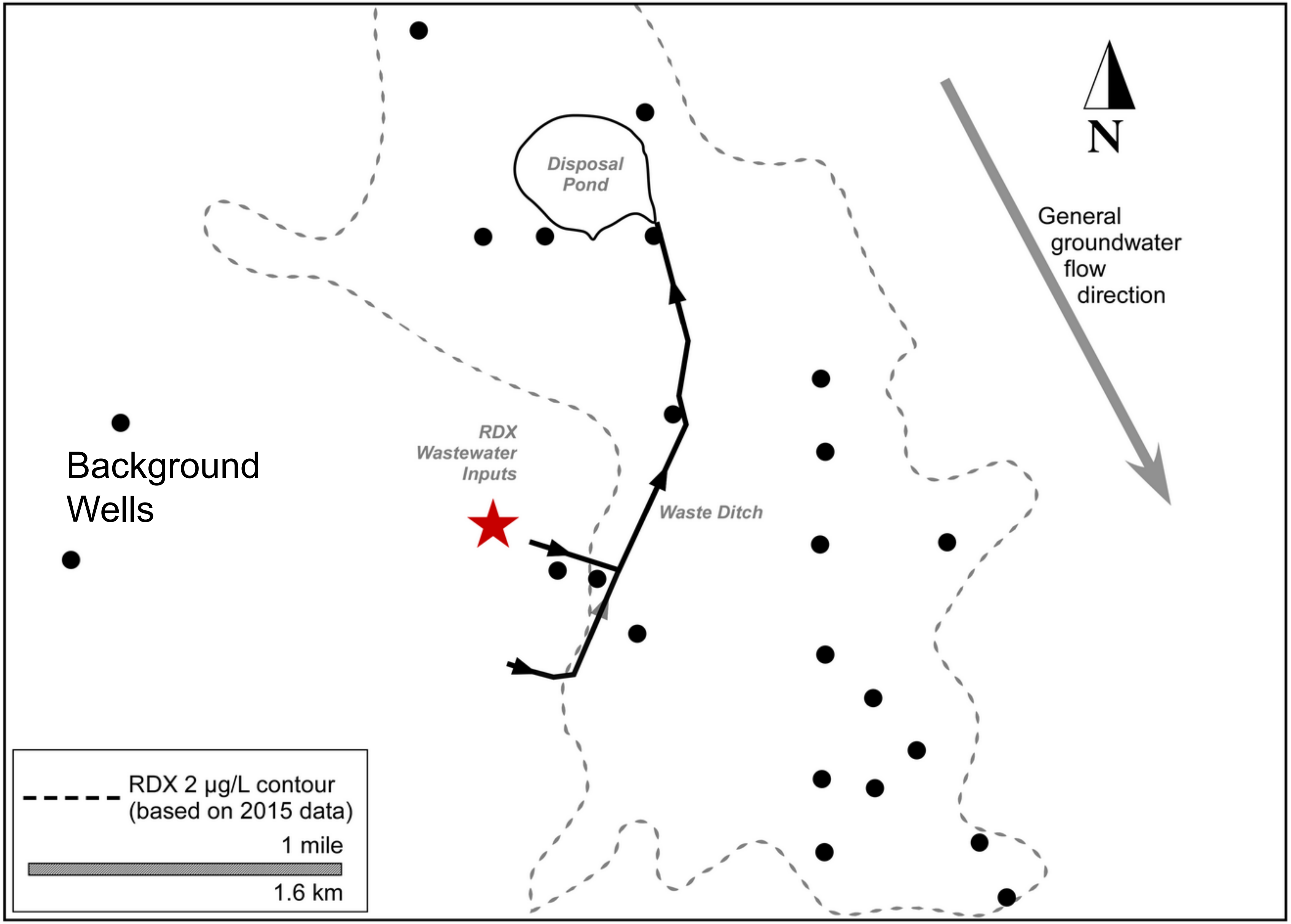
24 Wells Sampled



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Groundwater Analyses

- Anions
- Total Organic Carbon (TOC)
- Volatile Fatty Acids
- Dissolved Gases (methane)
- Dissolved TAL Metals
- RDX
- RDX Breakdown Products – NX's and NDAB

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- RDX Nitrogen CSIA – University of Delaware

- Metagenomics/Proteomics – Battelle

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Chemical Analyses – Geochemistry

- **Generally oxic**
  - Positive dissolved oxygen
  - Positive redox
    - ➢ few low redox/anoxic spots
    - ➢ BUT not correlated to dissolved oxygen
  - Low dissolved iron
  - Low dissolved methane

- **Low TOC** (< 1mg/L) = oligotrophic

- Neutral to **slightly alkaline pH** across the plume
  - ➢ Average 7.5 ± 0.5 **Max = 9.0**

Conditions supportive of:

✓ **Aerobic RDX biodegradation**
✓ **Possibility of localized anaerobic biodegradation**
✓ **Possibility of slow, localized alkaline hydrolysis**

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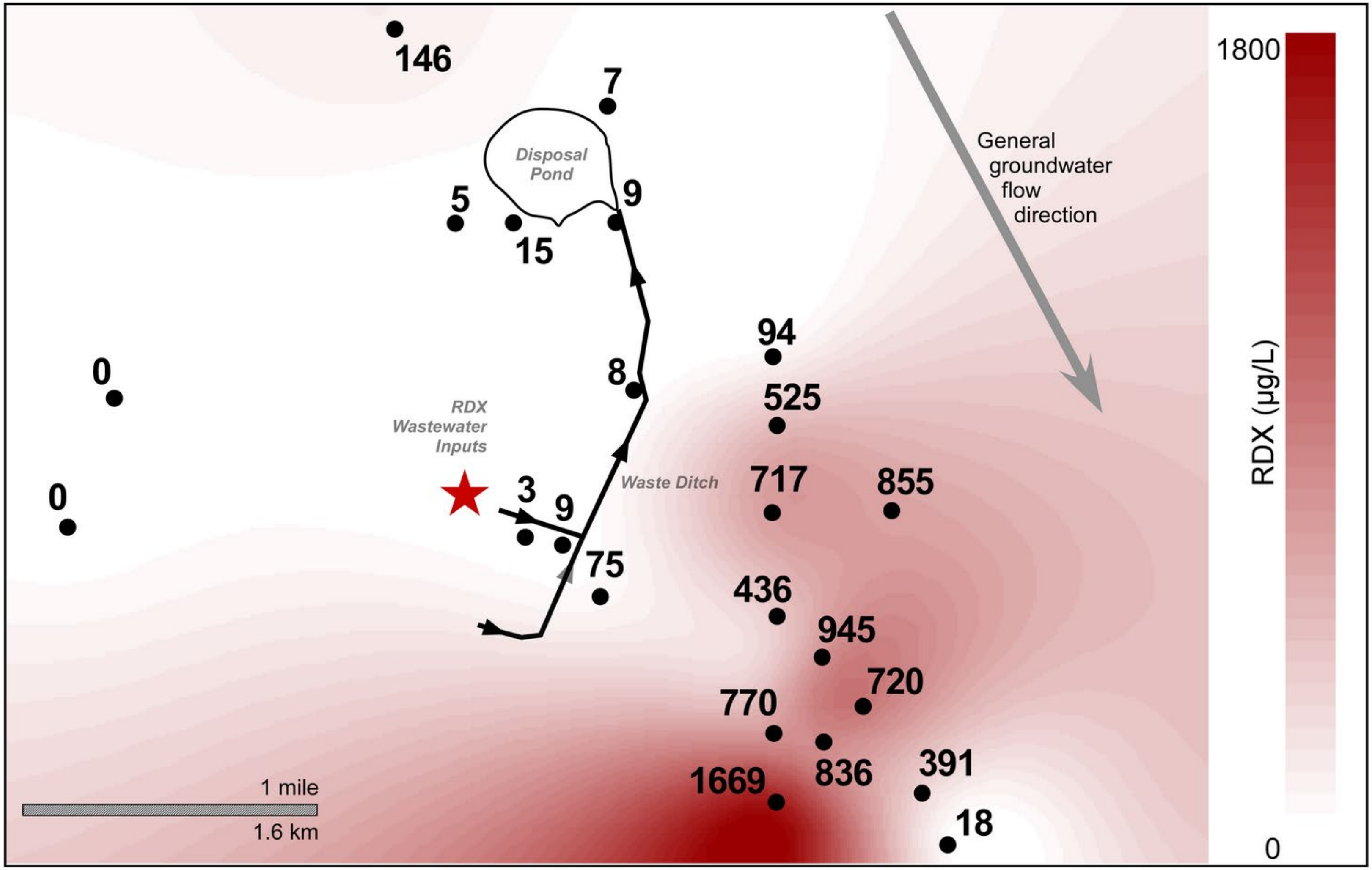
Chemical Analyses – RDX

Highest RDX (~1000 µg/L) currently in the southeastern portion of the plume



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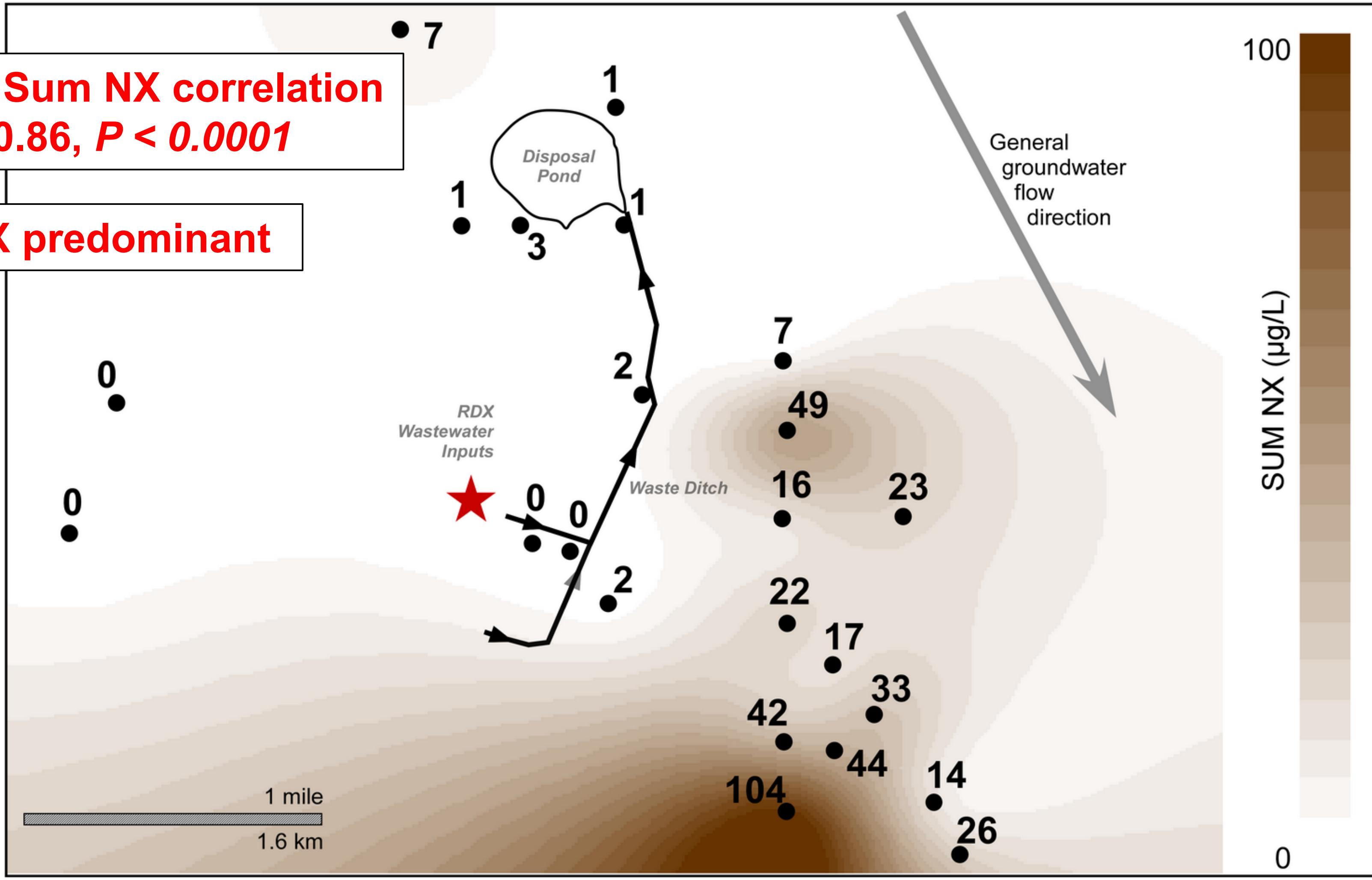
Chemical Analyses – Sum MNX/DNX/TNX

Highest anaerobic biodegradation nitroso products (up to 100 µg/L) co-located with RDX

RDX vs. Sum NX correlation
$r = 0.86$, $P < 0.0001$

TNX predominant



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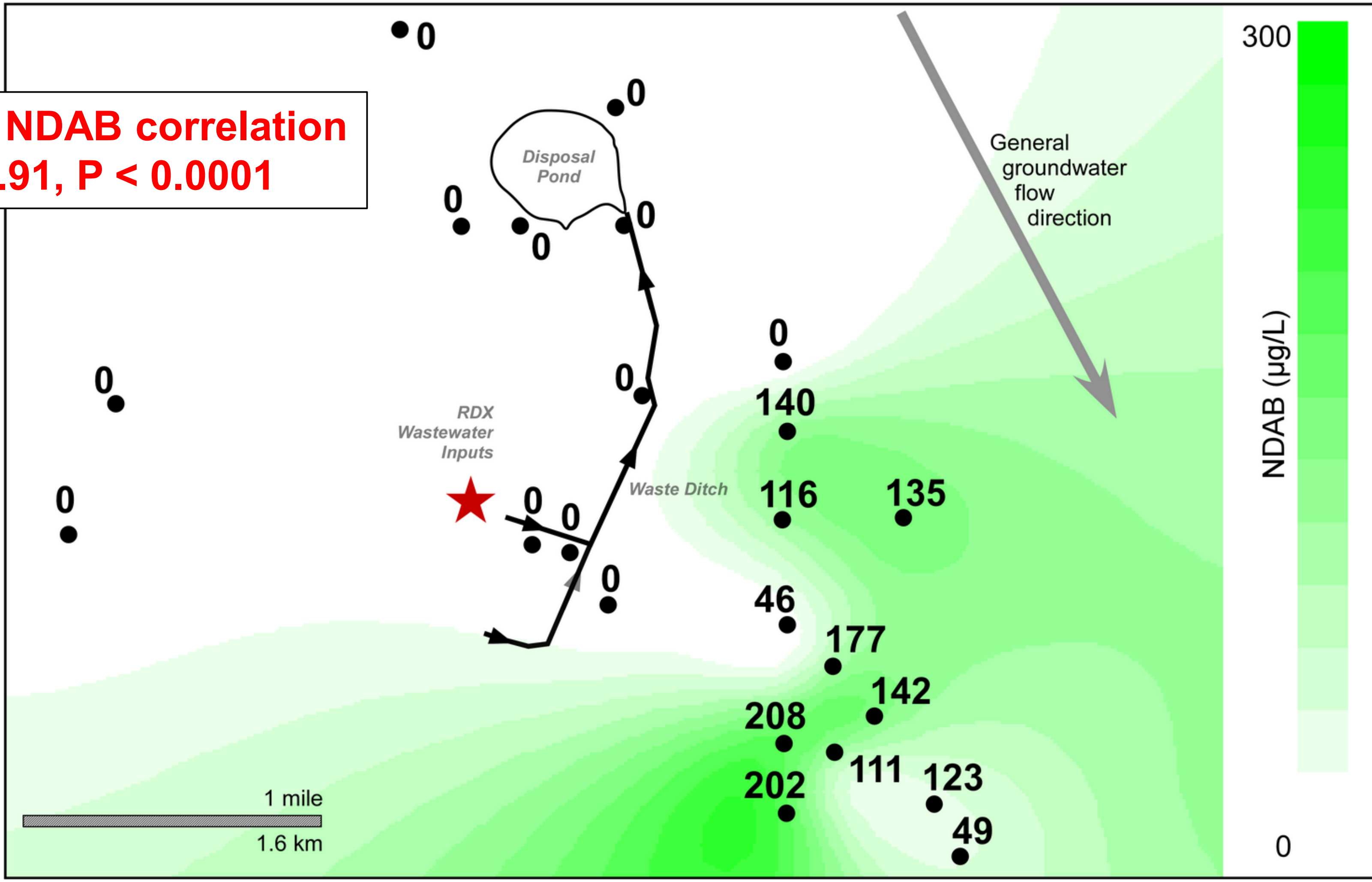
Chemical Analyses – NDAB

Highest aerobic biodegradation product NDAB (up to 200 µg/L) co-located with RDX

RDX vs. NDAB correlation
r = 0.91, P < 0.0001



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Chemical Analyses – Summary

✓ The groundwater is generally aerobic and oligotrophic

- Compatible with RDX **biodegradation**
- Probably limited by organic C

✓ RDX and RDX breakdown products are co-located

- Consistent with both **aerobic and anaerobic biodegradation**
- Possibly slow **alkaline hydrolysis**

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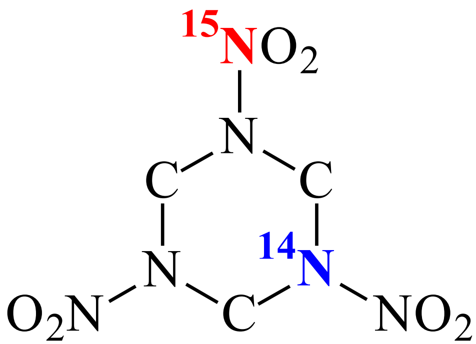
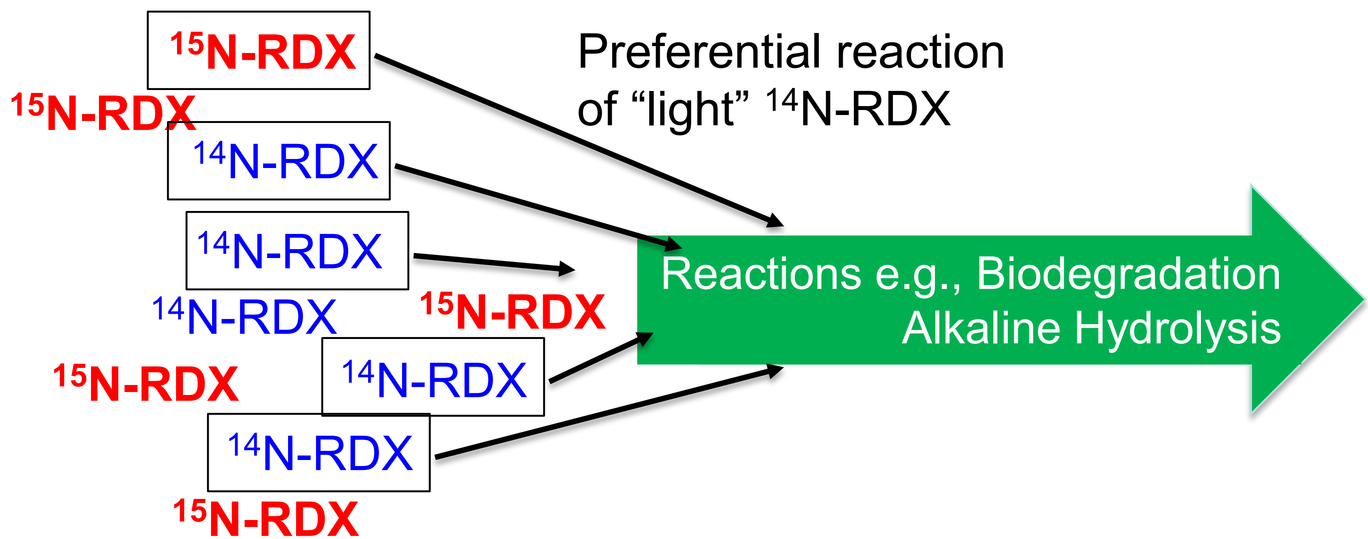
Compound Specific Isotope Analysis (CSIA)



Natural abundance of stable isotopes of nitrogen $^{14}N/^{15}N$ leads to synthesis of a certain portion of the RDX with heavier N in the molecular structure.

<u>Source RDX – Mixture of "heavy" and "light" molecules</u>



RDX $\delta^{15}N$ becomes <u>LESS NEGATIVE</u> or <u>MORE POSITIVE</u>

<u>Residual RDX – Enriched in "heavy" molecules</u>

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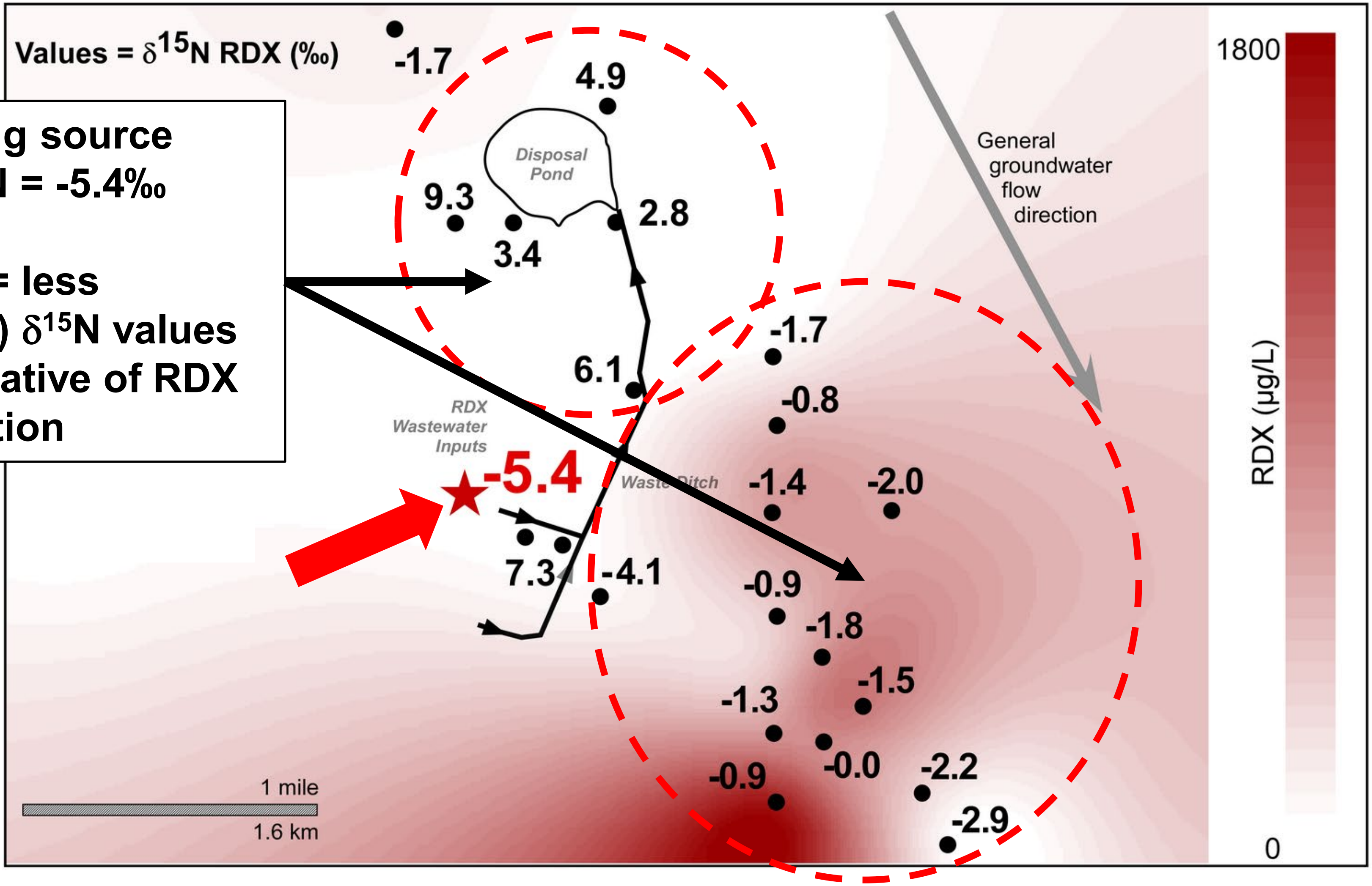
Compound Specific Isotope Analysis

$\delta^{15}N$ of RDX currently in groundwater

Assuming source RDX $\delta^{15}N$ = -5.4‰

Higher (= less negative) $\delta^{15}N$ values are indicative of RDX degradation



Compound Specific Isotope Analysis – Summary

✓ Isotope data is indicative with some level of RDX degradation

✓ Data is consistent with

- Both **aerobic and anaerobic biodegradation**
- Possibly slow **alkaline hydrolysis**

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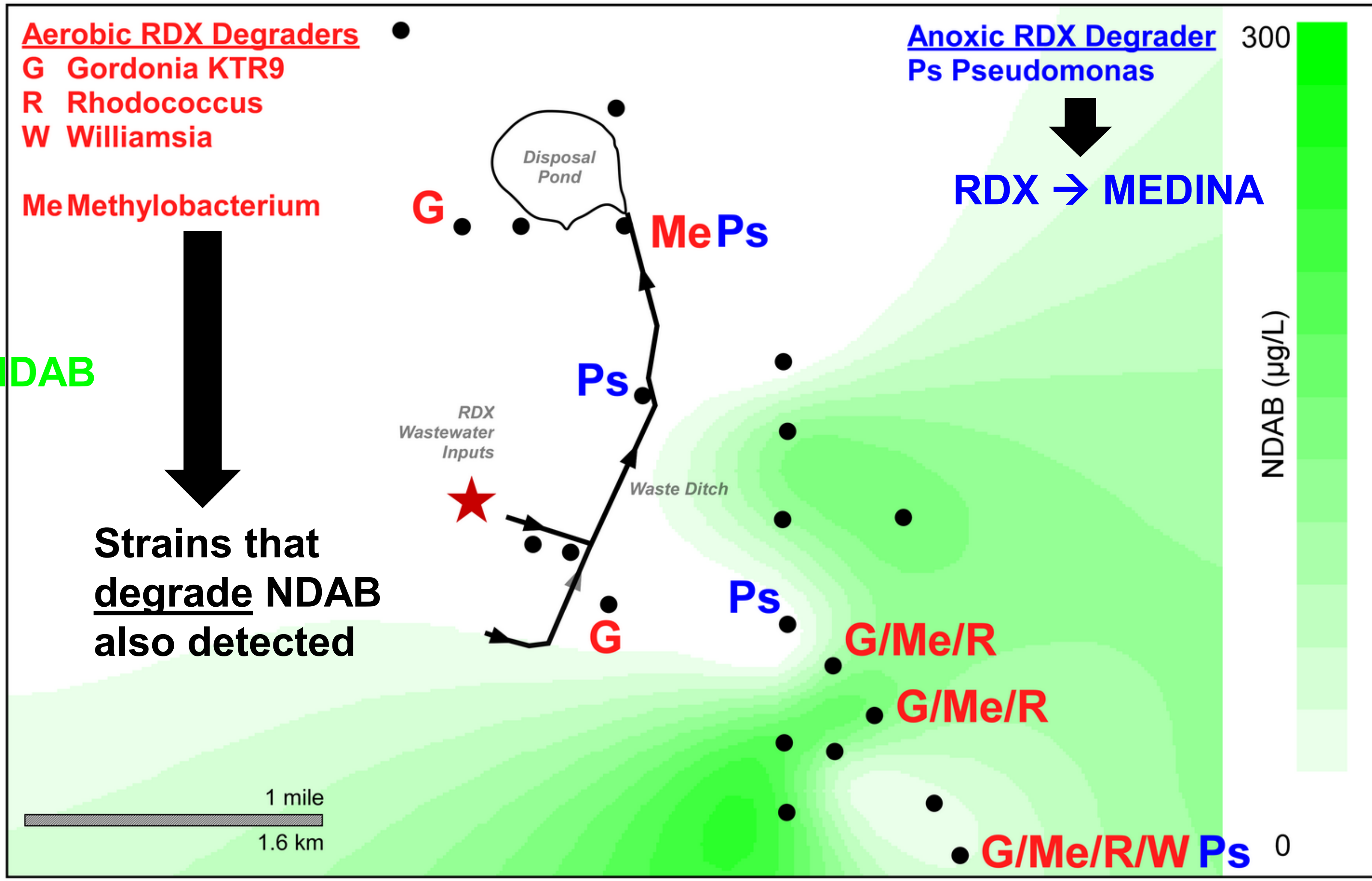
Metagenomics

Multiple detections (8 of 16 wells analyzed) of bacterial strains known to degrade RDX aerobically with production of NDAB, as well as detections of NDAB degraders

RDX → NDAB



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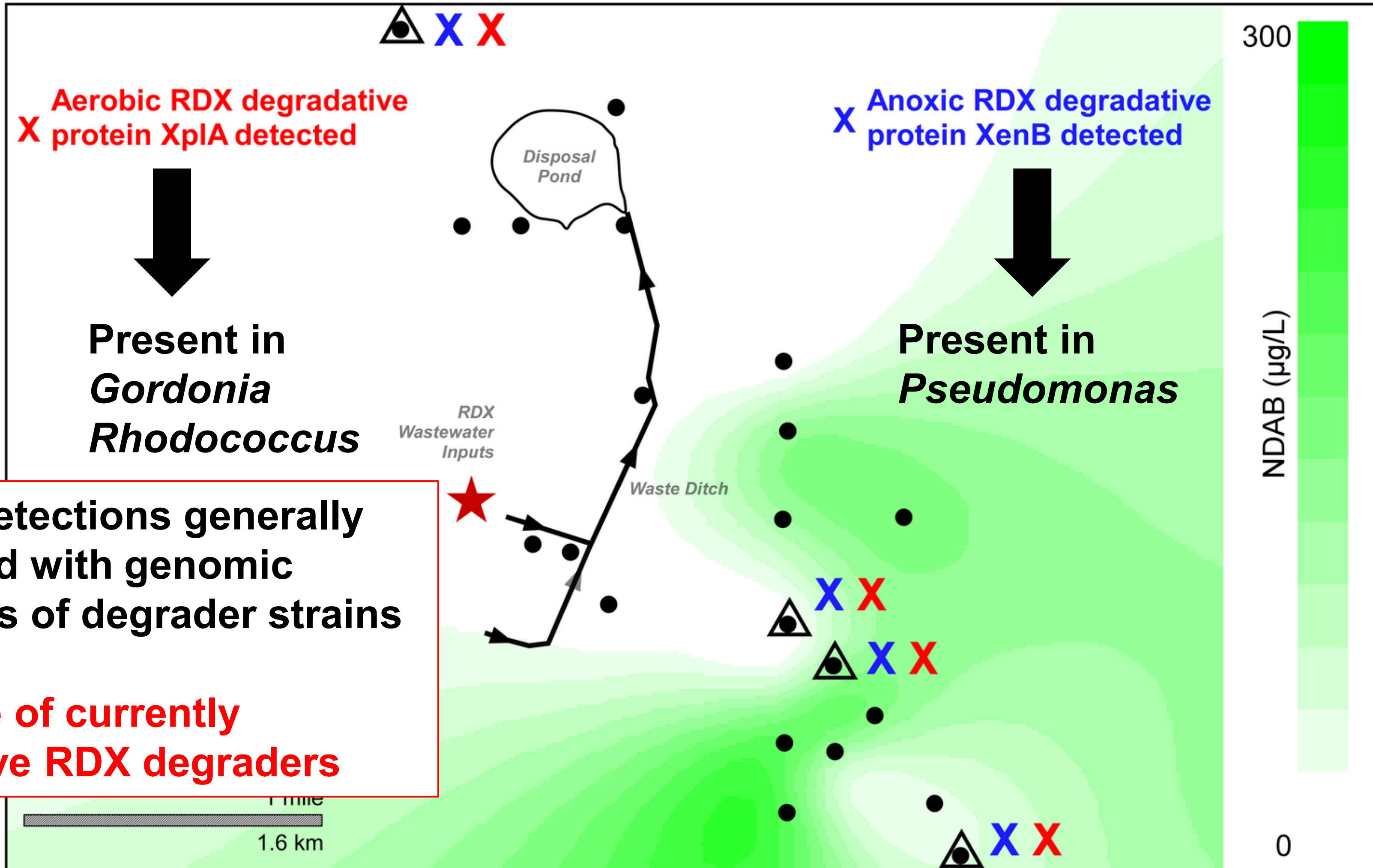
Proteomics

Detections of bacterial enzymes that degrade RDX aerobically via NDAB pathway and anoxically via MEDINA pathway



Protein detections generally co-located with genomic detections of degrader strains

Indicative of currently active/alive RDX degraders

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Metagenomics / Proteomics – Summary

✓ Genetic biomarkers of specific bacterial strains capable of RDX **biodegradation** were present.

✓ Protein biomarkers of aerobic and anoxic RDX degradative enzymes were present.

✓ The combined data indicate that bacterial strains capable of RDX biodegradation were likely alive and active.

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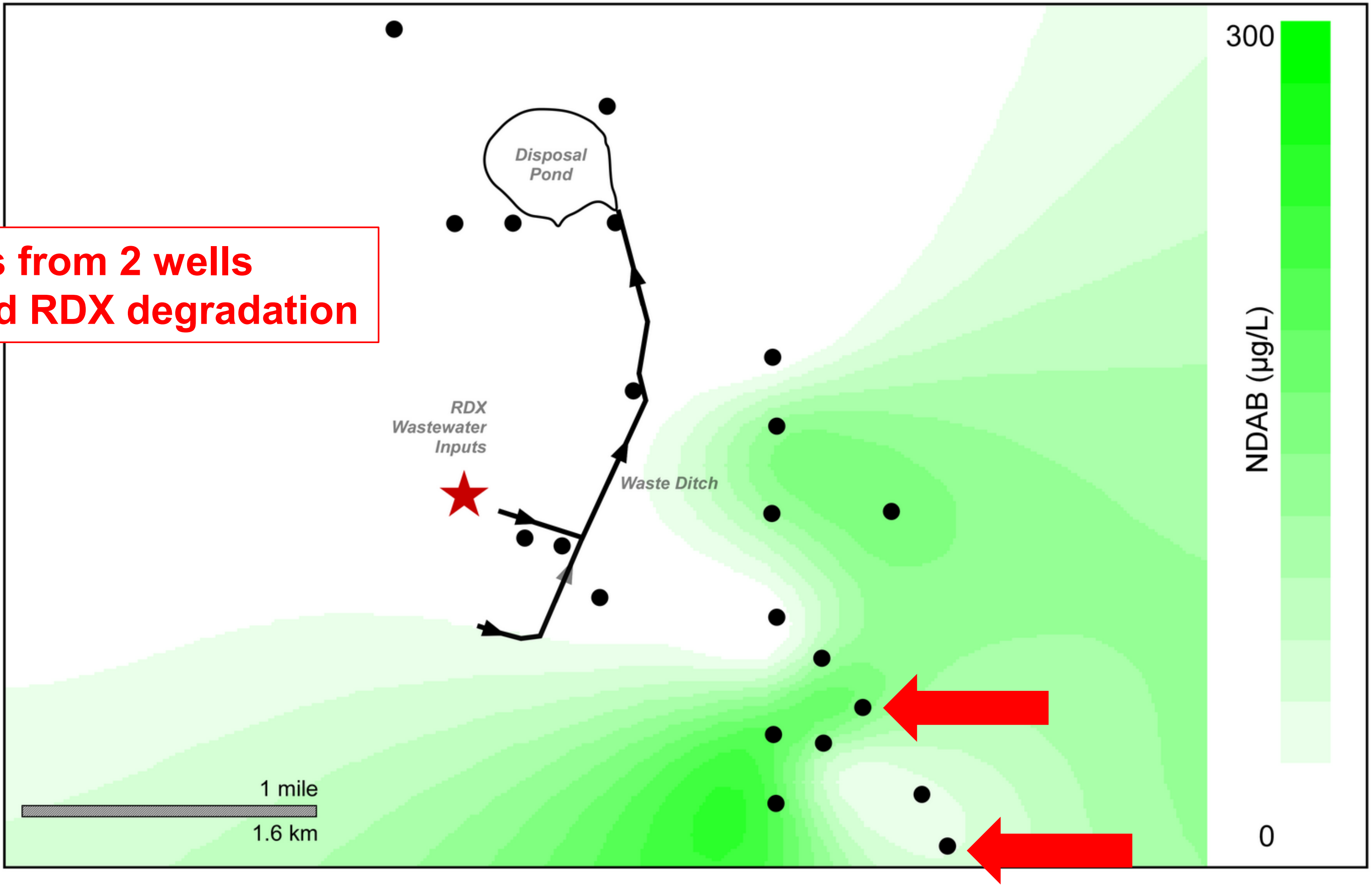
Aerobic RDX Biodegradation Potential

Groundwater inoculated into sterile medium containing RDX (~20 mg/L) plus organic carbon (500 mg/L each glucose + succinate + lactate)

Samples from 2 wells exhibited RDX degradation



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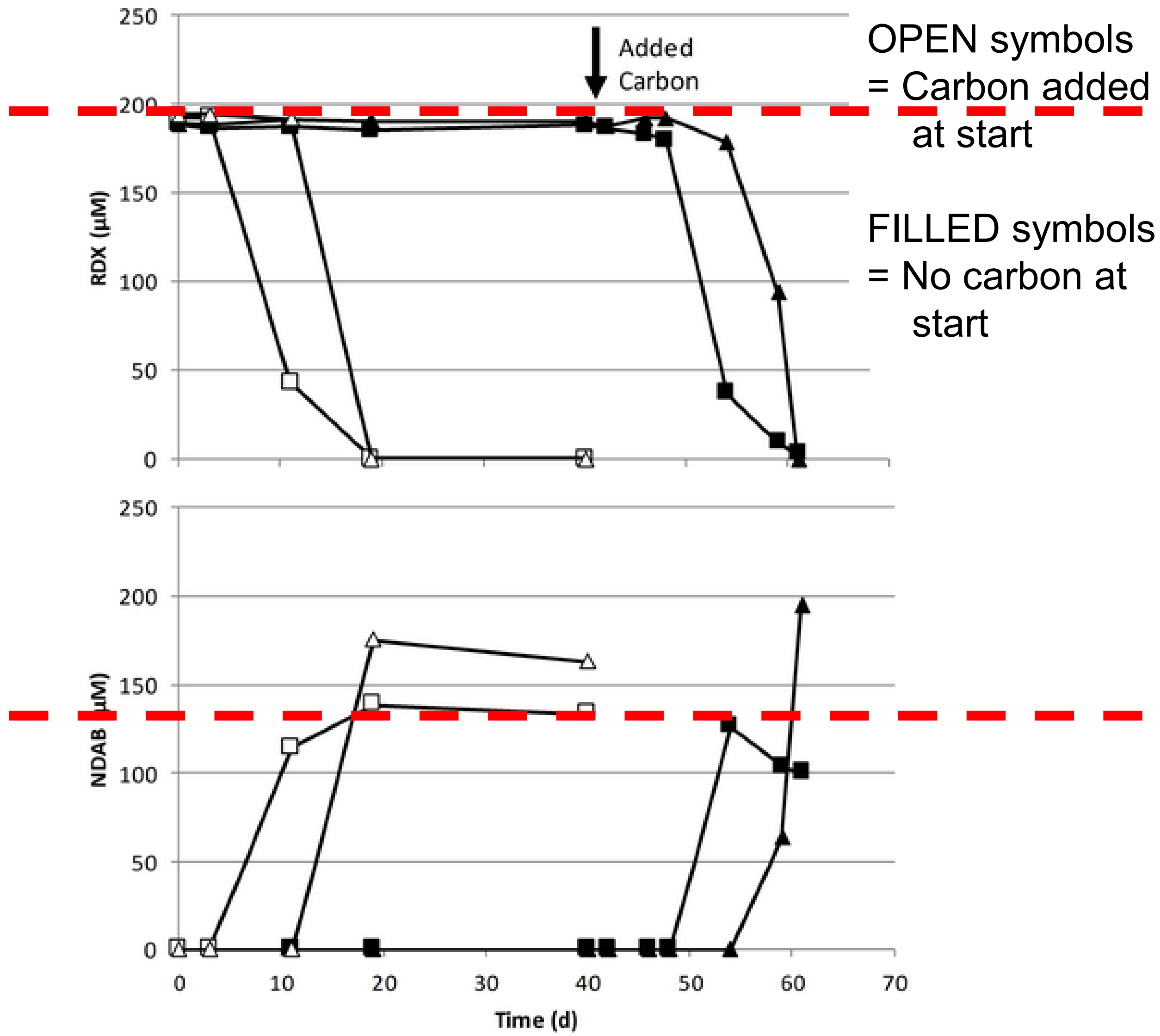
Aerobic RDX Biodegradation Potential

Two positive enrichments

- ✓ **Aerobically degraded RDX** only when carbon was added
- ✓ **Produced NDAB** from RDX
- ✓ Some indication of **NDAB degradation** given non-stoichiometric amounts of NDAB from RDX



OPEN symbols = Carbon added at start

FILLED symbols = No carbon at start

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Conclusions

- The perched aquifer has a geochemical composition compatible with both RDX **biodegradation** and **alkaline hydrolysis**

- Past or ongoing attenuation of RDX is supported by
  - ✓ Parent and breakdown products
  - ✓ Detectable fractionation of ^{15}N in residual RDX
  - ✓ Detection of strains and proteins associated with RDX biodegradation
  - ✓ Demonstration of RDX biodegradation in groundwater microcosms

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THANK YOU

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