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Finding a Better Way to Remove Nitrogen from Residential Cesspools Based on Microbiology and Microbial Ecology

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SoMAS, Stony Brook University

FAR BEYOND

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Figure from: Nutrient Attenuation in Chesapeake Bay Watershed Onsite Wastewater Treatment Systems - Final Report August 2016

THE PROBLEM: Most wastewater in Suffolk County is treated in on-site septic systems that do not remove nitrogen (N)…

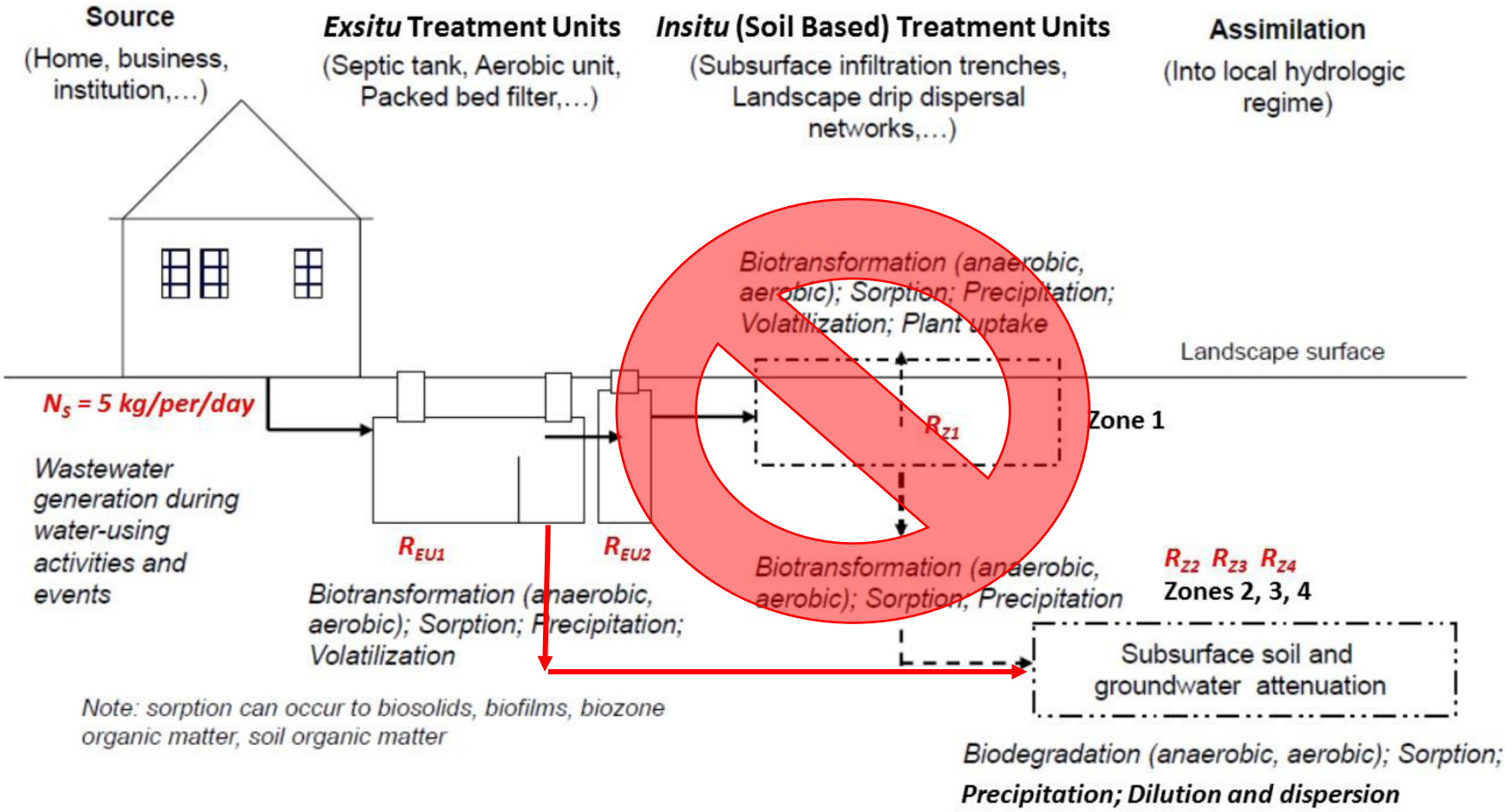

Figure 3. Nutrient Transformations associated with Treatment and Attenuation Zones (from Siegrist and Geza, 2014)

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…so nitrate concentrations are increasing in groundwater and coastal ecosystems are becoming N-enriched

Assume: residential wastewater, 5 kg TN/cap/year

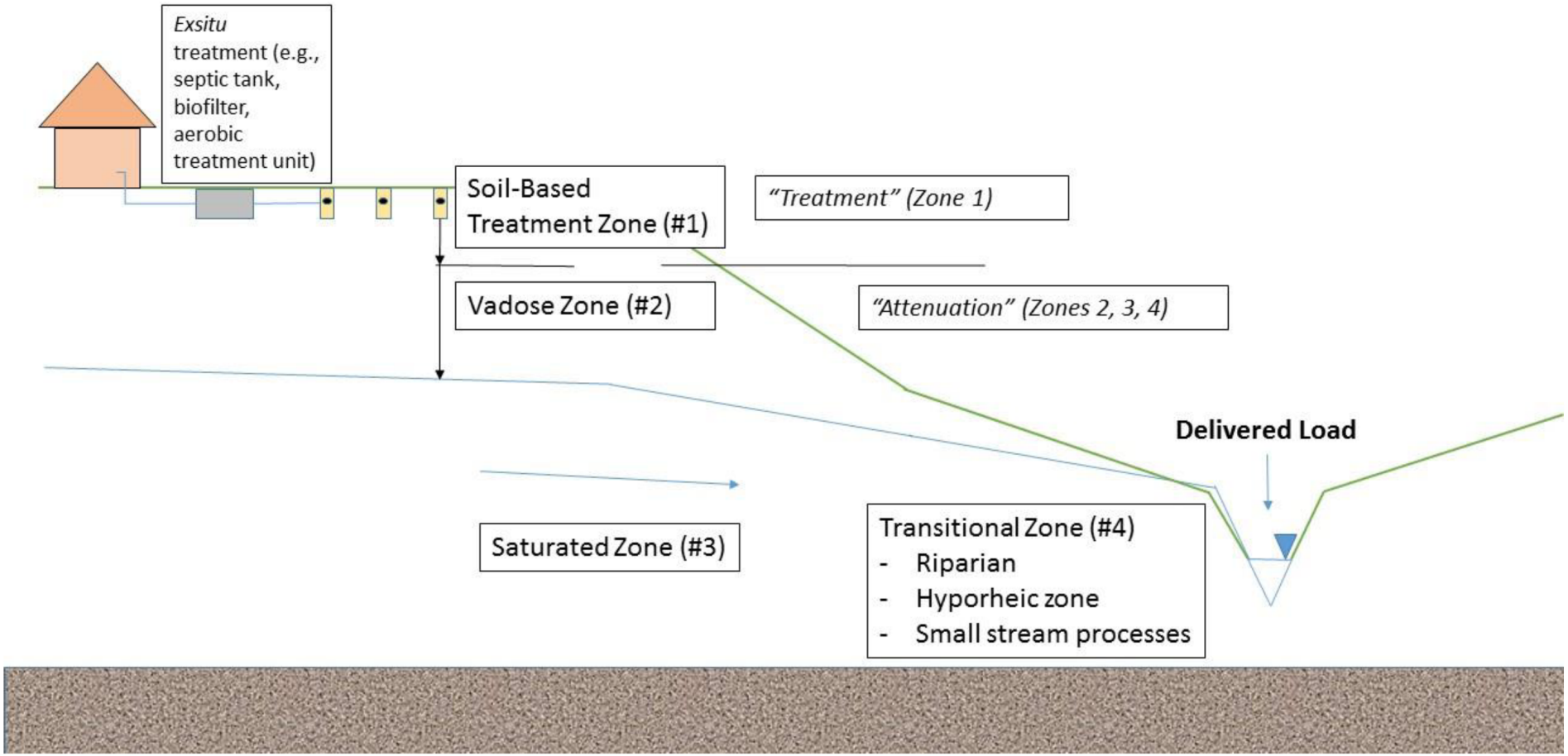

Figure 2. Onsite Wastewater Treatment and Attenuation Zones

The Center for Clean Water Technology (CCWT)

Our goal: develop onsite wastewater treatment systems (OWTS) that

- Cost less than $10,000 to install
  - Require minimal maintenance
- Reduce effluent N to < 10 mg per liter
- Function for at least 30 years

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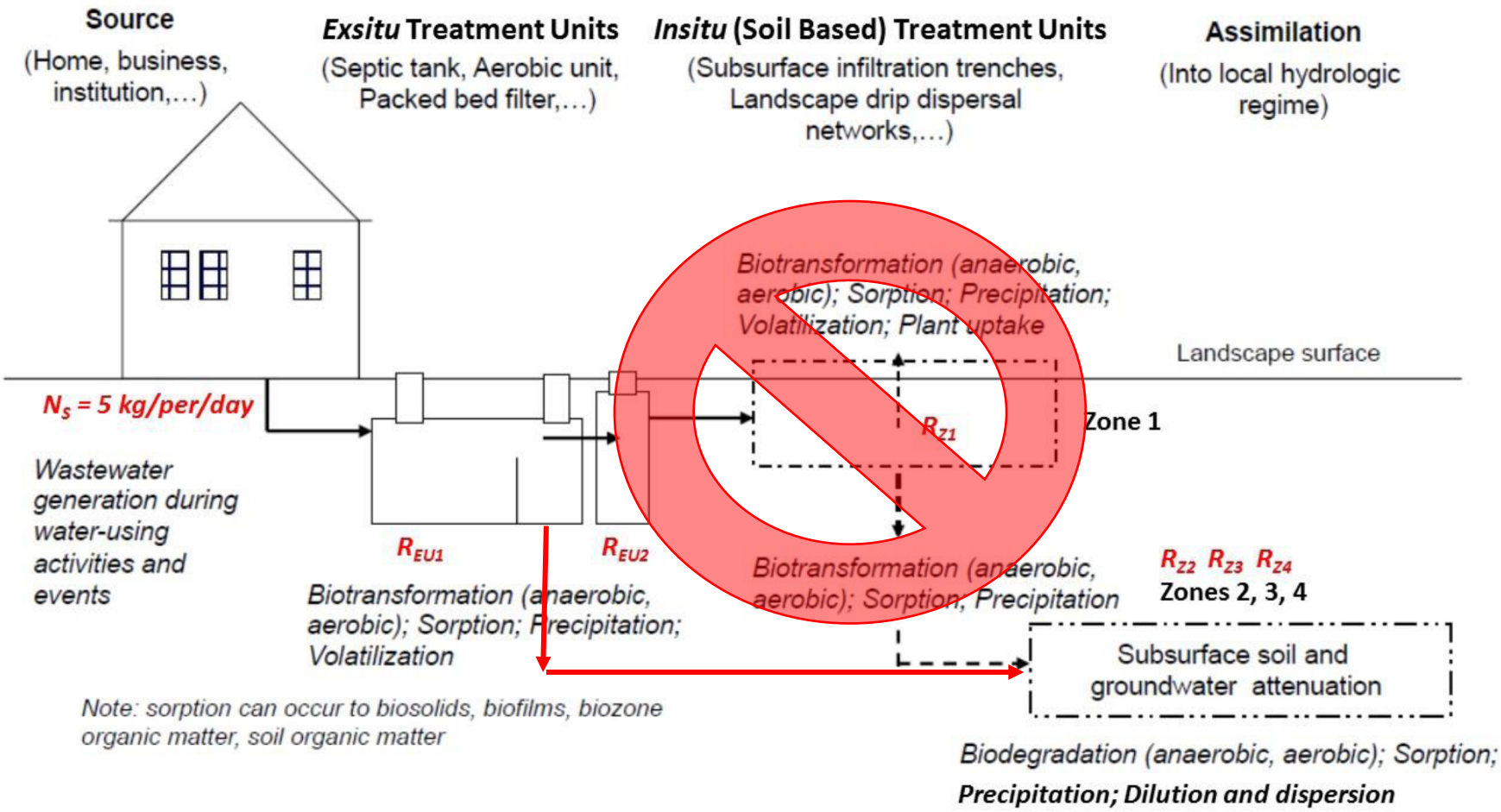
A SOLUTION?



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A SOLUTION?: Add passive nitrogen removing biofilters (NRBs) to existing OWTS

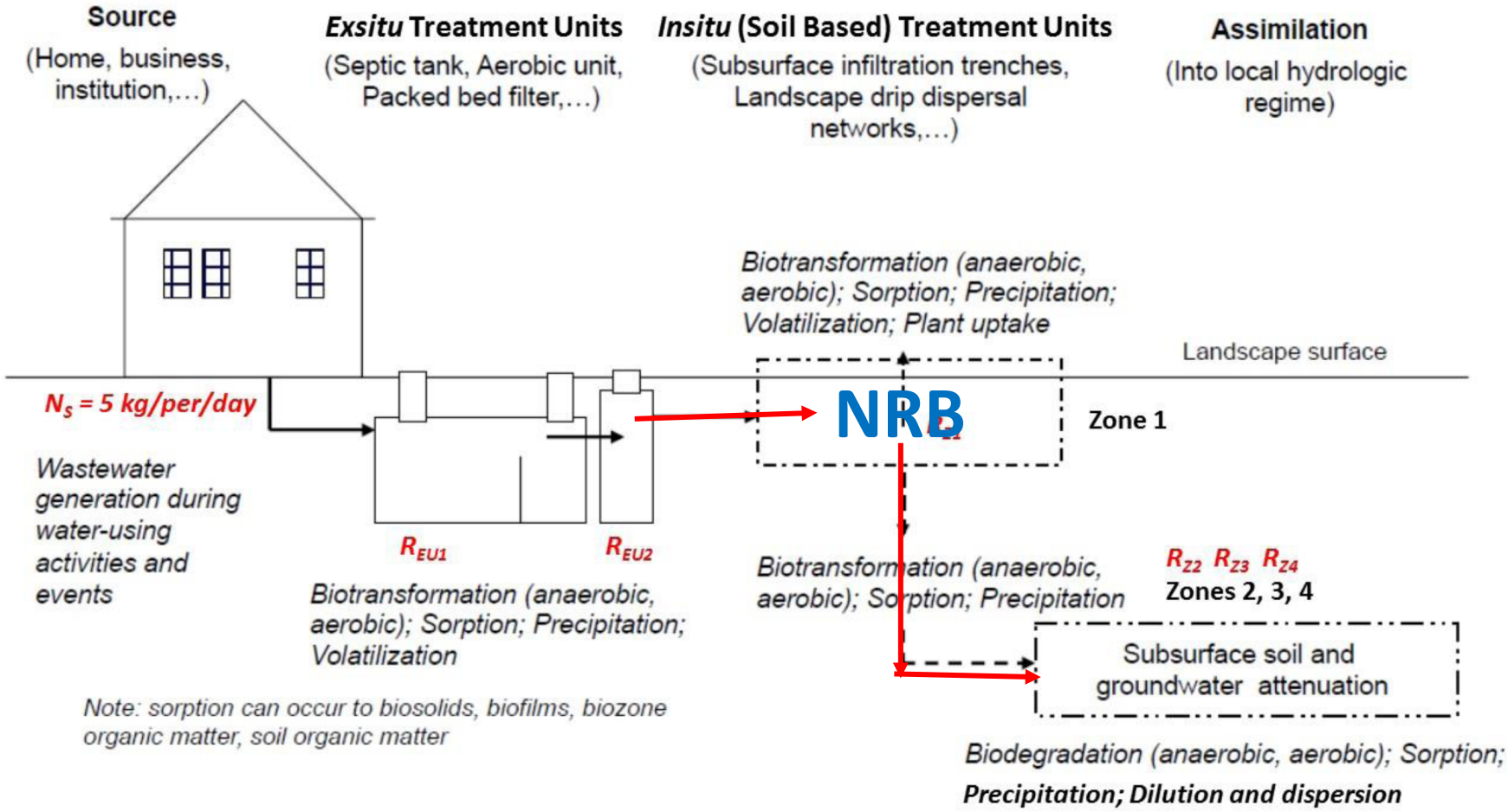
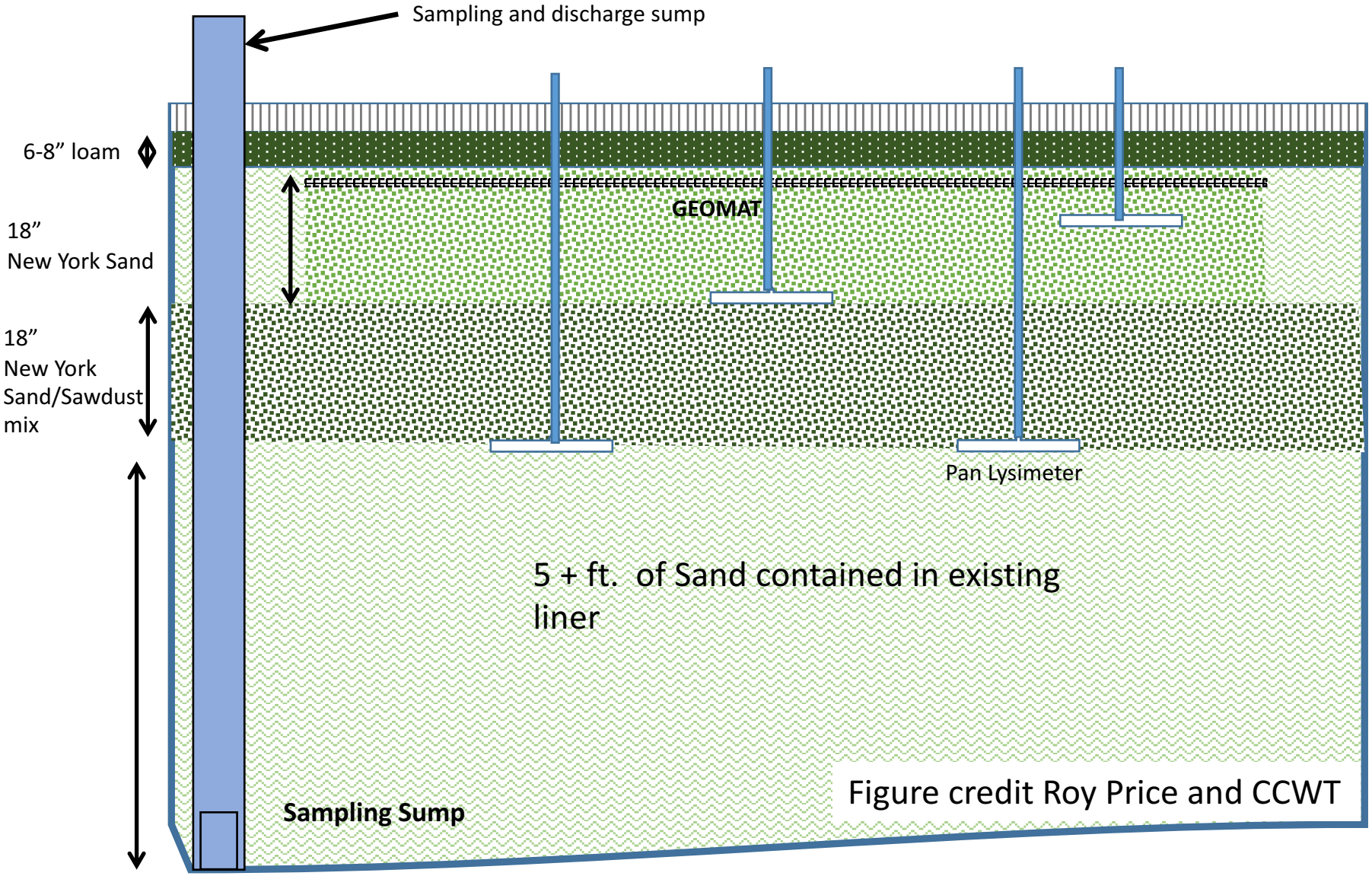


Figure 3. Nutrient Transformations associated with Treatment and Attenuation Zones (from Siegrist and Geza, 2014)

Schematic of NRB test systems installed at MASSTC

Massachusetts Alternative Septic System Test Center



Installing test NRB at MASSTC

Top left: bottom liner and collection tube

Top right: placing nitrification layer

Bottom left: placing influent distribution manifold

photo credits: Xinwei Mao, Max Grabinski, CCWT

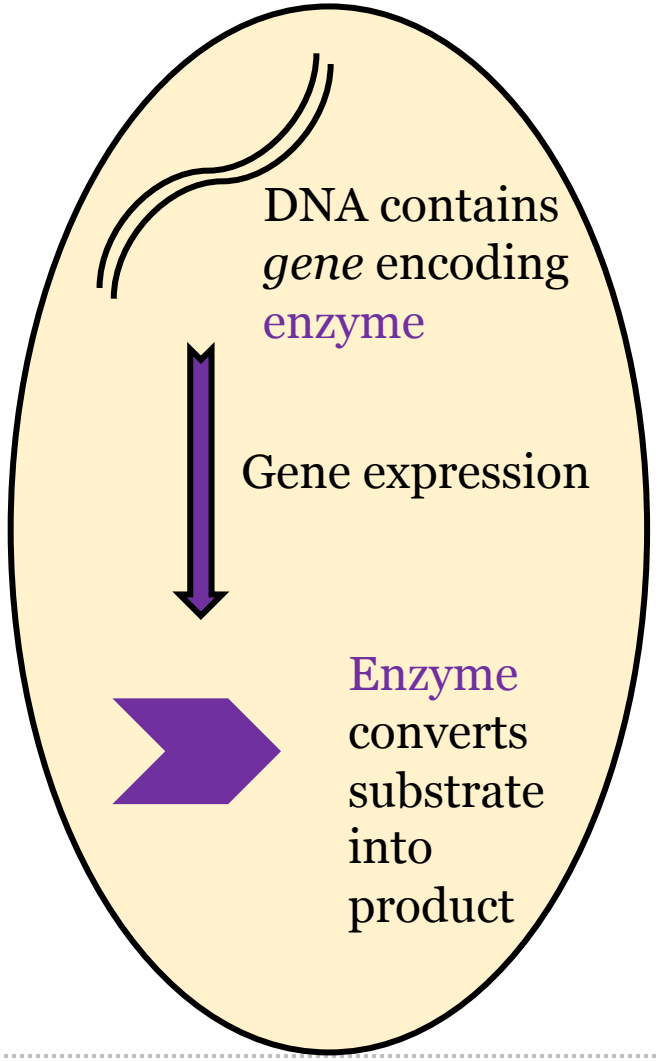
Nitrogen removal is…

- …the transformation of biologically available 'fixed' nitrogen into inert, harmless dinitrogen gas
  - ~78% of the atmosphere is N2 gas
- …catalyzed by microorganisms (mainly Bacteria, some Archaea) as part of their normal metabolism
- …complex: several pathways are known, and more are being discovered
  - These pathways, and the organisms driving them, may complement or compete with each other
  - = microbial ecology!

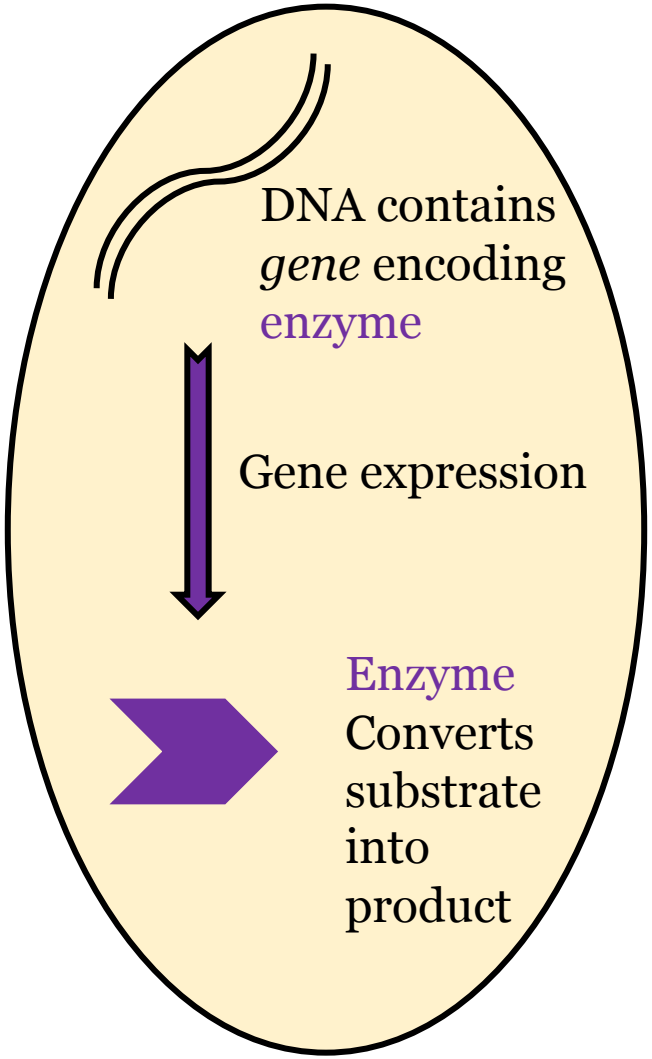
Effective Nitrogen Removal is Engineered Microbial Ecology

- Nitrogen transformations occurring in OWTS, particularly NRBs, are much less well understood than in large-scale, centralized WWTPs
- We can close the gap by using DNA amplification and sequencing to learn what microorganisms are present in different parts of existing OWTS
- We can use this information make more informed engineering choices about maximizing nitrogen removal during OWTS

Molecular Biology



Functional Genes

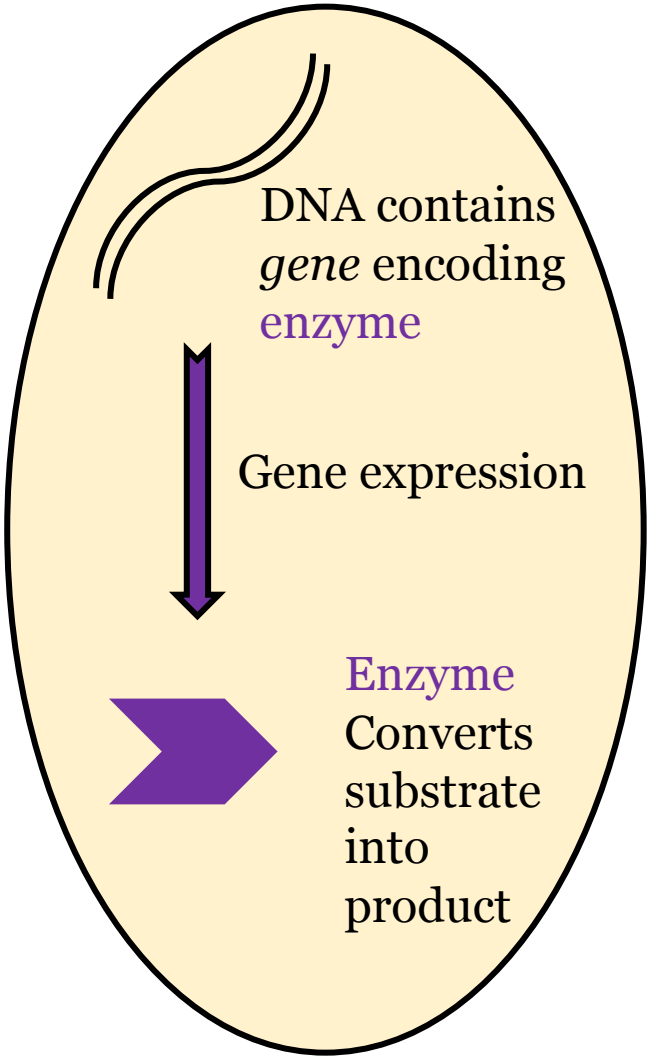


Example:
Denitrification

Gene = *nirS*

Enzyme NirS
(nitrite reductase)
NO_2^- -> -> N_2

Molecular Microbial Ecology



Specific regions of DNA can be amplified by PCR to detect presence of a particular enzymatic capacity

Sequences of amplified DNA can be analyzed to identify the organisms present

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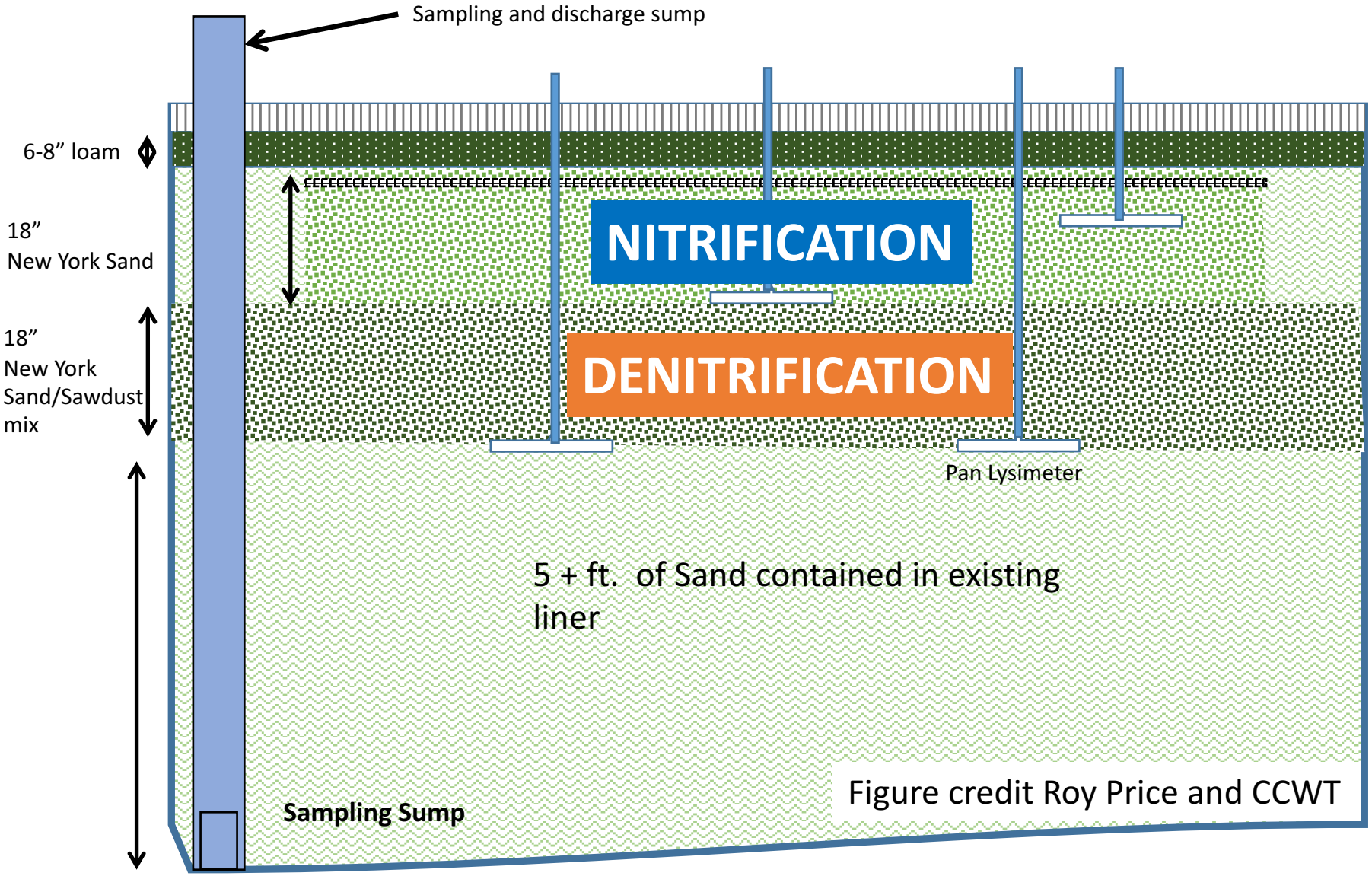
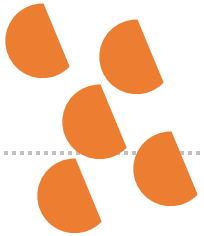


Classical denitrification

- Diverse bacteria can 'breathe' nitrate instead of oxygen
  - Requires organic carbon (as 'food'=electron donor) and anoxic or suboxic conditions
  - Generally facultative
  - aka nitrate respiration
- Denitrifiers can be identified by 'functional' genes
  - E.g., nitrate and nitrite reductases
  - we have detected *nirS* and *nirK*
  - we see diverse denitrifiers in every system, and different dominant types in nearly every sample



DENITRIFIERS:
Convert Nitrate to
dinitrogen gas (N_2)

dinitrogen gas (N_2)

DENITRIFICATION (nitrate respiration)

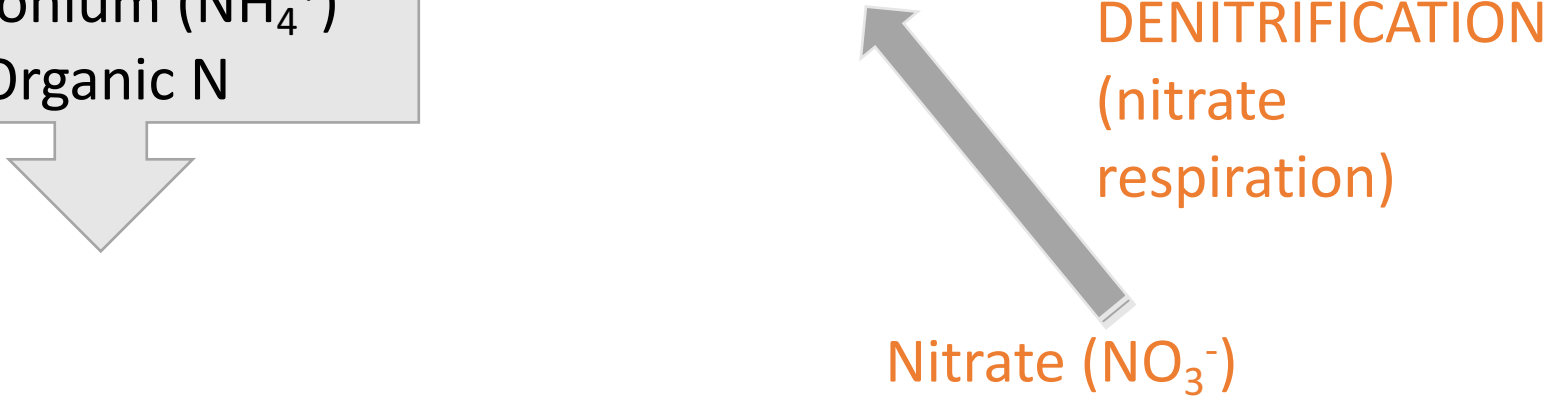
Nitrite (NO_2^-) is an intermediate

Nitrate (NO_3^-)

But nitrogen in wastewater is not initially in the form of nitrate

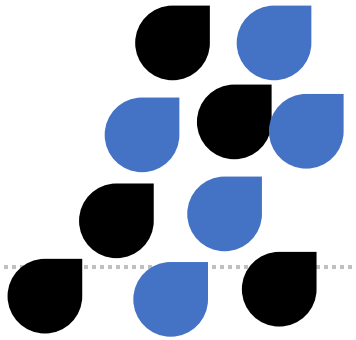
It's in the form of ammonium and organic compounds

INFLUENT:
Ammonium (NH_4^+)
Plus Organic N



Nitrification

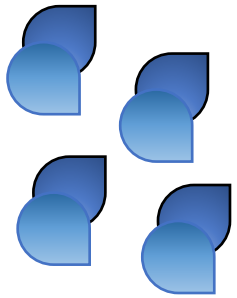
- Conversion of ammonium to nitrate
  - Requires oxygen but not organic carbon; usually obligate
  - Usually happens in two steps, done by different organisms
  - Nitrite is the intermediate between them
- Nitrifiers can be identified by 'functional' genes
  - Ammonium monooxygenase, nitrite oxidase
  - we have detected *amoA*, both bacterial and archaeal
  - we see diverse nitrifiers in every system, and different dominant types in nearly every sample



NITRIFIERS:
Convert
NH_4^+ to Nitrite (NO_2^-)
Then
Nitrite to Nitrate (NO_3^-)

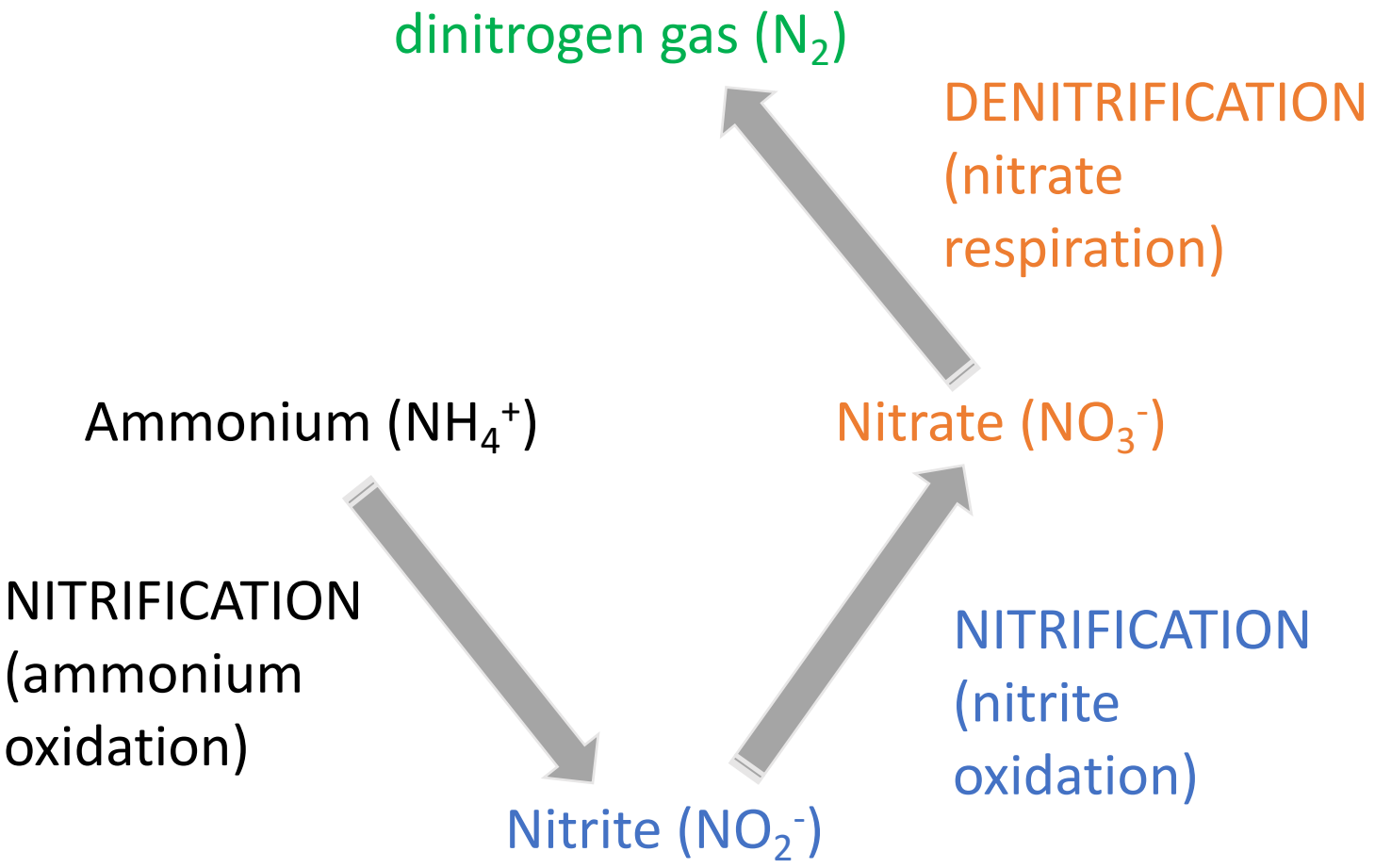
A New Form of Nitrification: Comammox

- Conversion of ammonium to nitrate by a single organism
  - rather than two; 'complete ammonia oxidation'
- Can be identified by 'functional' genes
  - we see *Nitrospira* in every sample, but very abundant in one

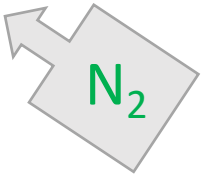


COMAMMOX:
Convert
NH_4^+ to Nitrate (NO_3^-)

Sequential nitrification-denitrification

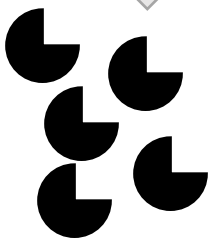


The standard approach: nitrogen removal by sequential nitrification/denitrification



DENITRIFIERS:
Convert Nitrate to dinitrogen gas (N_2)

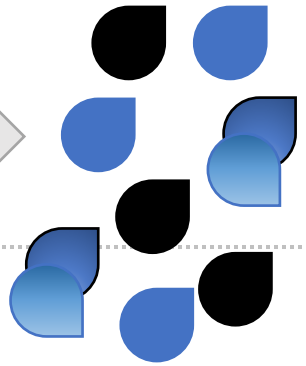
INFLUENT:
Ammonium (NH_4^+)
Plus Organic N



PRIMARY TREATMENT:
Converts Organic N to Ammonium (NH_4^+)

FROM NITRIFICATION:
Nitrate (NO_3^-)

FROM PRIMARY TREATMENT:
Ammonium (NH_4^+)



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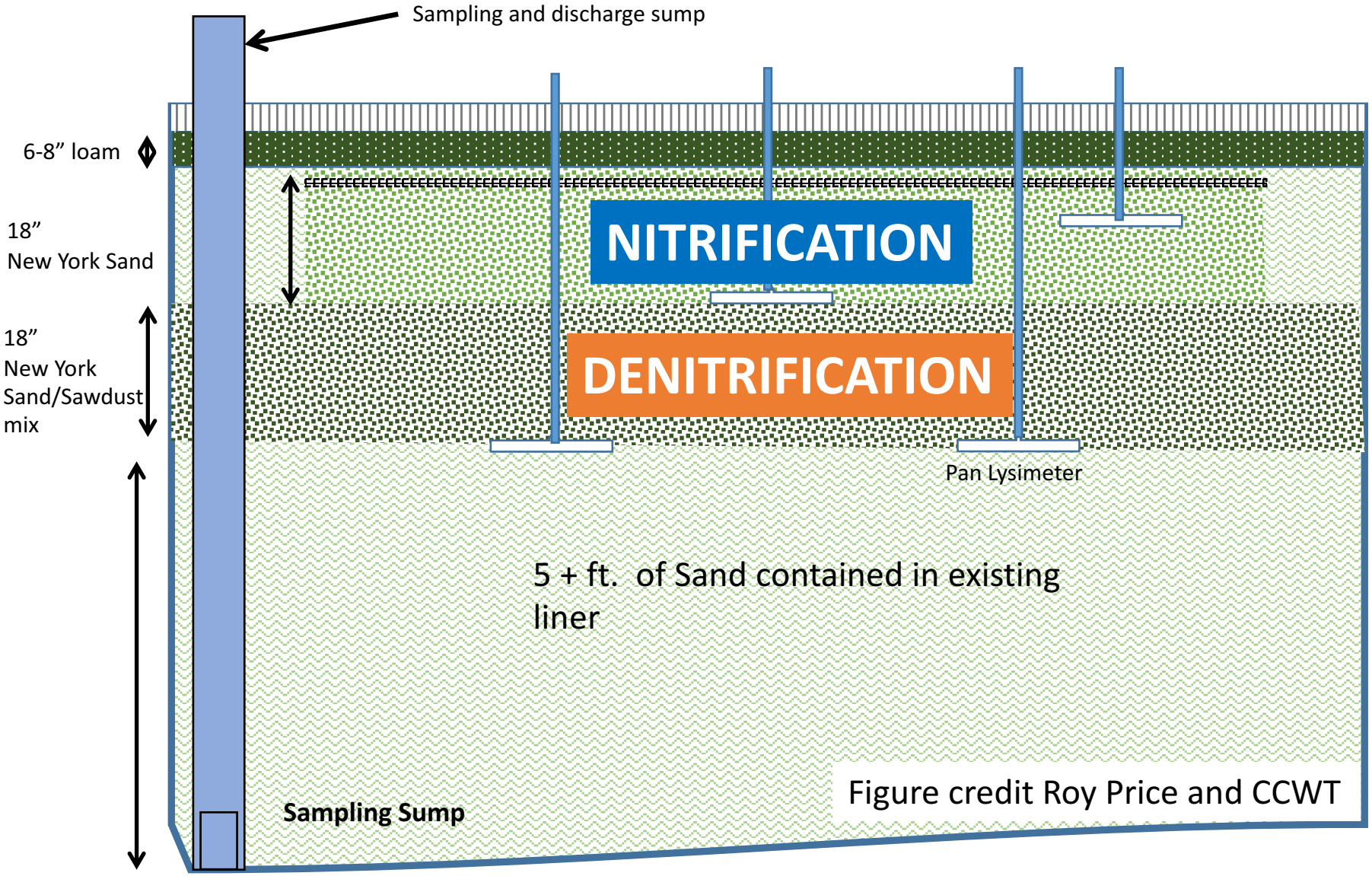
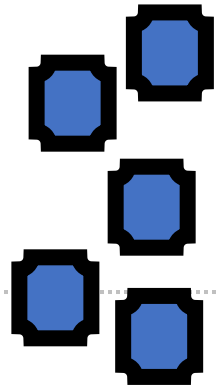


A New Paradigm: ANAMMOX

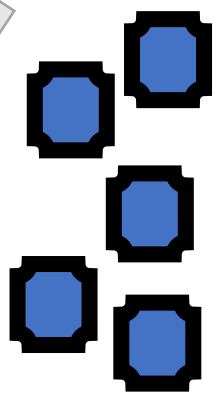
- Anaerobic ammonium oxidation
  - Aka partial nitritation/anammox (PNA)
- Ammonium and nitrite are the substrates
- Anammox bacteria can be identified by 'functional' genes
  - Hydrazine oxidoreductase
  - we have detected *hzo* at least in April
  - we have detected *Kuenenia*, *Jettenia*, *Brocadia*



ANAMMOX BACTERIA:
Convert
NH_4^+ plus Nitrite (NO_2^-)
to dinitrogen gas (N_2)
(and 15% nitrate)

A new paradigm: ANAMMOX

N_2



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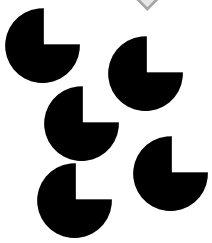
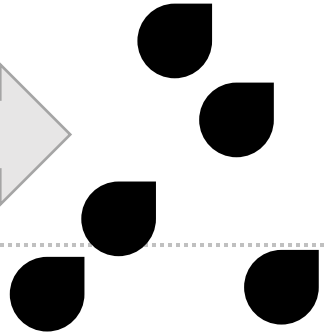


PRIMARY TREATMENT:
Converts Organic N to
Ammonium (NH_4^+)

FROM PARITAL NITRIFICATION:
Ammonium (NH_4^+)
Nitrite (NO_2^-)

FROM PRIMARY
TREATMENT:
Ammonium (NH_4^+)



NITRIFIERS:
Convert
NH_4^+ to Nitrite (NO_2^-)

Undesirable intermediates

- Some of these pathways, particularly classical nitrification and denitrification, involve intermediates like nitrous oxide (N_2O)
  - N_2O is ~300X stronger greenhouse gas than carbon dioxide and also depletes ozone layer
  - Can build up and be released under suboptimal conditions
- Engineered nitrogen removal systems must consider this and other potential side reactions and products

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As always, big thanks to our sponsors!

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