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Decoding the microbial ‘black box’ of onsite wastewater treatment (OWTS)

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FAR BEYOND

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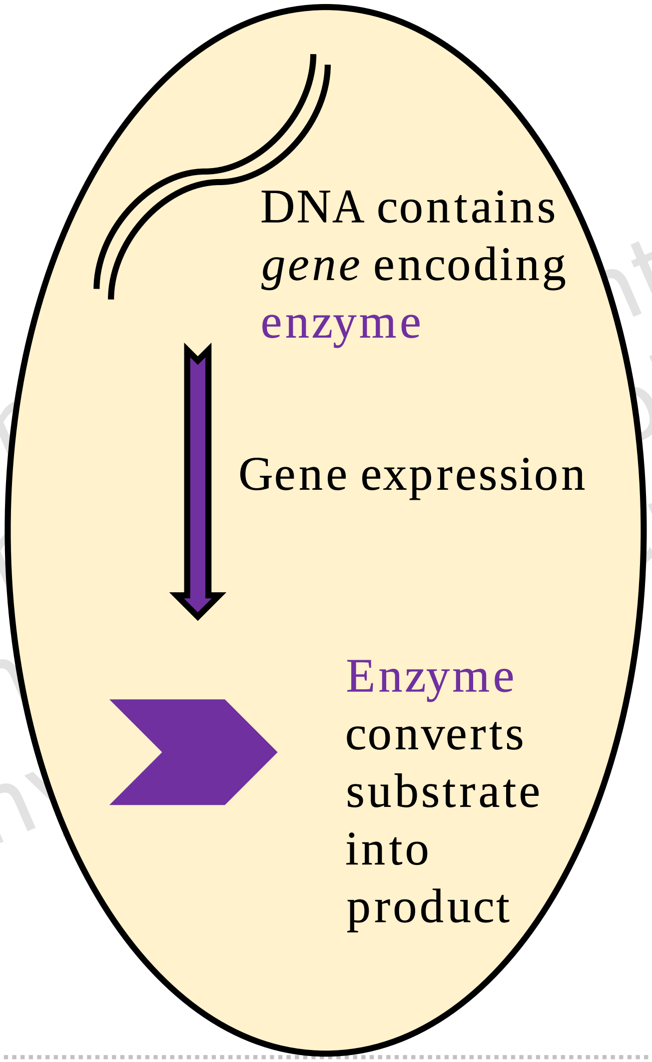
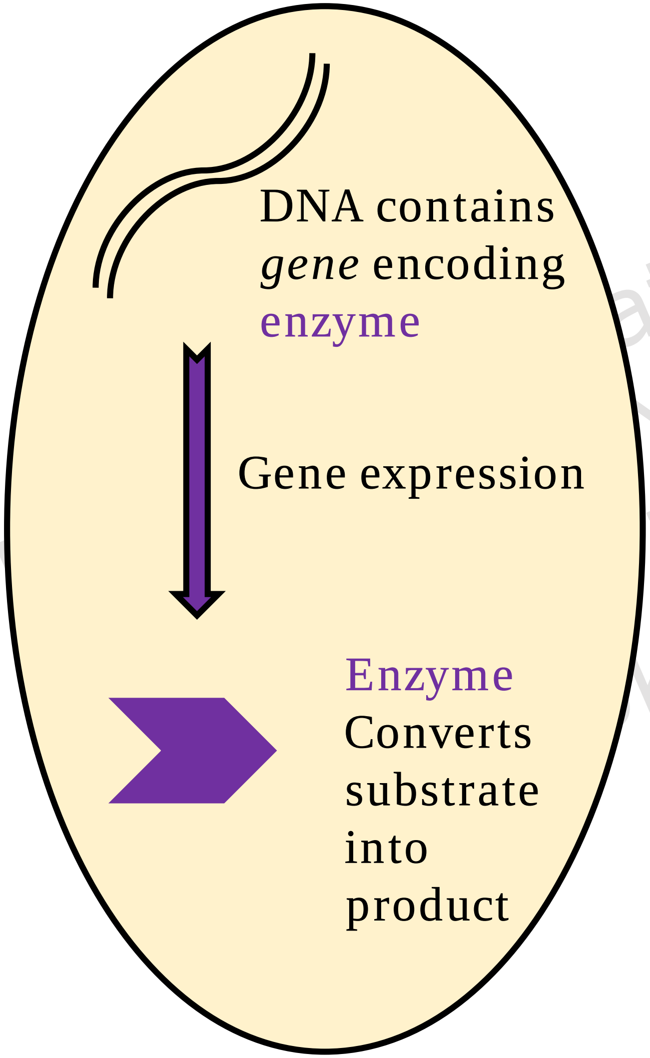
Effective Nitrogen Removal is Engineered Microbial Ecology

- Nitrogen transformations occurring in OWTS 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 how to maximize nitrogen removal during OWTS

Molecular Biology



Functional Genes

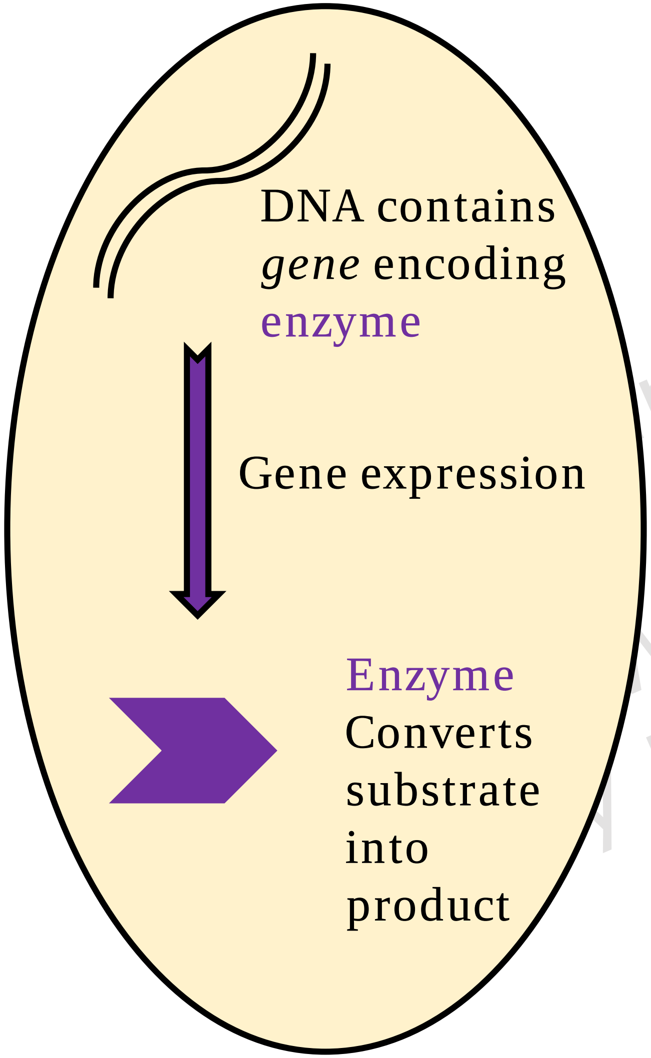


Example:
Denitrification

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 particular enzymatic capacity

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

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Nitrogen removal is…

- …the transformation of biologically available 'fixed' nitrogen into inert, harmless dinitrogen gas
    - ~78% of the atmosphere is N_2 gas
- …catalyzed by microorganisms (mainly Bacteria) 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!

Classical denitrification

- Diverse bacteria can 'breathe' nitrate instead of oxygen
  - Requires organic carbon (as 'food'=electron donor) and anoxic conditions
  - Nitrate respiration
- Denitrifiers can be identified by 'functional' genes
  - E.g., nitrate and nitrite reductases
  - -we have detected *nirS* and *nirK*

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

dinitrogen gas (N_2)

DENITRIFICATION (nitrate respiration)

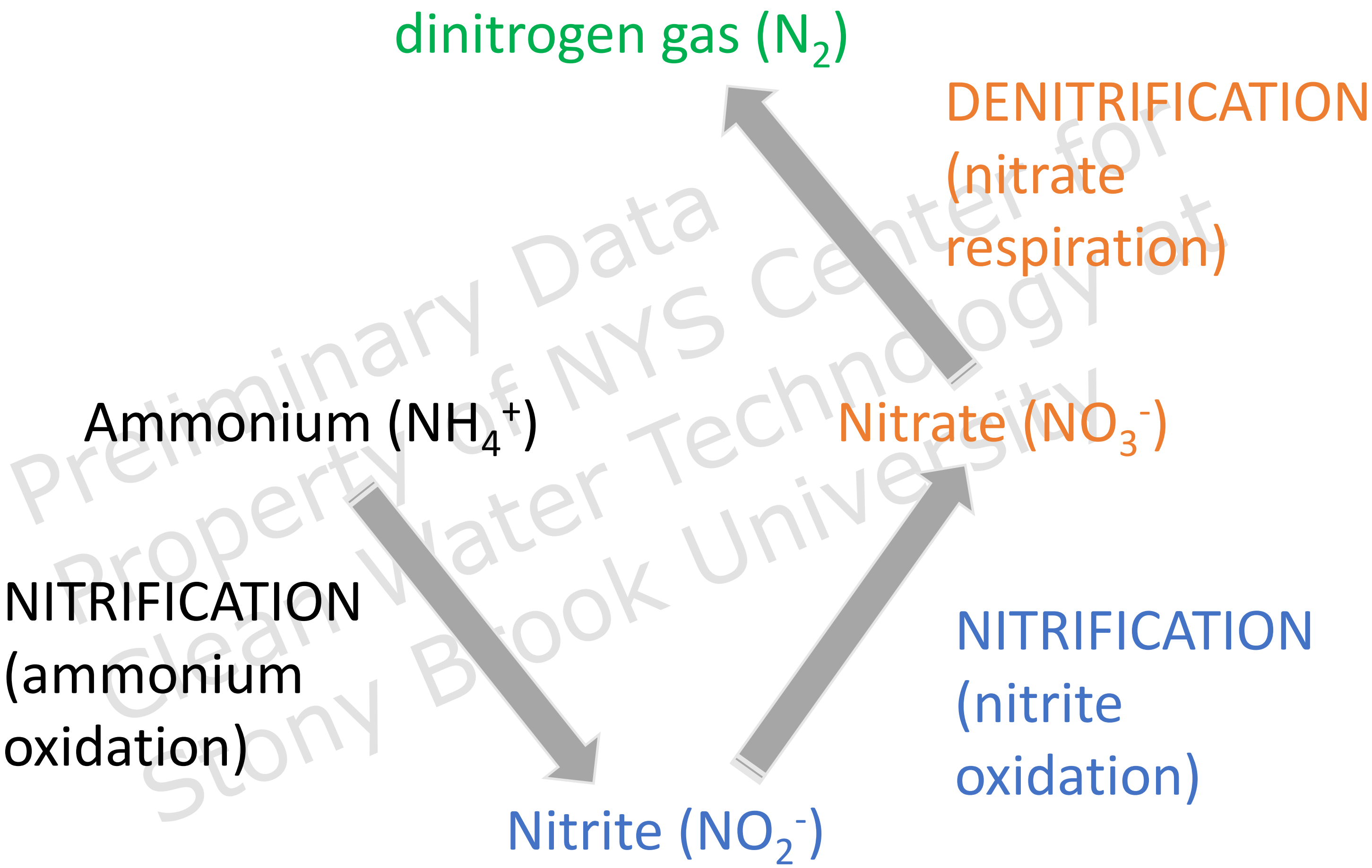
Nitrate (NO_3^-)

Nitrification

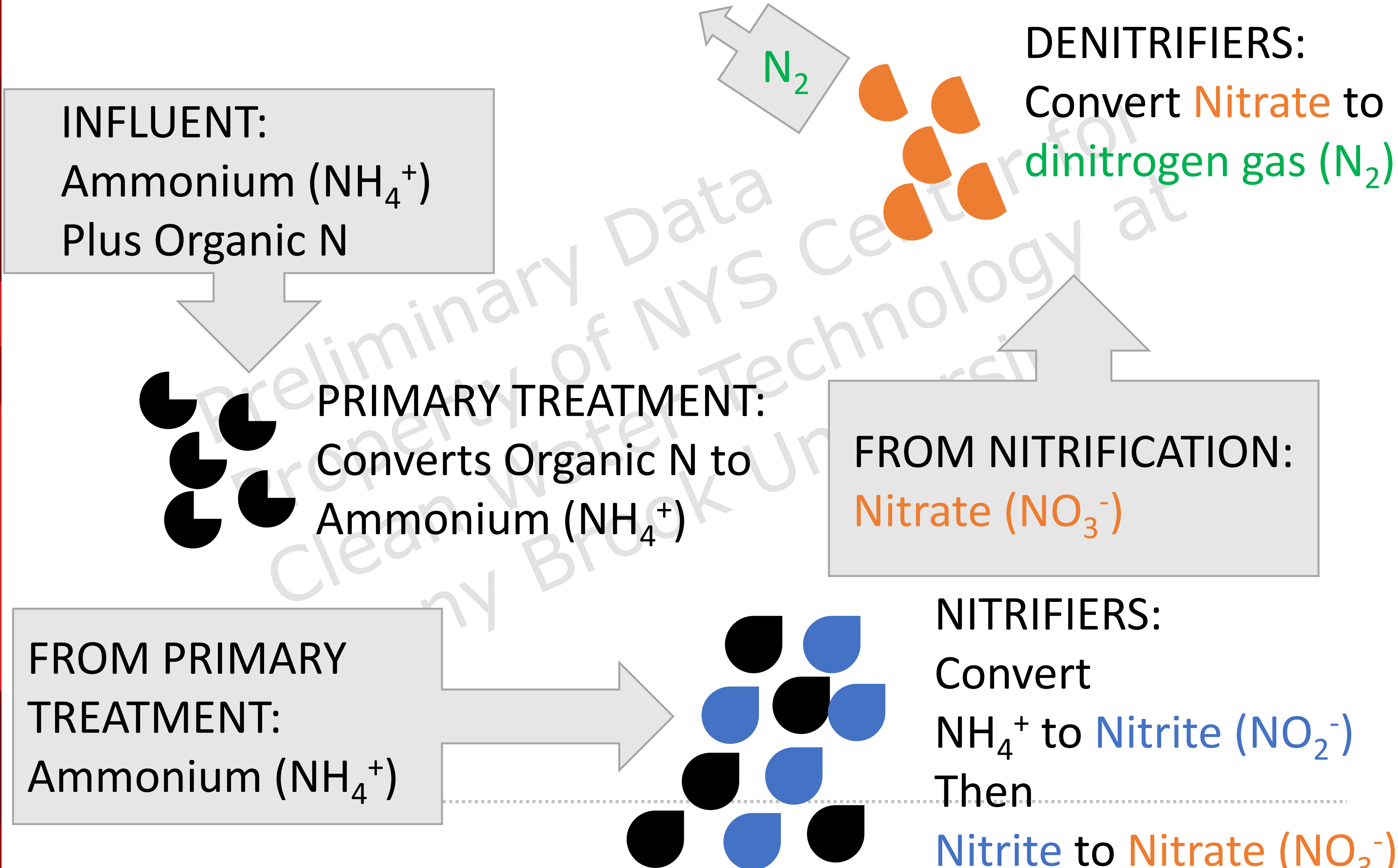
- Conversion of ammonium to nitrate
  - Requires oxygen but not organic carbon
  - Actually happens in two steps, done by different organisms
  - Nitrite is the intermediate between them
- Nitrifiers can be identified by ‘functional’ genes
  - Ammonium monooxygenase, nitrite oxidase
  - -we have detected *amoA*, both bacterial and archaeal

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

Sequential nitrification-denitrification



The standard approach: nitrogen removal by sequential nitrification/denitrification



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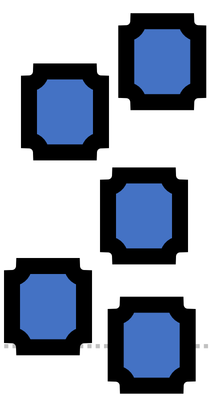
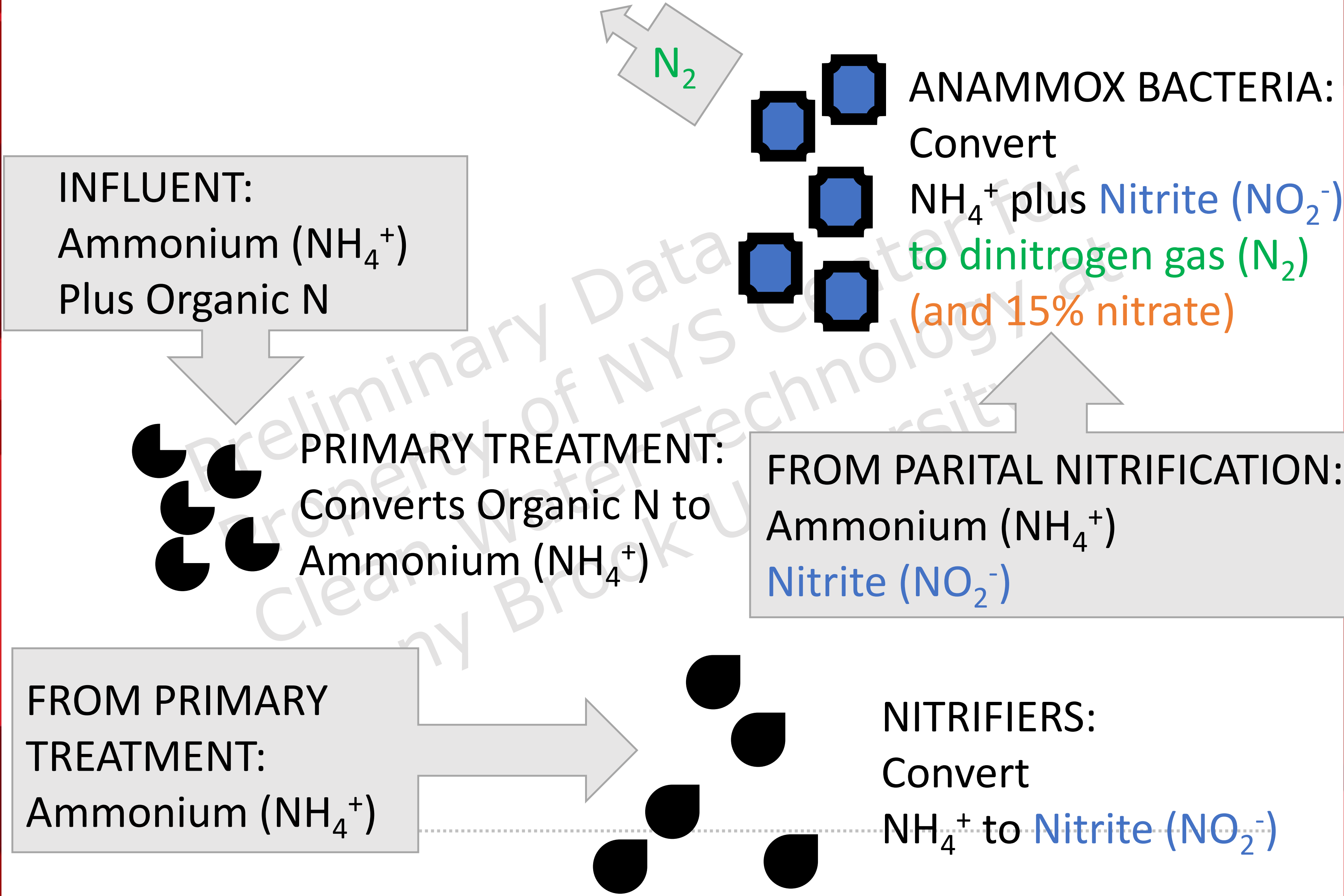
A New Paradigm: ANAMMOX

- 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



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

A new paradigm: ANAMMOX



‘HNR’: Another new paradigm maybe too good to be true?

INFLUENT:
Ammonium (NH_4^+)
Plus Organic N

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

N_2

FROM PRIMARY TREATMENT:
Ammonium (NH_4^+)

‘HNR’ BACTERIA:
Convert NH_4^+
to dinitrogen gas (N_2)

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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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