Metropolitan Water Reclamation
District of Greater Chicago

WELCOME
TO THE DECEMBER EDITION
OF THE 2019
M&R SEMINAR SERIES

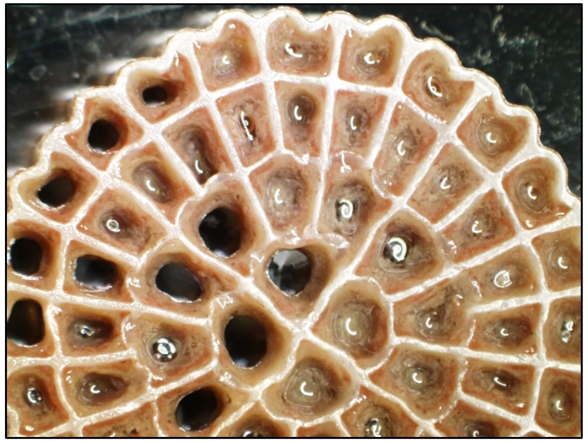
BEFORE WE BEGIN

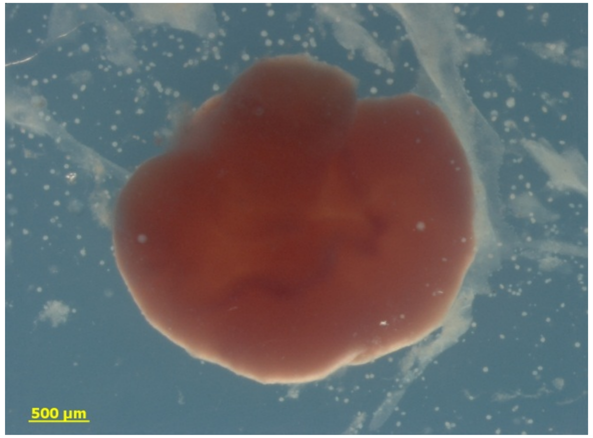
- **SAFETY PRECAUTIONS**
  - PLEASE FOLLOW EXIT SIGN IN CASE OF EMERGENCY EVACUATION
  - AUTOMATED EXTERNAL DEFIBRILLATOR (AED) LOCATED OUTSIDE
- **PLEASE SILENCE CELL PHONES AND/OR SMART DEVICES**
- **QUESTION AND ANSWER SESSION WILL FOLLOW PRESENTATION**
- **PLEASE FILL EVALUATION FORM**
- **SEMINAR SLIDES WILL BE POSTED ON MWRD WEBSITE** (https://mwrd.org/seminars)
- **STREAM VIDEO WILL BE AVAILABLE ON MWRD WEBSITE** (https://mwrd.org/seminars - after authorization for release is arranged)

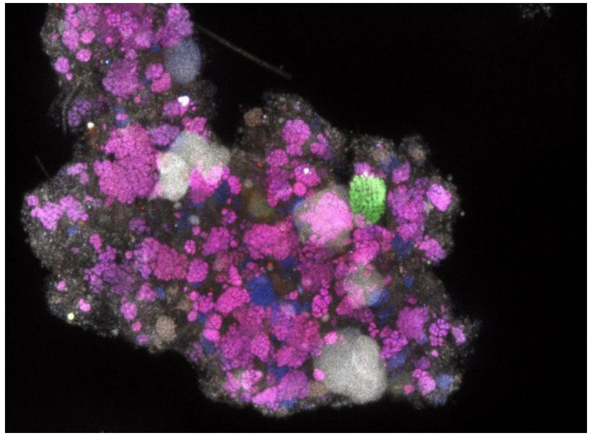
George F. Wells, Ph.D.

- Dr. Wells is an Assistant Professor in the Department of Civil and Environmental Engineering at Northwestern University, where he directs the Environmental Biotechnology and Microbial Ecology Laboratory. His primary research interests are microbial nitrogen and phosphorus cycling and shortcut biological nutrient removal processes, resource and energy recovery from wastewater, microbial ecology of engineered and impacted natural systems, sustainable biological wastewater treatment, and microbial greenhouse gas production. George collaborates extensively with utilities and practitioners to develop and test feasibility of sustainable biological wastewater treatment processes, with a strong focus on energy efficient shortcut nitrogen removal and phosphorus removal and recovery bioprocesses.
- Prior to joining Northwestern University, George spent nearly 2.5 years as a postdoctoral scholar under Dr. Eberhard Morgenroth at the Swiss Federal Institute of Aquatic Science and Technology (near Zürich, Switzerland).
- George received his B.S. in Chemical Engineering and B.A. in Environmental Engineering from Rice University, Houston, Texas, and MS and PhD from Department of Civil and Environmental Engineering at Stanford University, under Dr. Craig Criddle and Dr. Chris Francis.

Towards Integrated Mainstream Shortcut Nitrogen and Biological Phosphorus Removal







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The Wells Research Group at Northwestern

Microbial Ecology

Environmental Biotechnology

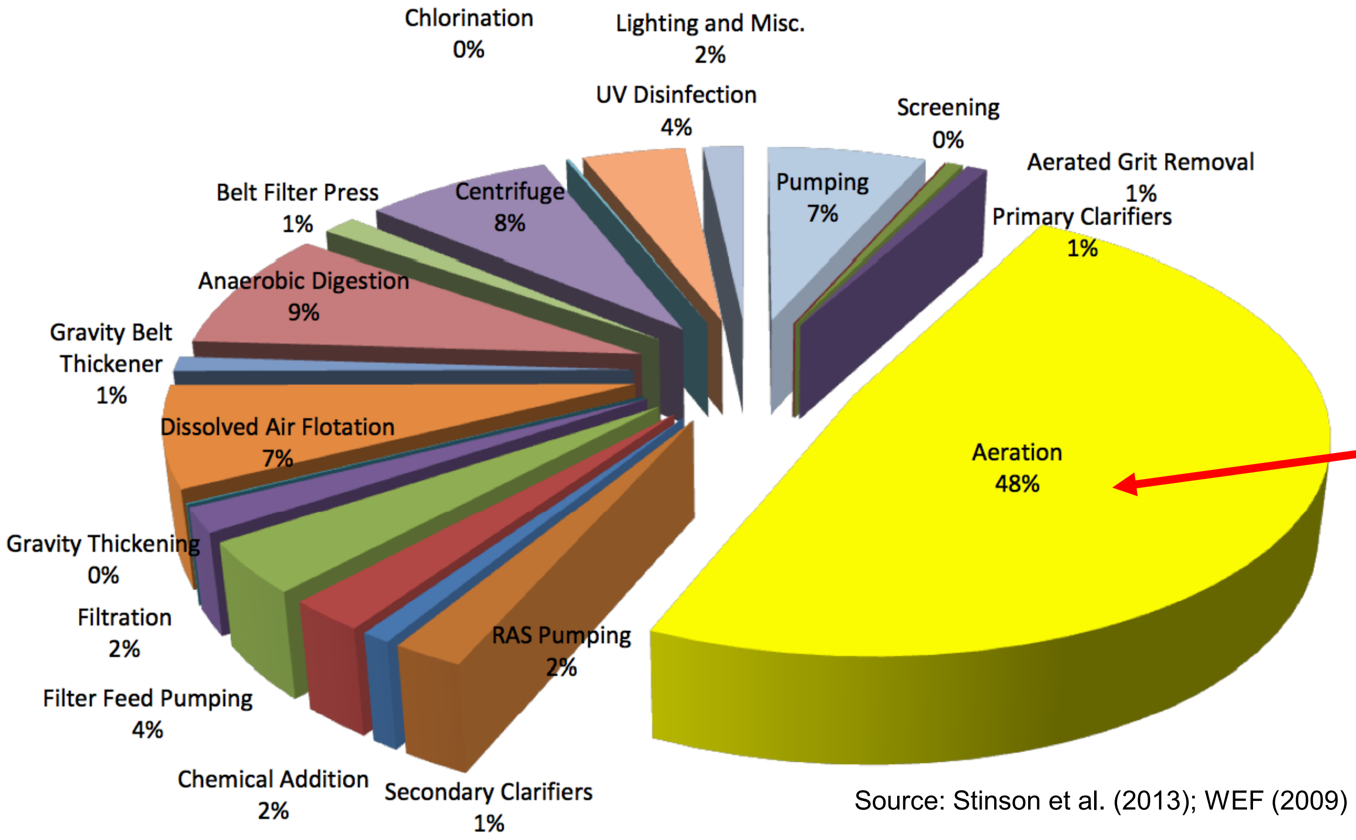
Sustainable Environmental and Public Health Protection *and* Resource Recovery from Urban “Waste” Streams

Specific Focus: Microbial Nitrogen and Phosphorus Cycling and Nutrient Pollution Management

Website: wells.northwestern.edu

Conventional nitrogen (N) and phosphorus (P) removal bioprocesses at wastewater treatment plants are largely successful, but also highly **energy intensive**

Wastewater treatment accounts for ~3% of nationwide electricity use (~15 GW)[1]



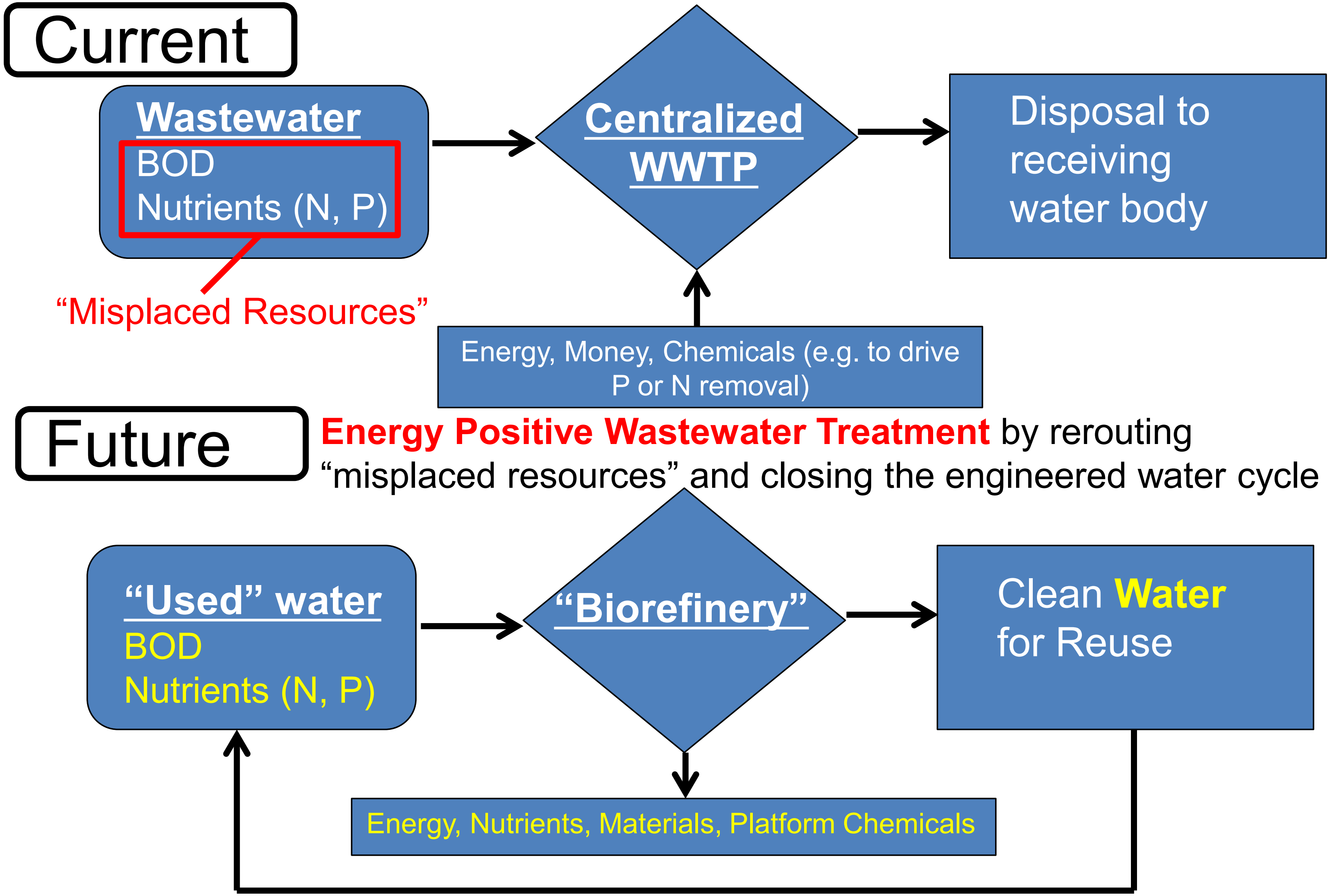
Source: Stinson et al. (2013); WEF (2009)

~50% of energy use for oxygen supply (aeration)

Conversely, organic-rich municipal, industrial, and agricultural wastewater contains potential energy equivalent to ~17 GW of power[2]

1. McCarty et al. 2011. *Environ. Sci. Technol.* 2011, 45, 7100–7106.
2. Logan et al. 2012. *Science* 2012, 337, 686-690.

A Paradigm Shift Towards Resource Recovery



Current

Wastewater

BOD

Nutrients (N, P)

"Misplaced Resources"

Centralized WWTP

Disposal to receiving water body

Energy, Money, Chemicals (e.g. to drive P or N removal)

Future

Energy Positive Wastewater Treatment by rerouting "misplaced resources" and closing the engineered water cycle

"Used" water

BOD

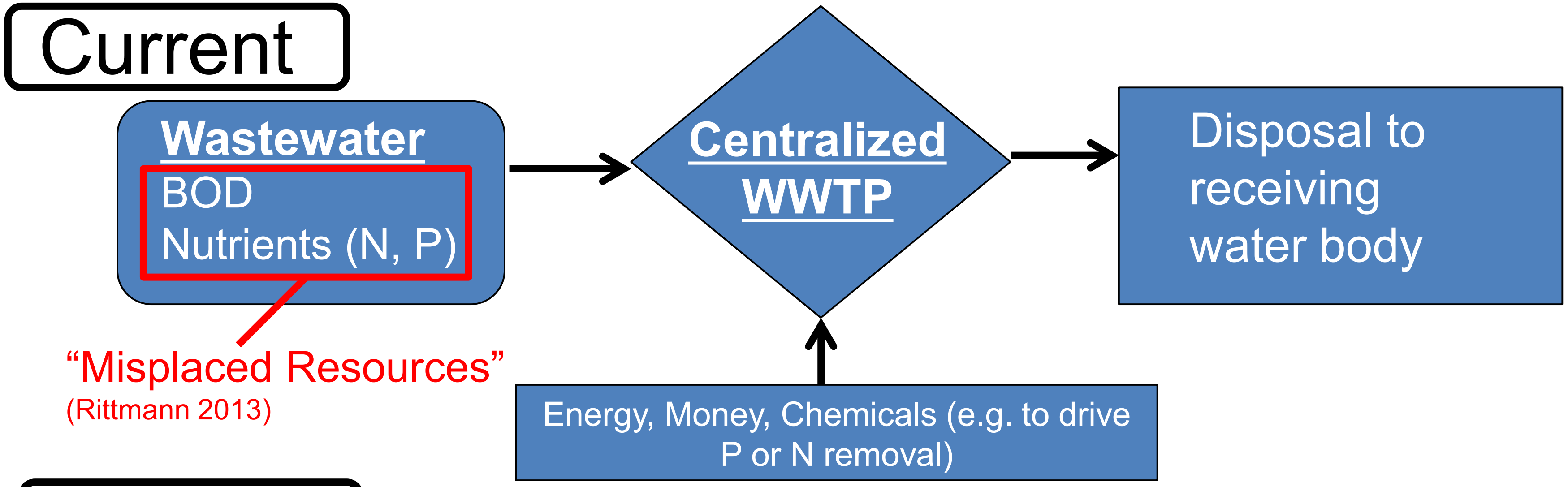
Nutrients (N, P)

"Biorefinery"

Clean **Water** for Reuse

Energy, Nutrients, Materials, Platform Chemicals

A Paradigm Shift Towards Resource Recovery



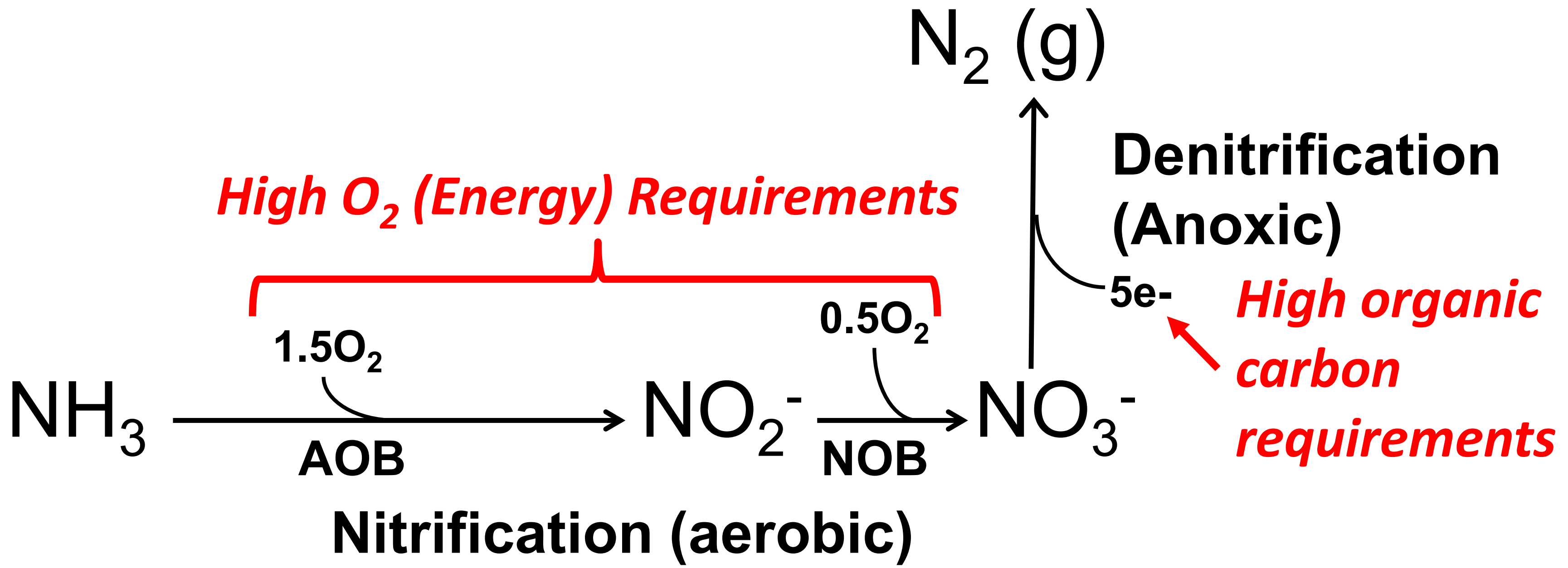
Future

Energy Positive Wastewater Treatment by rerouting "misplaced resources" and closing the engineered water cycle

Given that conventional nutrient removal processes are highly energy intensive, ***it is unlikely that energy positive wastewater treatment targeting resource recovery can be achieved without new innovations in N and P removal bioprocesses***

Shortcut N Removal Processes: A Critical Opportunity for Sustainable Wastewater Treatment

Conventional Biological N Removal: Nitrification/ Denitrification



N_2 (g)

Denitrification (Anoxic)

5e-

High organic carbon requirements

High O_2 (Energy) Requirements

$$NH_3 \xrightarrow[\text{AOB}]{1.5O_2} NO_2^- \xrightarrow[\text{NOB}]{0.5O_2} NO_3^-$$

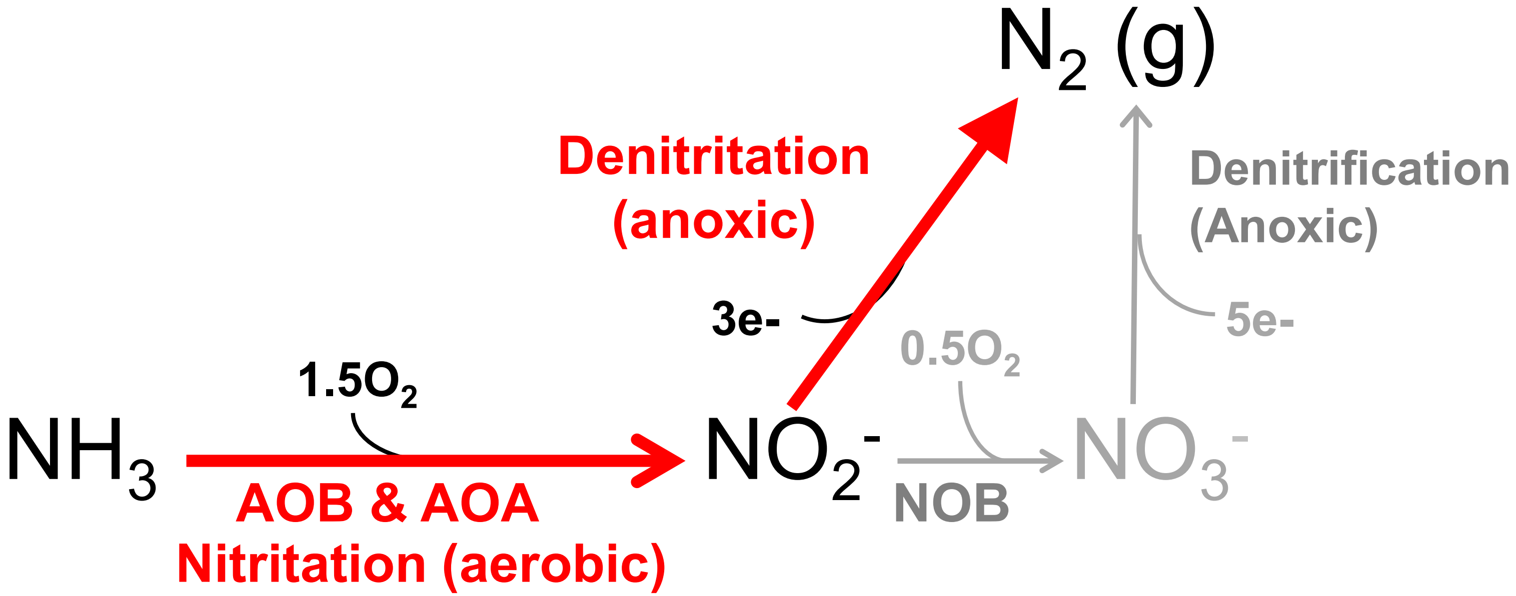
Nitrification (aerobic)

AOB: Ammonia-Oxidizing Bacteria
NOB: Nitrite-Oxidizing Bacteria

Shortcut N Removal Processes: A Critical Opportunity for Sustainable Wastewater Treatment

Shortcut 1: Nitritation-Denitritation

(Also called Nitrite Shunt)



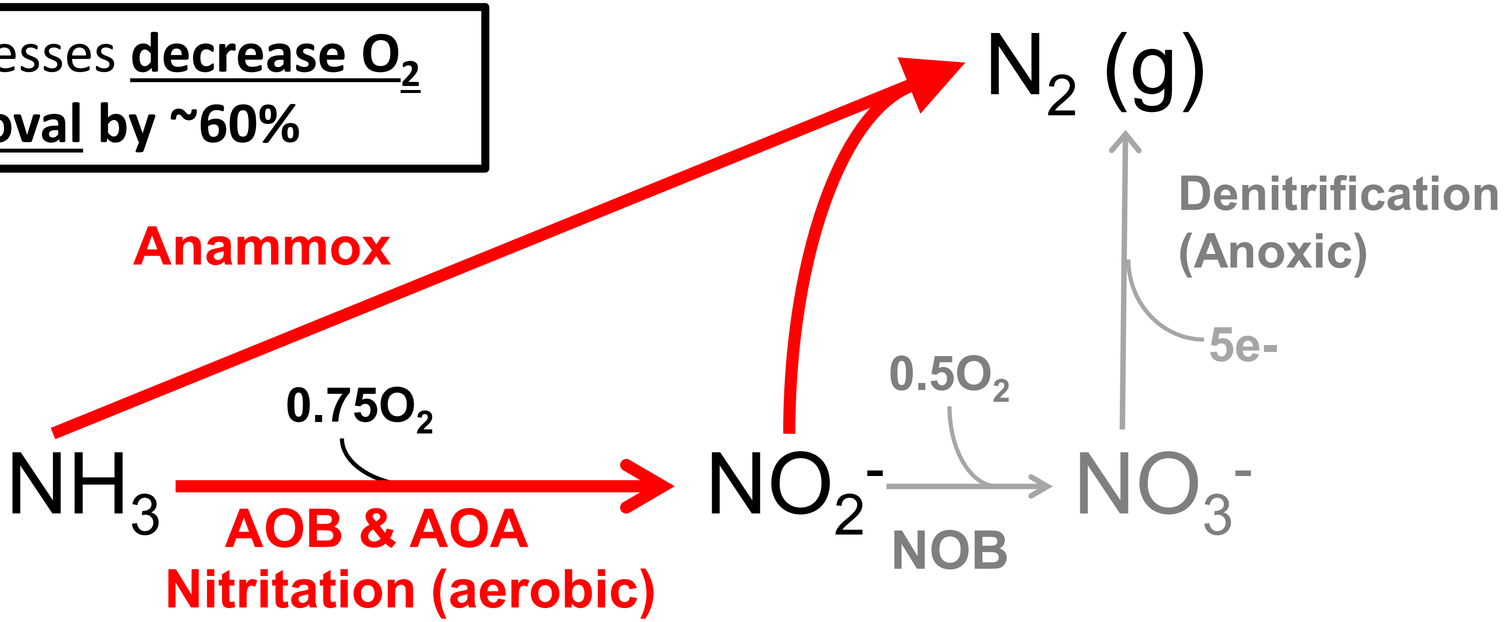
- 25% reduction in O_2 demand
- 40% reduction in carbon demand (potentially redirected to bio P)

Shortcut N Removal Processes: A Critical Opportunity for Sustainable Wastewater Treatment

Shortcut 2: Deammonification

(or Partial Nitritation/Anammox [PNA])

Deammonification processes **decrease O_2 requirement for N removal** **by ~60%**

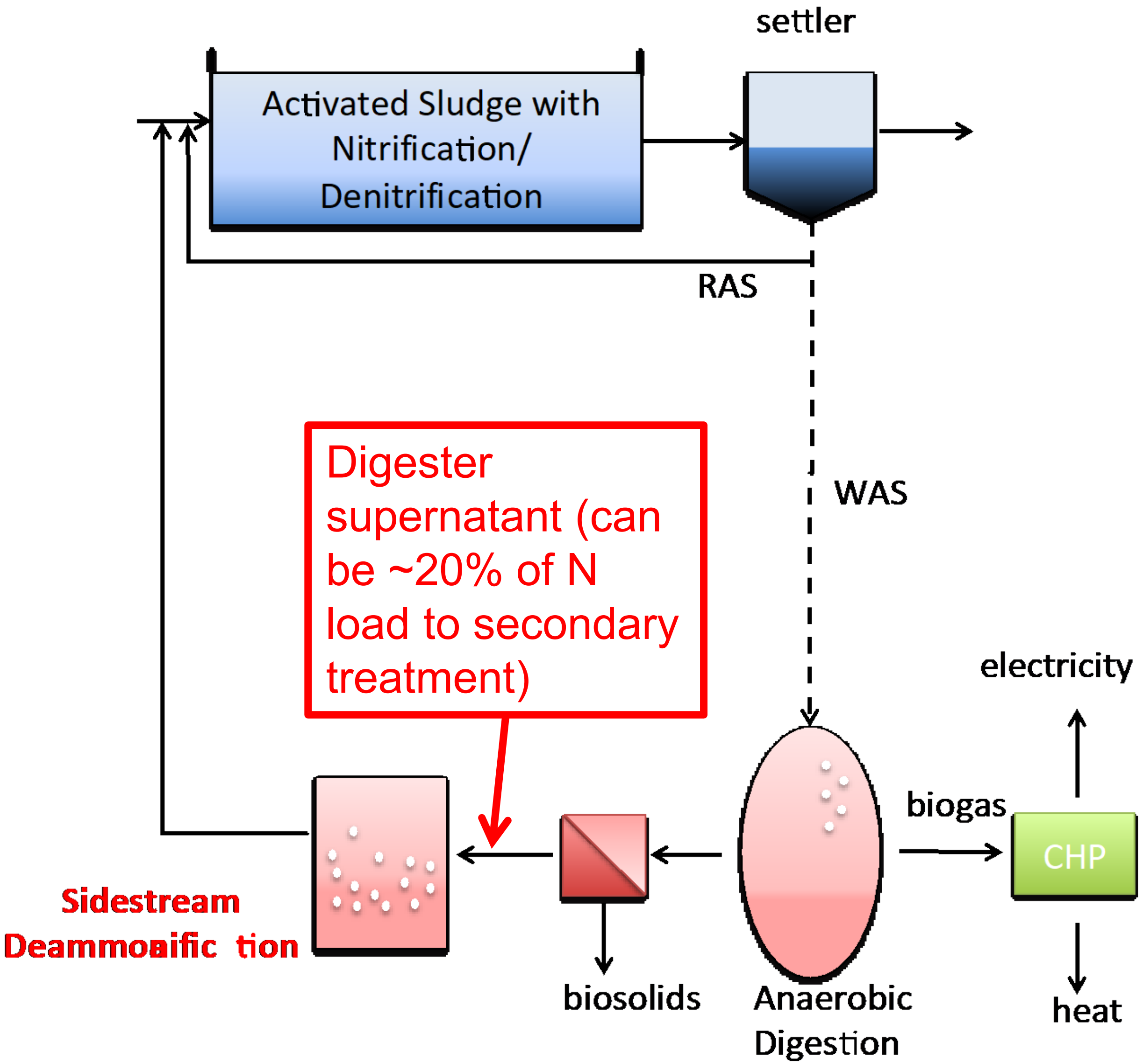


Deammonification processes ***decouple C and N removal***, thereby thereby **enabling efficient use of C for bioP removal** or enhanced energy recovery

Initial Development of Shortcut N Removal Processes has focused on *sidestream* treatment of anaerobic digester supernatant

Sidestreams are characterized by:

- *High temperature (~30°C)*
- *High NH_4^+ (~500-1000 mgN/L)*

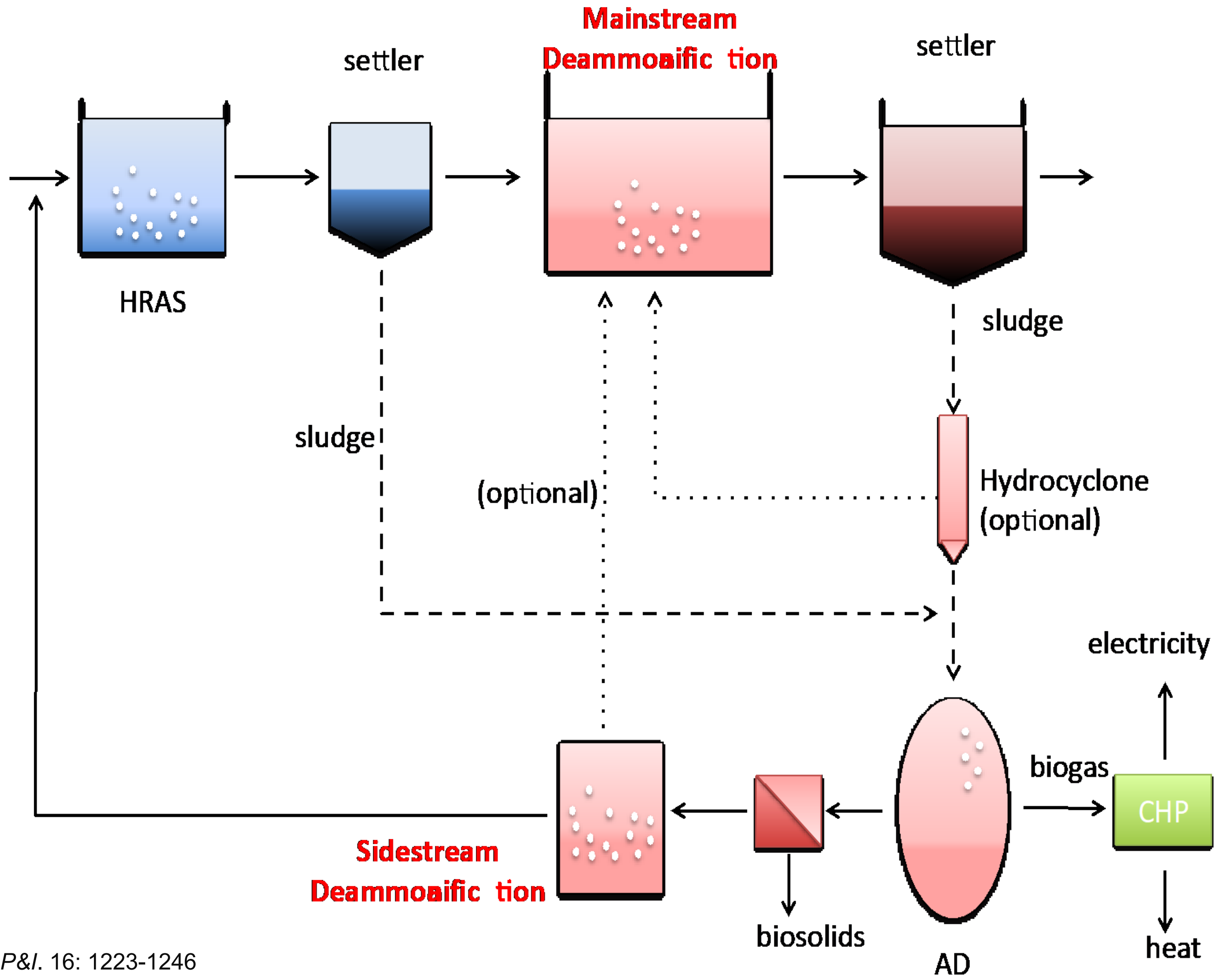


Digester supernatant (can be ~20% of N load to secondary treatment)

Key Research Question: How can we apply shortcut N removal bioprocesses in the **mainstream**?

The mainstream is characterized by:

- *Low temperatures (~10-20°C)*
- *Low* NH_4^+ *(~20-30 mgN/L)*
- *Dynamic process conditions*



Source: Gao, Scherson, & Wells (2014) *ES:P&I*. 16: 1223-1246

Critical Challenges to Mainstream Shortcut N Removal

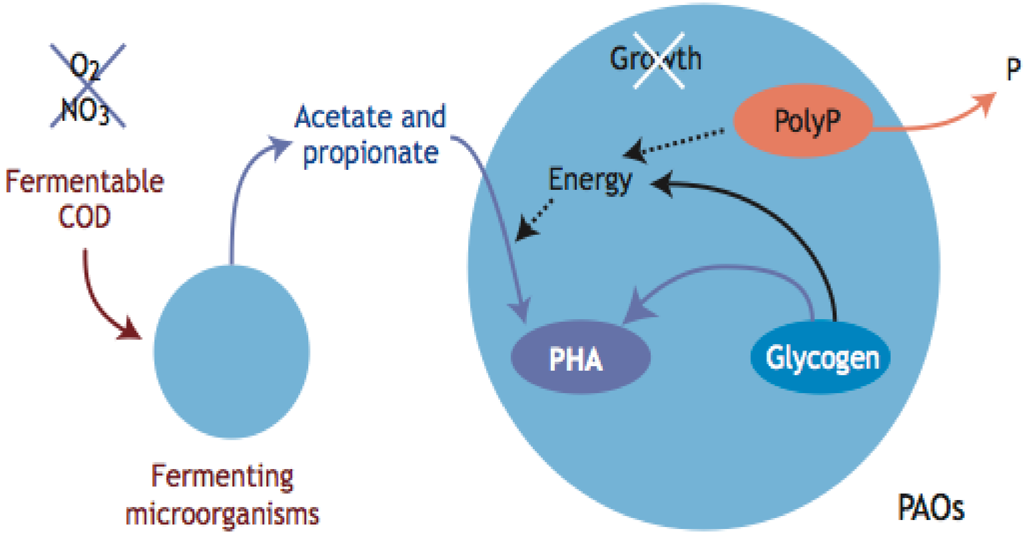
1. Robust and stable ***suppression of NOB***
2. Maintenance of ***high levels of slow growing anammox biomass and activity***
3. ***Robust process performance and stability*** under dynamic conditions expected in the mainstream
4. Integrated shortcut N and ***biological P removal***

Polyphosphate Accumulating Organisms (PAOs)

PAOs are a critical microbial functional guild in processes that facilitate ***Enhanced Biological Phosphorus Removal processes (BioP or EBPR).*** *Most PAOs are thought to affiliate with as-yet-uncultivated* ***Candidatus 'Accumulibacter phosphatis'****.*

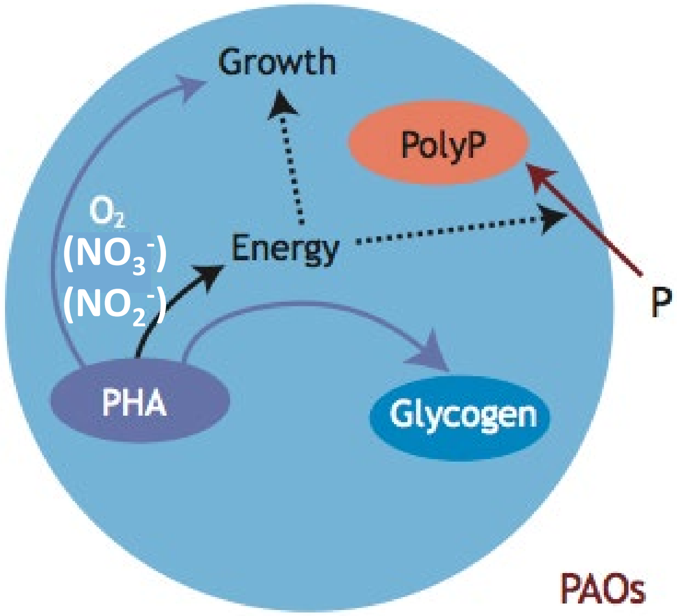
Anaerobic Conditions:

P Release from Polyphosphate



Aerobic (or denitrifying?) Conditions:

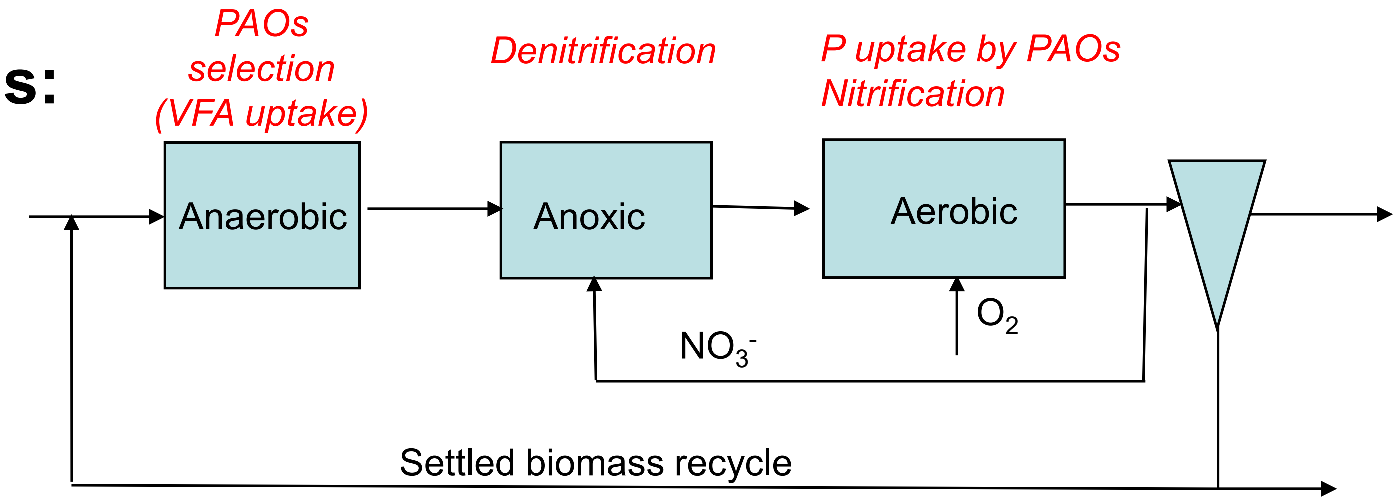
P Uptake for Polyphosphate Synthesis



Source: Wentzel et al. (2008) "Phosphorus Removal" in Henze, van Loosdrecht, Ekama, and Brdjanovic, Ed. Biological Wastewater Treatment: Principles, Modeling, and Design. IWA Publishing: London, UK

Conventional Routes for Integrated BioP and N removal

A^2O Process:



- Conventional BioP and N removal processes are **energy intensive** due to the need to provide O_2 for both nitrifiers and PAOs
- In addition, they are often **carbon limited** because bCOD is needed to drive both PAO and denitrifier activity. Carbon limitations can lead to poor bioP performance.

→These deficiencies can potentially be addressed by integrating carbon and energy efficient shortcut N removal with bio-P

Shortcut Biological N and BioP Removal Research Station

O’Brien Water Reclamation Plant (Chicago, IL, USA)

- Reactor operation on multiple parallel treatment trains began in Spring 2016
- Uninterrupted Access to Primary Effluent

Broad Objective: Evaluate strategies for mainstream shortcut N removal coupled to biological P removal

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

Shortcut Biological N and BioP Removal Research Station

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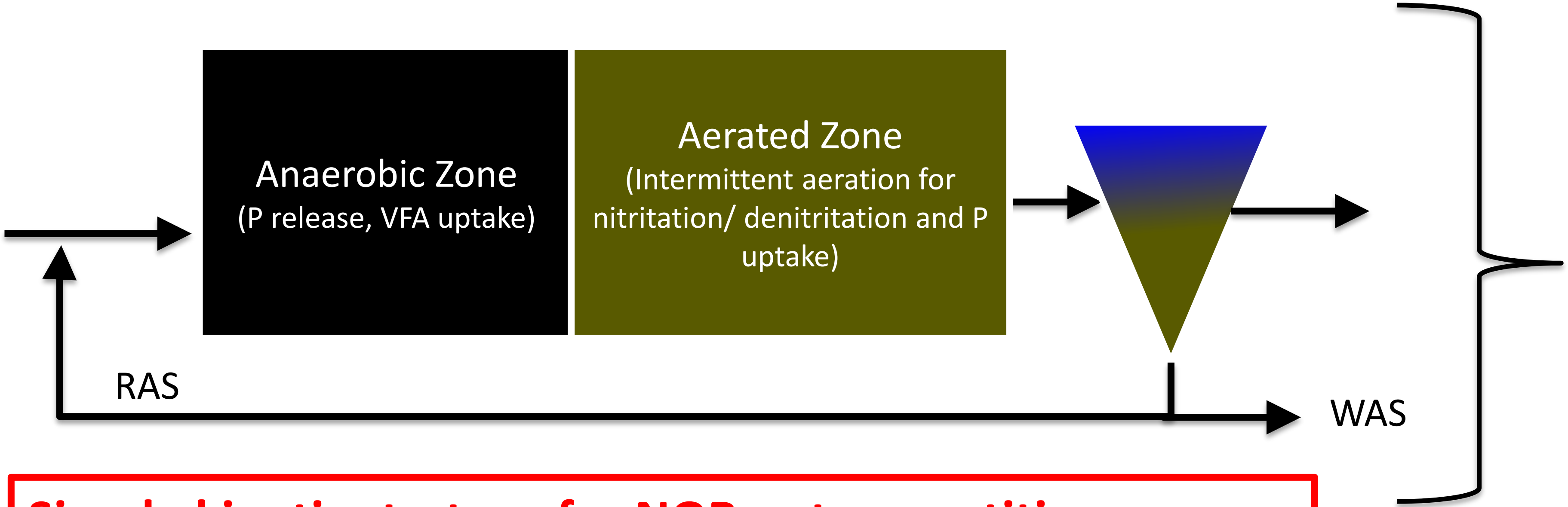
Broad Objective: Evaluate strategies for mainstream shortcut N removal coupled to biological P removal

1. Integrated **Nitritation/ Denitritation with BioP**
2. **High rate BioP** followed by **Mainstream Deammonification**
   - **2a. IFAS** (Biofilm + Suspended Growth)
   - **2b. Suspended Growth**

Strategy 1: Integrated Nitritation/Denitritation + BioP

- Single sludge process for energy and COD efficient N and P removal
- Well-suited for modification of existing MWRD infrastructure (e.g. Kirie WRP)



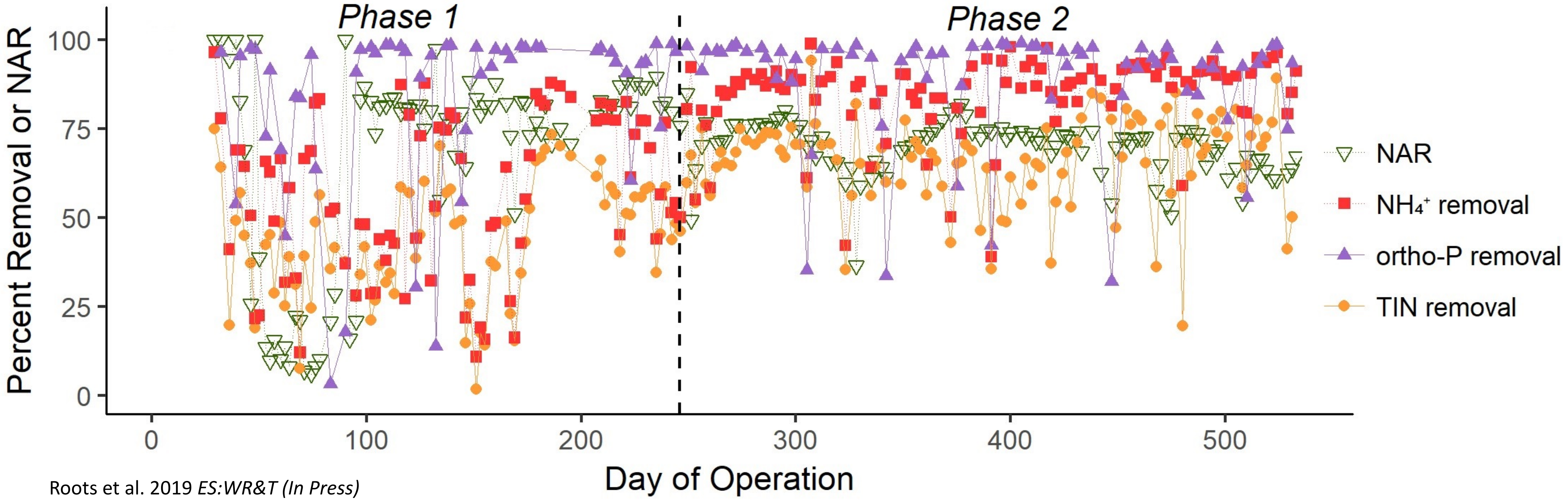
56 L SBR

Operated with primary effluent as feed for >500 days

Simple kinetic strategy for NOB outcompetition:

- Intermittent aeration
- Robust “nitrite sink” (denitrifiers)
- Tight SRT control for NOB washout

Robust High Rate N, C, and P Removal



Roots et al. 2019 *ES:WR&T (In Press)*

NAR=Nitrite Accumulation Ratio= $\frac{NO_2^-}{NO_2^- + NO_3^-}$

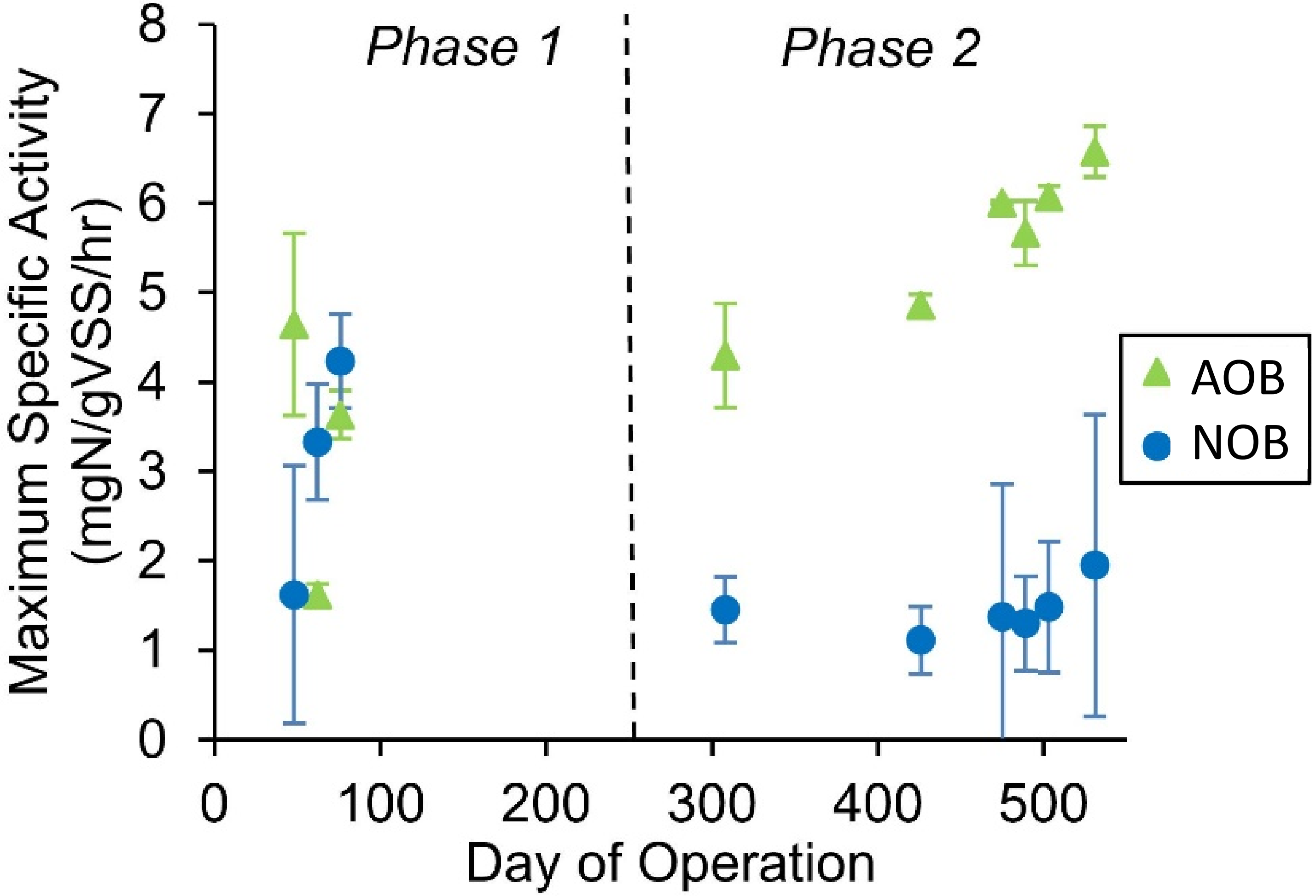
→Key indicator of suppression of NOB activity

Phase 2:

- Total Inorganic Nitrogen (TIN) removal = 68%
- NH_4^+ removal = 87%
- **Ortho-P removal = 91%**
- **NAR= 70%**

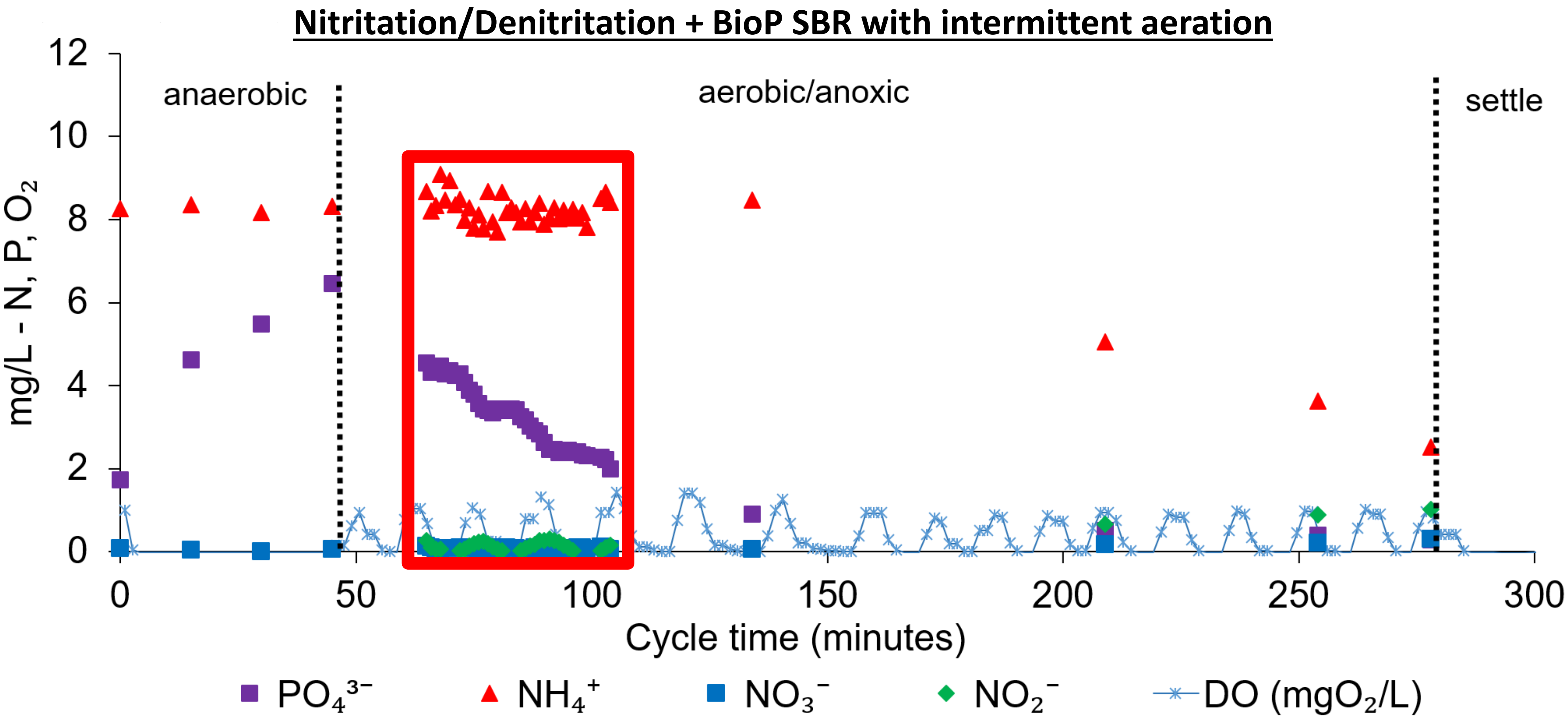
Maximum Activity Assays Confirm NOB Suppression

AOB and NOB Maximum Activity Assays



Roots et al. 2019 *ES:WR&T (In Press)*

NOB Suppression is Also Apparent in Within-Cycle Profiling



Roots et al. 2019 *ES:WR&T (In Press)*

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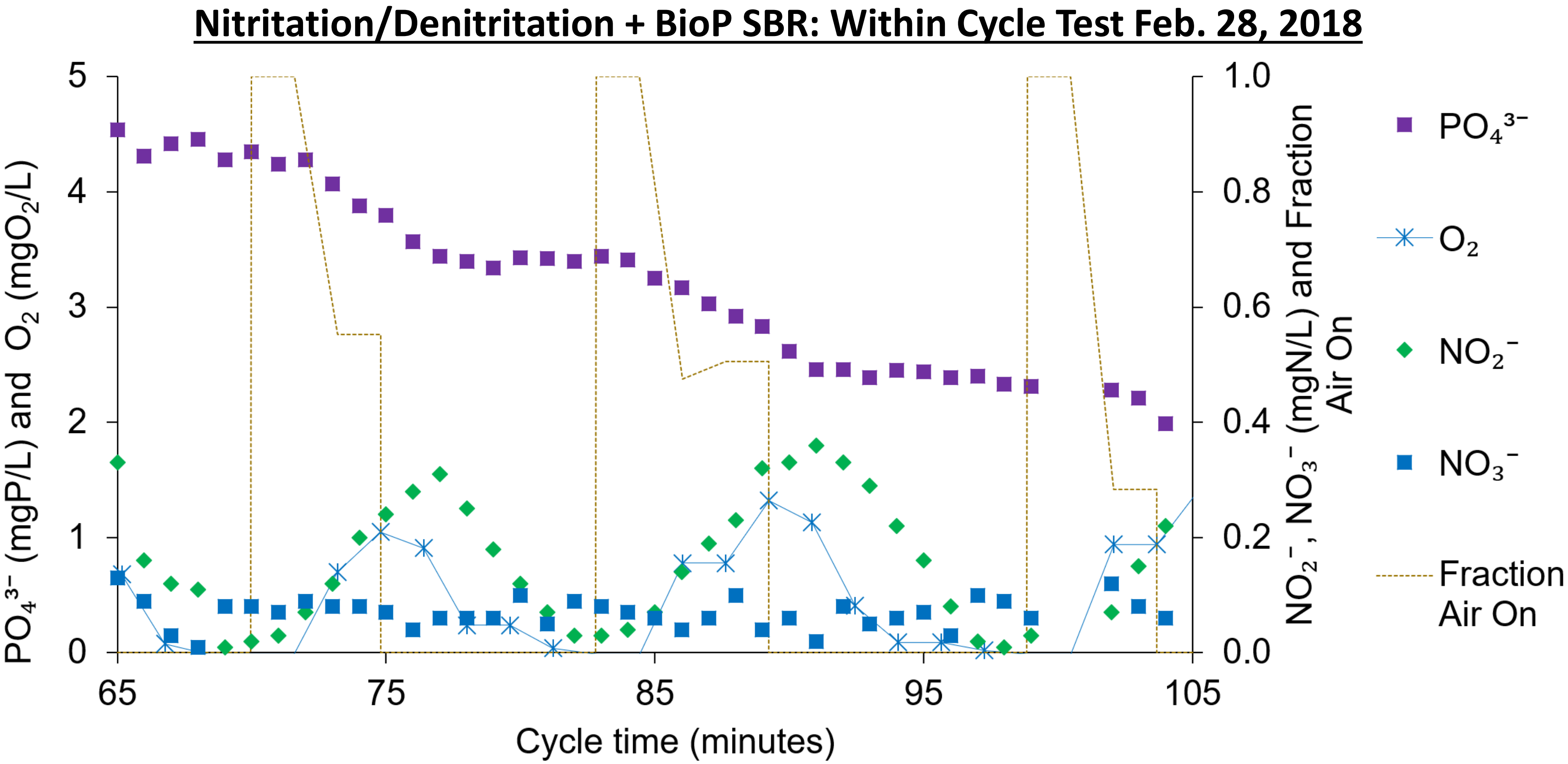
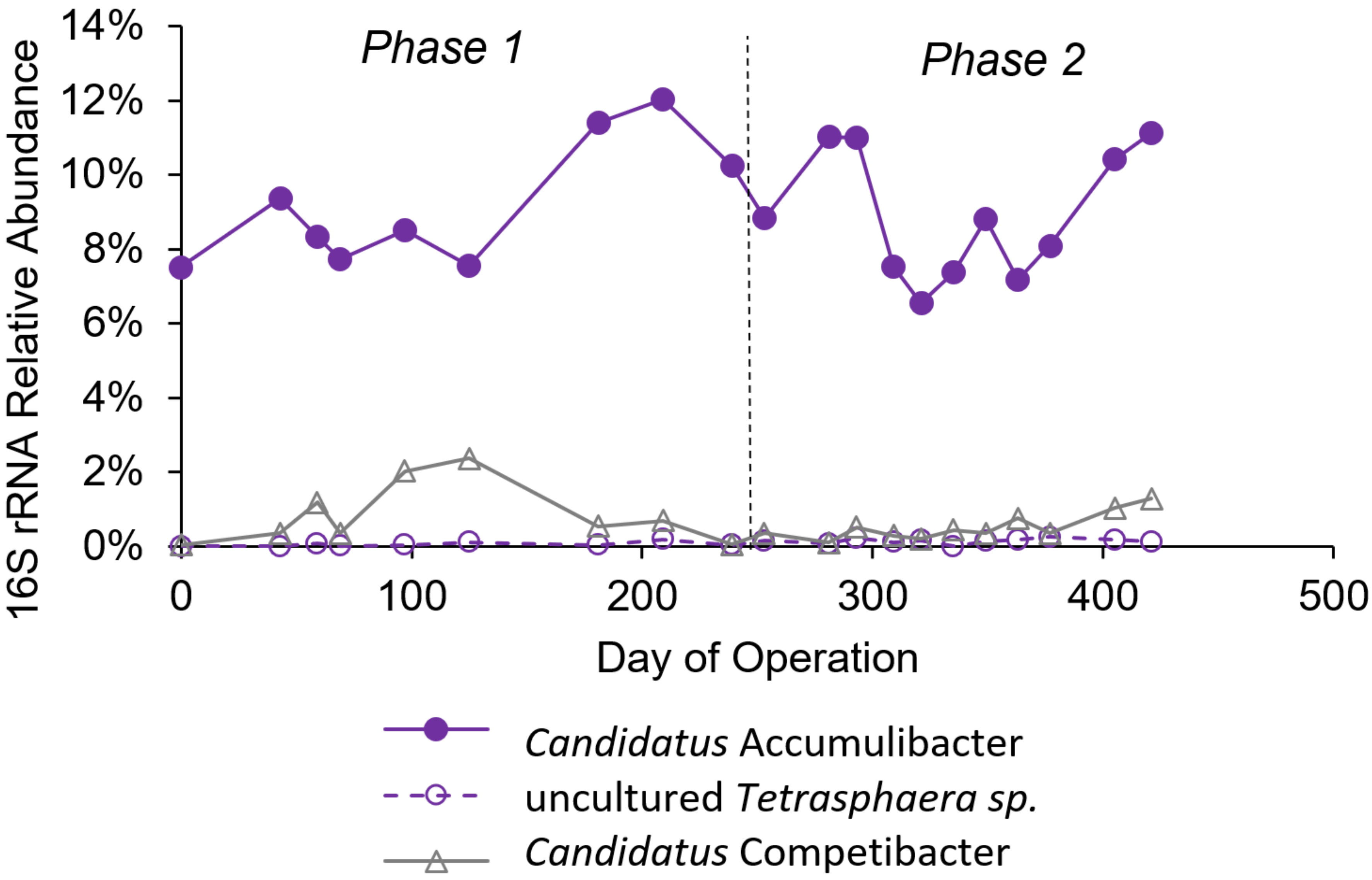


Nitrite, but not nitrate, accumulates during aerated periods, and is consumed during anoxic periods

Roots et al. 2019 *ES:WR&T (In Press)*

Intermittent aeration for NOB suppression is compatible with selection for a robust Accumulibacter PAO population for biological P removal



Strategy 1: Integrated Nitritation/Denitritation + BioP

Lessons Learned:

- Carbon and energy efficient **nitritation/denitritation with biological P removal is feasible and robust** under dynamic mainstream process conditions→ well suited to scale up in existing infrastructure

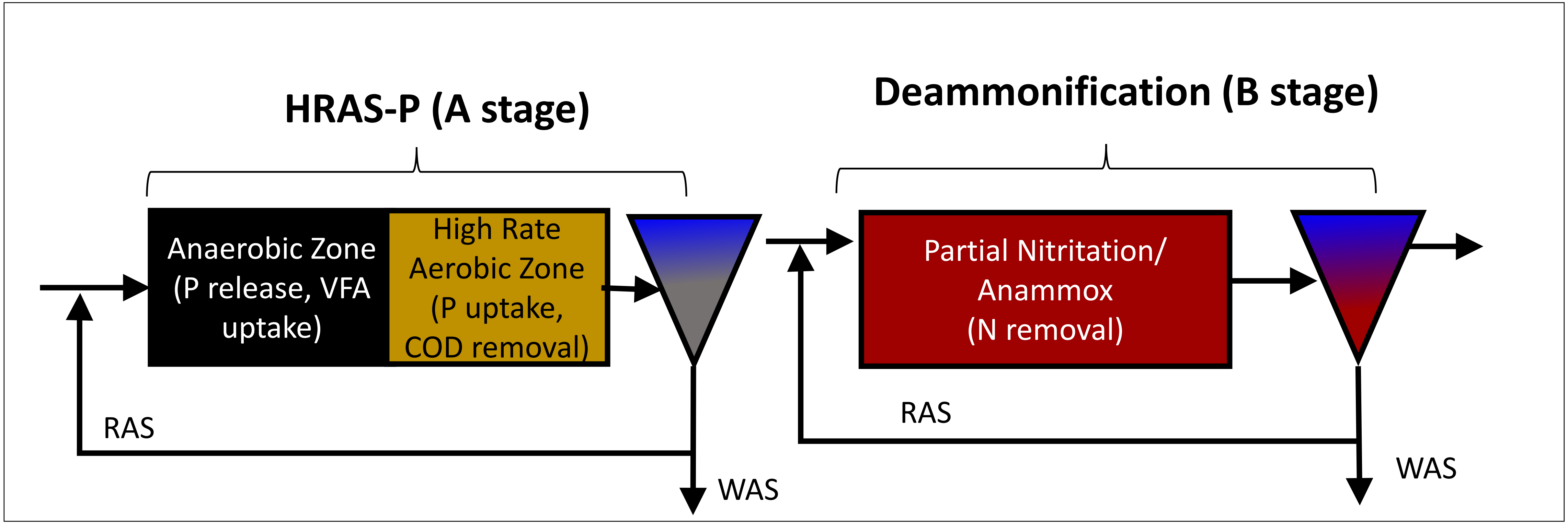
- A simple kinetic strategy based on minimizing substrate availability enables **effective NOB suppression without negatively impacting PAO activity**

Strategy 2: High Rate BioP followed by Deammonification

AB process:

A-Stage: High-Rate Activated Sludge *and* biological P removal (HRAS-P)

B-Stage: Deammonification



Two stage (two sludge) system enables high-rate removal of C and P in stage A (for sidestream resource recovery), and shortcut low energy total N removal in stage B decoupled from carbon management

Strategy 2: High Rate BioP followed by Deammonification

Suite of SBRs operated with primary effluent as feed for >500 days at $20^{o}C$

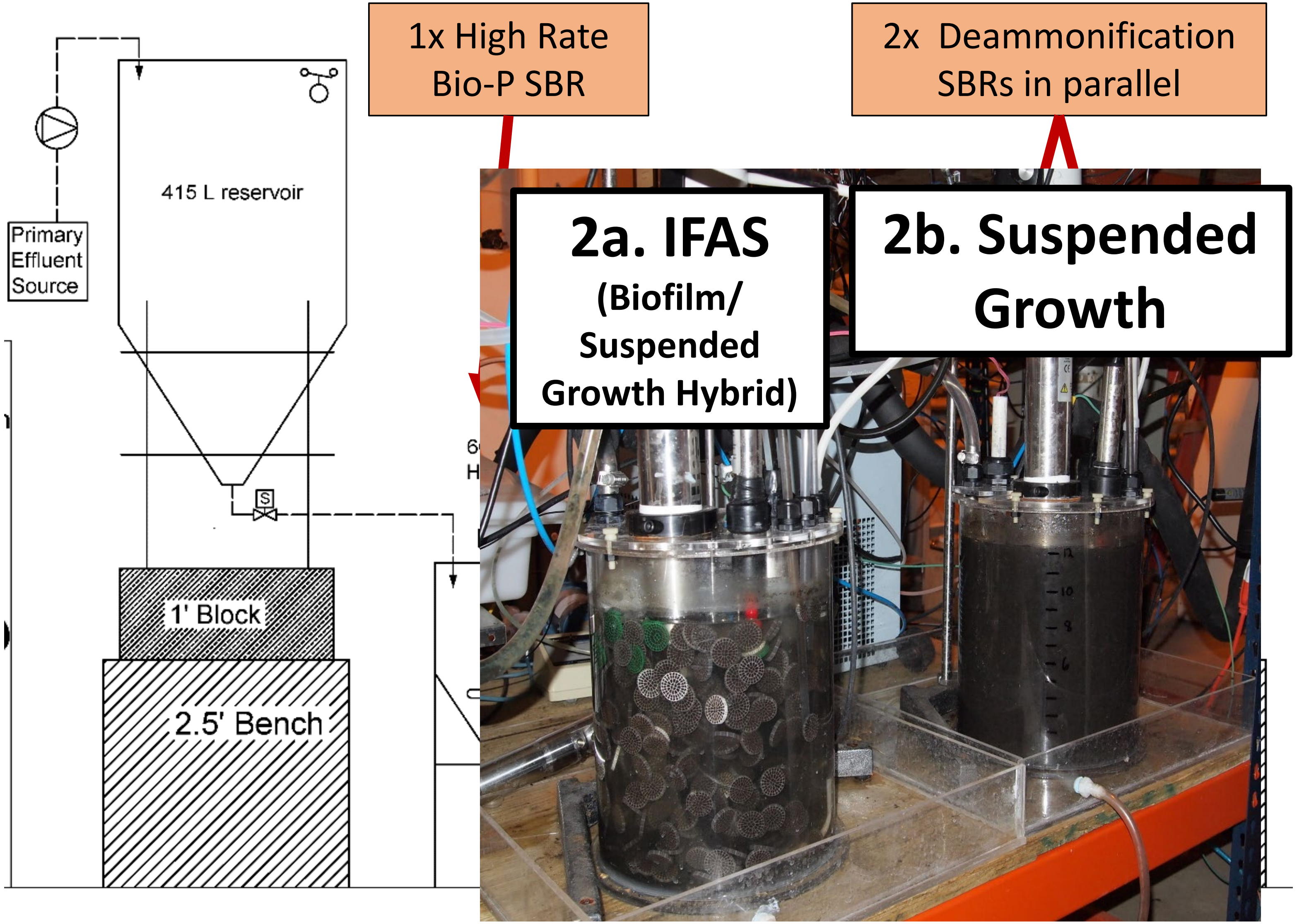
Treatment Objectives:

1) High Rate BioP:

- <1 mg Ortho-P/L
- 70% sCOD removal

2) Deammonification

- N removal via anammox
- Suppress NOB with low DO



1x High Rate Bio-P SBR

2x Deammonification SBRs in parallel

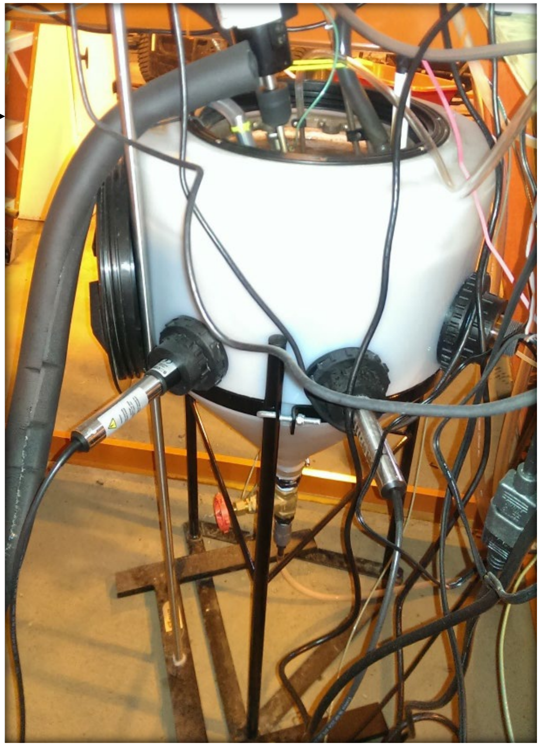
2a. IFAS (Biofilm/ Suspended Growth Hybrid)

2b. Suspended Growth

A-Stage:
High-Rate Activated Sludge with Phosphorus removal (HRAS-P)

Low **CO_2**

Influent:
High **COD**
NH_4^+ & **P**



Effluent:
Low **COD** & **P**
High **NH_4^+**

Waste:
High **COD**
& **P**

SRT: 2.3 days
Aerobic SRT: 1.4 days
HRT: ~4.2 hrs
Ortho-P Removal: 82% (<0.5 mgP/L

Roots et al. (In prep)

Strategy 2: High Rate BioP followed by Deammonification

Suite of SBRs operated with primary effluent as feed for >500 days at 20^{o}C

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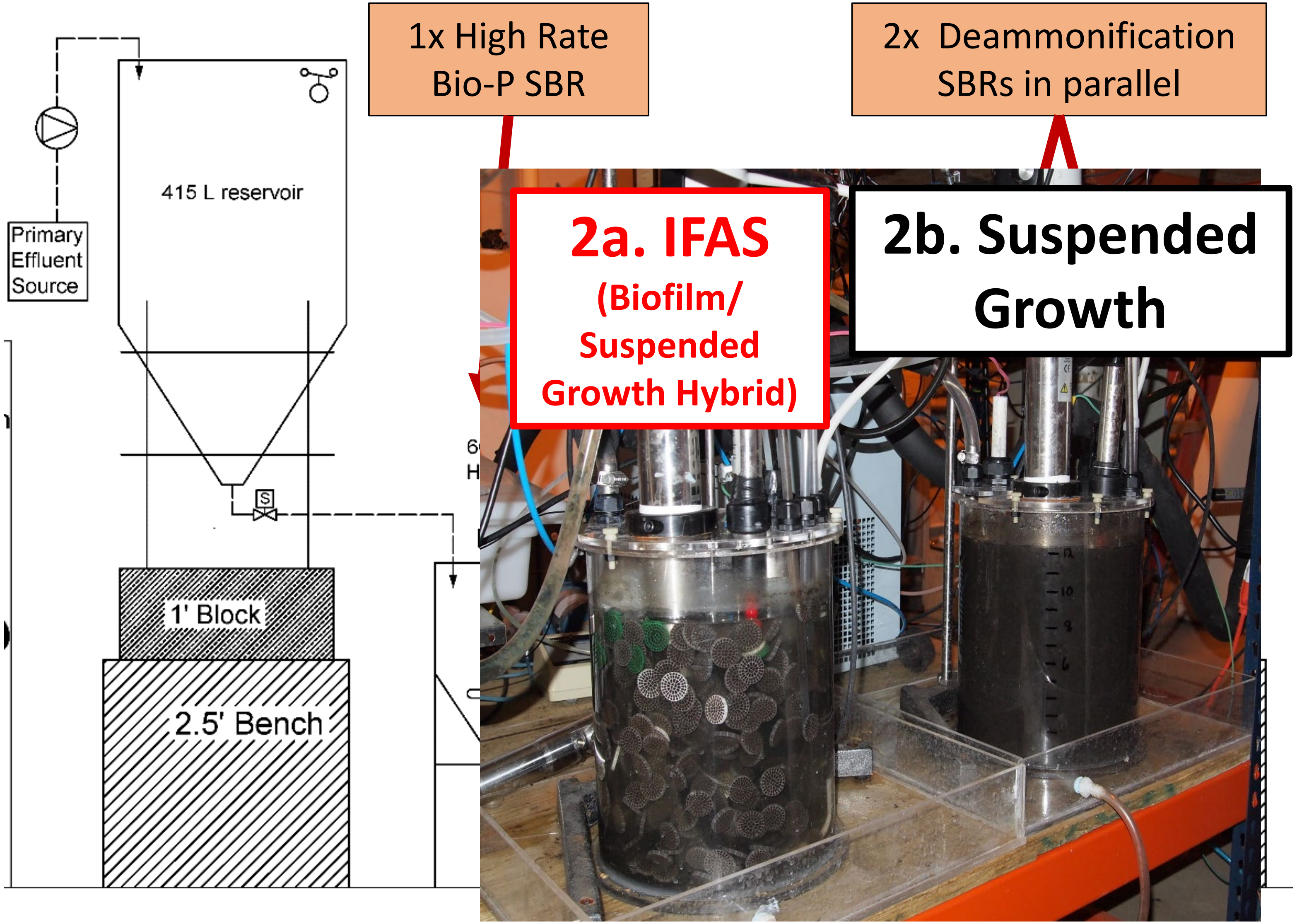

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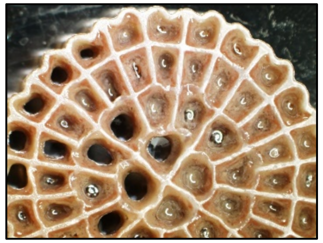
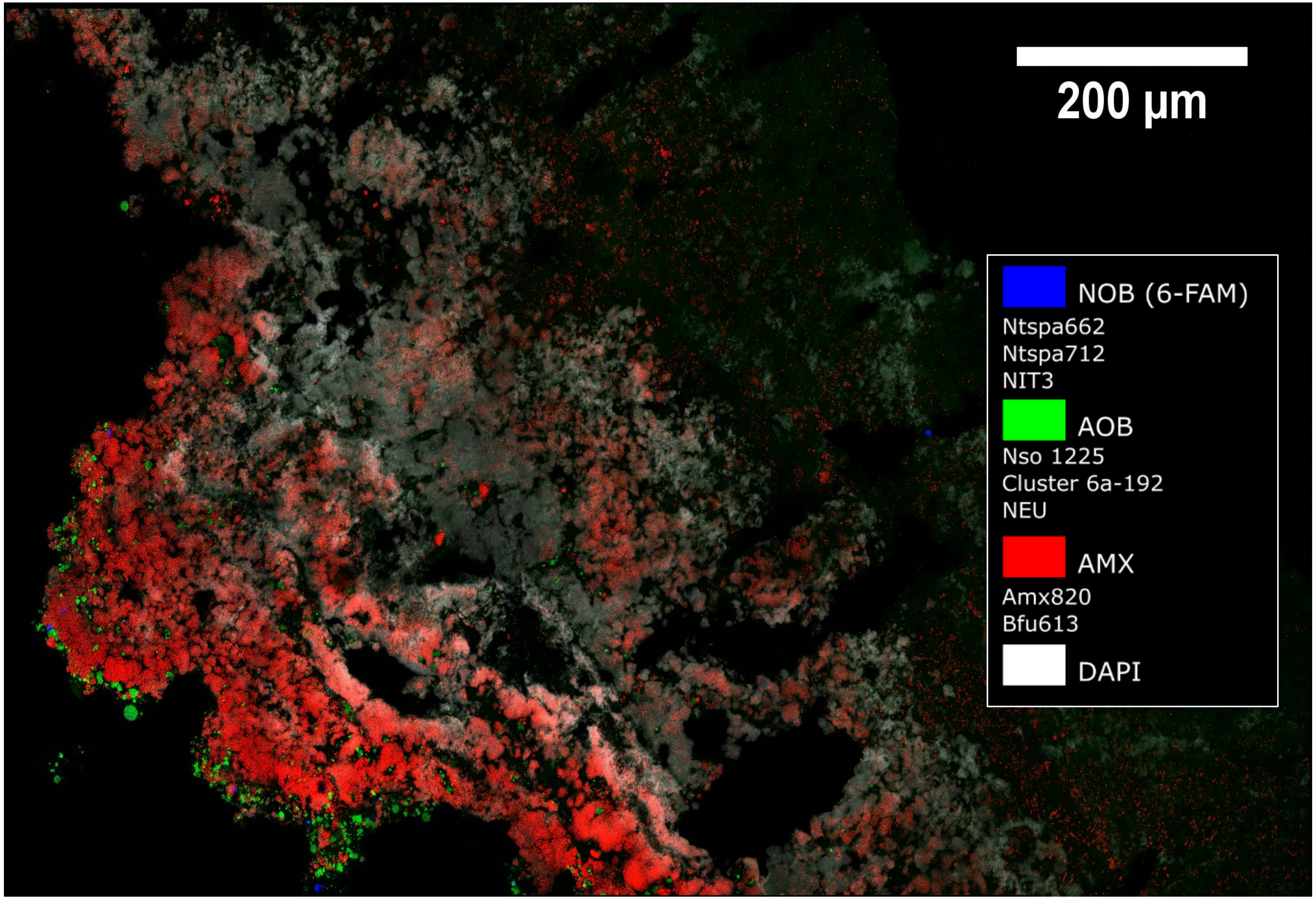


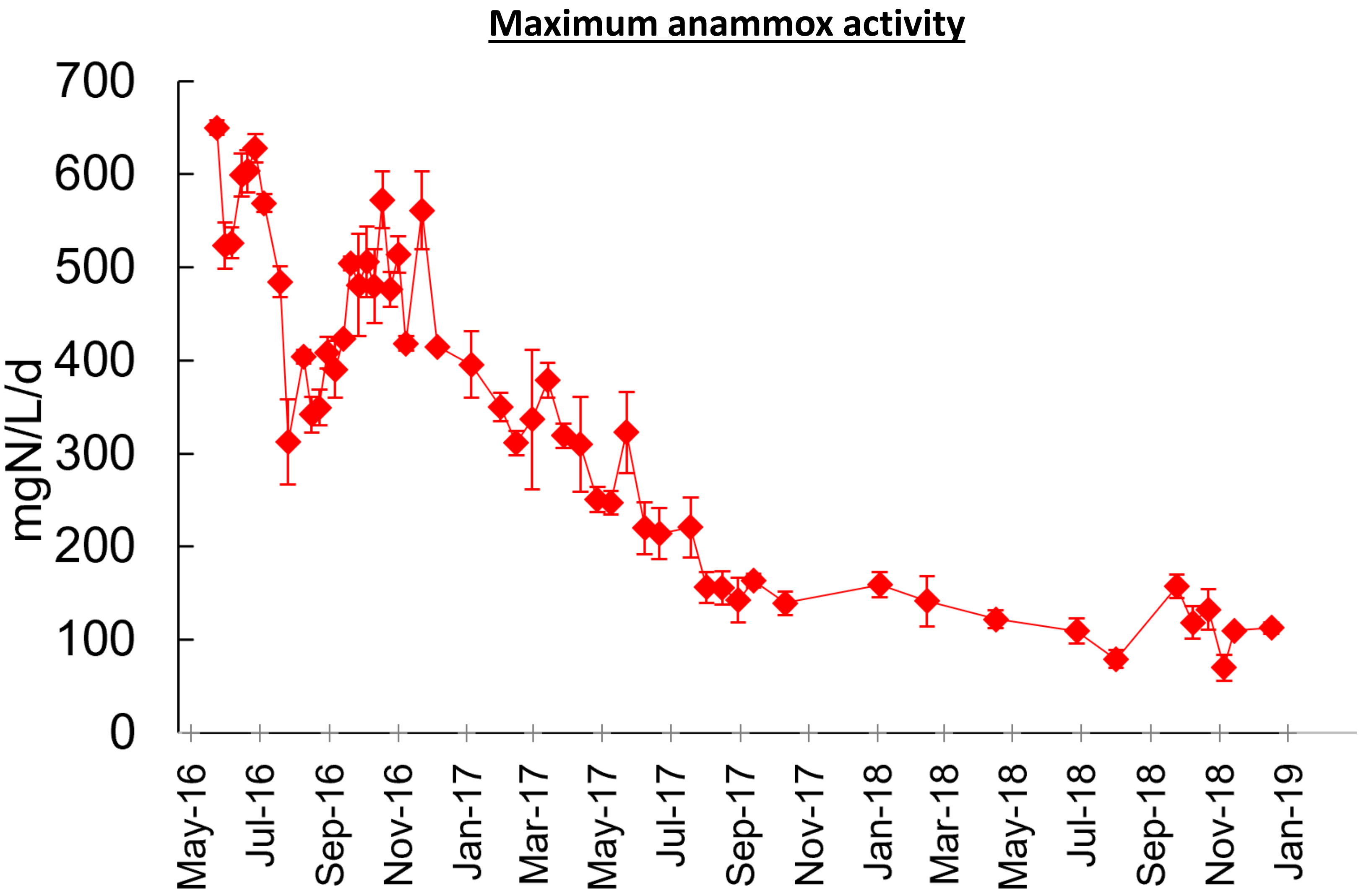
B-Stage: IFAS Deammonification Reactor

Initial Condition

- Sidestream enriched biofilm
  (ANITA Mox K5 biofilm carriers)
- Dense underlying layer of anammox microcolonies
- Selective enrichment of AOB at the bulk liquid interface



IFAS Deammonification Reactor:
Robust Retention of Anammox Biomass and Activity



Observed decline then stabilization of maximum anammox activity

Aug 2017 – Dec 2018

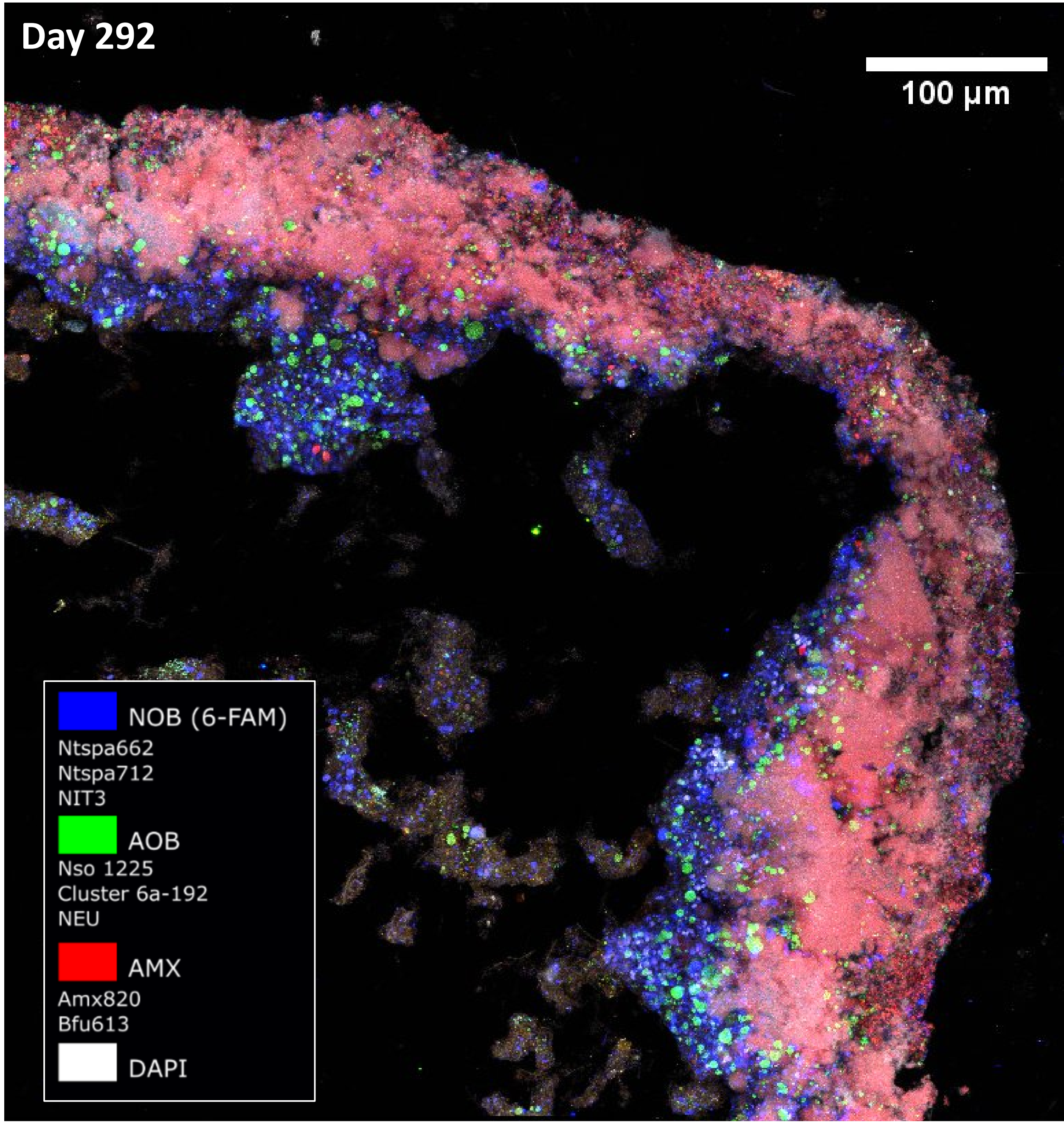
Max anammox activity:
129 ± 28 mg N/L/d

Average N loading:
68 mg TKN/L/d

O'Brien N loading :
≈ 65 mg TKN/L/d

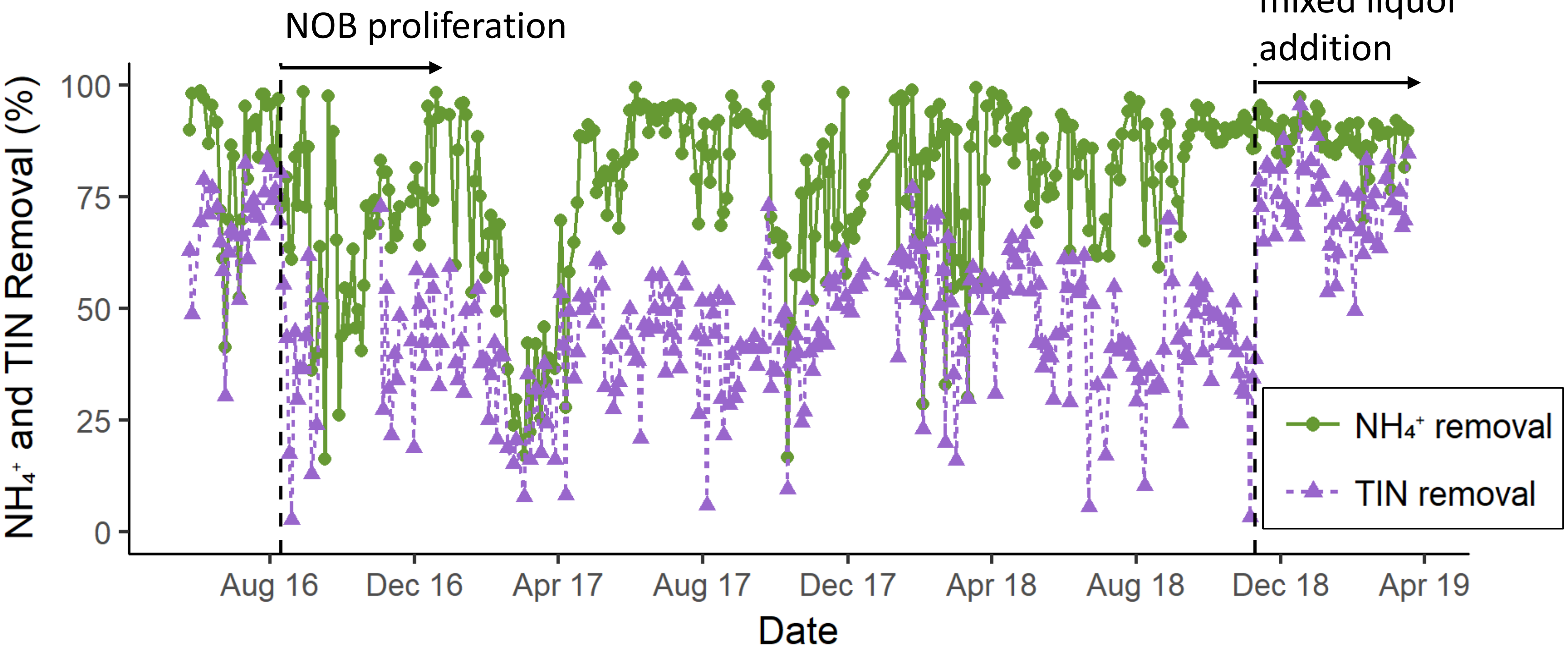
IFAS Deammonification Reactor: NOB proliferation

- Nitrogen removal efficiency declined
- Effluent nitrate increased
- **Accumulation of NOB observed**



From NOB proliferation to suppression

	May ‘16 to Aug ‘16	Aug ‘16 to Nov ‘18
% NH_4^+ removal	84	77
% TIN removal	68	42
NO_3^- : NH_4^+ ratio	0.07	0.43



<u>Nov 9, 2018</u>

Added 10% PE to feed, influent tCOD ↑ **35%**

(45 to 61 mgCOD/L)

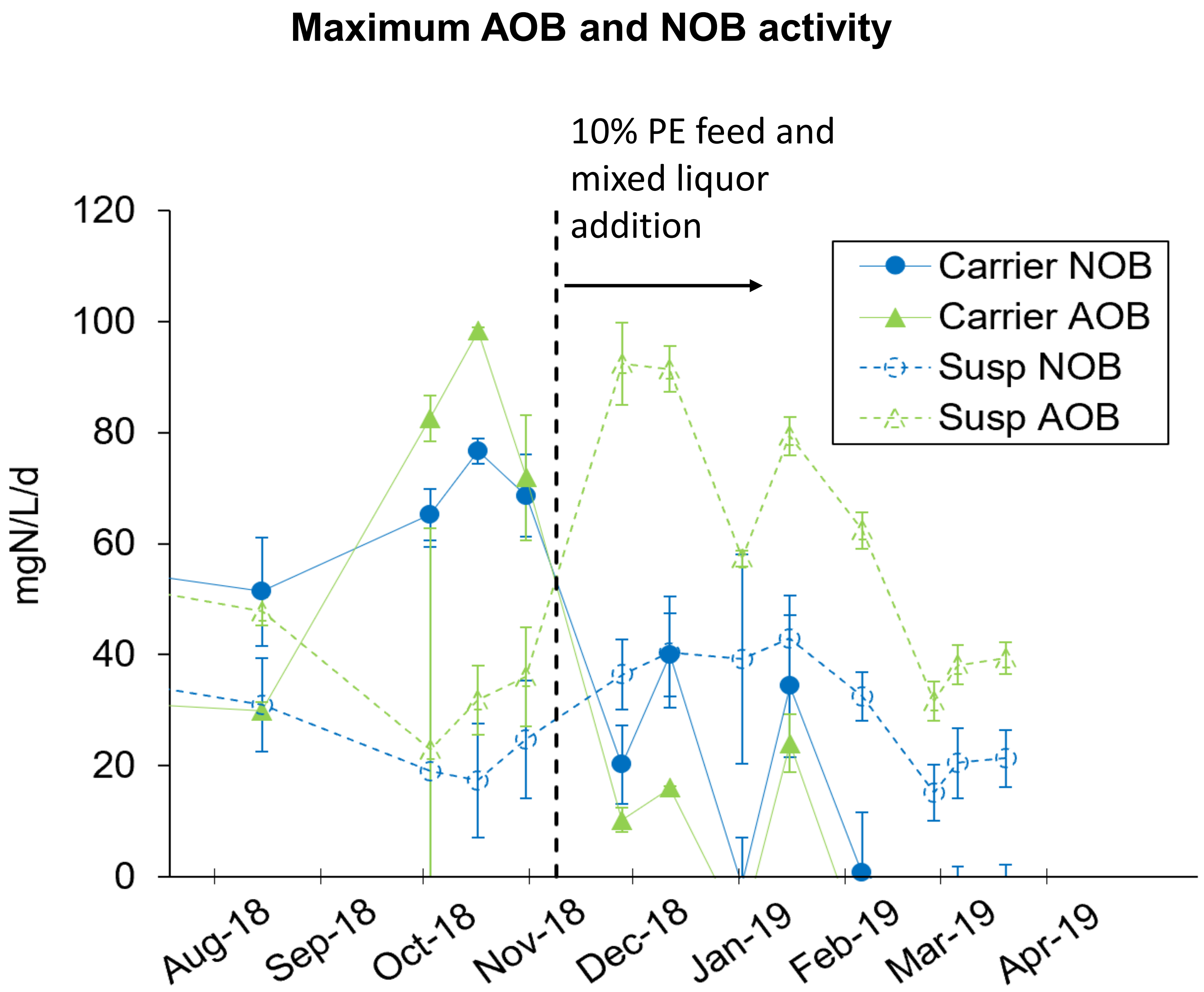
MLVSS ↑ 152%

(250 to 630 mgVSS/L)

<u>Conservative estimate of anammox contribution:</u>

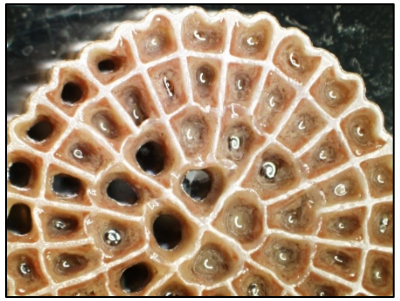
If all tCOD removal went to denitrification, **~50-60% of N removal is from anammox**

Nitrifier shift from carriers to suspension



This is important because NOB are more easily washed out from suspension via SRT than from carriers

Strategy 2: High Rate BioP followed by Deammonification



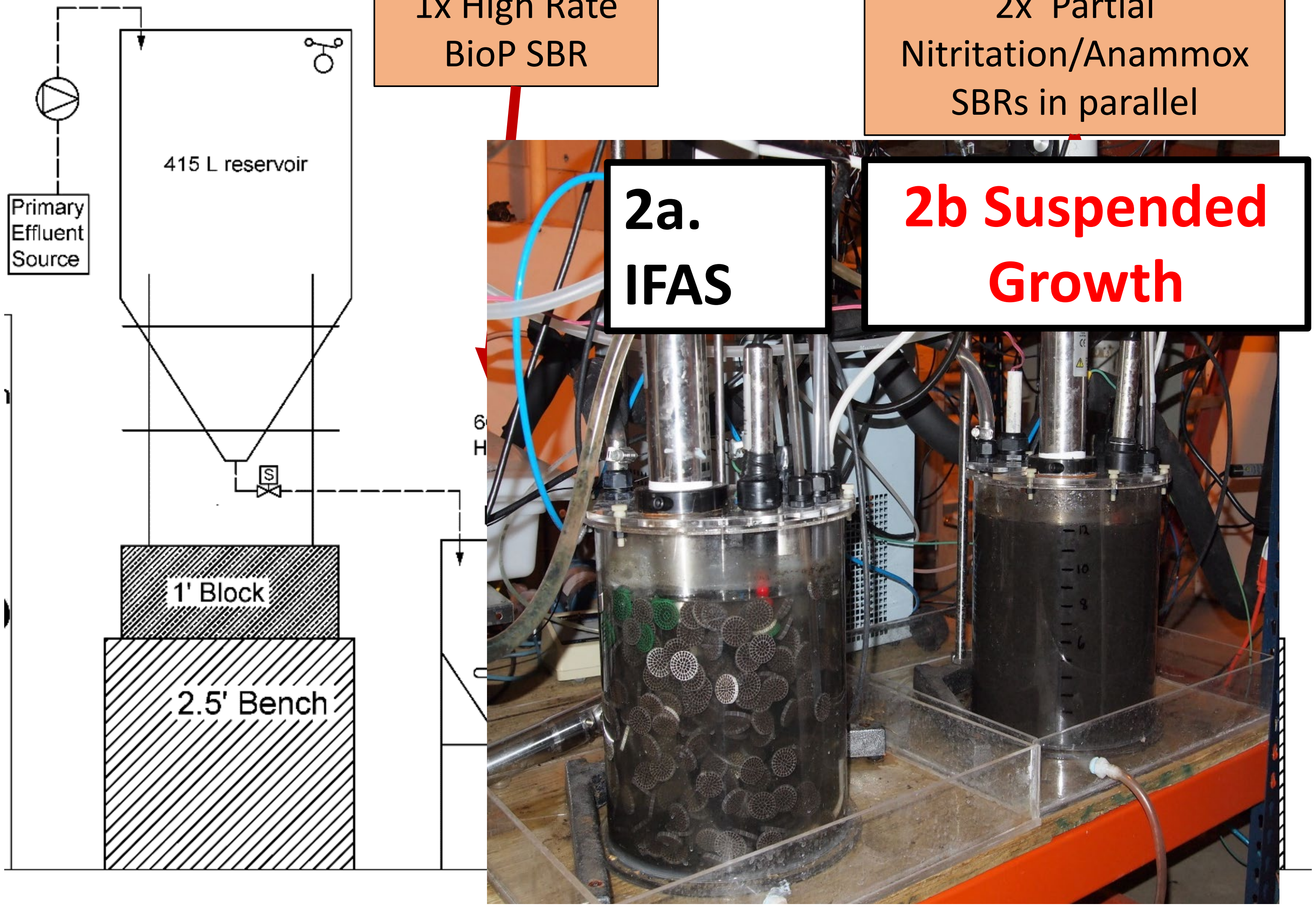
Lessons Learned:

Mainstream deammonification is feasible but depends strongly on **aggregate architecture**

- Performance is greatly aided by hybrid systems with elevated **suspended growth biomass** and small amounts of influent COD
- *Future efforts are warranted to increase anammox contribution to total N removal, and to integrate bioP directly in anammox processes*

Strategy 2: High Rate BioP followed by Deammonification

Is mainstream deammonification feasible in a suspended growth bioprocess?



1x High Rate BioP SBR

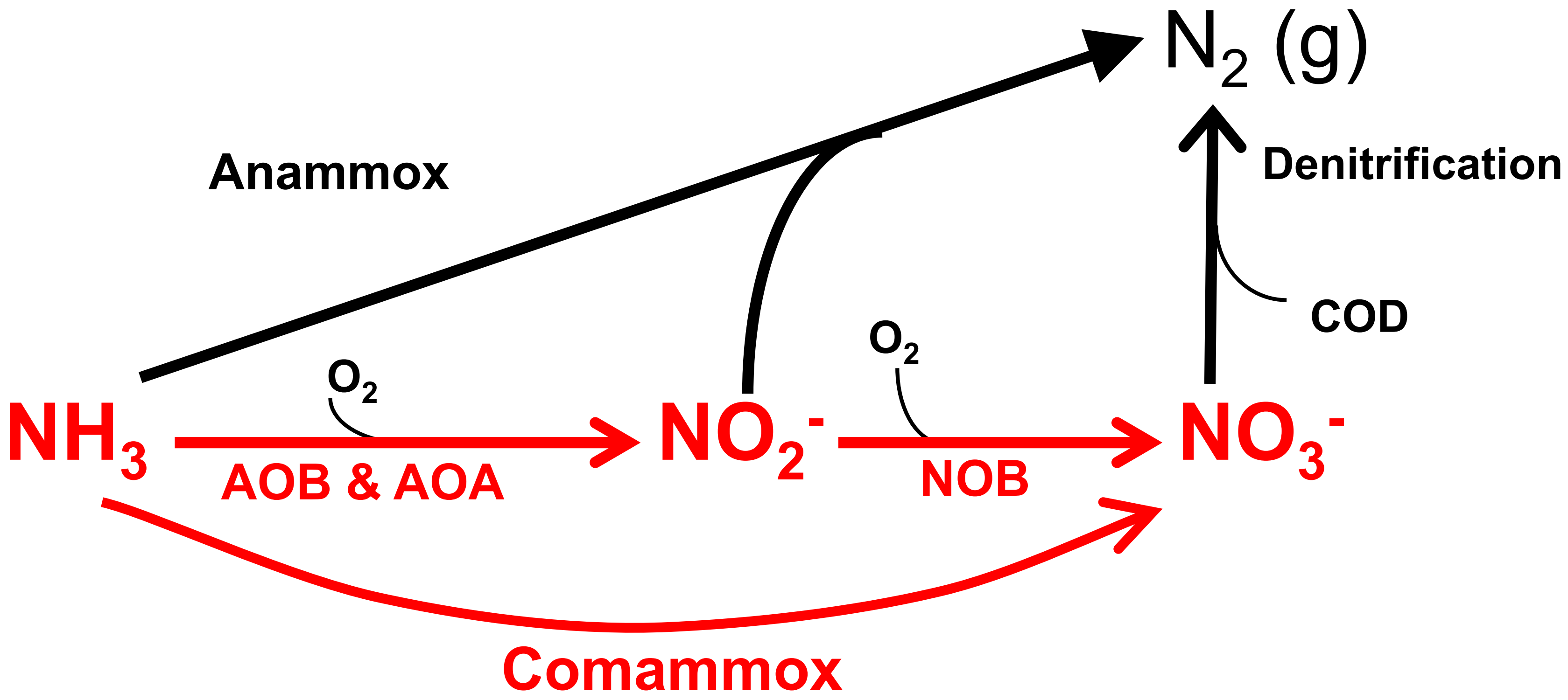
2x Partial Nitritation/Anammox SBRs in parallel

2a. IFAS

2b Suspended Growth

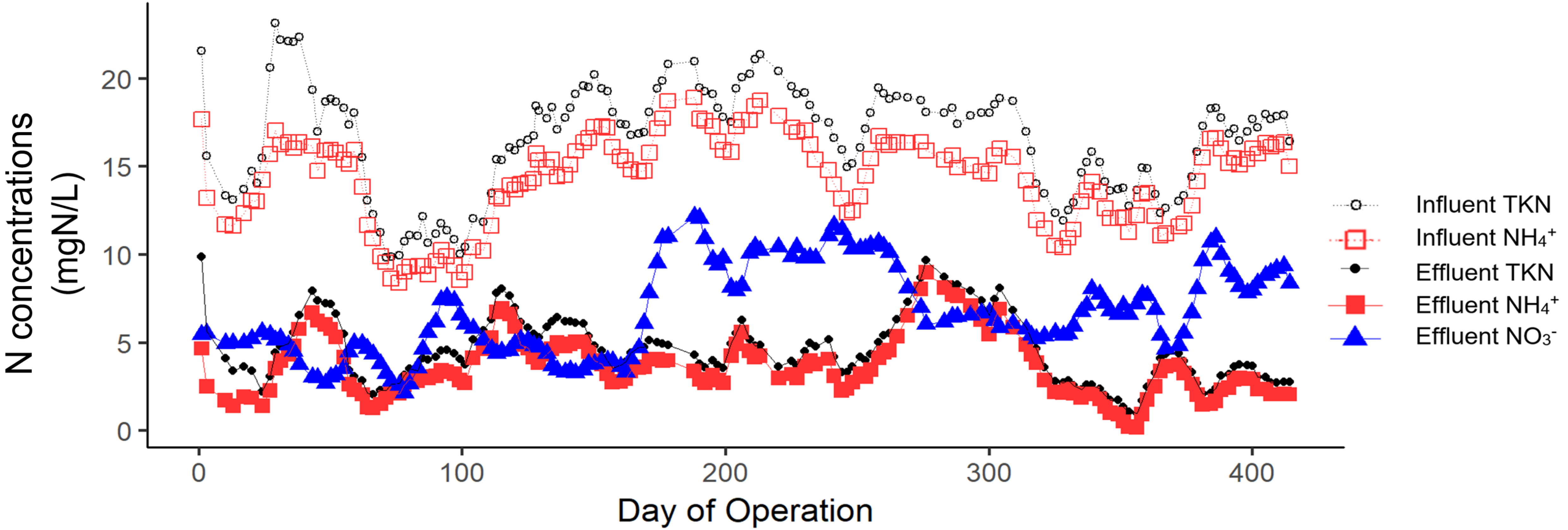
Complete Ammonia Oxidation (Comammox): A new twist in the microbial nitrogen cycle

Nitrification has been viewed as a 2 step process for >100 years:



AOB: Ammonia-Oxidizing Bacteria (*Nitrosomonas, Nitrosospira)*
AOA: Ammonia-Oxidizing Archaea
NOB: Nitrite-Oxidizing Bacteria *(Nitrospira, Nitrobacter, Nitrolancea, Nitrotoga)*
Comammox: Complete Ammonia Oxidizing Bacteria (*Nitrospira)*

The Suspended Growth SBR was initially operated for deammonification, but transitioned to low DO full nitrification after ~90 days

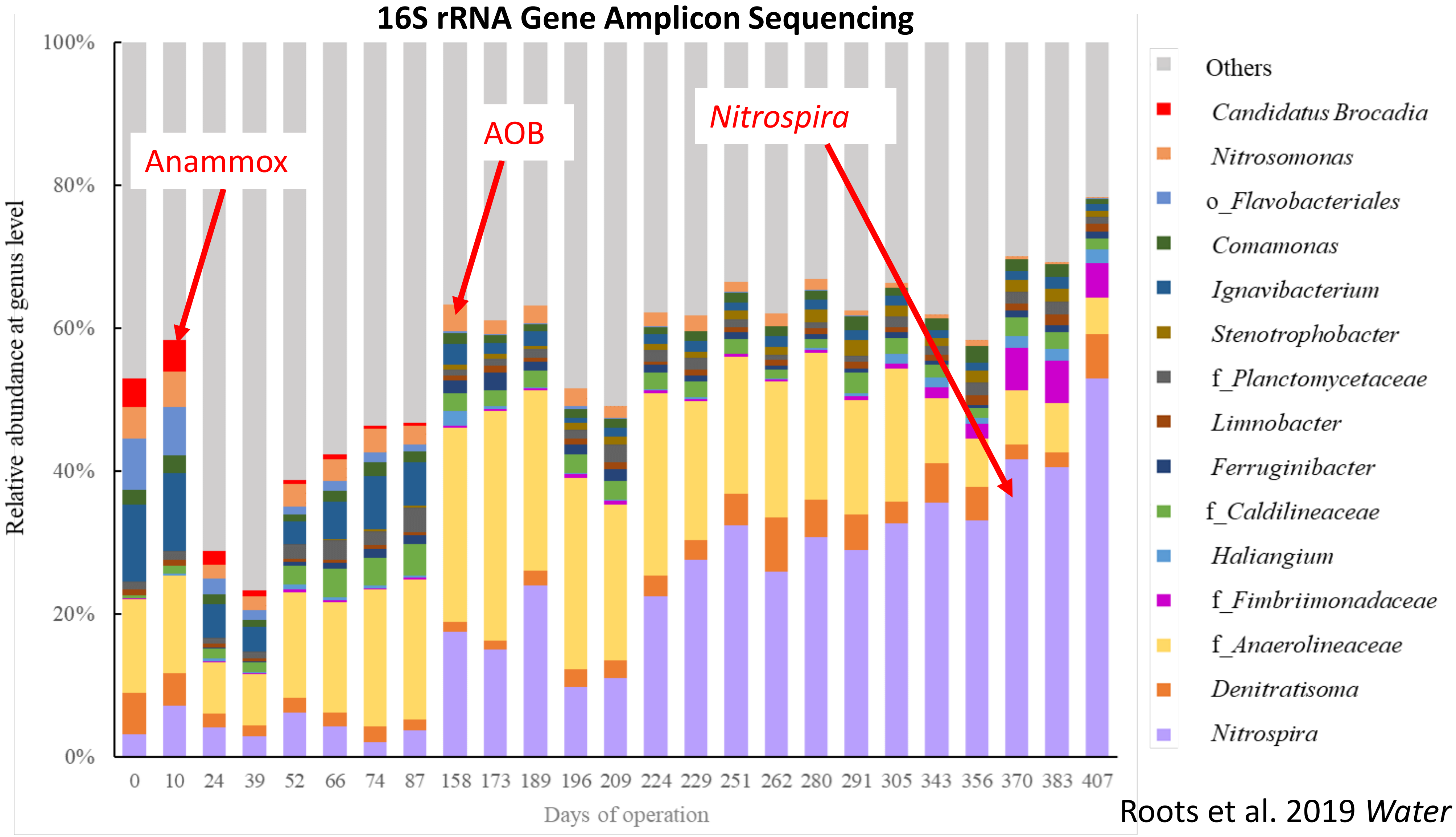


50-60% TIN removal, $\Delta NO_x/\Delta NH_4^+=0.24$

~20% TIN removal, $\Delta NO_x/\Delta NH_4^+=0.6-0.7$

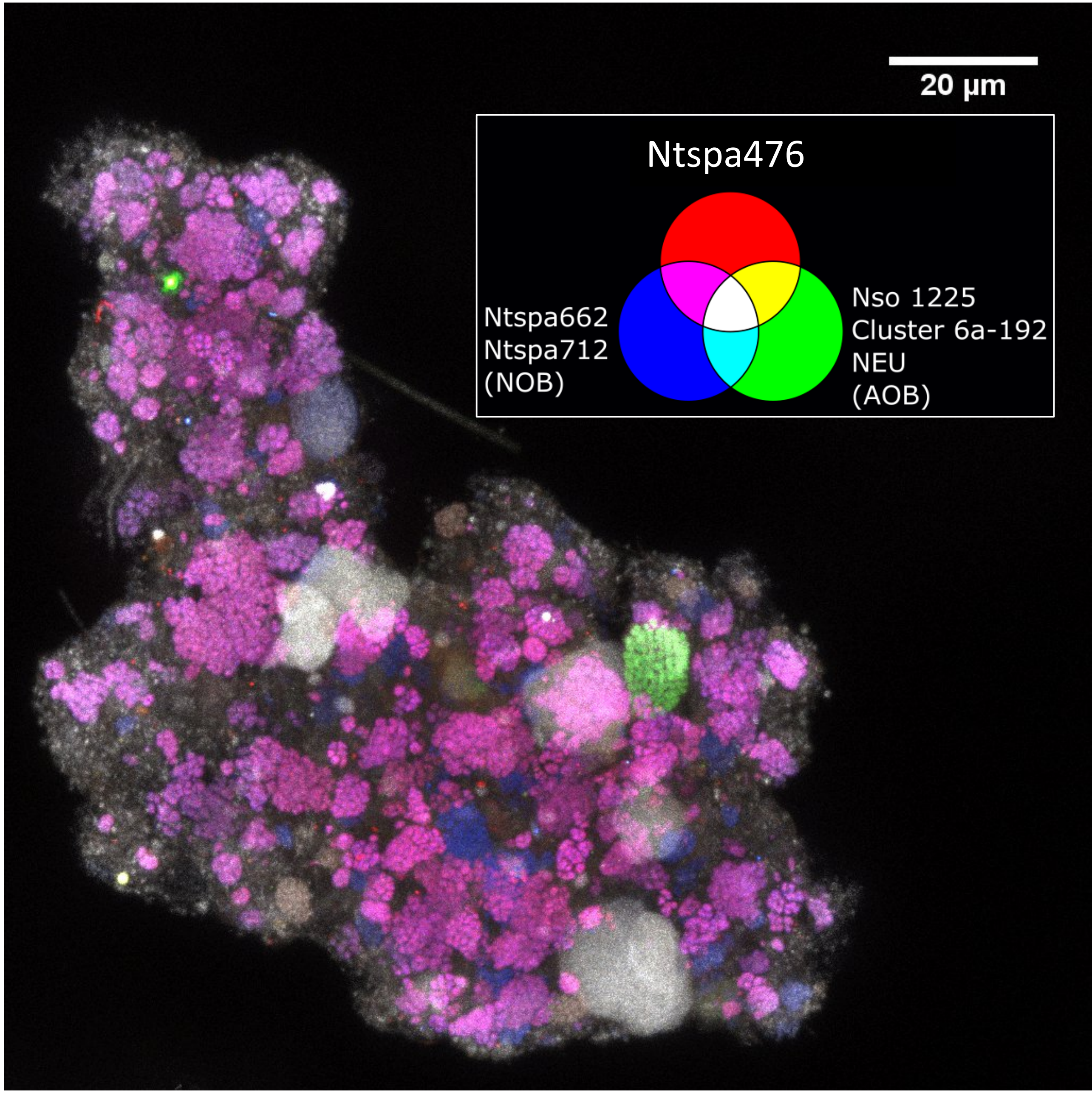
Roots et al. 2019 *Water Research*

Nitrospira increased in abundance to 53% of the overall microbial community



Roots et al. 2019 *Water Research*

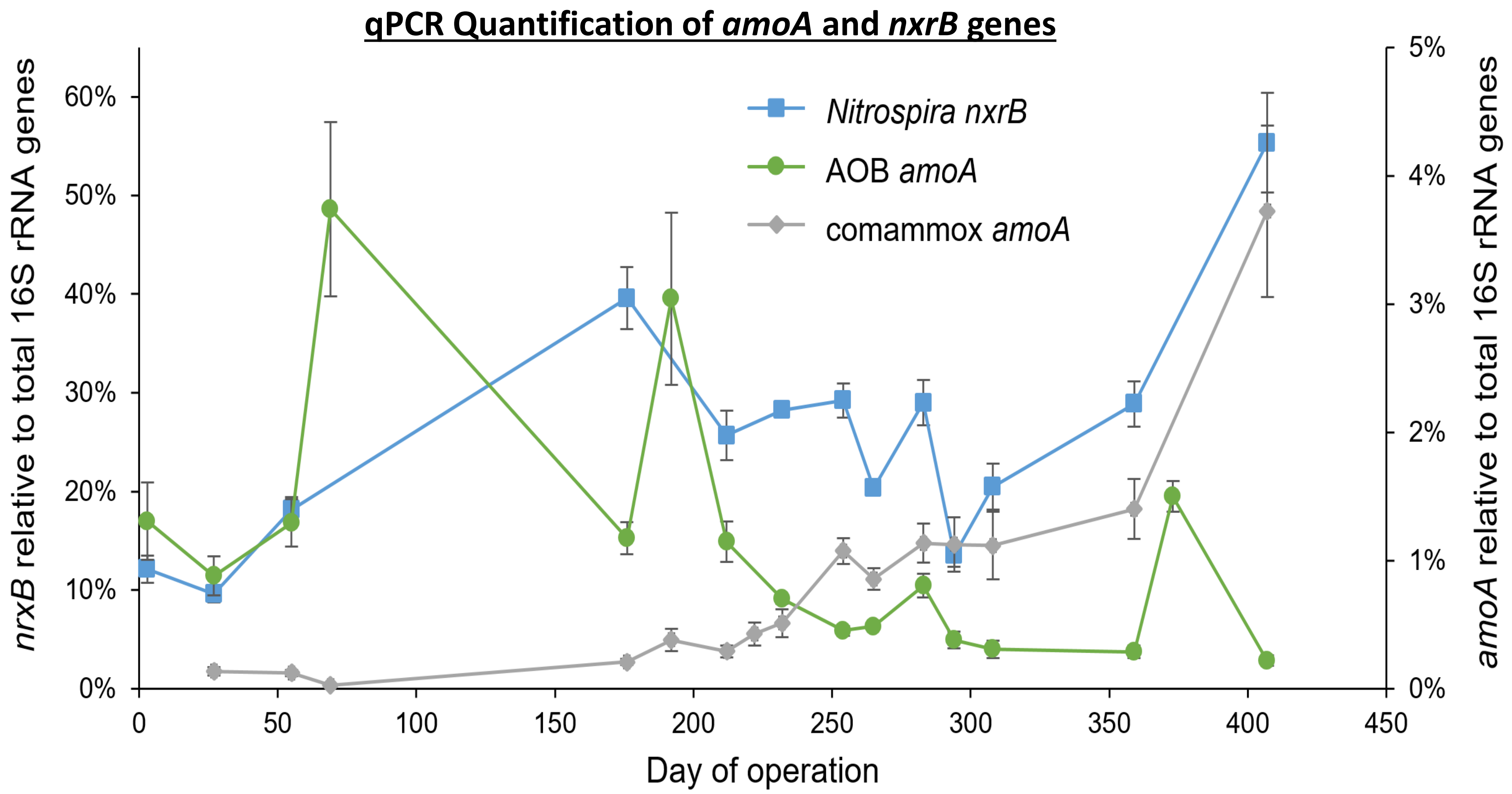
Strong enrichment of *Nitrospira* and decline in abundance of AOB was confirmed by FISH



Probe Ntspa476 (magenta) targets a subset of lineage II *Nitrospira* that includes comammox

Roots et al. 2019 *Water Research*

Comammox dominates the ammonia-oxidizing community in this low DO nitrification reactor



By day 407, comammox accounted for **94% of *amoA* gene copies** in the reactor

Roots et al. 2019 *Water Research*

Ammonia Removal Rate Comparison to Parallel Full-Scale Nitrifying Activated Sludge Bioreactor

	Low DO Nitrification Reactor	**O’Brien Water Reclamation Plant (Full-scale)**
DO (mg/L)	**0.2-1**	3-5*
Average NH_4+ Removal Rate (mg NH_4^+/L-d)	**59**	50

Low DO nitrification with comammox could be an energy-saving alternative to conventional high DO nitrification systems

* *End of basin. Effluent NH_4^+ concentrations differ between O’Brien and the low DO nitrification reactor.*

Take Home Points

Integrated mainstream shortcut N and biological P removal is a promising emerging route for resource and energy efficient nutrient management

- Both **Nitritation/ Denitritation + bioP and Deammonification are robust and stable** in the mainstream under appropriate operational conditions
- **Comammox *Nitrospira* may be well-suited to energy-efficient low DO processes** for complete nitrification

Acknowledgements

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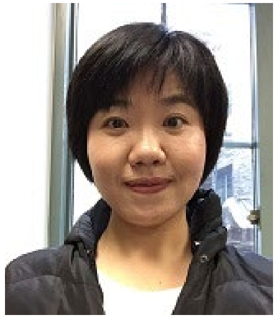
Microbial Ecology

Environmental Biotechnology


Paul Roots

Alex Rosenthal

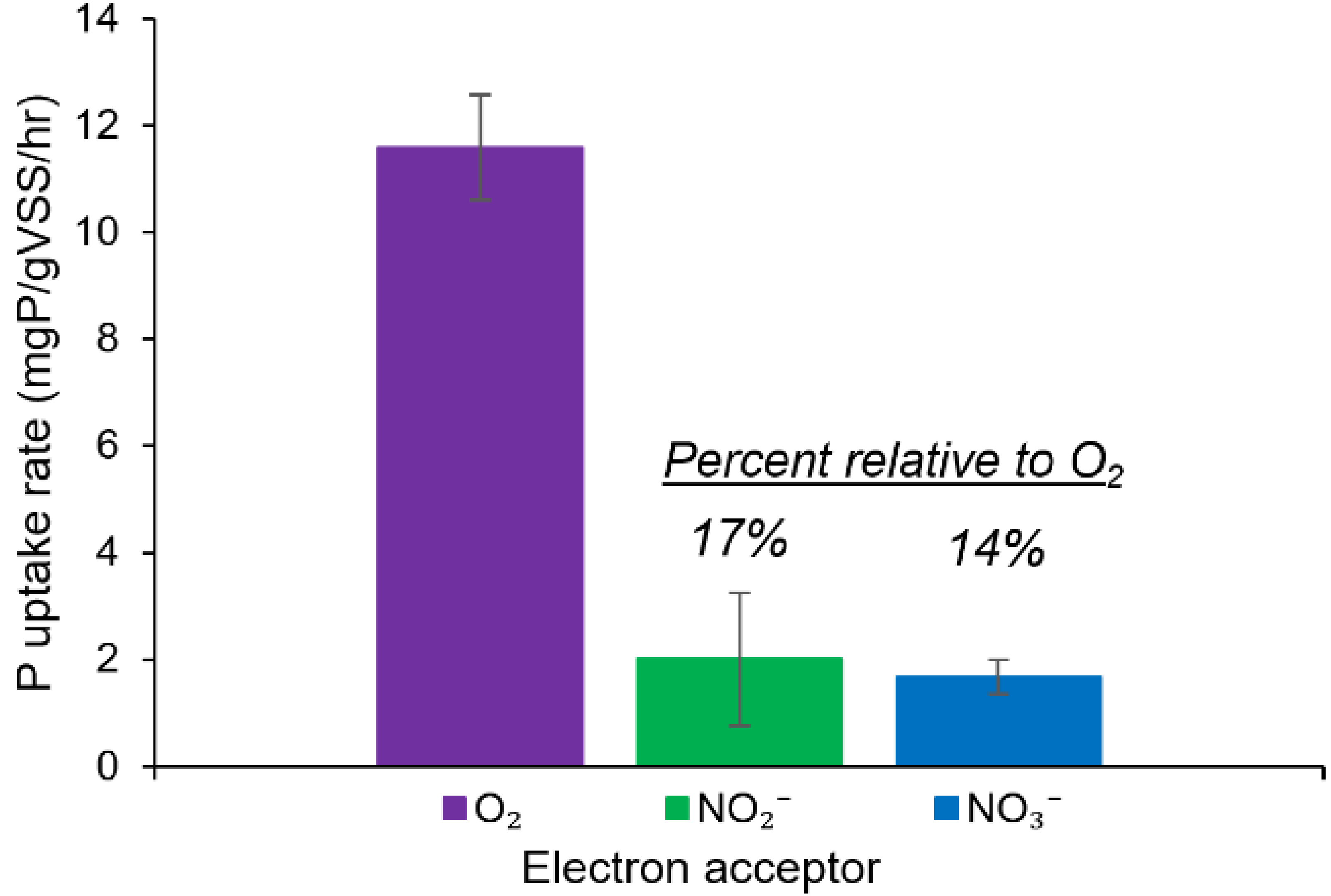

Yubo Wang


Fab Sabba

MWRDGC

District SCBNR Project Team: Monitoring & Research, Maintenance & Operations, and Engineering Departments

However, P removal was driven by aerobic PAOs, rather than C-efficient DPAOs



Take Away Points and Future Directions

Mainstream shortcut N removal has extraordinary promise, but is in its infancy, with key remaining challenges to be addressed

1. **Mainstream NOB outselection** is feasible, but remains a considerable challenge
2. Reliability at **low temperatures** (10-15°C) is uncertain
3. Operational conditions often used in shortcut N removal processes may inadvertently select for **comammox** and associated complete nitrification

- Conversely, **comammox *Nitrospira* may be well-suited to energy-efficient low DO processes** for complete nitrification

Summary and Discussion Points

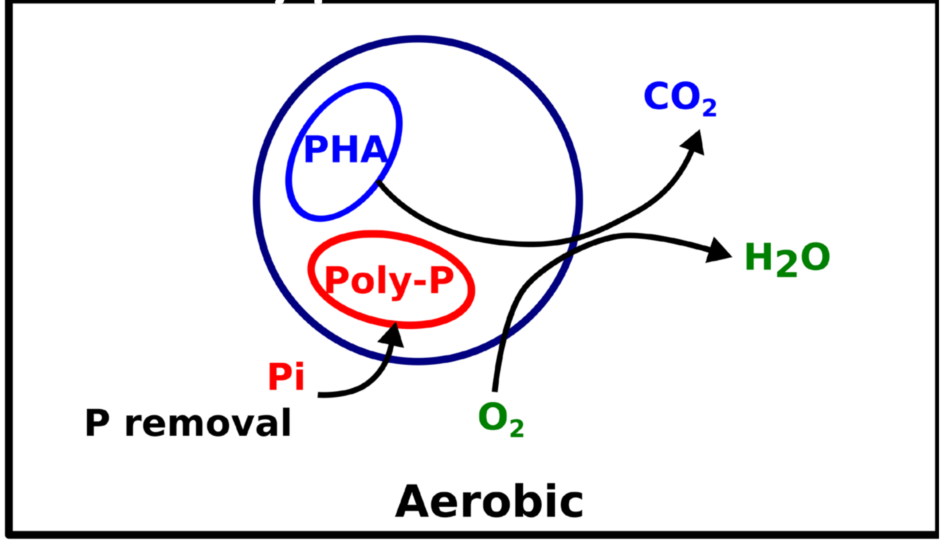
Robust P and shortcut N removal via two different EBPR processes without supplemental carbon:

- NiDeMA-P:
  - Robust P removal with intermittent aeration to 1 mg O_2/L
  - Compatible with N removal via nitritation-denitritation
  - P removal transiently impacted by heavy wet-weather flows
- HRAS-P:
  - Excellent P and sCOD removal at a 2.3 day SRT
  - RAS fermenter (via higher SRT) stabilized P removal during wet-weather flows
  - Compatible with downstream N removal via deammonification
- P and COD removal performance was maintained in both processes down to ~10 ºC

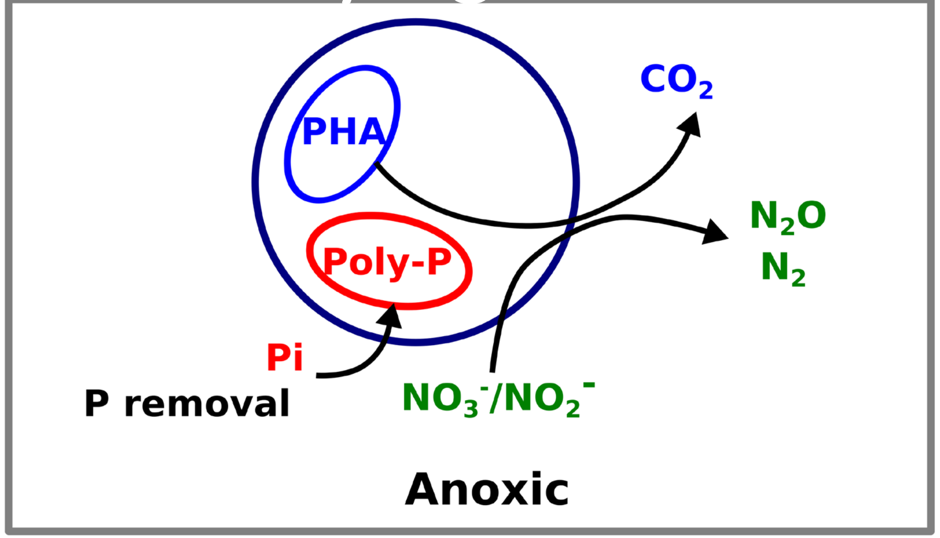
The Promise of Denitrifying PAOs

*Some (but not all) Accumulibacter clades are capable of **denitrification**. These so-called **DPAOs** are little understood, but offer a critical opportunity to couple N removal to P accumulation and recovery.*

Typical PAOs



Denitrifying PAOs



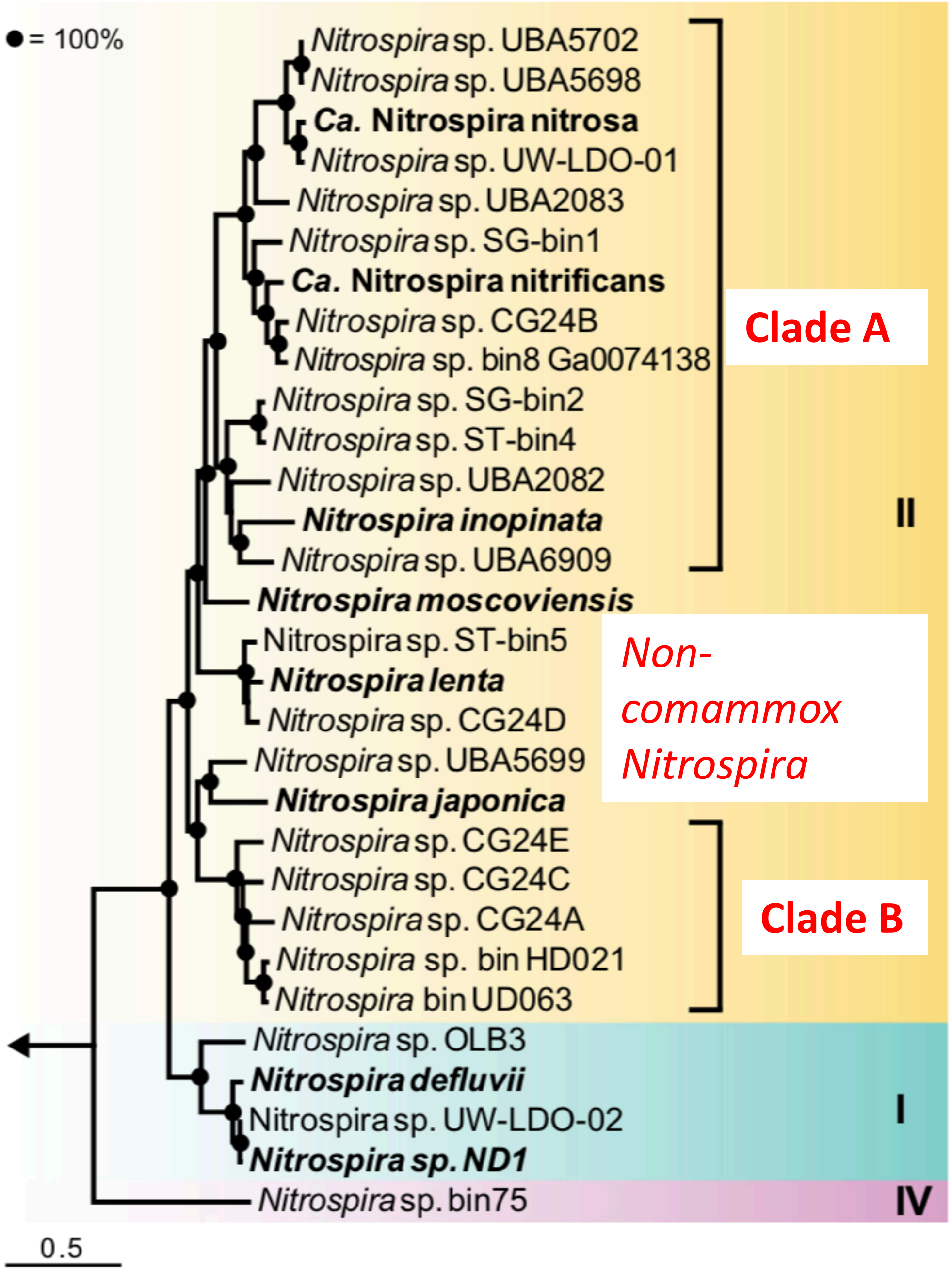
Advantages of DPAO-based Bioprocesses relative to Aerobic PAOs:

1. Potential for dramatic energy savings due to decreased aeration requirements
2. Optimized use of organic matter for combined N and P removal

Key research question: Can we exploit new knowledge of **metabolic versatility of PAOs** to link N removal, P recovery, & bioenergy production?

Comammox Diversity, Putative Niche, and Relevance to Practice

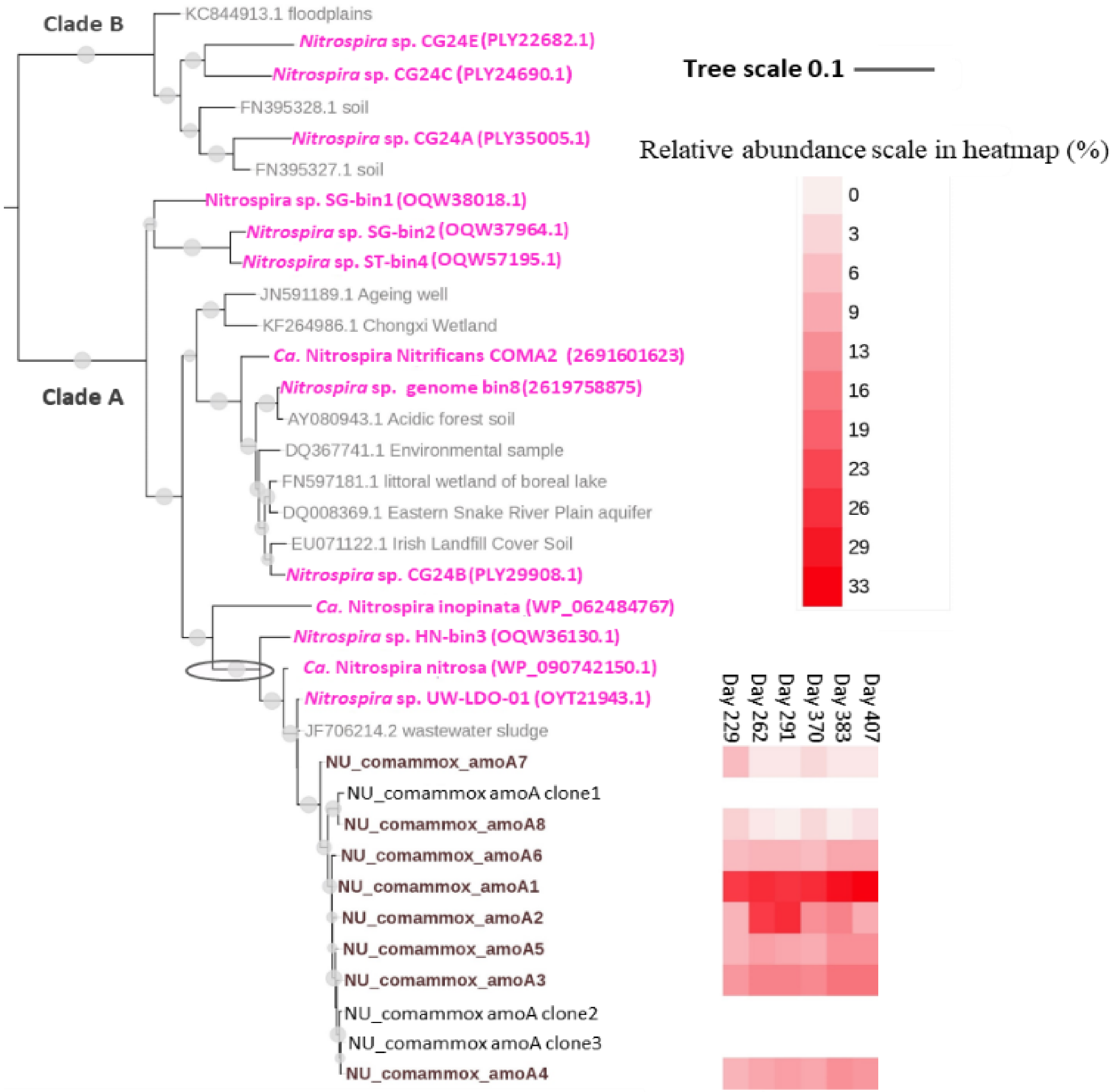
- Comammox form two distinct clades within the genus *Nitrospira* [1]
- Comammox *Nitrospira* appear to be adapted to an oligotrophic lifestyle with low NH_4^+, and possibly also low dissolved oxygen [2]
- **Early reports suggested that comammox are absent or present at low abundance in nitrifying activated sludge, and thus may not be important from a functional standpoint [3,4]**



Koch et al. 2018 *AMAB* (doi.org/10.1007/s00253-018-9486-3)

1. Daims et al. 2015 *Nature* 428: 504-509
2. Kits et al. 2017 *Nature* 549: 269-272.
3. Gonzalez-Martinez et al. 2016 *Environ. Sci. Pollut. Res.* 23:25501-25511
4. Annavajhala et al. 2018 *ES&T Letters* 5(2): 110-116

Comammox affiliated with *Ca* 'Nitrospira nitrosa'

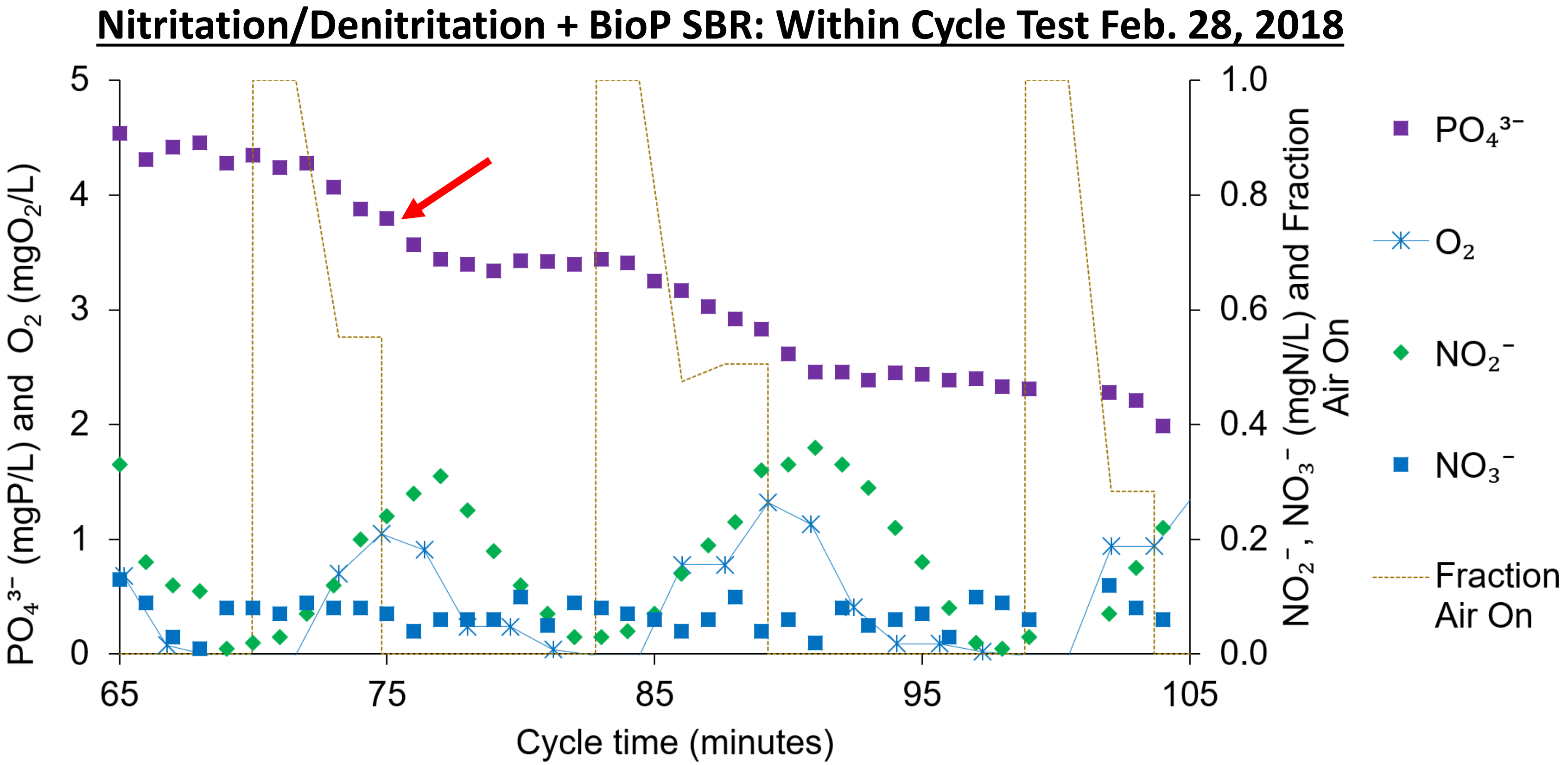


Comammox *amoA* Phylogenetic Tree

Results from *amoA* gene amplicon sequencing coupled to genome resolved shotgun metagenomics

Roots et al. 2019 *Water Research*

NOO Suppression is Also Apparent in Within-Cycle Profiling….
but P uptake is limited to aerated periods



In the absence of O_2 and presence of NO_2^-, P uptake is not observed → P uptake by **Denitrifying Polyphosphate Accumulating Organisms (DPAOs)** is surprising limited

Roots et al. 2019 (*In review*)

Strategy 1: Integrated Nitritation/Denitritation + BioP

Future Direction: How can **Denitrifying Polyphosphate Accumulating Organisms (DPAOs)** be effectively selected for in integrated shortcut N and P removal processes?

- DPAOs offer the opportunity to decrease aeration (energy) requirements and optimize use of organic matter for combined N and P removal
- Conditions and process configurations that select for high DPAO activity (in conventional or shortcut BNR) aren’t well understood

Advantages of Shortcut N Removal Bioprocesses[a]

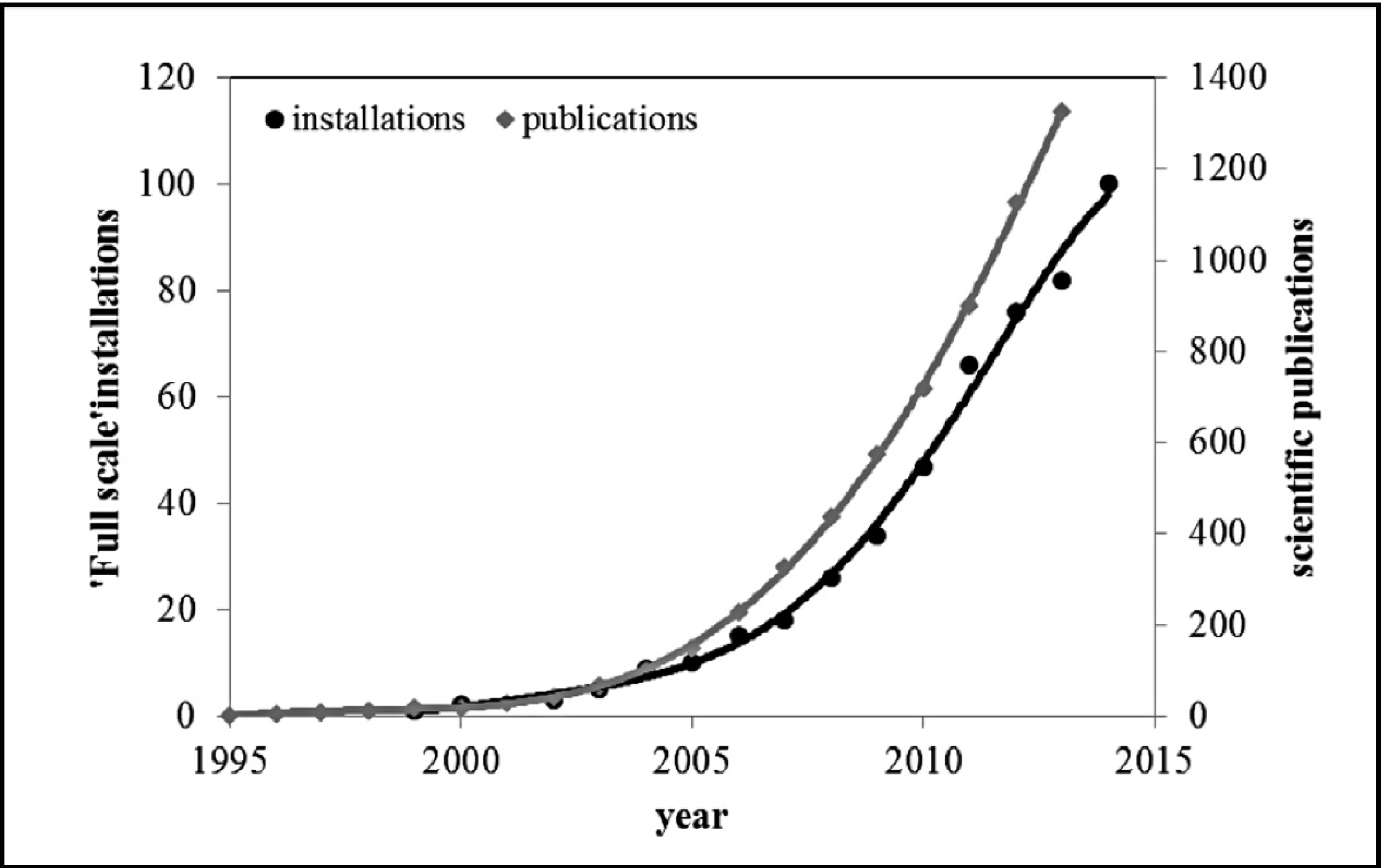
	Nitrification/ Denitrification	Nitritation/ Denitritation	Partial Nitritation/ Anammox
O_2 (mole)	1.8	1.3	0.7
Reducing Equivalents from Organics (e^-)	9	5.5	1
Biomass Produced (g VSS)[b]	28	18	7

[a] Per mol ammonia. Calculations based on reported biomass yield and typical SRT for each unit operation (Rittmann & McCarty, 2001).

[b] Value includes biomass produced from ammonia oxidation and NO_x reduction

Source: Gao, Scherson & Wells (2014) *Environ. Sci: Processes Impacts* 16:1223-1246

While challenges remain to be addressed, particularly regarding process stability, sidestream deammonification is a rapidly maturing technology



Source: Lackner et al. 2014. *Water Research*, 55 (2014) 292-303.

OUTLINE

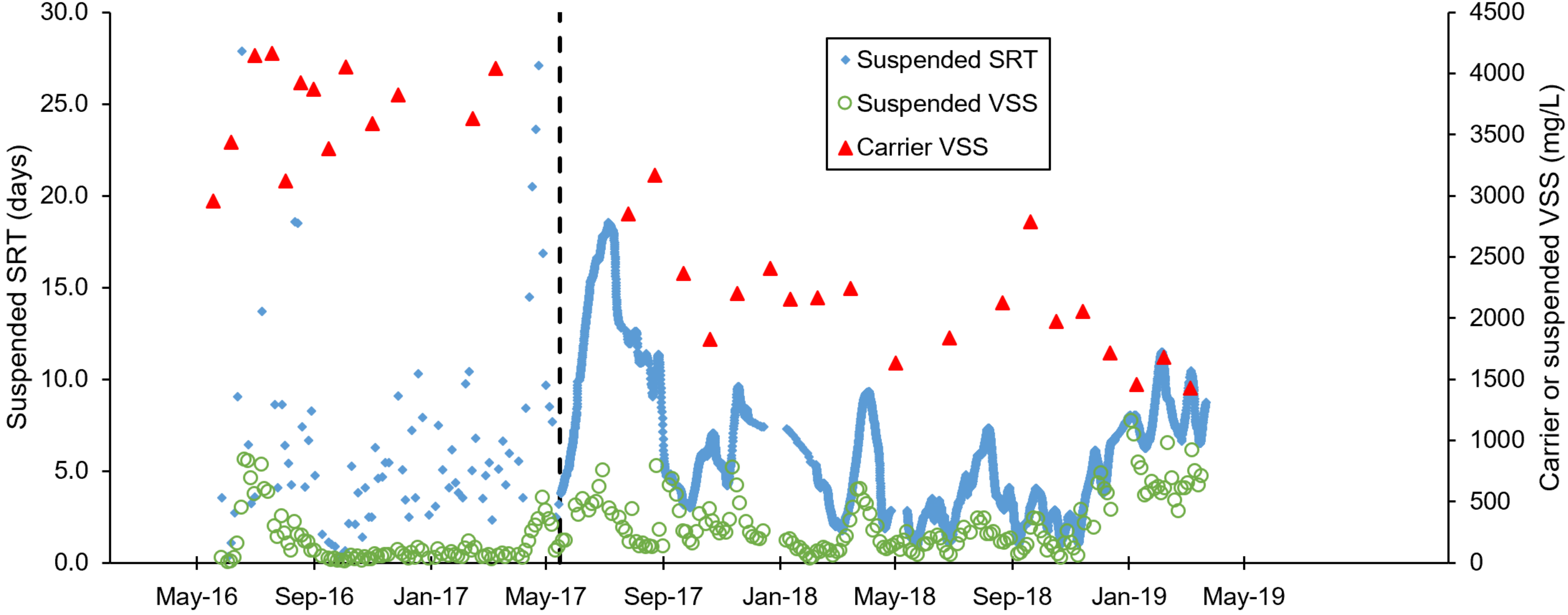
OUTLINE (40 min total, aim for 35 min-- ~35 slides):

1) N removal intro– why a concern [may remove or shrink quite a bit– could easily cut slides 5-7]]
2) Conventional vs. shortcut N removal– intro to nitrite shunt, PN/A
3) key challenges
4) Our approach: Shortcut N removal research station with MWRD, 2 overall strategies
5) Nitrite shunt with bioP
6) IFAS PN/A
7) SG comammox

TO DO:

- Standardize NOB to NOO, AOB to AOO
- Standardize terminology (nitrite shunt vs. nitritation/ denitritiation, deammonification vs. PN/A)

IFAS PN/A SRTs



IFAS Reactor

Performance History

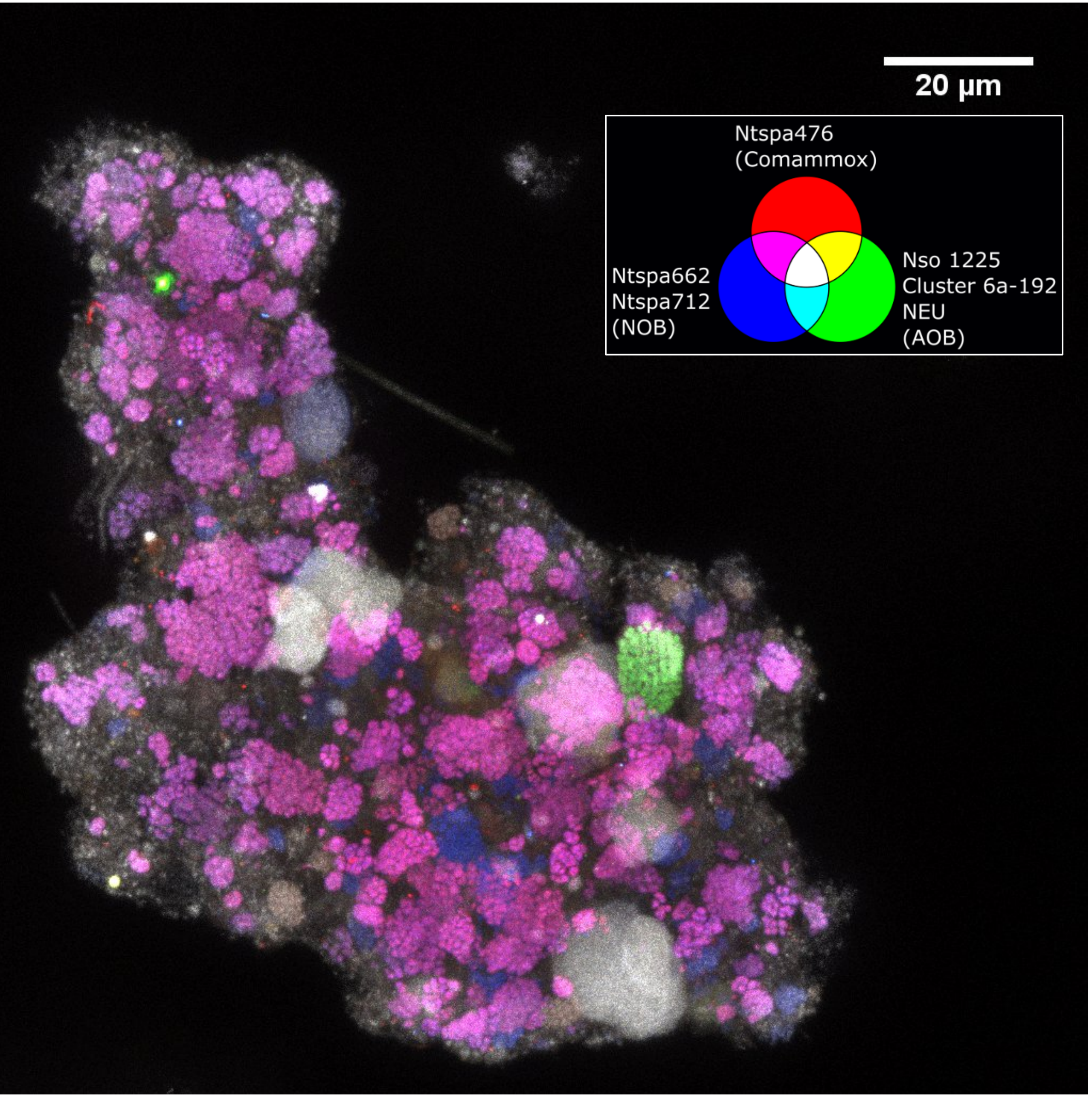
July 2017: Switched from intermittent aeration to continuous low-DO (0.1 mg/L) operation

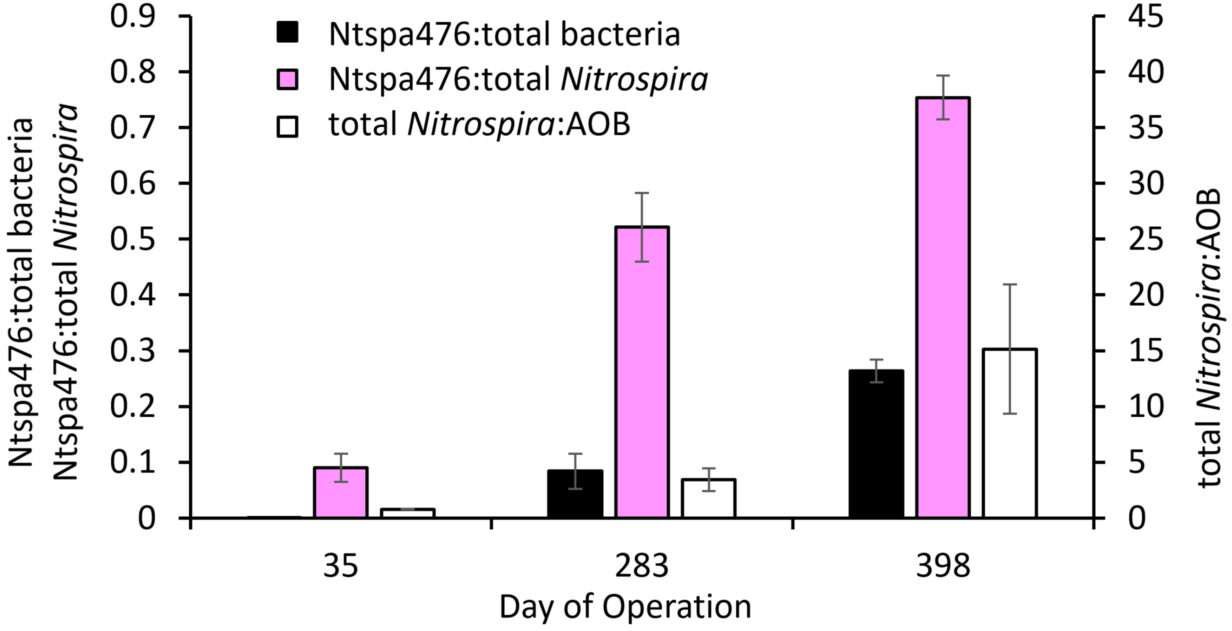
	May 16 – Aug 16	Aug 16 – Dec 17	Dec 17 – Mar 18
% NH_4^+ removal	85	75	**77**
% TIN removal	68	39	**55**
NO_X/NH_4^+ ratio	0.09	0.47	**0.21**

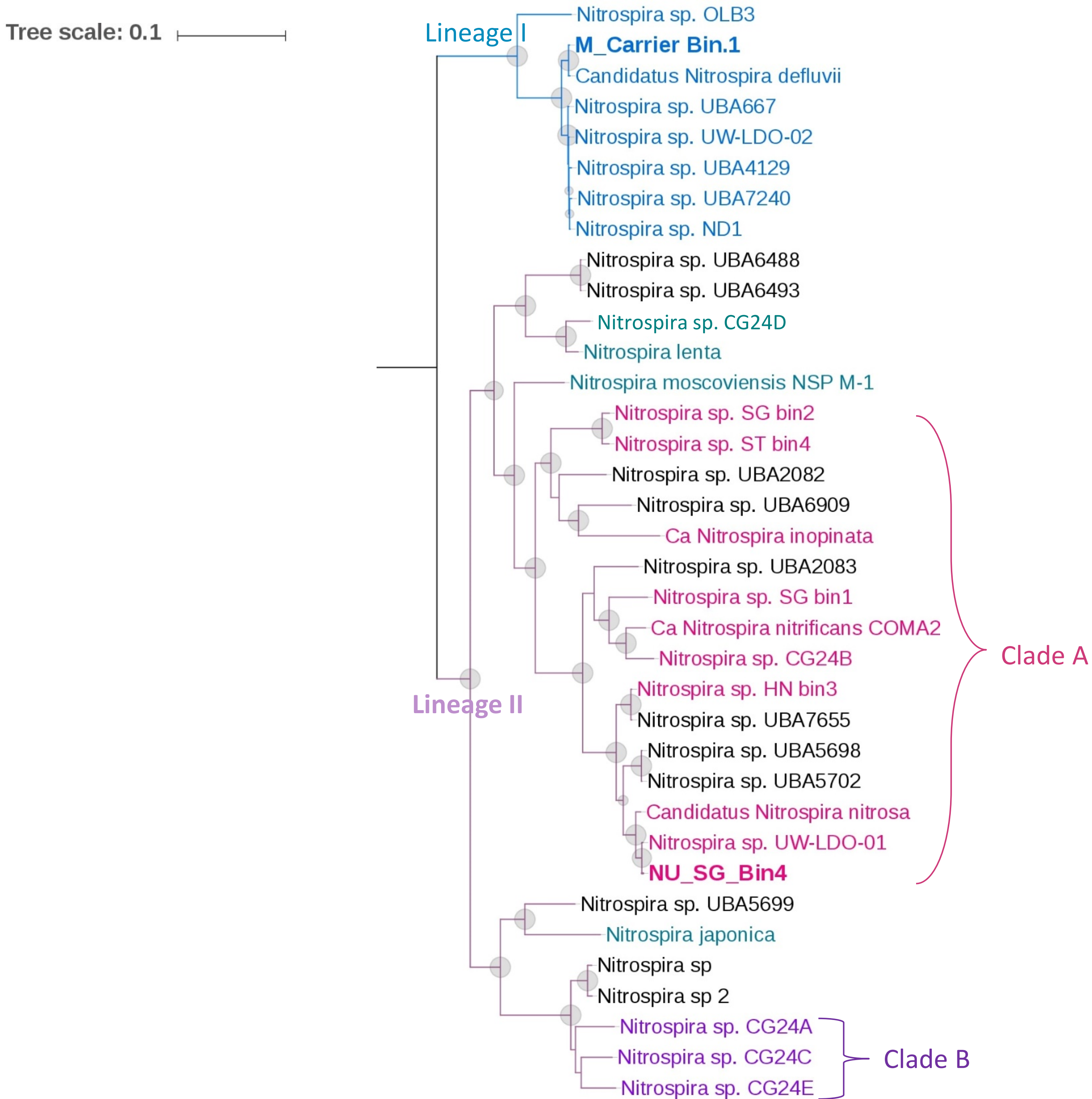
December 2017 – March 2018

Effluent NH_4^+ = 2.9 ± 2.5 mgN/L
HRT = 6.0 ± 1.2 hr
MLVSS = 133 ± 66 mg/L
SRT ≈ 4 d (suspended growth)

qFISH demonstrates strong enrichment of putative comammox







- 108 Essential single copy gene based phylogenetic association of Nitrospira genomes recovered from the metagenome dataset;

- Genome of *Nitrobacter hamburgensis* was applied as the outgroup genome here;

- Bootstrap value > 0.7 was displayed as circle at each branch, and the size of the circle was in positive relationship with the bootstrap value;

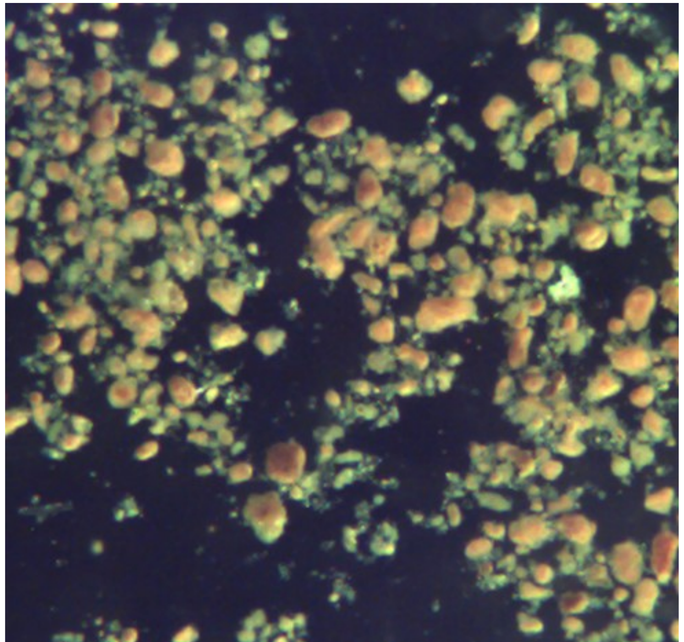
PN/A SBRs

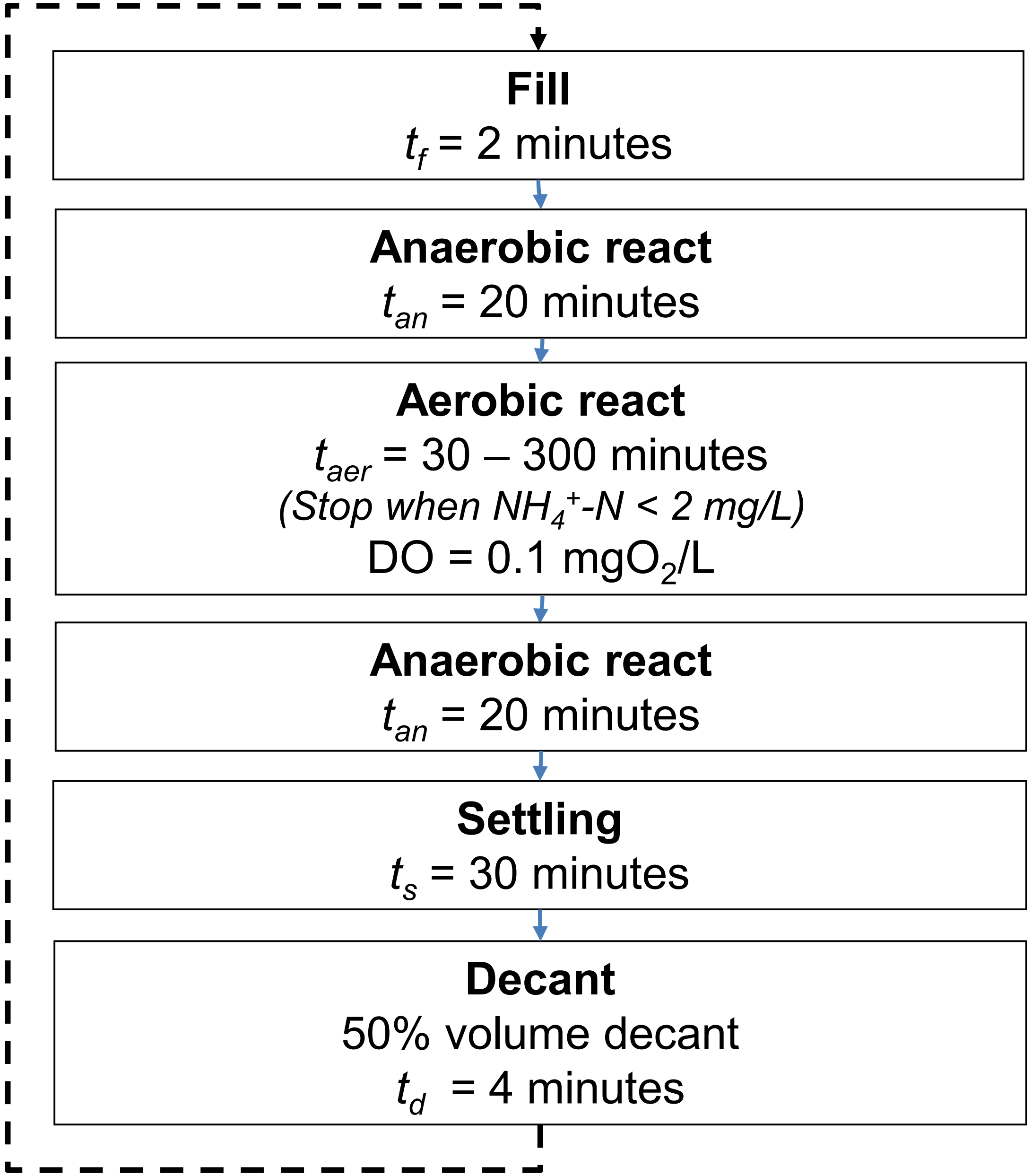
Bio-P Reactor Effluent
30 mg sCOD/L
16 mg NH_x-N/L
0.3 mg Ortho-P/L
10mg TSS/L

- Biofilm reactor seeded with sidestream enriched anammox carriers from the James River treatment plant (HRSD)
- Suspended growth reactor seeded with sidestream enriched DEMON sludge from the York River treatment plant (HRSD)

Suspended Growth SBR operation

- Biomass not intentionally wasted to preserve anammox, though biomass was lost through the effluent
  - Very long SRT: 50-99 days
  - HRT: 9 hrs until day ~300, then 5.4 hrs
- Two primary DO strategies:
  - Intermittent aeration, target DO = 1 mgO_2/L
  - Continuous aeration, target DO = 0.1 mgO_2/L





Fill
t_f = 2 minutes

Anaerobic react
t_{an} = 20 minutes

Aerobic react
t_{aer} = 30 – 300 minutes
(Stop when NH_4^+-N < 2 mg/L)
DO = 0.1 mgO_2/L

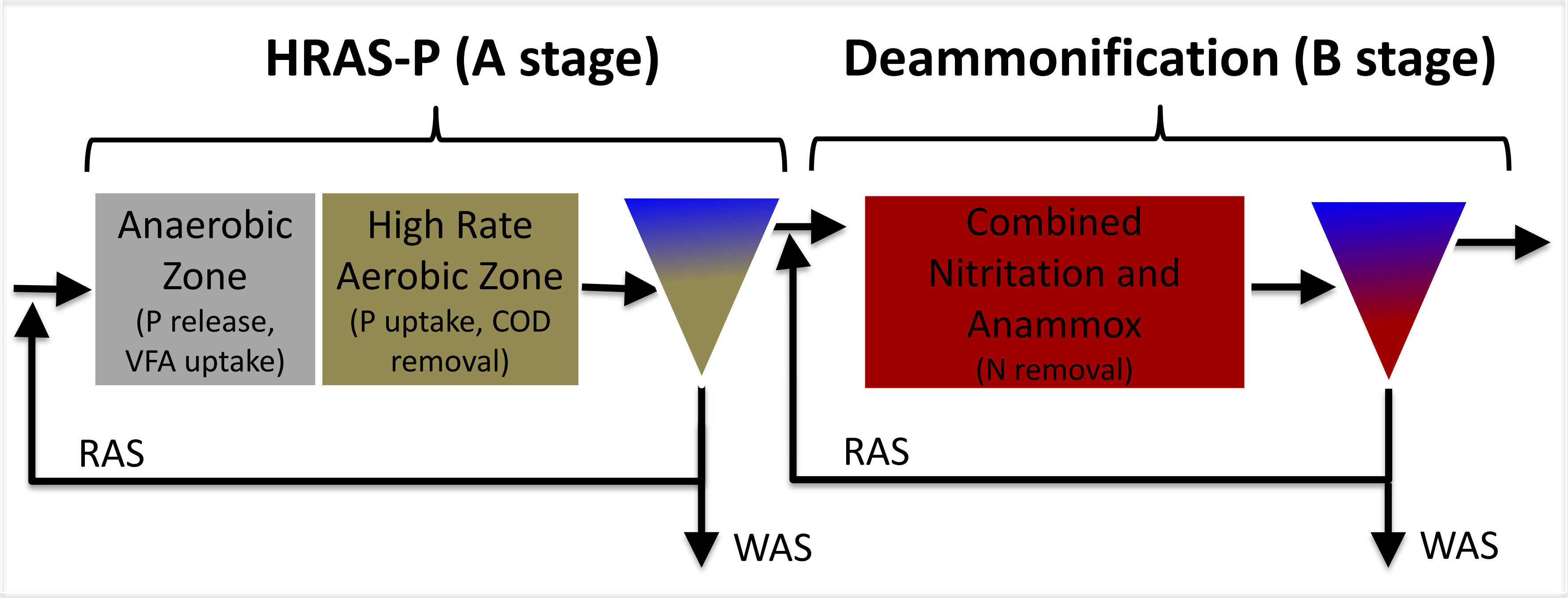
Anaerobic react
t_{an} = 20 minutes

Settling
t_s = 30 minutes

Decant
50% volume decant
t_d = 4 minutes

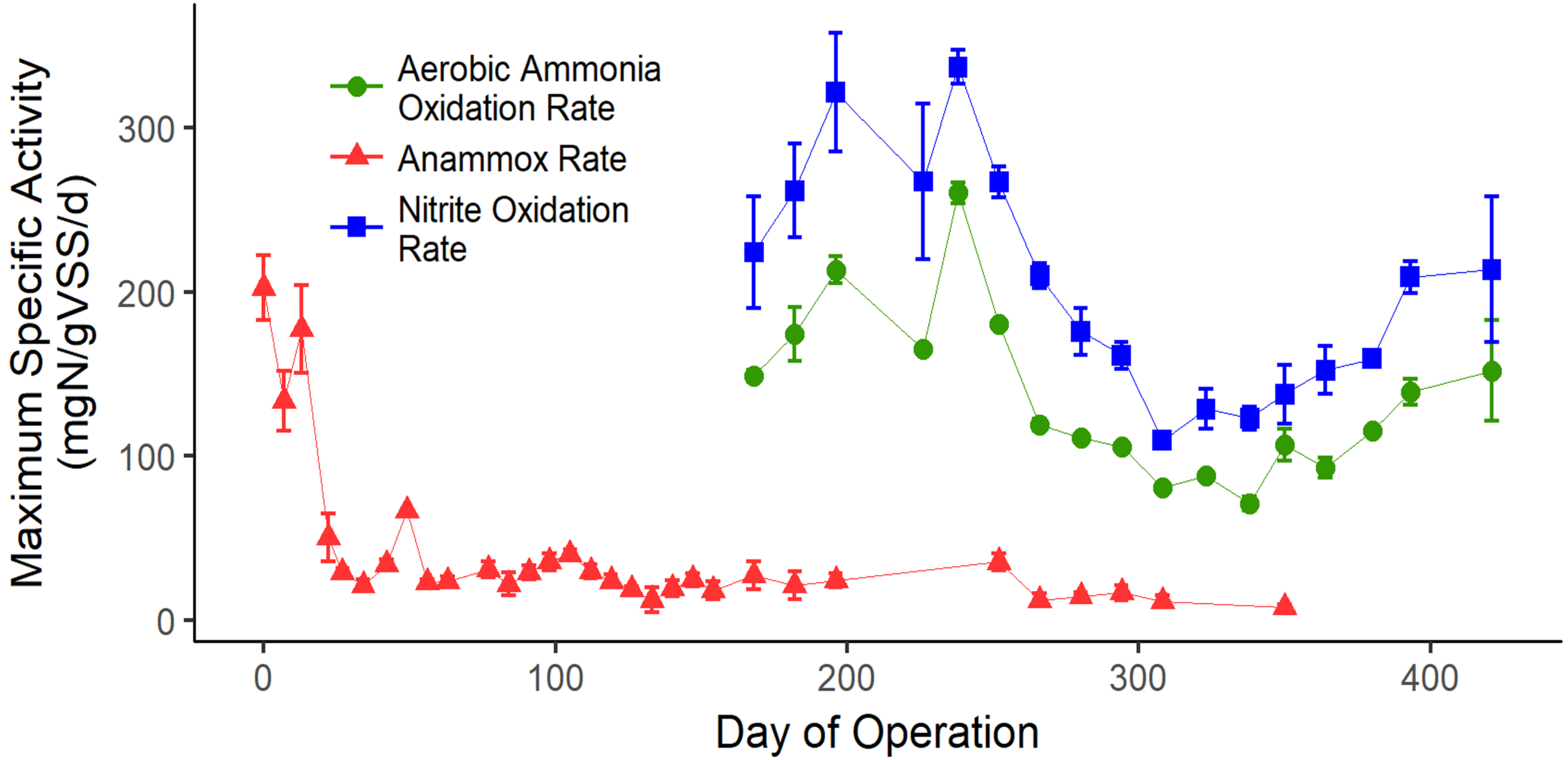
Process Option 1: HRAS-P + Deammonification

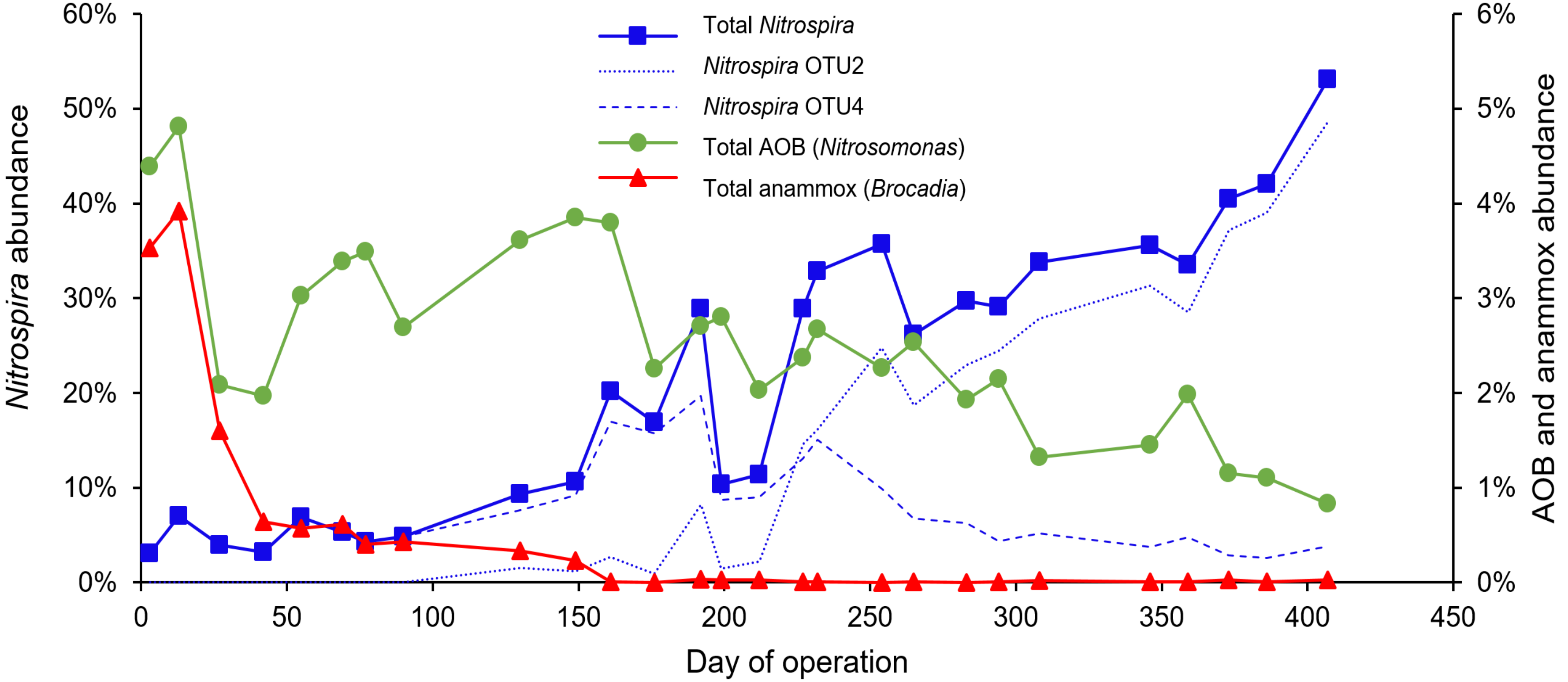
(High-Rate Activated Sludge (HRAS) and biological P removal (stage A) and Deammonification (stage B)



- This is an alternative approach to dPAOs for addressing carbon limitations for combined N and P removal from mainstream wastewater

[paralleled by steep drop in anammox activity]




Nitrospira abundance
60%
50%
40%
30%
20%
10%
0%
AOB and anammox abundance
6%
5%
4%
3%
2%
1%
0%
0
50
100
150
200
250
300
350
400
450
Day of operation
Total *Nitrospira*
Nitrospira OTU2
Nitrospira OTU4
Total AOB (*Nitrosomonas*)
Total anammox (*Brocadia*)

Mainstream Deammonification Challenges

Carbonaceous, Cold, Dilute, Dynamic

	Sidestream	Mainstream (Chicago)	Mainstream Consequence
cBOD:N	<1	>4	-Heterotrophic competition
Temperature (°C)	30-35	10-23	-Lower growth rates -Lower selective pressure against nitrite oxidizing organisms
$TKN_{x,Influent}$	500-1000	7-21	-Lower surface loading rate -Lower selective pressure against nitrite oxidizing organisms

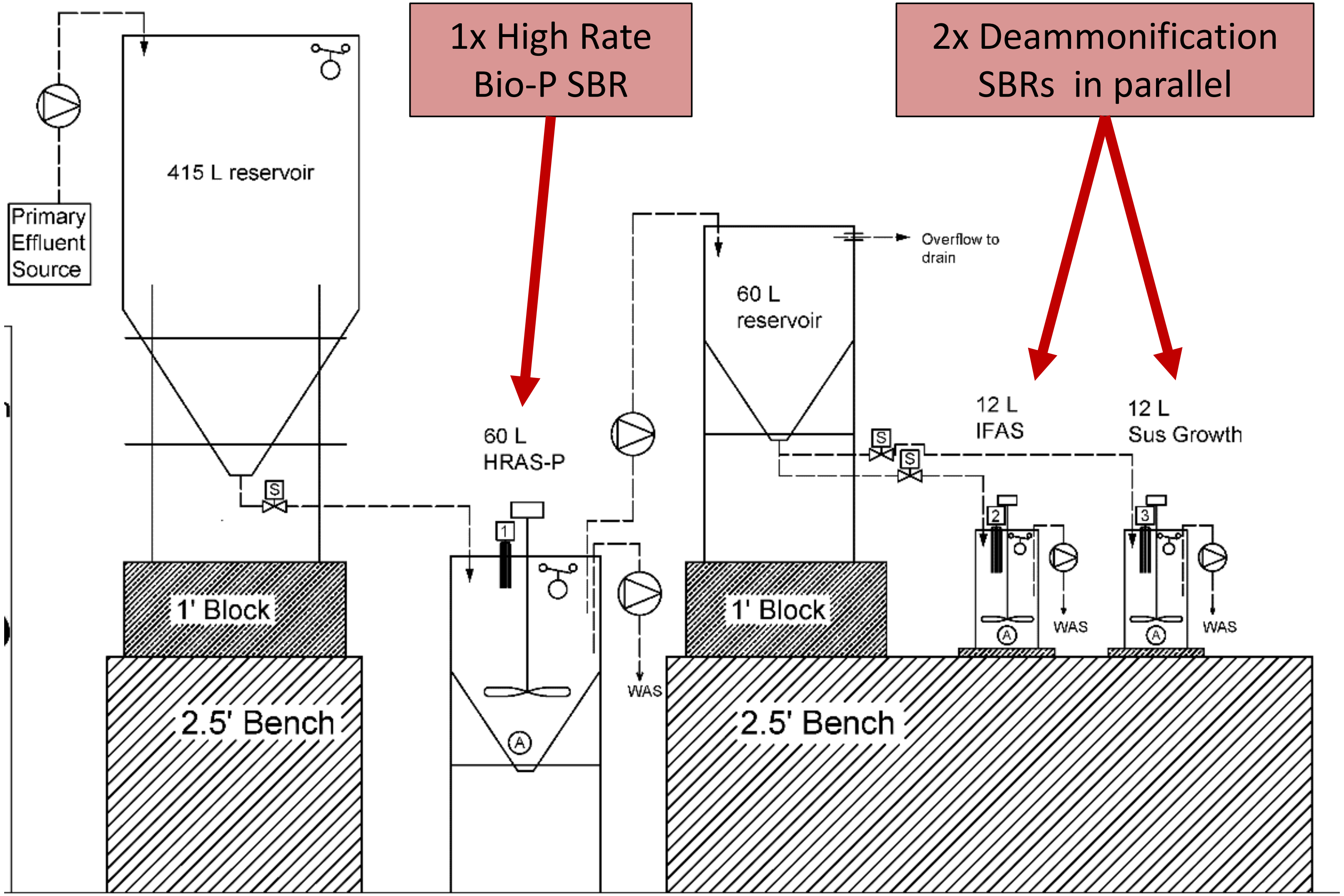
	Primary effluent			A-stage effluent			Reactor effluent			O'Brien effluent		
TKN (mgN/L)	20.6	±	4.4	16.5	±	4.7	4.5	±	2.7	1.9	±	0.2
NH_4^+ (mgN/L)	15.5	±	3.6	14.3	±	3.8	3.6	±	2.6	0.7	±	0.1
NO_X (mgN/L)	---[a]	±	---	0.5[b]	±	0.7	7.2	±	3.3	7.4	±	2.1
COD (mgCOD/L)	141	±	43	42	±	32	24	±	17	*not available*[c]		
sCOD (mgCOD/L)	84	±	21	29	±	11	20	±	7	*not available*		
alkalinity (meq/L)	4.7	±	0.5	4.6	±	0.5	3.3	±	0.6	*not available*		
TSS (mg/L)	45	±	25	15	±	35	7	±	8	6		

[a] NO_X in primary effluent was at or below detection limit of 0.15 mgN/L in 93% of samples

[b] NO_X in A-stage effluent was at or below detection limit of 0.15 mgN/L in 54% of samples

[c] COD not measured, but BOD_5 in O'Brien WRP effluent = 5.7 ± 2.9 mgBOD/L

HRAS-P/Deammonification 2-Sludge Process

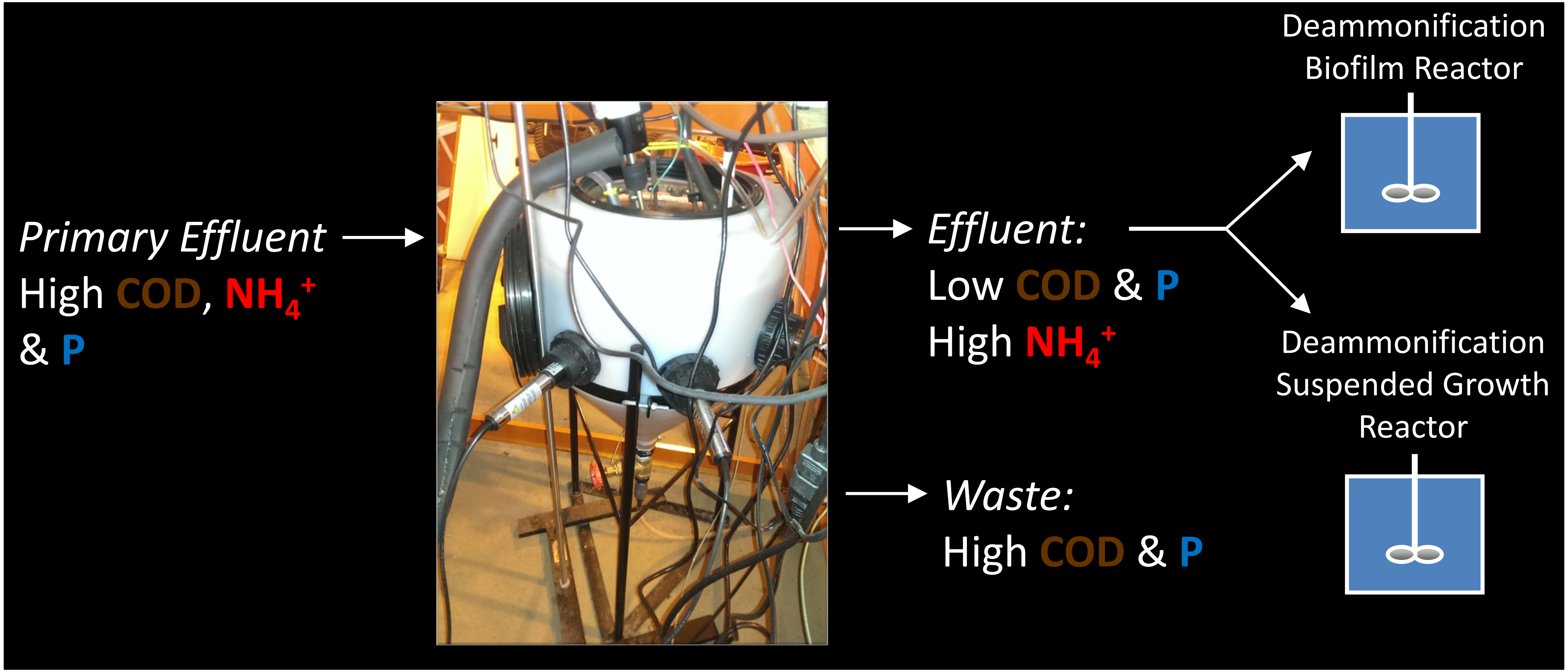


1x High Rate Bio-P SBR

2x Deammonification SBRs in parallel

Challenge 1: Carbonaceous BOD (and P) Removal

Tested Solution: Remove the carbon in a high rate Bio-P Reactor



Primary Effluent
High COD, NH_4^+
& P

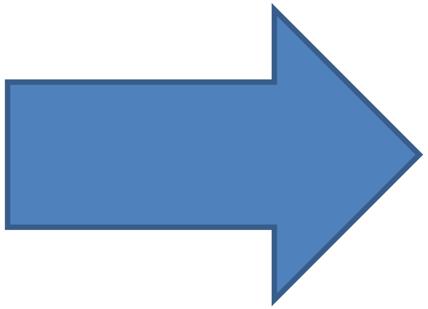
Effluent:
Low COD & P
High NH_4^+

Waste:
High COD & P

Deammonification
Biofilm Reactor

Deammonification
Suspended Growth
Reactor

Deammonification SBRs



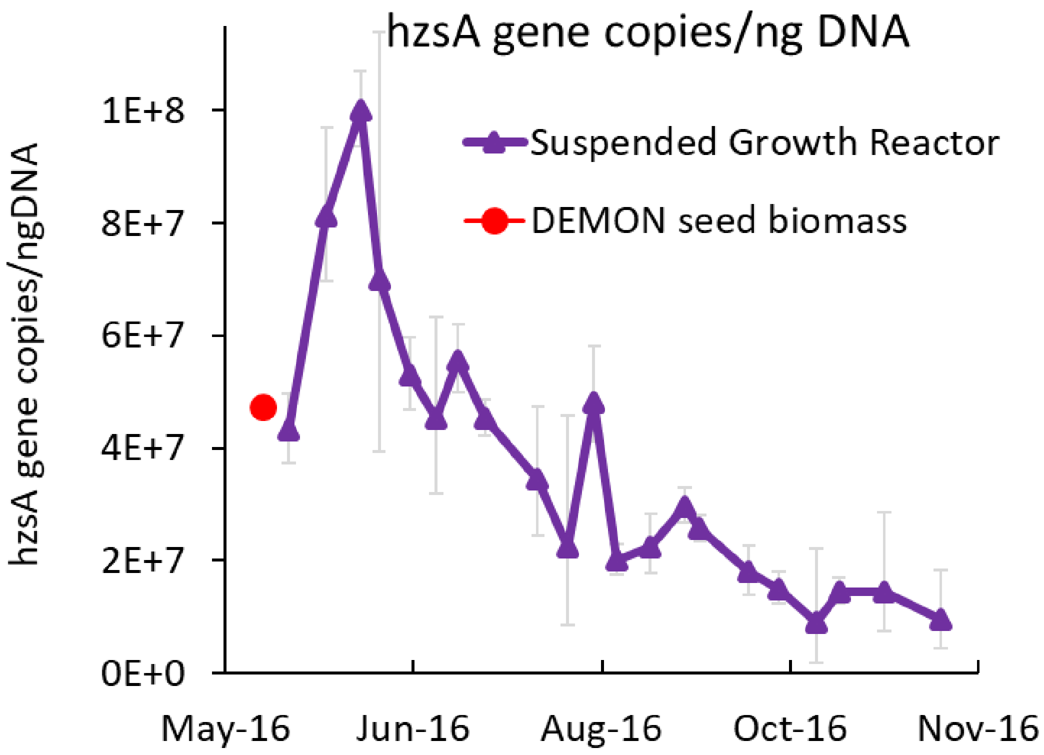
<u>Bio-P Reactor Effluent</u>

30 mg sCOD/L
16 mg NH_x-N/L
0.3 mg Ortho-P/L
10mg TSS/L

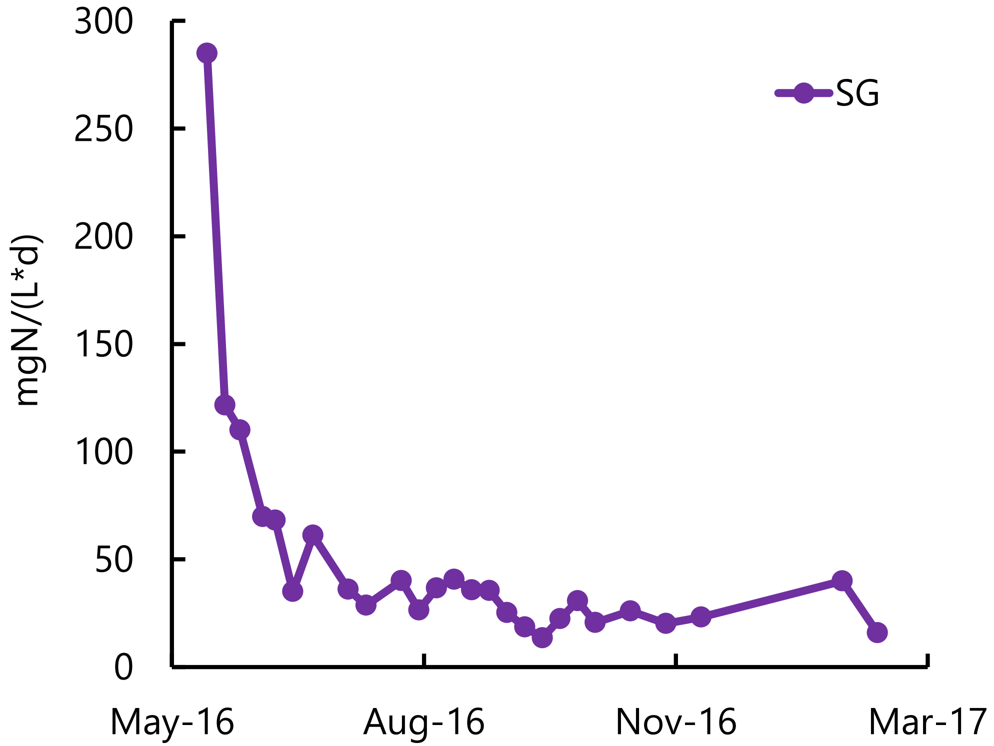
- Biofilm reactor seeded with sidestream enriched anammox carriers from the James River treatment plant (HRSD)
- Suspended growth reactor seeded with sidestream enriched DEMON sludge from the York River treatment plant (HRSD)

Suspended Growth Reactor

Decline in Anammox Abundance and Activity



Quantification of anammox abundance via qPCR of the hzsA gene



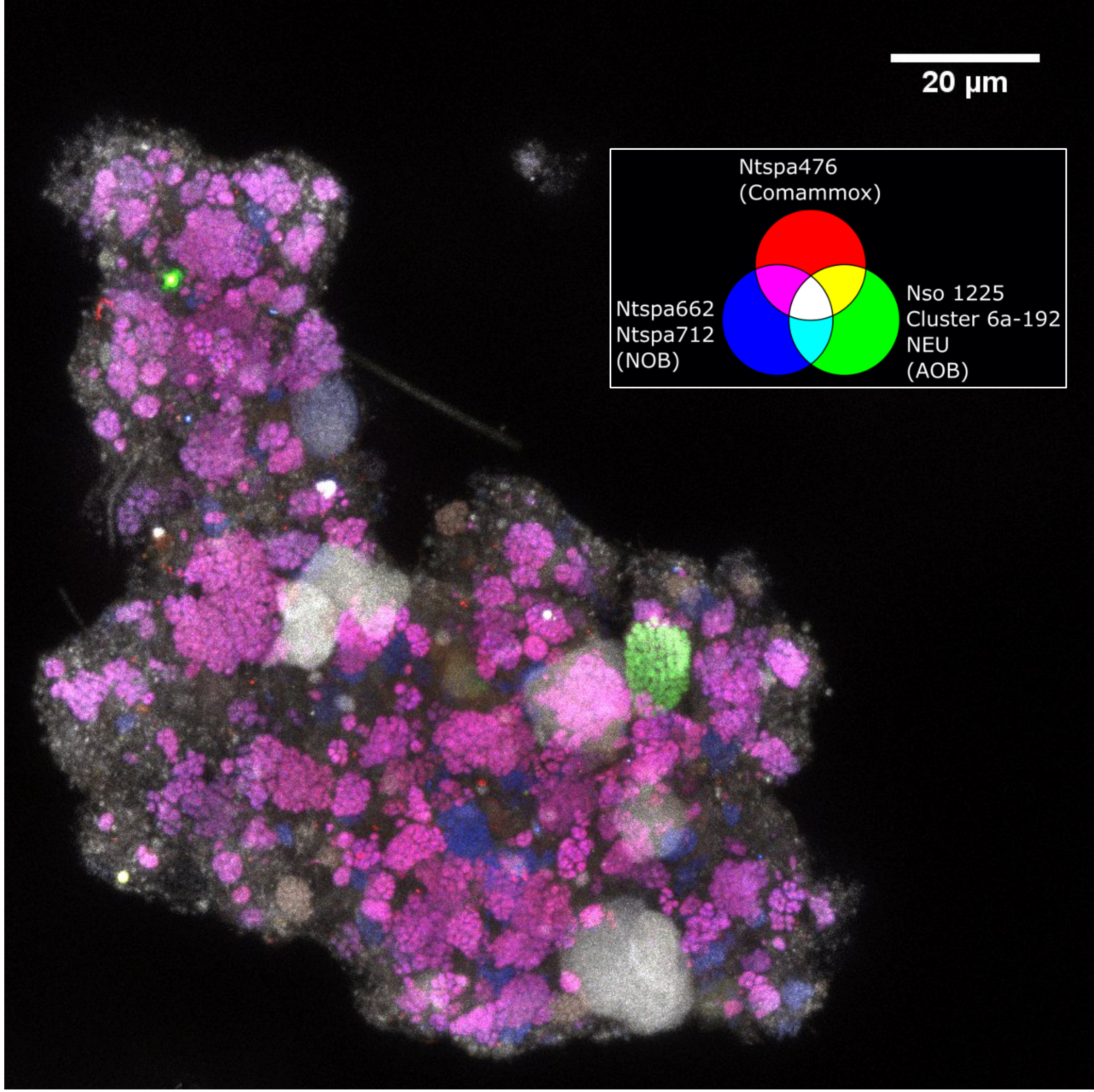
Maximum *in situ* anammox activity

…Comammox!

Supporting Evidence

- 16s sequencing data
  - High nitrospira
  - Almost no AOB
- FISH
  - High Nitrospira
  - High Nitrospira Ntspa476

    (“comammox” probe from van Kessel *et al.* 2015)
  - Almost no AOB
- qPCR
  - ~10% Comammox



Final Suspended Growth Reactor Performance

Target DO = 0.1 mg/L (occasionally 0.05 mg/L)

HRT = 6.3 ± 2.3 hr

Effluent NH_4^+ = 3.6 ± 3.2 mgN/L

NH_4^+ removal = 75%

		Chicago Plants		
	SG	O'Brien	Kirie	Stickney
N loading rate (mg TKN/L/d)	**83**	65	64	81

Low-DO nitrification with comammox could be an energy-saving alternative to conventional high-DO nitrification systems