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Complete ammonia oxidisers
a new pathway in the nitrification process

the international
water association

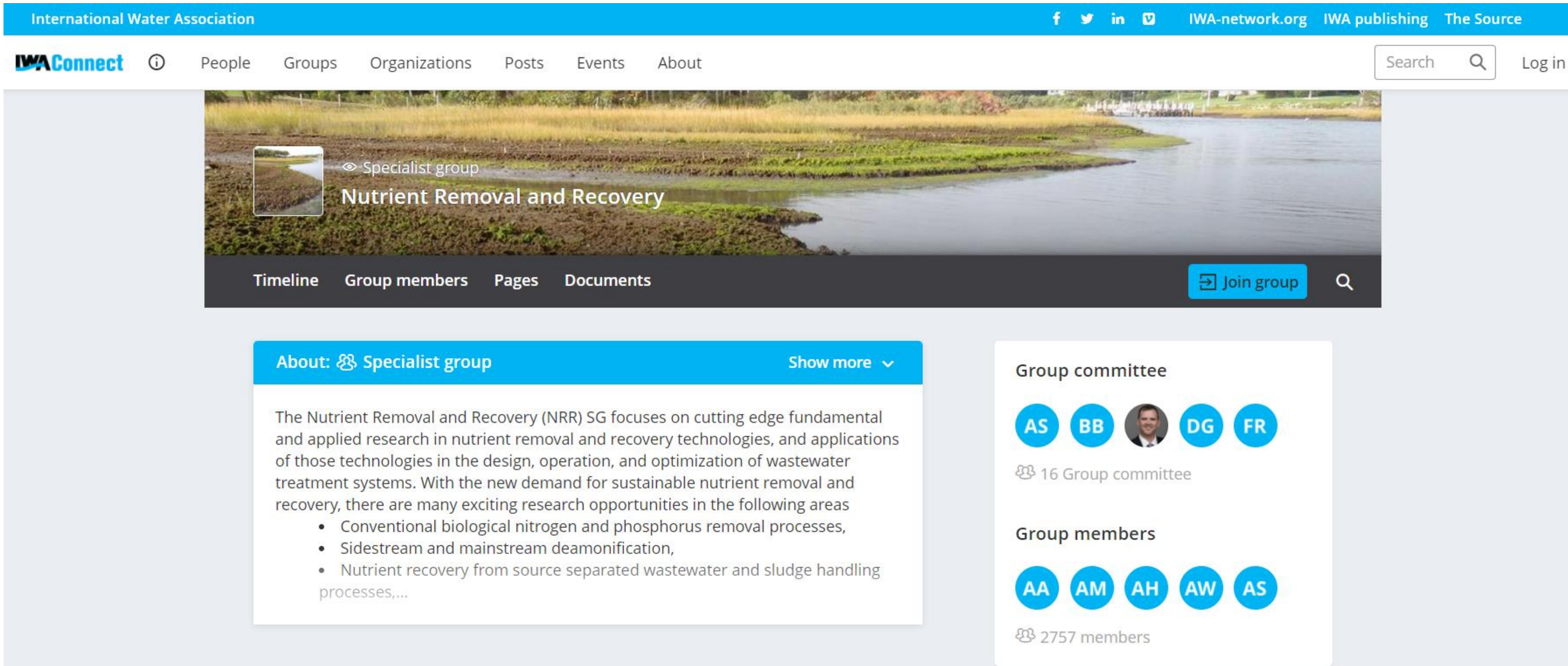
WEBINAR

25 May 2022 | 14:00 BST
iwa-network.org/webinars

inspiring change

IWA NUTRIENT REMOVAL AND RECOVERY (NRR) SG

the international water association



The IWA NRR SC focuses on cutting edge fundamental and applied research in nutrient removal and recovery technologies.

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https://iwa-connect.org/group/nutrient-removal-and-recovery?searchFor=all

IWA World Water Congress & Exhibition

Register at
https://worldwatercongress.org/

WEBINAR INFORMATION

- This webinar will be **recorded and made available "on-demand"** on the IWA website, with presentation slides, and other information.
- The **speakers** are responsible for **securing copyright permissions** for any work that they will present of which they are not the legal copyright holder.
- The opinions, hypothesis, conclusions or recommendations contained in the presentations and other materials are the **sole responsibility of the speaker(s)** and do not necessarily reflect IWA opinion.

WEBINAR INFORMATION

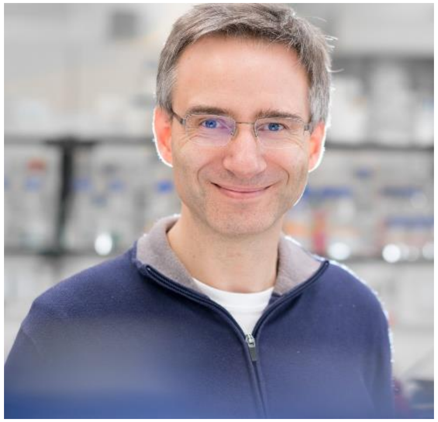
- **‘Chat’ box**: please use this for general requests and for interactive activities.
- **‘Q&A’ box**: please use this to send questions to the panelists.
  (We will answer these during the discussions)

Please Note: *Attendees’ microphones are muted. We cannot respond to ‘Raise Hand’.*

AGENDA

- Welcome, introduction, housekeeping rules
  Sudhir Murthy
- Discovery of complete nitrification
  Sebastian Lücker
- Physiology of Comammox: Key Features of a 'Green Microbe'
  Holger Daims
- Model development for the Comammox process
  Jacek Mąkinia
- Potential applications of Comammox *Nitrospira* in wastewater treatment systems
  Jianhua Guo
- Q&A Panel Discussion
  Sudhir Murthy (moderator)
- Final remarks and conclusion
  Jianhua Guo

MODERATOR & PANELISTS



Holger Daims
University of Vienna,
Austria

Sebastian Lücker
Radboud University,
The Netherlands

Jianhua Guo
University of Queensland,
Australia

Jacek Mąkinia
Gdańsk University of Technology
Poland

Sudhir Murthy
NEWhub Corp,
USA

LEARNING OBJECTIVES

1. To learn more about the discovery, physiology, kinetic and metabolic properties of Comammox Nitrospira;
2. To enhance their understanding of the role of Comammox Nitrospira in biological nitrogen removal processes in wastewater treatment plants;
3. To explore potential applications of Comammox Nitrospira in wastewater treatment and contaminant removal;

SHARE YOUR THOUGHTS ON SOCIAL MEDIA

the international water association

Tag @IWAHQ on social media and tell us:

Why COMAMMOX is important for nitrification?

How does it affect your life?

What is the main contribution to the SDG6 and the 2030 Agenda?

Don’t forget to include the hashtags #IWA & #COMAMMOX.

Discovery of complete nitrification

SEBASTIAN LÜCKER, RADBOUD UNIVERSITY, THE NETHERLANDS

inspiring change

OUTLINE

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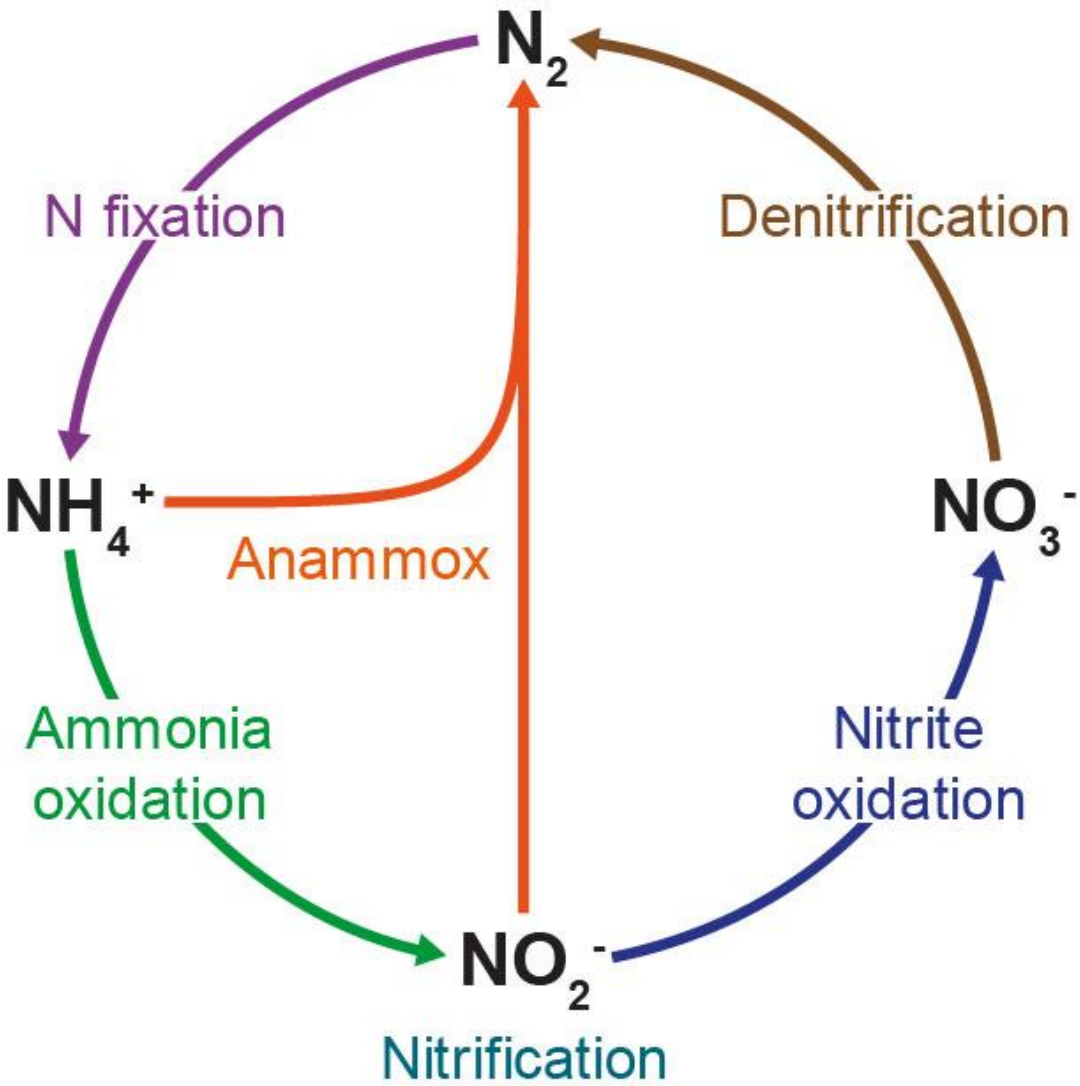
- **Complete nitrification by *Nitrospira***
- ***In situ* detection of ammonia-oxidizing bacteria**

THE BIOGEOCHEMICAL NITROGEN CYCLE



Opinion TRENDS in Microbiology Vol.14 No.5 May 2006
Full text provided by www.sciencedirect.com

Why is metabolic labour divided in nitrification?

Engràcia Costa[1], Julio Pérez[1] and Jan-Ulrich Kreft[2]

AOB: High growth rates, low yield

r strategist

Comammox: Low growth rates, high yield

K strategist

LETTER

doi:10.1038/nature16459

Complete nitrification by a single microorganism

Maartje A. H. J. van Kessel[1], Daan R. Speth[1], Mads Albertsen[2], Per H. Nielsen[2], Huub J. M. Op den Camp[1], Boran Kartal[1,3], Mike S. M. Jetten[1,4] & Sebastian Lücker[1]

ARTICLE

doi:10.1038/nature16461

the international water association

Complete nitrification by *Nitrospira* bacteria

Holger Daims[1], Elena V. Lebedeva[2], Petra Pjevac[1], Ping Han[1], Craig Herbold[1], Mads Albertsen[3], Nico Jehmlich[4], Marton Palatinszky[1], Julia Vierheilig[1], Alexandr Bulaev[2], Rasmus H. Kirkegaard[3], Martin von Bergen[4,5], Thomas Rattei[6], Bernd Bendinger[7], Per H. Nielsen[3] & Michael Wagner[1]

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AMERICAN SOCIETY FOR MICROBIOLOGY | mSphere™

OBSERVATION
Applied and Environmental Science

Metagenomic Evidence for the Presence of Comammox *Nitrospira*-Like Bacteria in a Drinking Water System

Ameet J. Pinto,[a] Daniel N. Marcus,[b,c] Umer Zeeshan Ijaz,[a]
Quyen Melina Bautista-de lose Santos,[a] Gregory J. Dick,[b] Lutgarde Raskin[c]

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The ISME Journal (2016), 1–13

www.nature.com/ismej

ORIGINAL ARTICLE

Metagenomic analysis of rapid gravity sand filter microbial communities suggests novel physiology of *Nitrospira* spp.

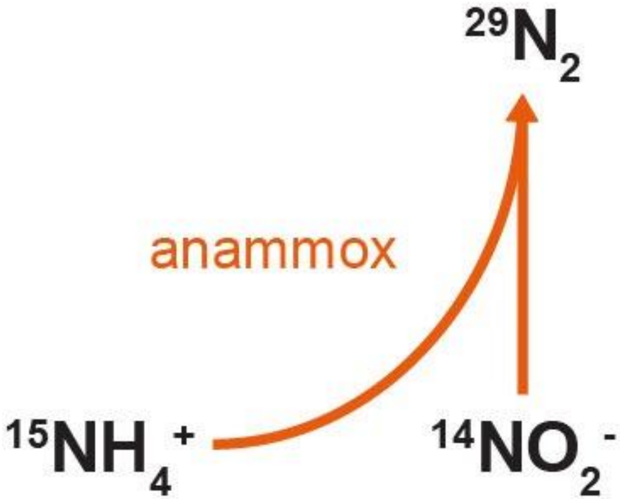
Alejandro Palomo[1,2], S Jane Fowler[1], Arda Gülay[1], Simon Rasmussen[2], Thomas Sicheritz-Ponten[2] and Barth F Smets[1]

BIOREACTOR ENRICHMENT CULTURE

- Inoculum:
  - Biofilm from aquaculture biofilter
- Medium:
  - Aquaculture water, supplemented with NH_4^+, NO_2^-, NO_3^-
  - No extra carbon source
  - Hypoxic conditions (≤3.1 µM O_2)

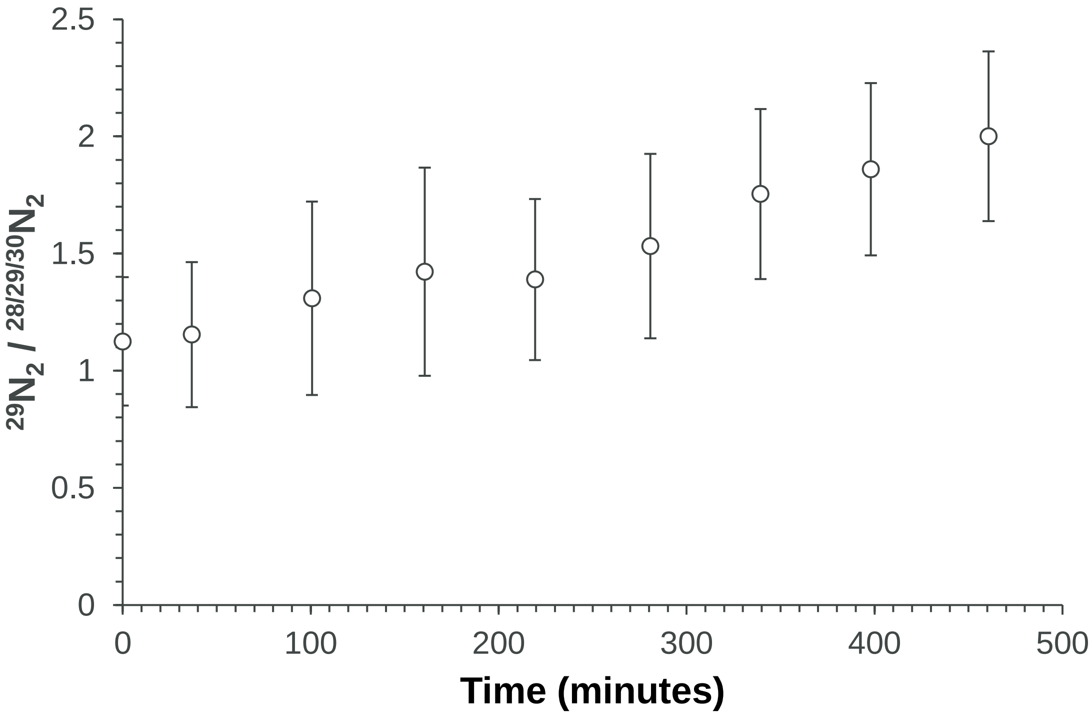
→ optimal conditions for anammox bacteria

MEASURING ANAMMOX ACTIVITY



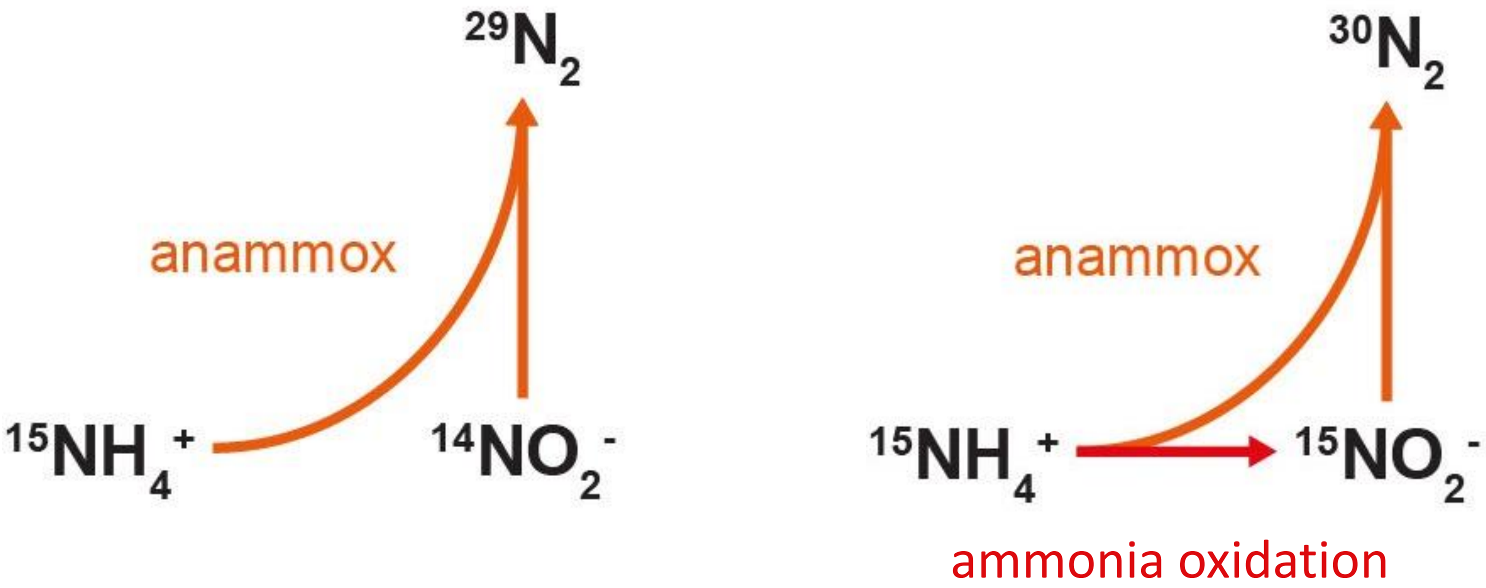
ANAMMOX ACTIVITY ASSAYS

- Formation of $^{29}N_2$ in incubations with ^{15}N-labelled NH_4^+ confirms anammox activity



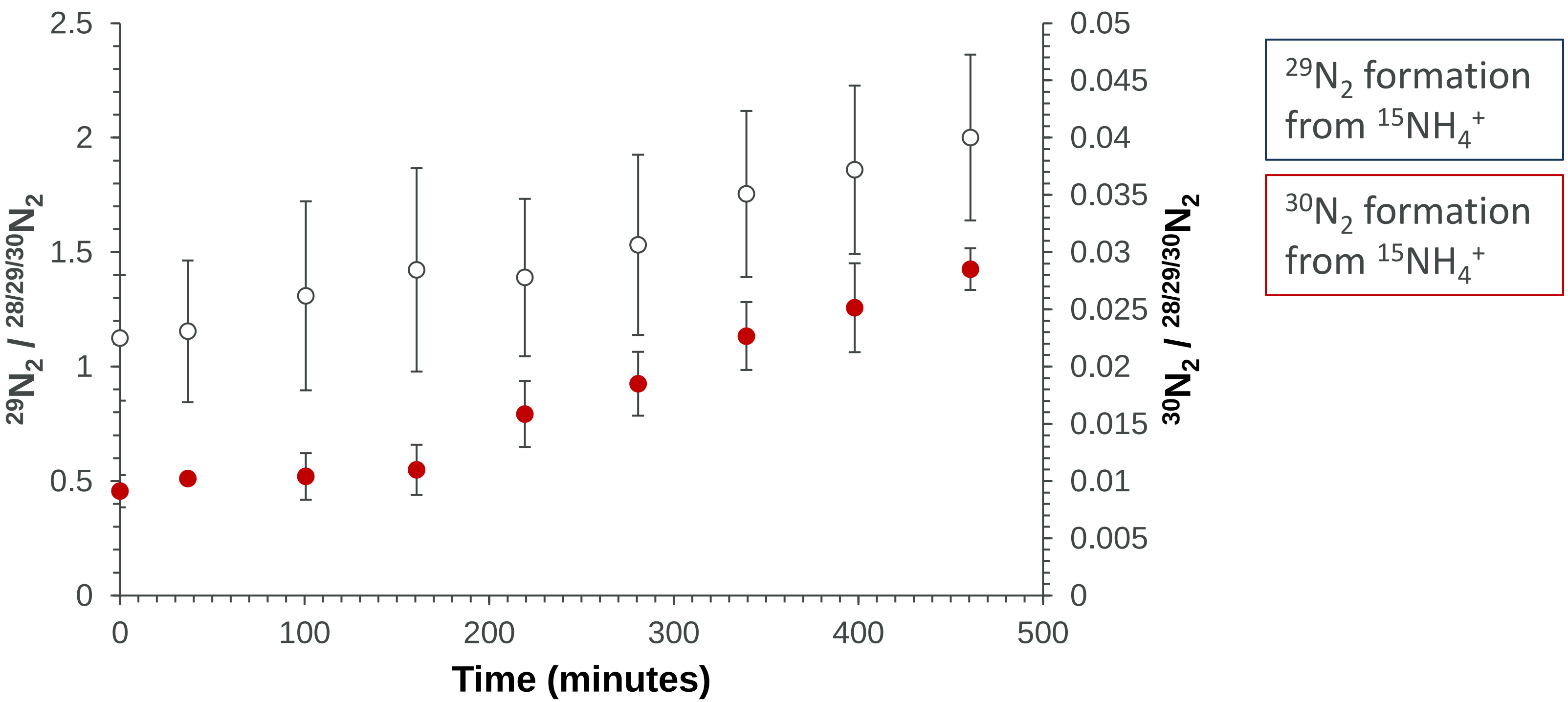
$^{29}N_2$ formation from $^{15}NH_4^+$

MEASURING ANAMMOX ACTIVITY



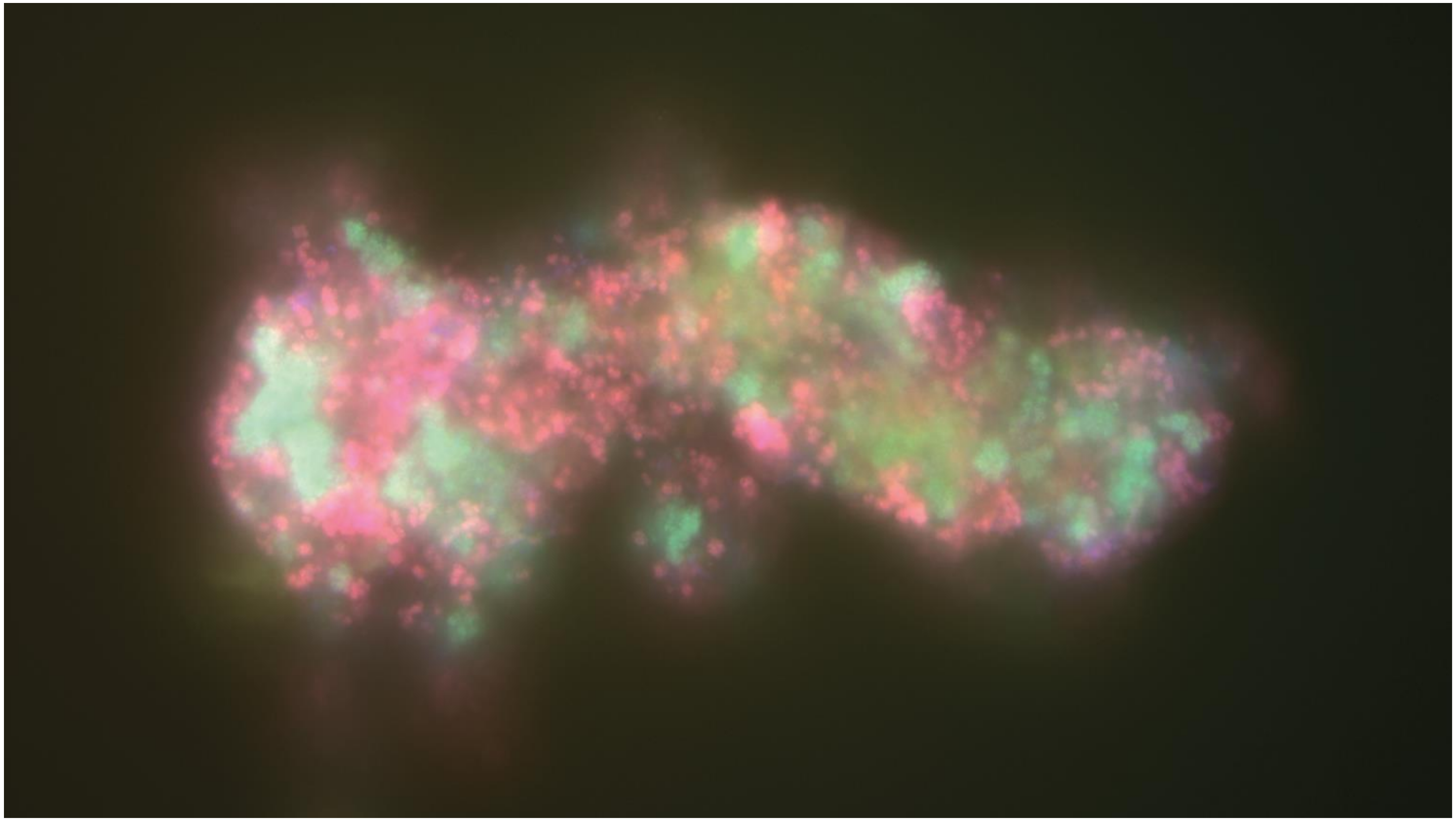
ANAMMOX ACTIVITY ASSAYS

- Formation of $^{30}N_2$ from $^{15}NH_4^+$ indicates ammonia oxidation



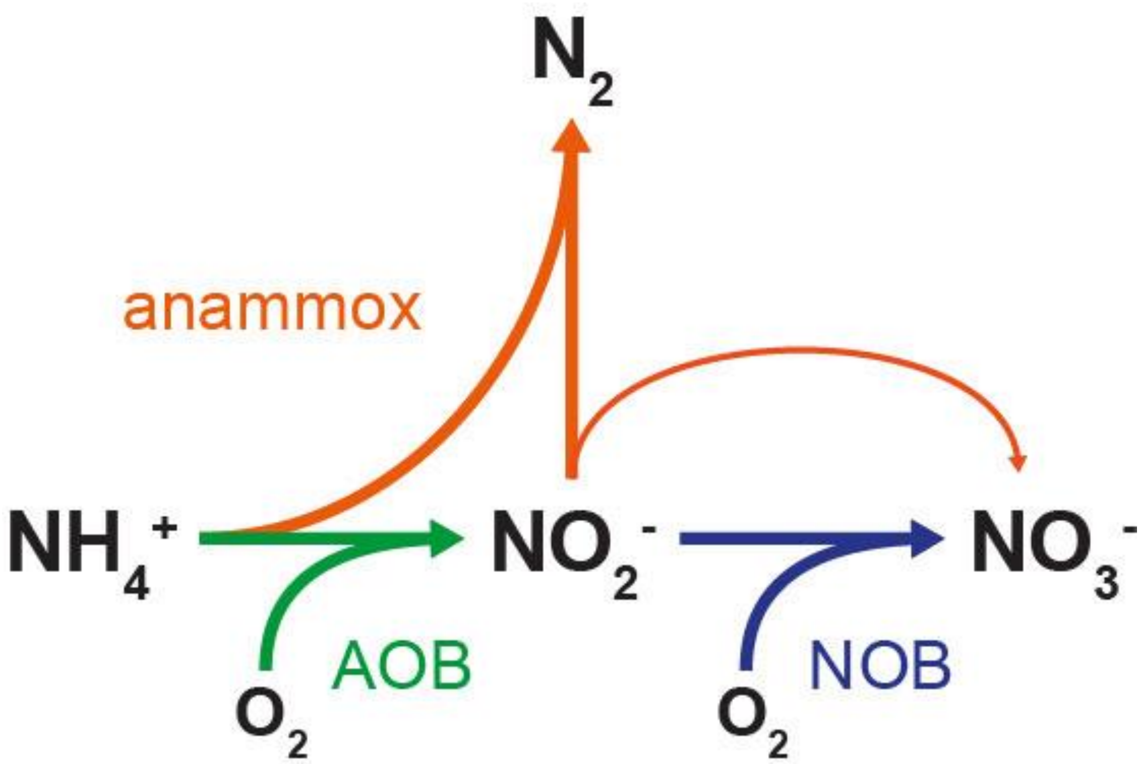
FISH ON BIOREACTOR ENRICHMENT

- *Nitrospira* are always present in flocs with anammox (*Brocadia*)
- Stable coculture



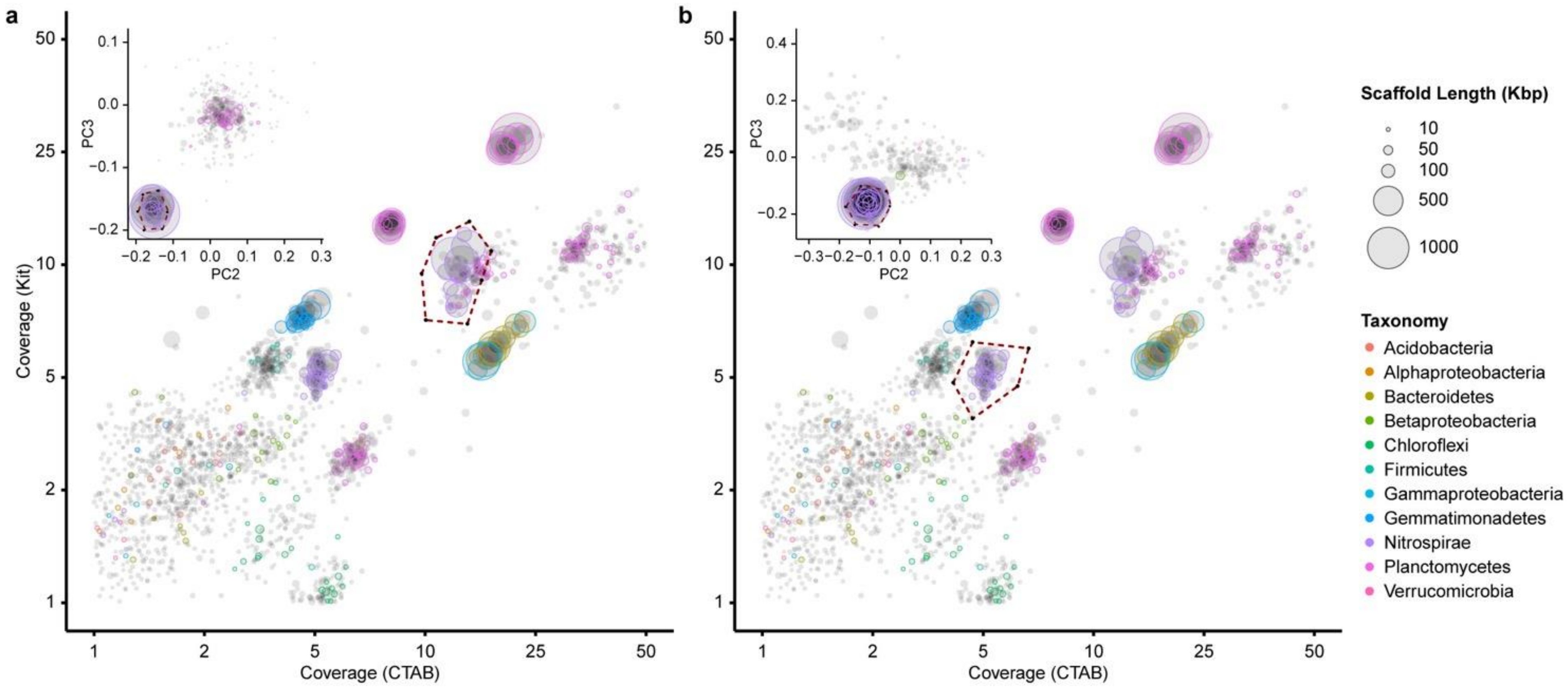
red = anammox; green = *Nitrospira*; blue= all bacteria

INTERACTION AND COMPETITION BETWEEN ANAMMOX AND NITRIFIERS



METAGENOMIC SEQUENCING

- Recovery of two high quality *Nitrospira* genomes

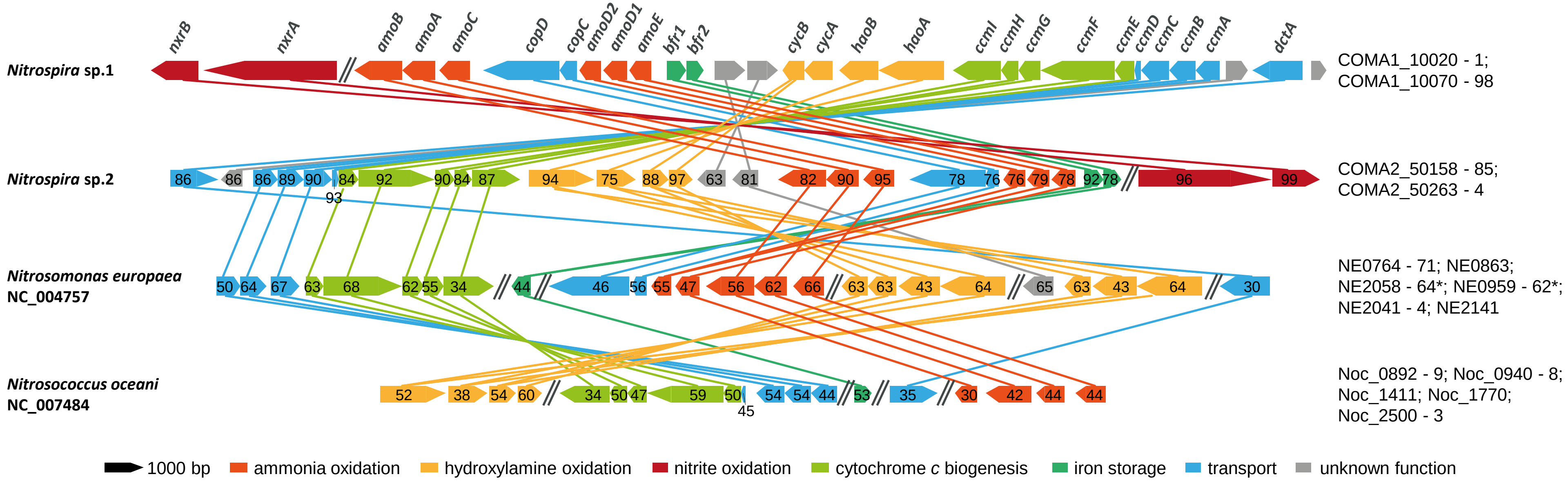


METAGENOMIC ANALYSES

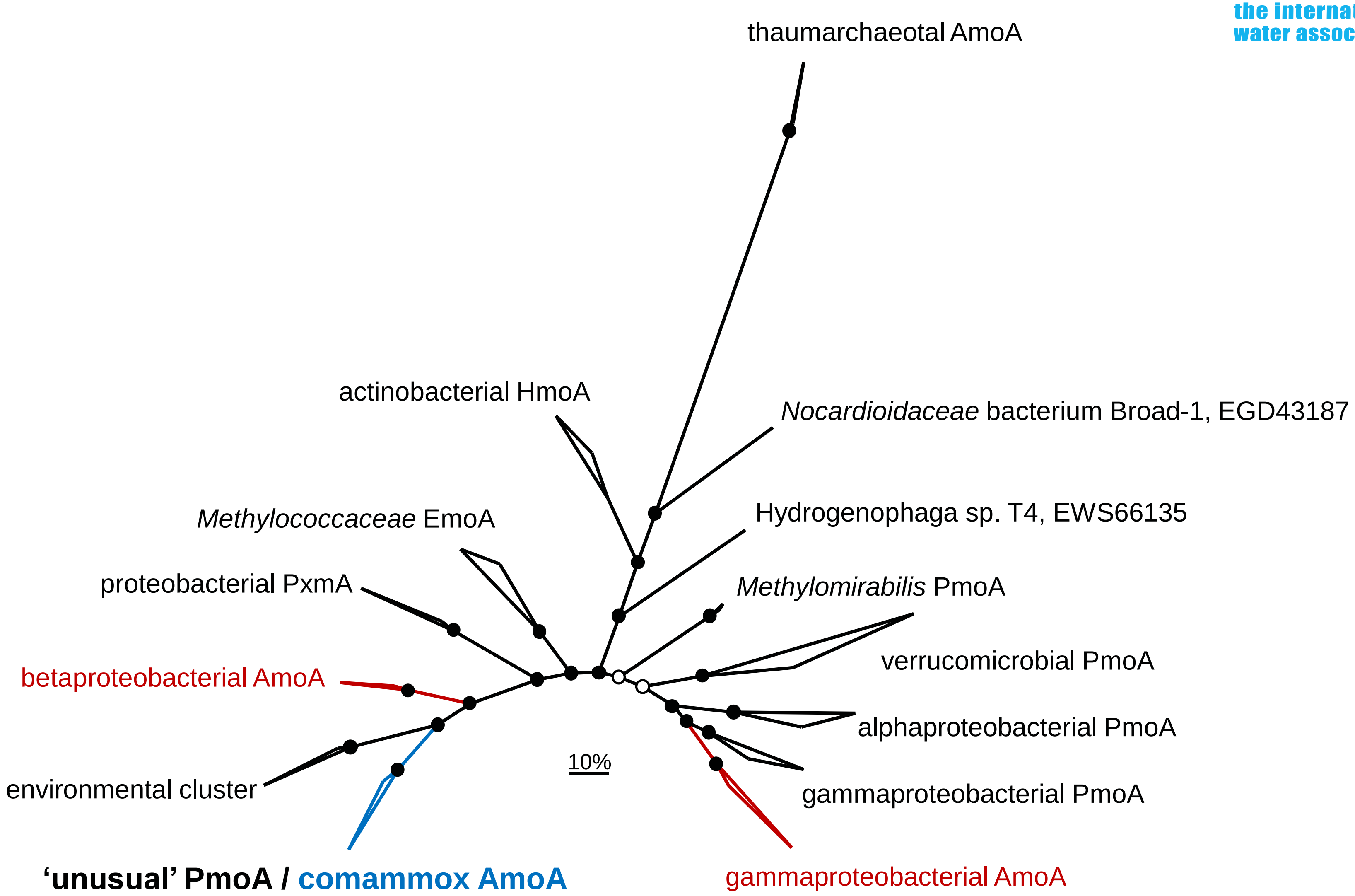
- Both *Nitrospira* spp. genomes contain genes for
  - Ammonia monooxygenase
  - Hydroxylamine dehydrogenase
  - Nitrite oxidoreductase

Only in ammonia oxidizers

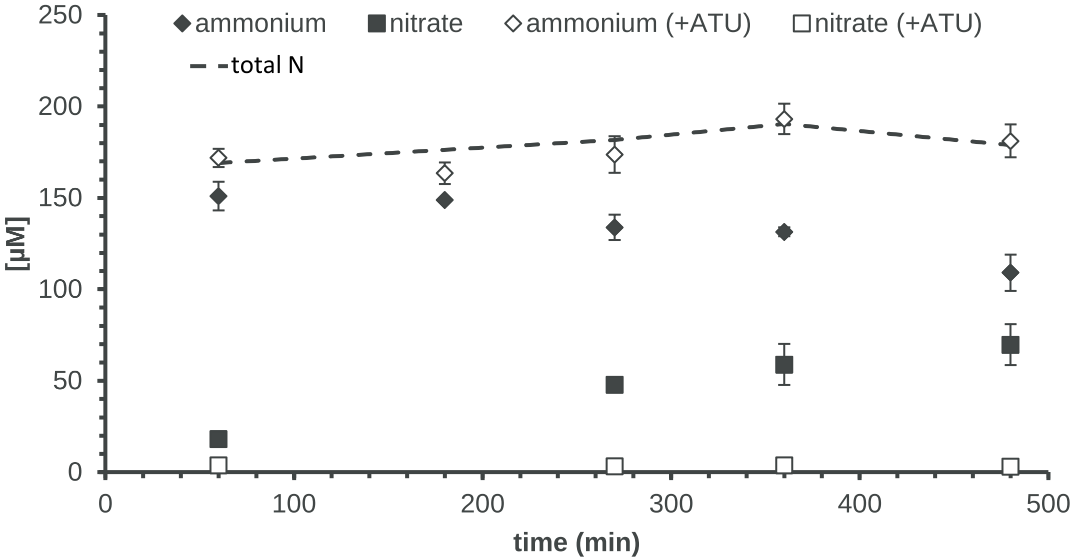
Only in nitrite oxidizers



AMOA PHYLOGENY



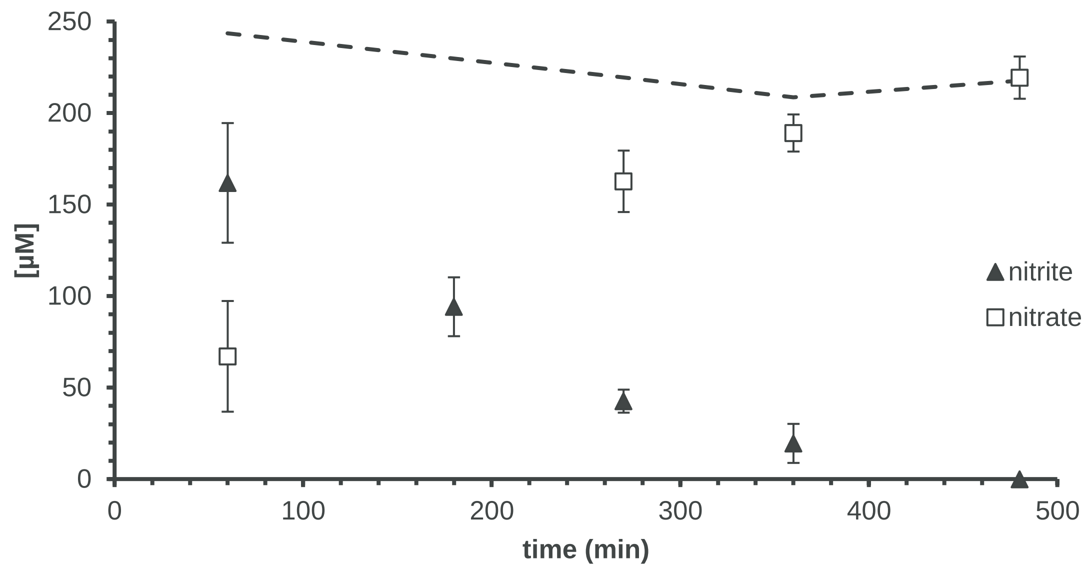
AEROBIC BATCH INCUBATION ASSAYS



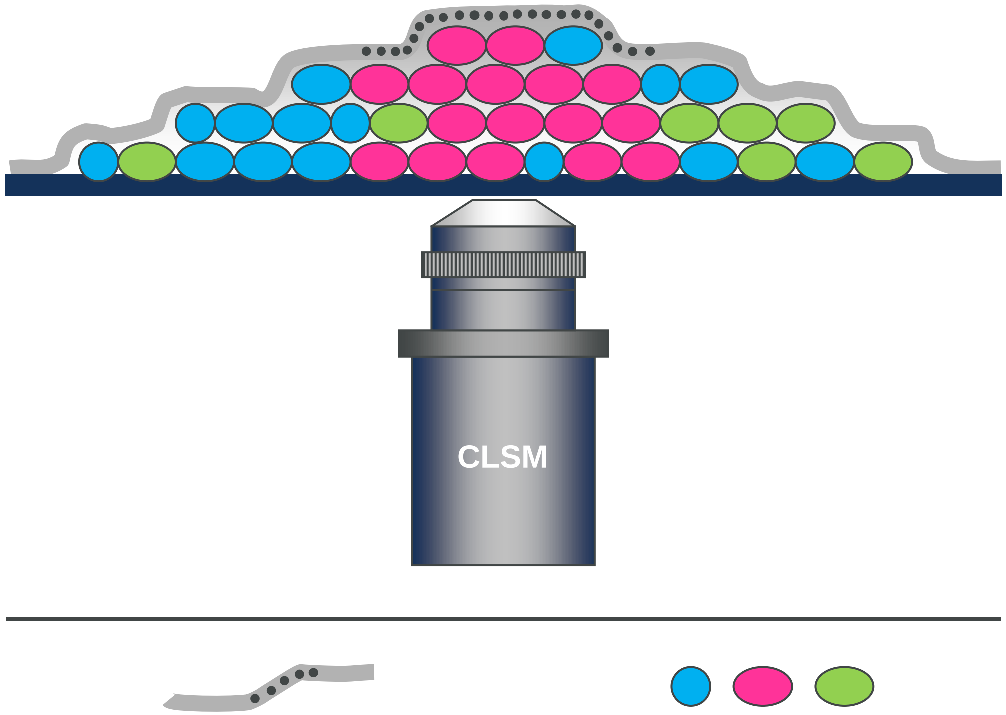
NH_4^+ + ATU

NH_4^+ oxidation

NO_2^- oxidation

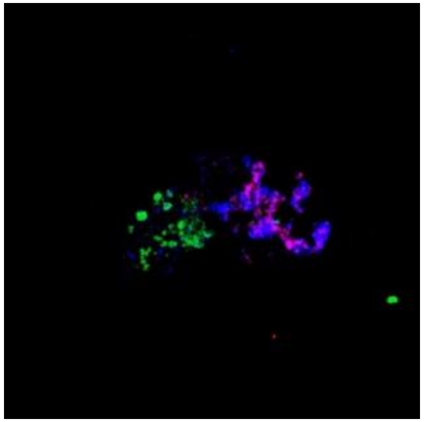


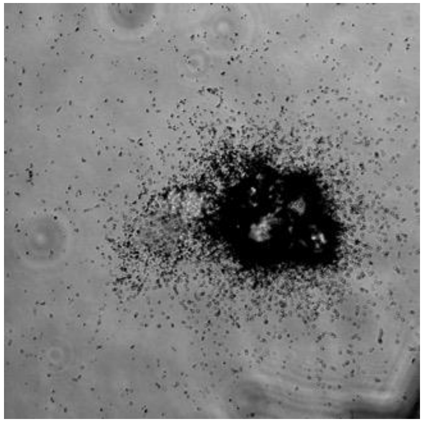
RADIOISOTOPE INCORPORATION (FISH-MAR)

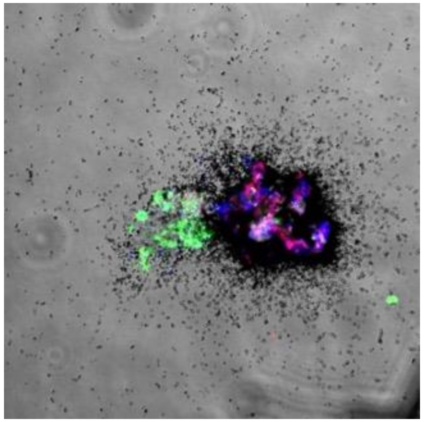


MAR: Cells which took up radioactively labeled substrate induce silver grain formation.

FISH: Bacteria stained with fluorescently labeled probes



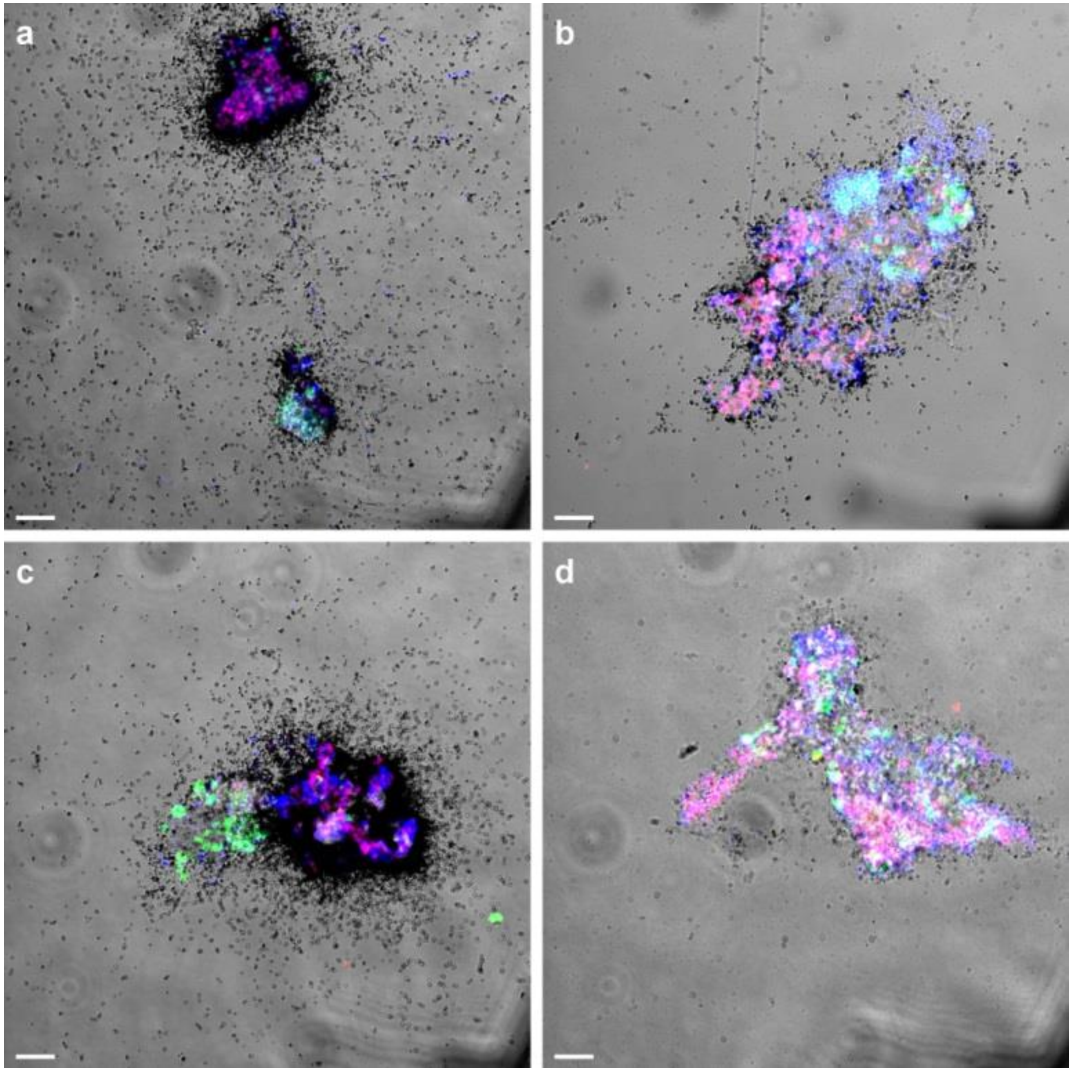




FISH-MAR ON BIOREACTOR ENRICHMENT

NH_4^+

NO_2^-



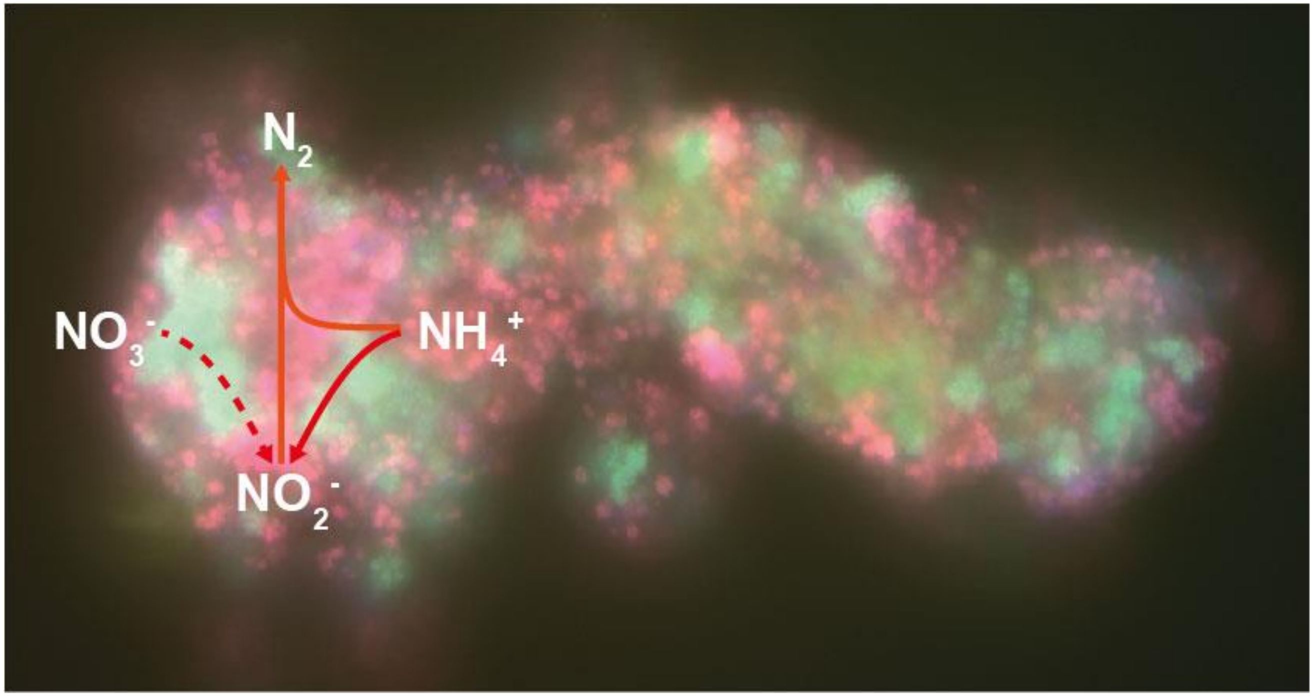
NH_4^+ + ATU

no substrate

red= *Nitrospira*; green = anammox; blue= all bacteria

CONCLUSIONS I

- Novel *Nitrospira* spp. are complete nitrifiers
- Cooperation between anammox and comammox possible



red = anammox; green = *Nitrospira;* blue= all bacteria

Nitrospira:

$NH_4^+ + O_2 \rightarrow NO_2^-$

$NO_3^- + \text{org. C} \rightarrow NO_2^-$

$NH_4^+ + NO_3^- \rightarrow 2\ NO_2^-$

Anammox:

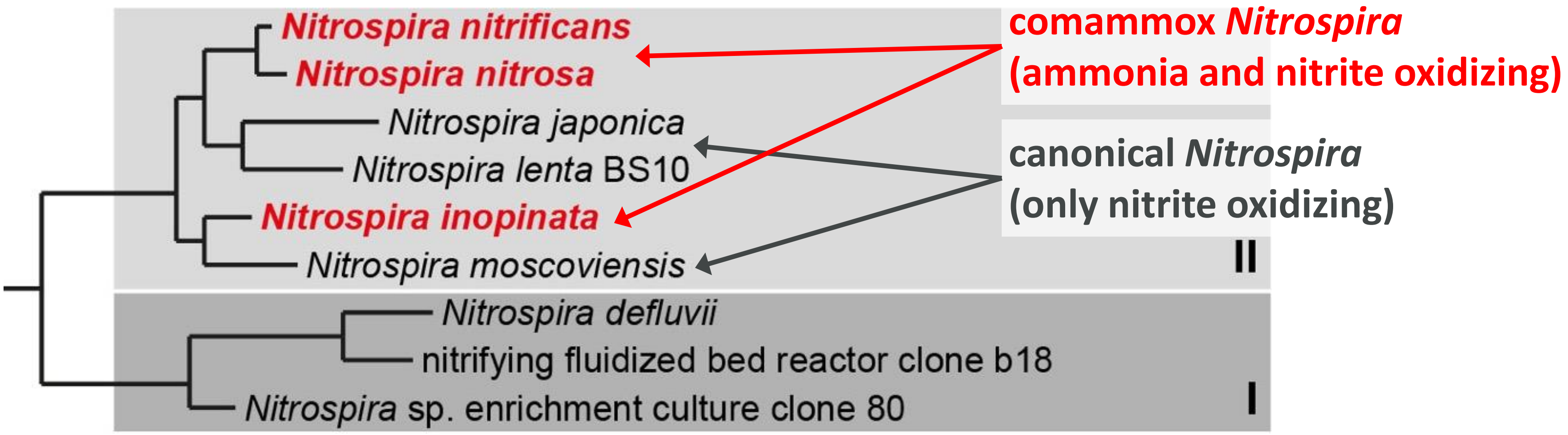
$NH_4^+ + NO_2^- \rightarrow N_2$

OUTLINE

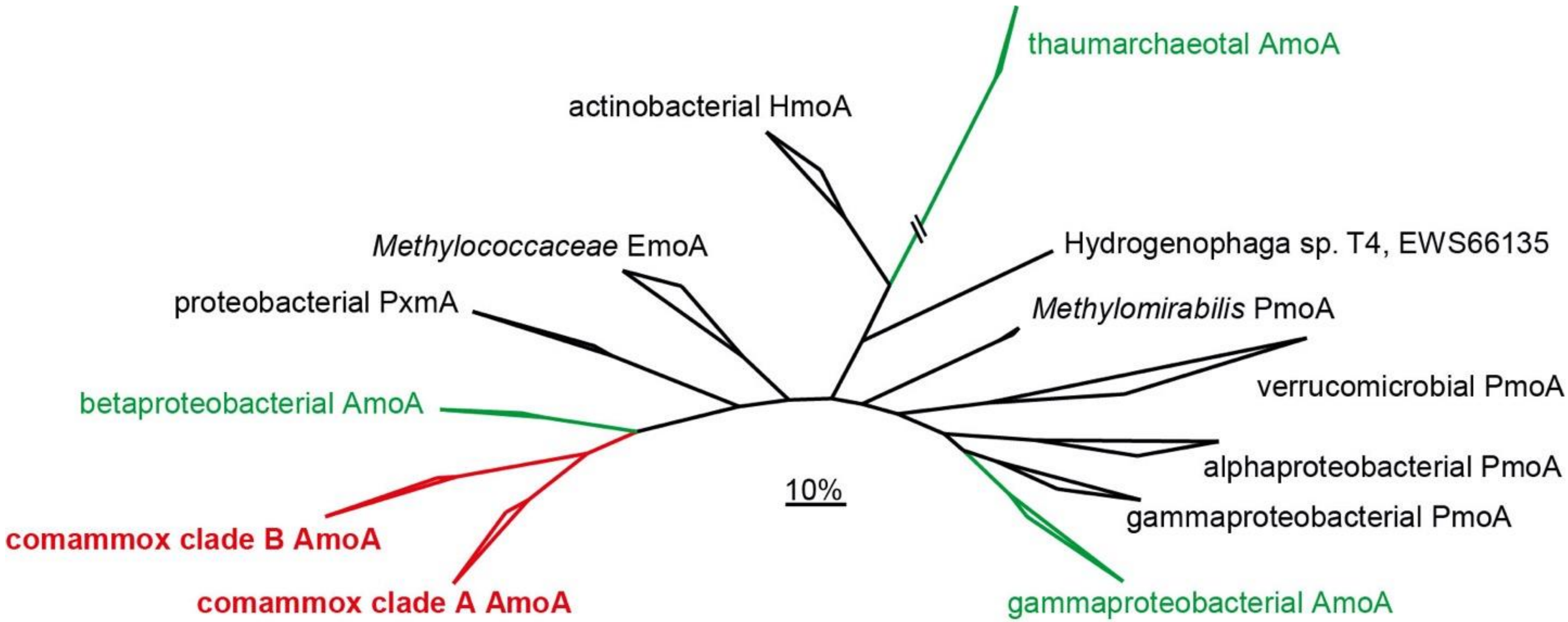
- Complete nitrification by *Nitrospira*
- *In situ* detection of ammonia-oxidizing bacteria

COMAMMOX AND NITRITE-OXIDIZING *NITROSPIRA*

ARE INDISTINGUISHABLE BASED ON 16S rRNA



AMMONIA MONOOXYGENASE SUBUNIT A (AMOA) PHYLOGENY



AMOA-BASED COMAMMOX DETECTION

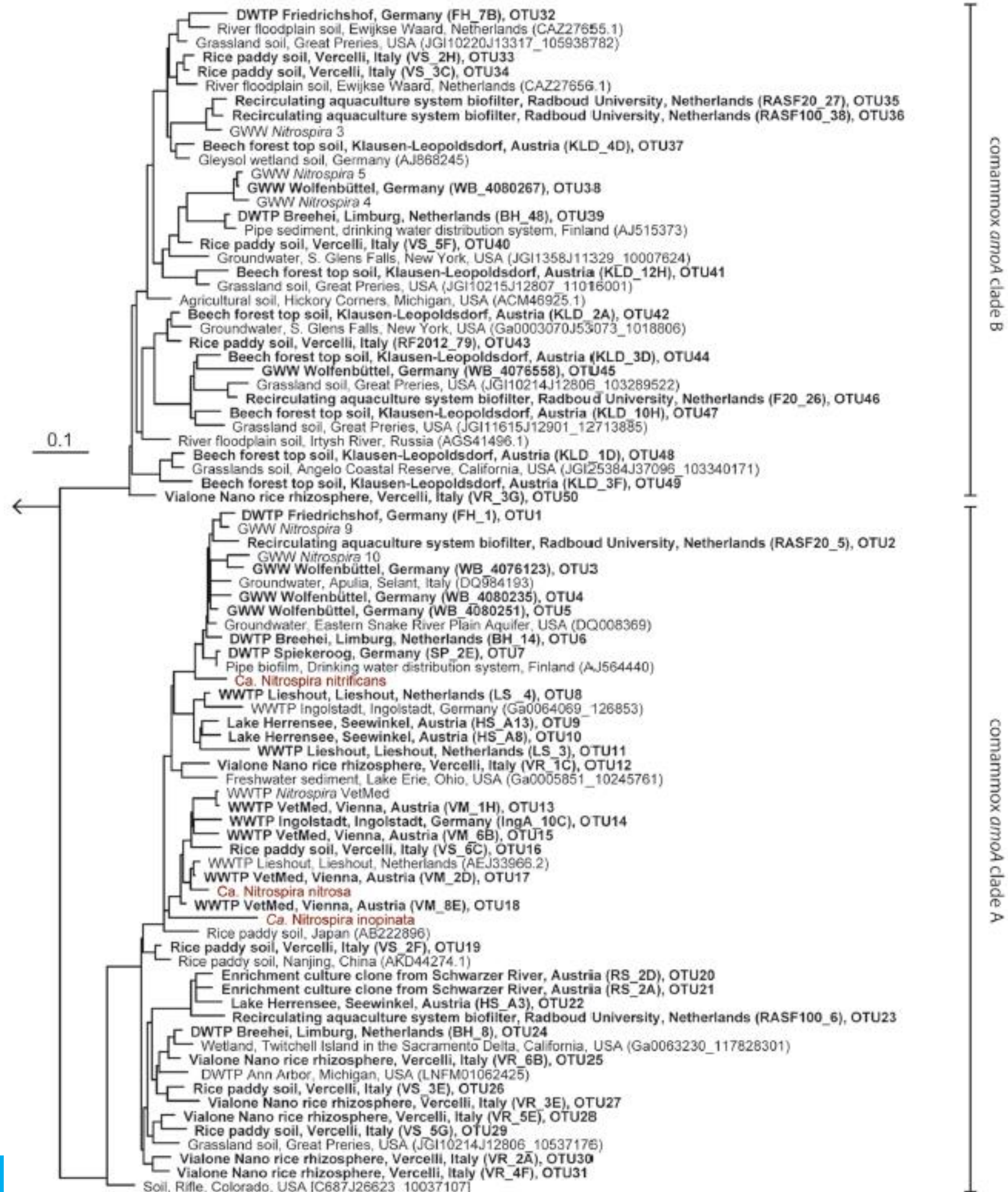
the international water association

frontiers in Microbiology

METHODS
published: 04 August 2017
doi: 10.3389/fmicb.2017.01508

AmoA-Targeted Polymerase Chain Reaction Primers for the Specific Detection and Quantification of Comammox *Nitrospira* in the Environment

Petra Pjevac[1], Clemens Schauberger[1†], Lianna Poghosyan[2†], Craig W. Herbold[1], Maartje A. H. J. van Kessel[2], Anne Daebeler[1], Michaela Steinberger[1], Mike S. M. Jetten[2], Sebastian Lücker[2*], Michael Wagner[1] and Holger Daims[1*]*



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IN SITU AMO PROTEIN LABELING

www.nature.com/ismej

ISME

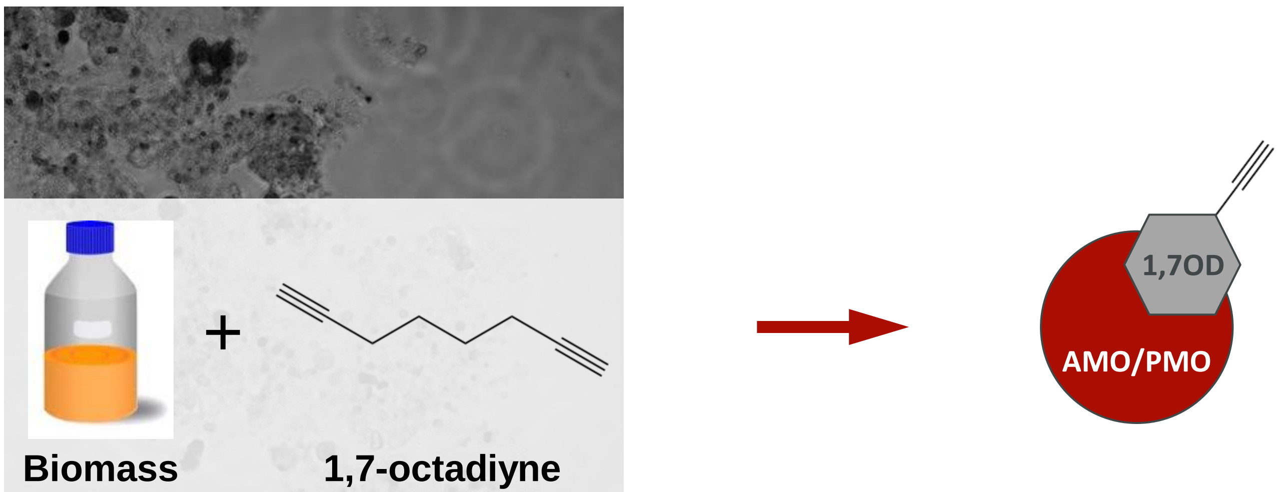
ARTICLE OPEN

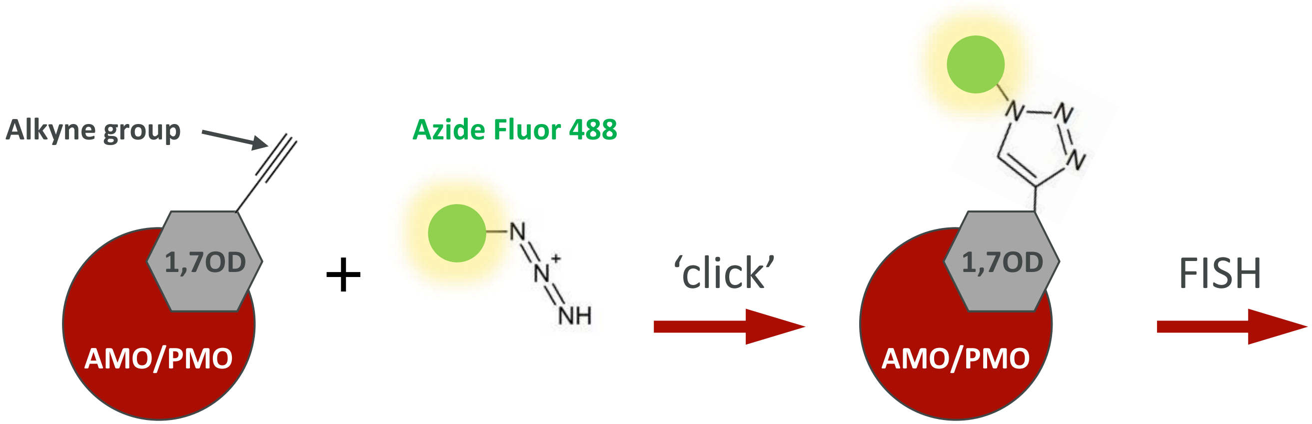
Universal activity-based labeling method for ammonia- and alkane-oxidizing bacteria

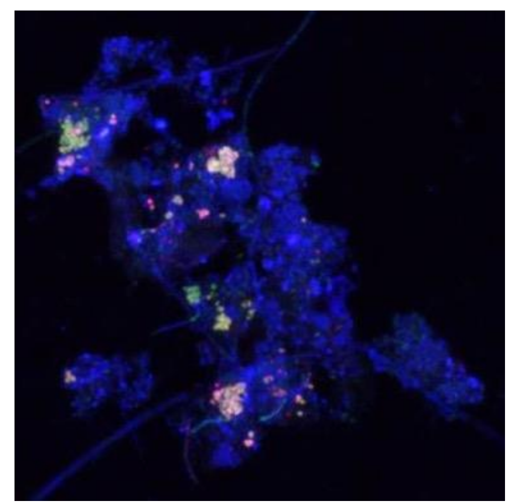
Dimitra Sakoula[1,3], Garrett J. Smith[1], Jeroen Frank[1,2], Rob J. Mesman[1], Linnea F. M. Kop[1], Pieter Blom[1], Mike S. M. Jetten[1,2], Maartje A. H. J. van Kessel[1] and Sebastian Lücker[1]

The ISME Journal (2022) 16:958–971; https://doi.org/10.1038/s41396-021-01144-0

IN SITU DETECTION OF AMMONIA MONOOXYGENASE (AMO)

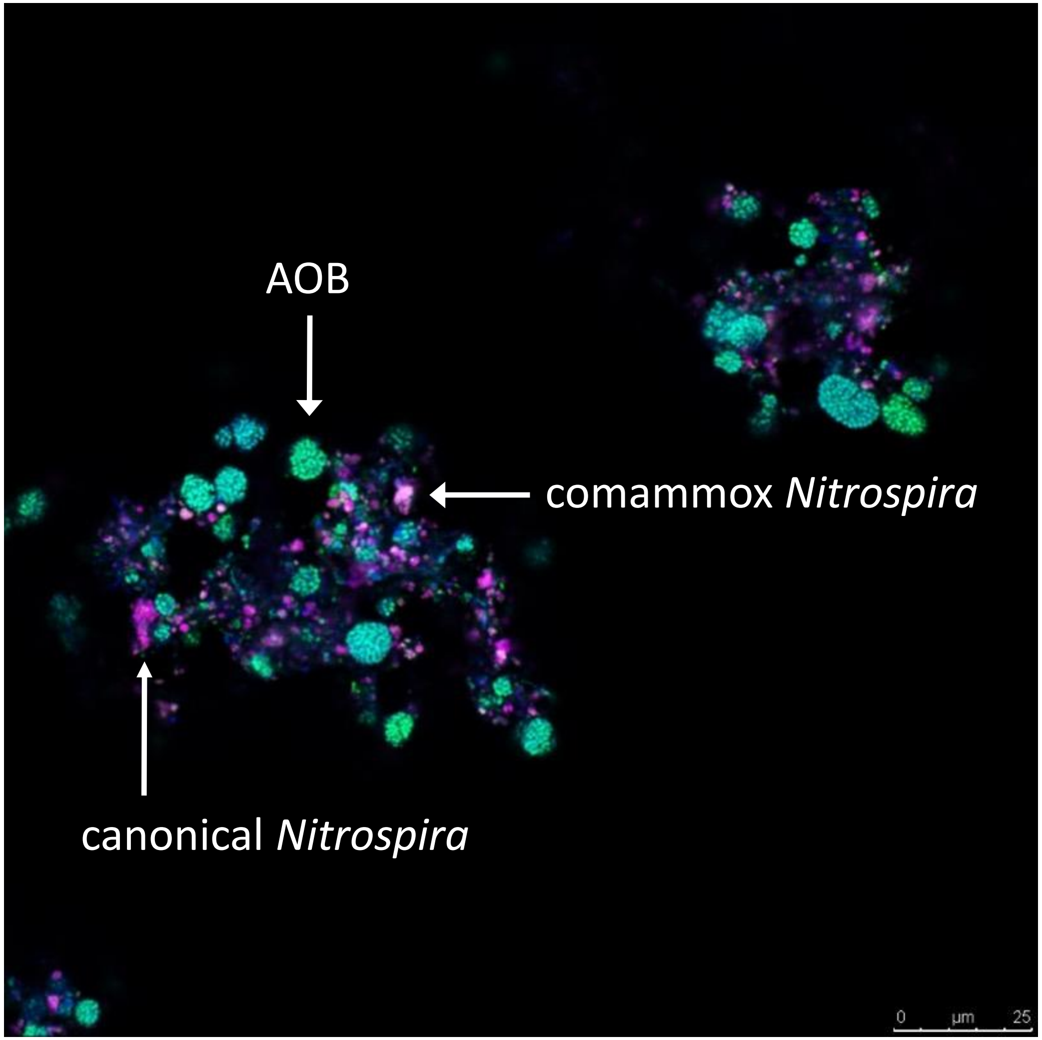






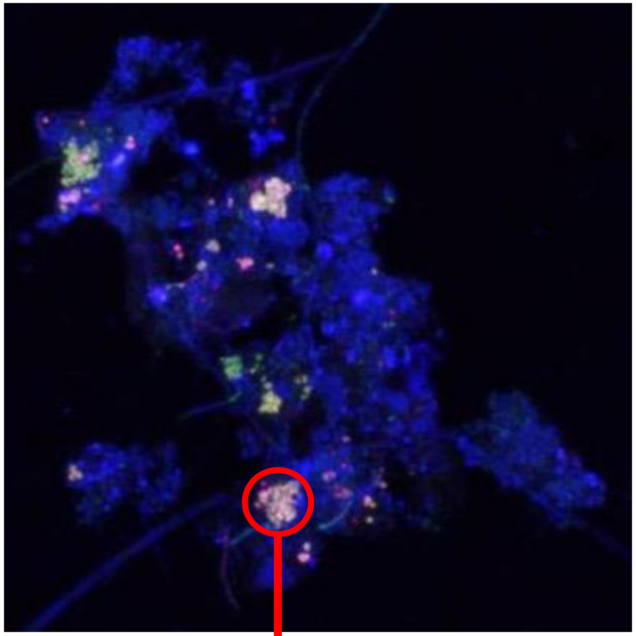
adapted from Bennett *et al.* (2016), AEM 82 (8): 2270-2279

LINKING FUNCTION (AMO LABELING) AND IDENTITY (FISH)



AMO, *Nitrospira*, all bacteria

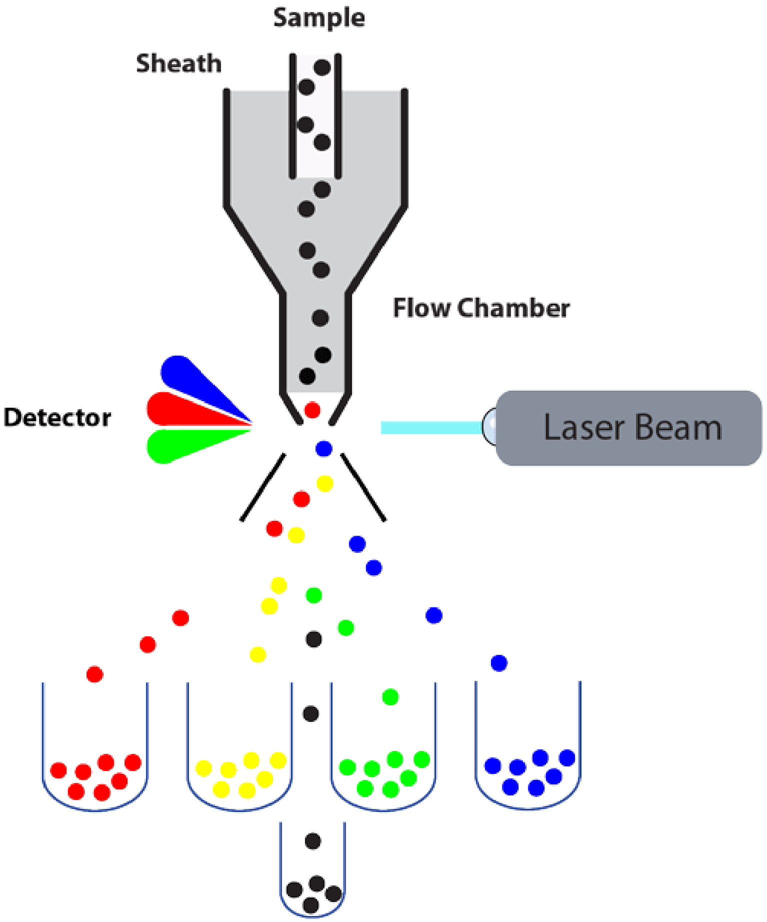
AMO LABELING IN COMBINATION WITH CELL SORTING



Targeted metagenomics

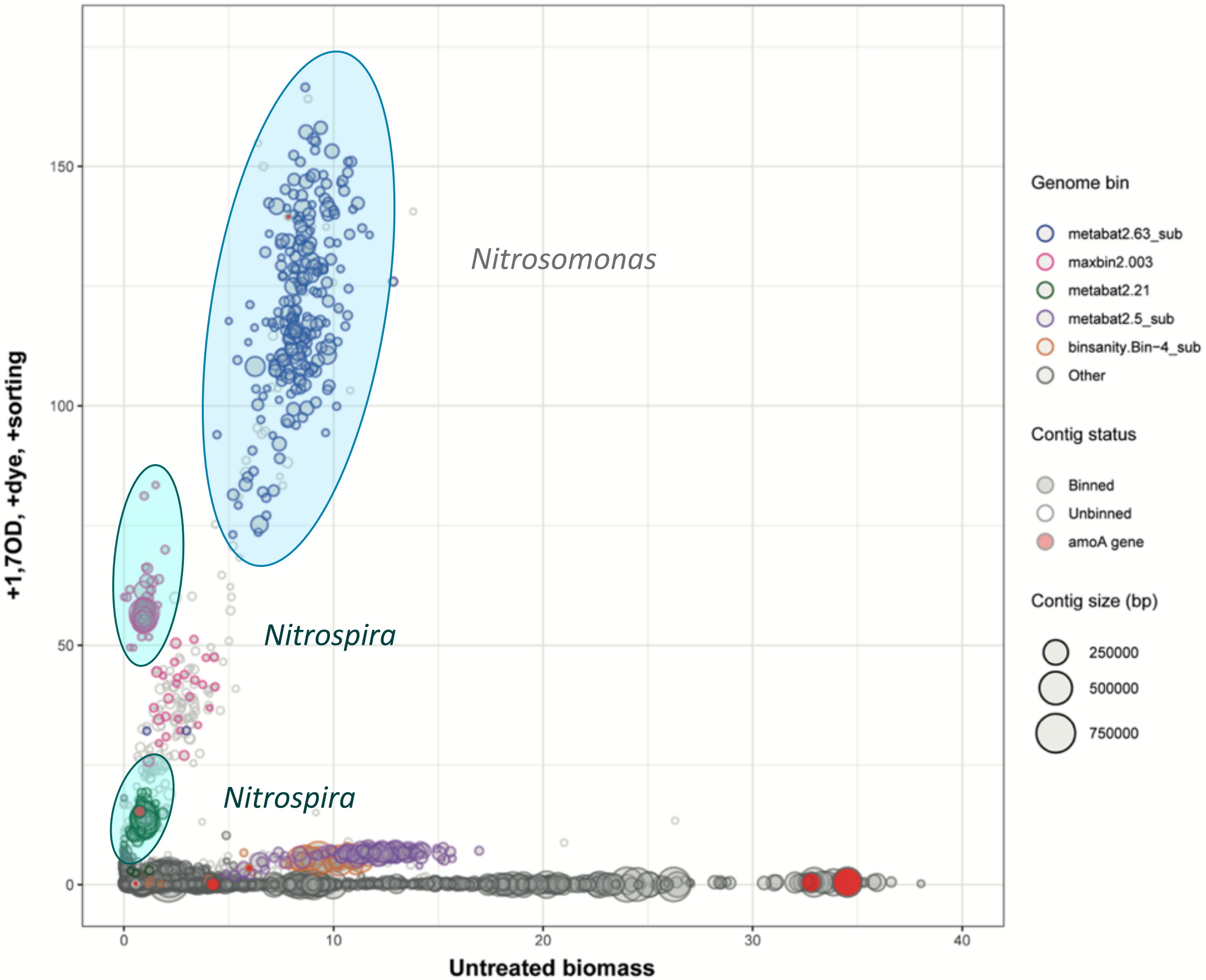
+

Fluorescence-activated cell sorting



https://www.tes.com/lessons/Wpg6sEfF7jdPgg/electrical-impedance

TARGETED METAGENOMICS – NITRIFYING ENRICHMENT CULTURE

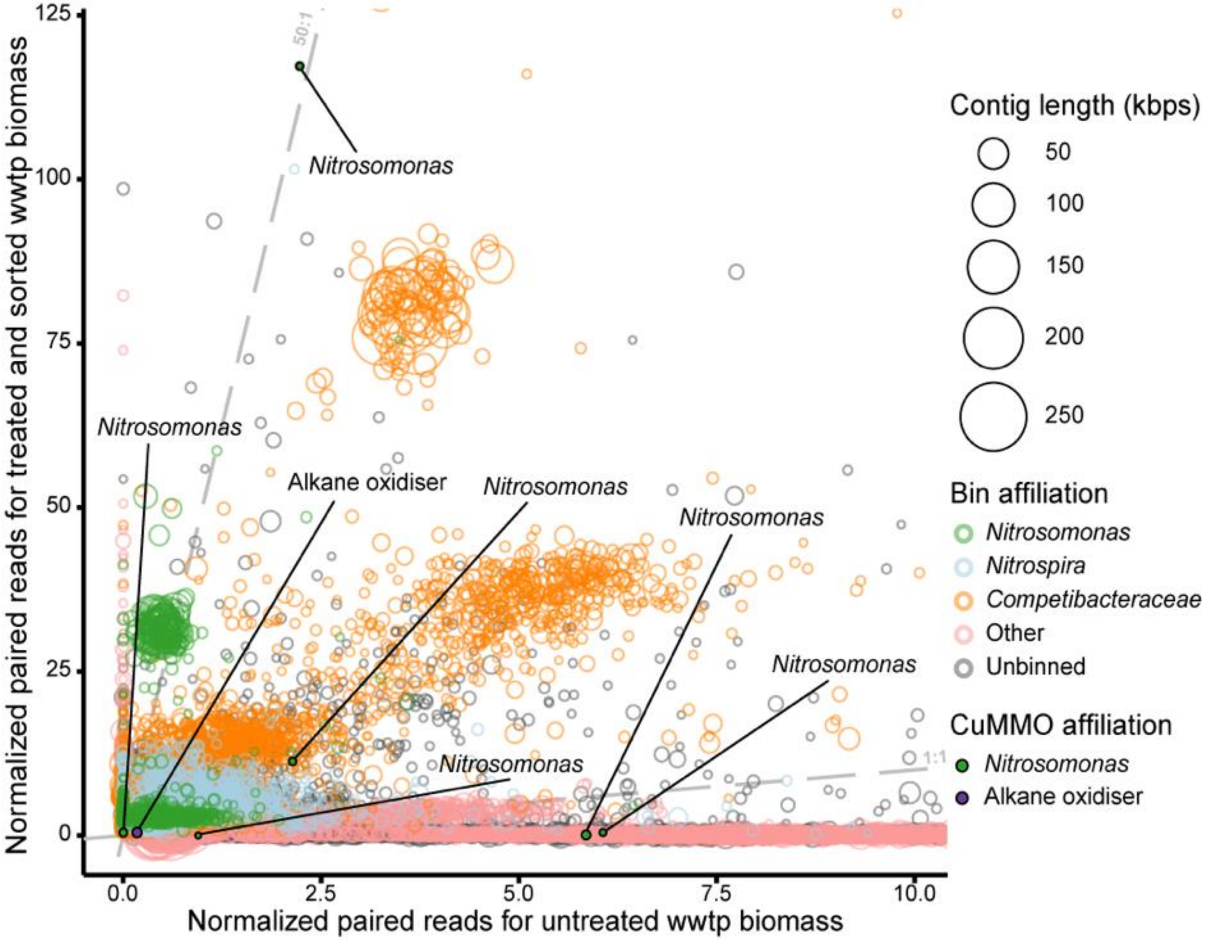


3 high quality MAGs

>92% completeness

<3.7% redundancy

TARGETED METAGENOMICS – ACTIVATED SLUDGE



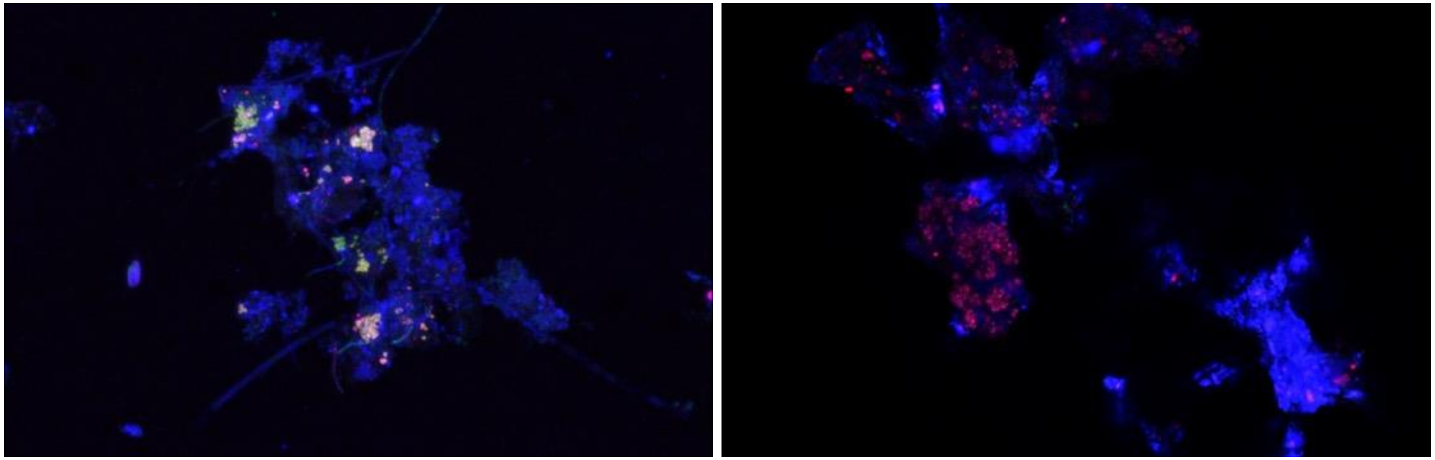
Very low abundance of ammonia oxidizers in original sample
(0.03% of total reads)

Nitrosomonas **high quality MAG**
(188-fold enrichment)

5 MAGs → *Competibacteraceae*

CONCLUSIONS II

- ABPP-based protocol allows
  - specific detection of AMO (and PMO) containing bacteria
  - phylogenetic identification in combination with FISH
  - targeted retrieval of enriched metagenomes



+ 1,7-Octadiyne   - 1,7-Octadiyne

ACKNOWLEDGEMENTS

Radboud Universiteit Nijmegen

Dimitra Sakoula
Maartje van Kessel
Garrett Smith
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Jeroen Frank
Pieter Blom

Guylaine Nuijten
Theo van Alen
Huub Op den Camp
Mike Jetten

universität wien

Petra Pjevac
Holger Daims
Michael Wagner

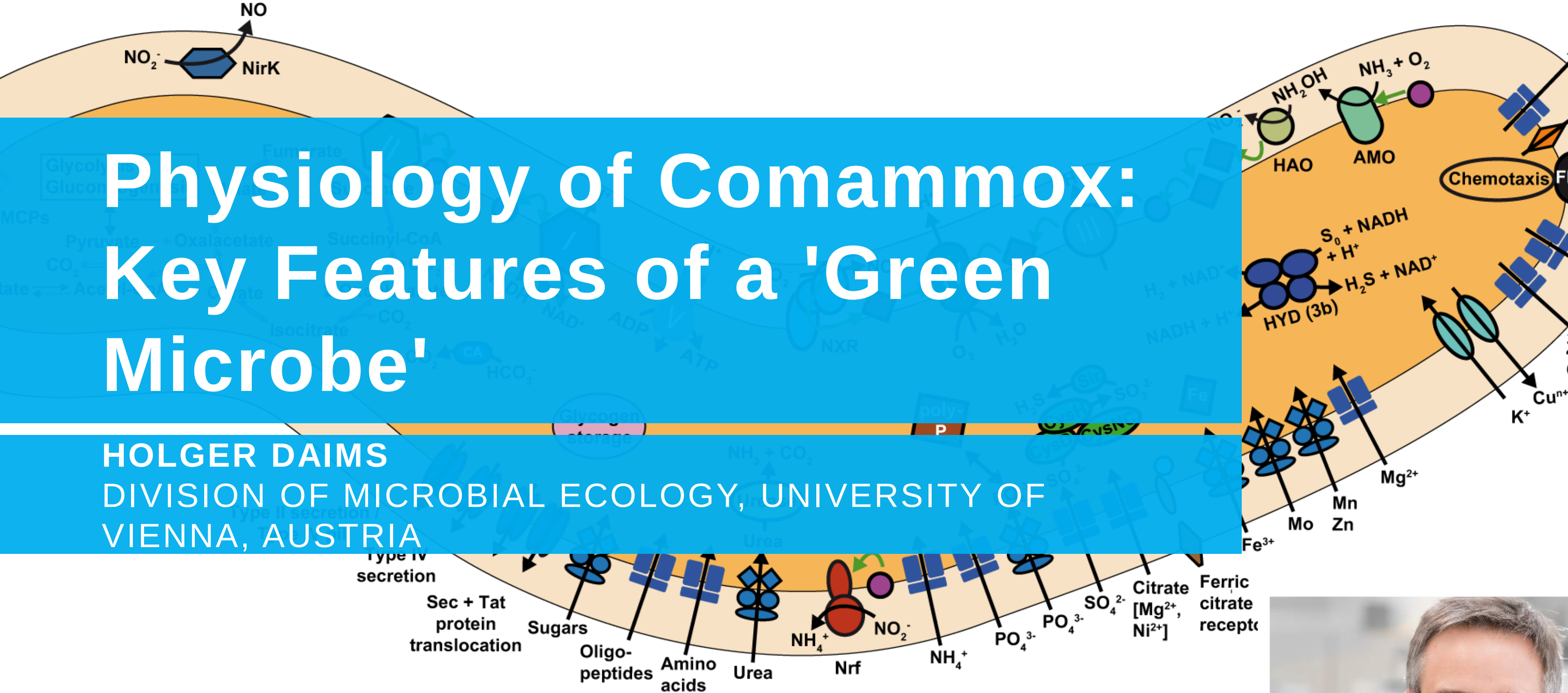
AALBORG UNIVERSITY DENMARK

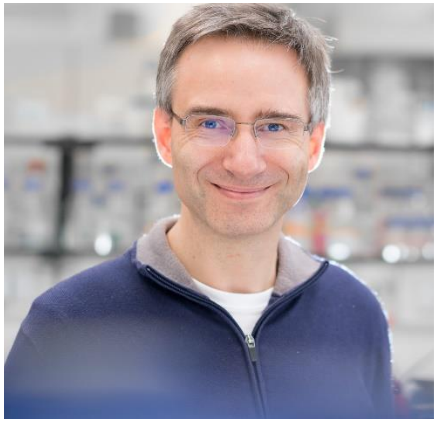
Mads Albersen
Per Halkjær Nielsen

NWO erc SIAM

Physiology of Comammox: Key Features of a 'Green Microbe'

HOLGER DAIMS
DIVISION OF MICROBIAL ECOLOGY, UNIVERSITY OF VIENNA, AUSTRIA





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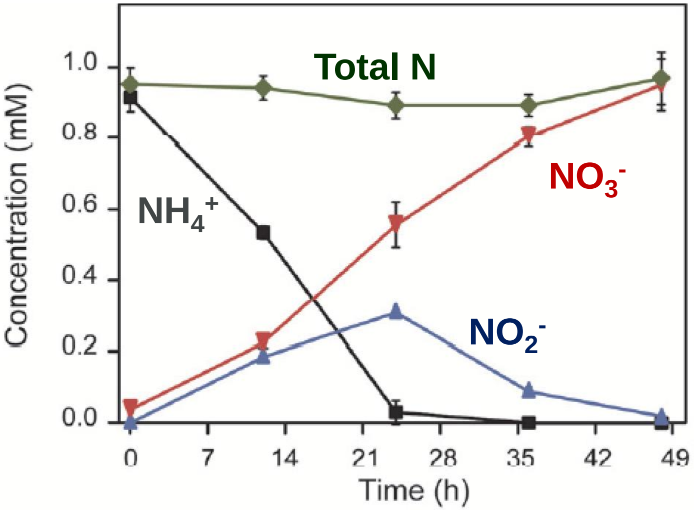
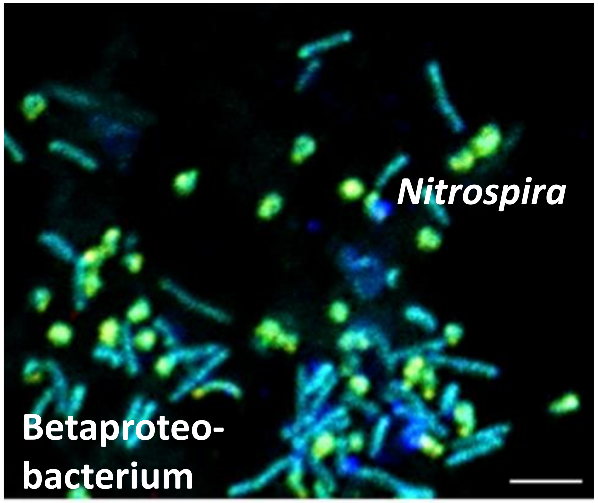
DISCOVERY OF COMAMMOX

Elena Lebedeva

Groundwater from a 1,200 m deep oil exploration well

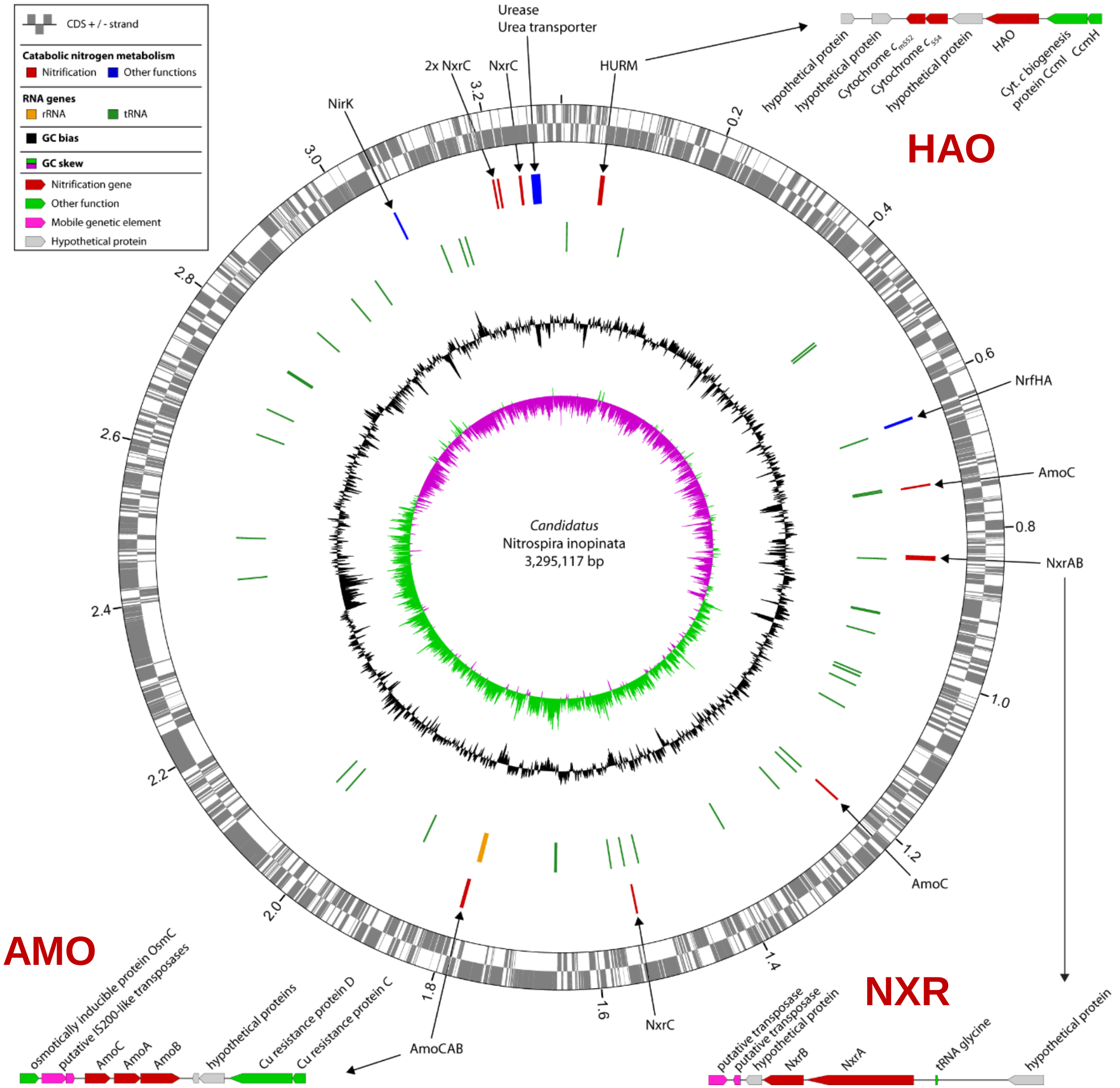
Moderately thermophilic (50°C), nitrifying biofilm





Daims et al. Nature 528: 504-509 (2015)

DISCOVERY OF COMAMMOX



Nitrospira inopinata
Comammox =
Complete ammonia
oxidizer

Daims et al. Nature 528: 504-509 (2015)
Kits et al. Nature 549: 269–272 (2017)

COMAMMOX: SOME PRESSING QUESTIONS

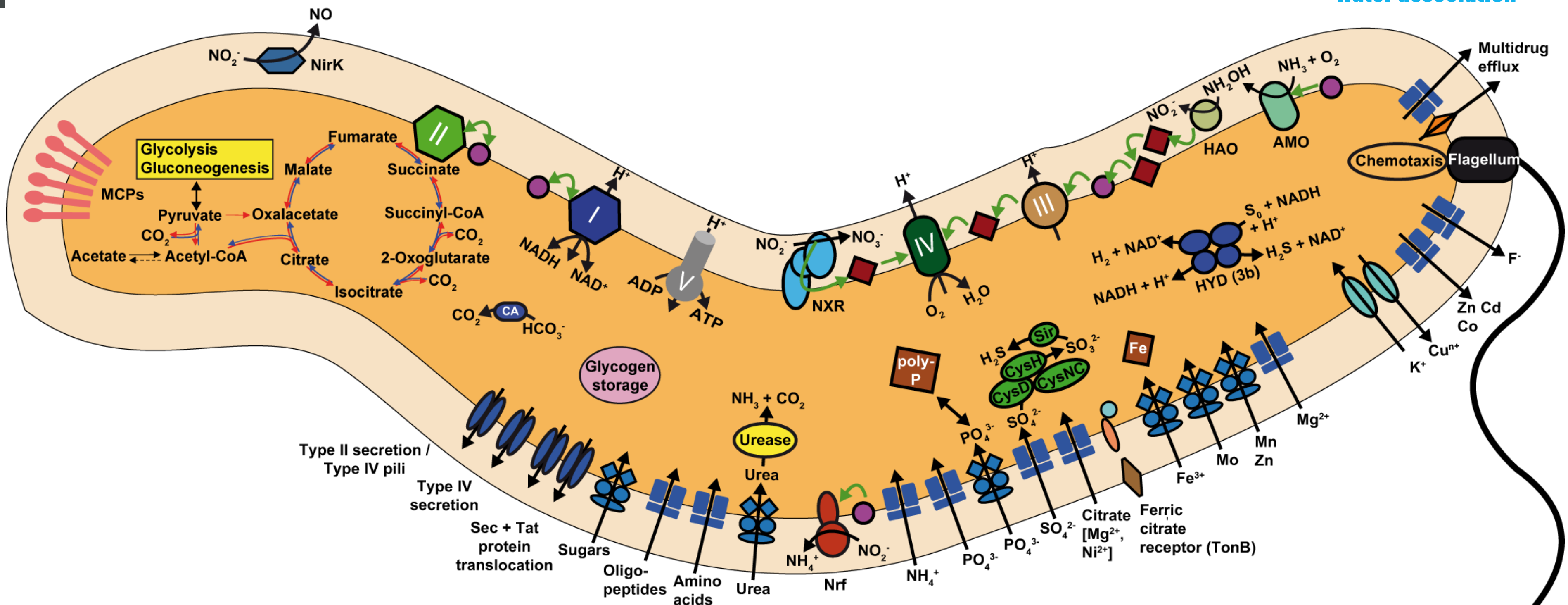


Importance of comammox in agriculture, natural ecosystems, and water treatment plants ? Applications in engineered systems ?

Comammox and greenhouse gas (N_2O) emissions ?

Biochemistry of comammox ? Structure and properties of its key enzymes ?

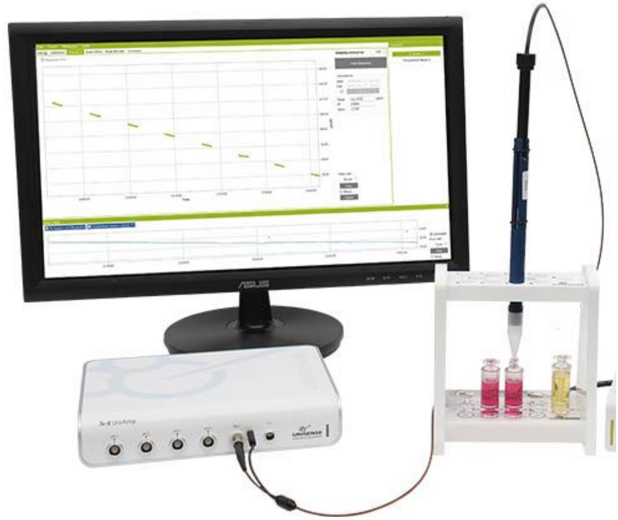
NITROSPIRA INOPINATA METABOLISM



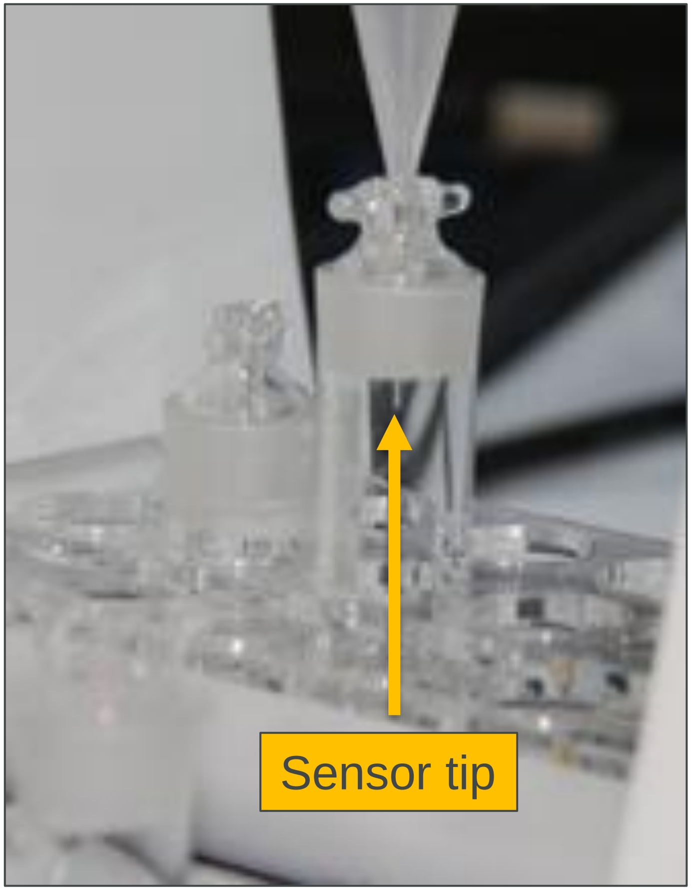
- Complete NH_3 and NO_2^- oxidation pathways
- Can use urea as source of NH_3 and CO_2
- CO_2 fixation by rTCA cycle (O_2 tolerant version)
- Cannot grow on NO_2^- alone
- Potentially capable of respiratory ammonification
- Can oxidize formate (but: no growth, poor affinity)
- Can form glycogen and poly-P storage

Daims et al. Nature 528: 504-509 (2015)

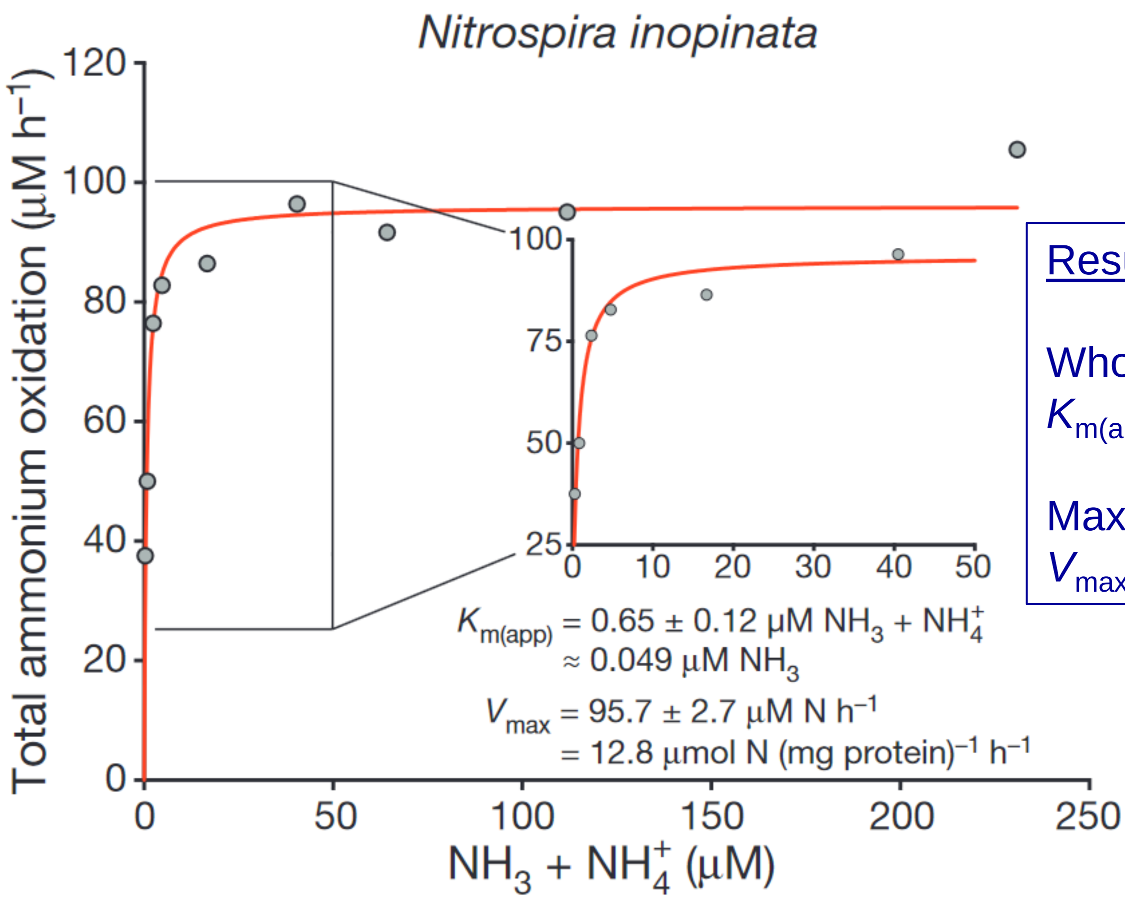
MICRORESPIROMETRY TO MEASURE NITRIFICATION KINETICS



Additional port for adding substrates



NH_3 OXIDATION KINETICS



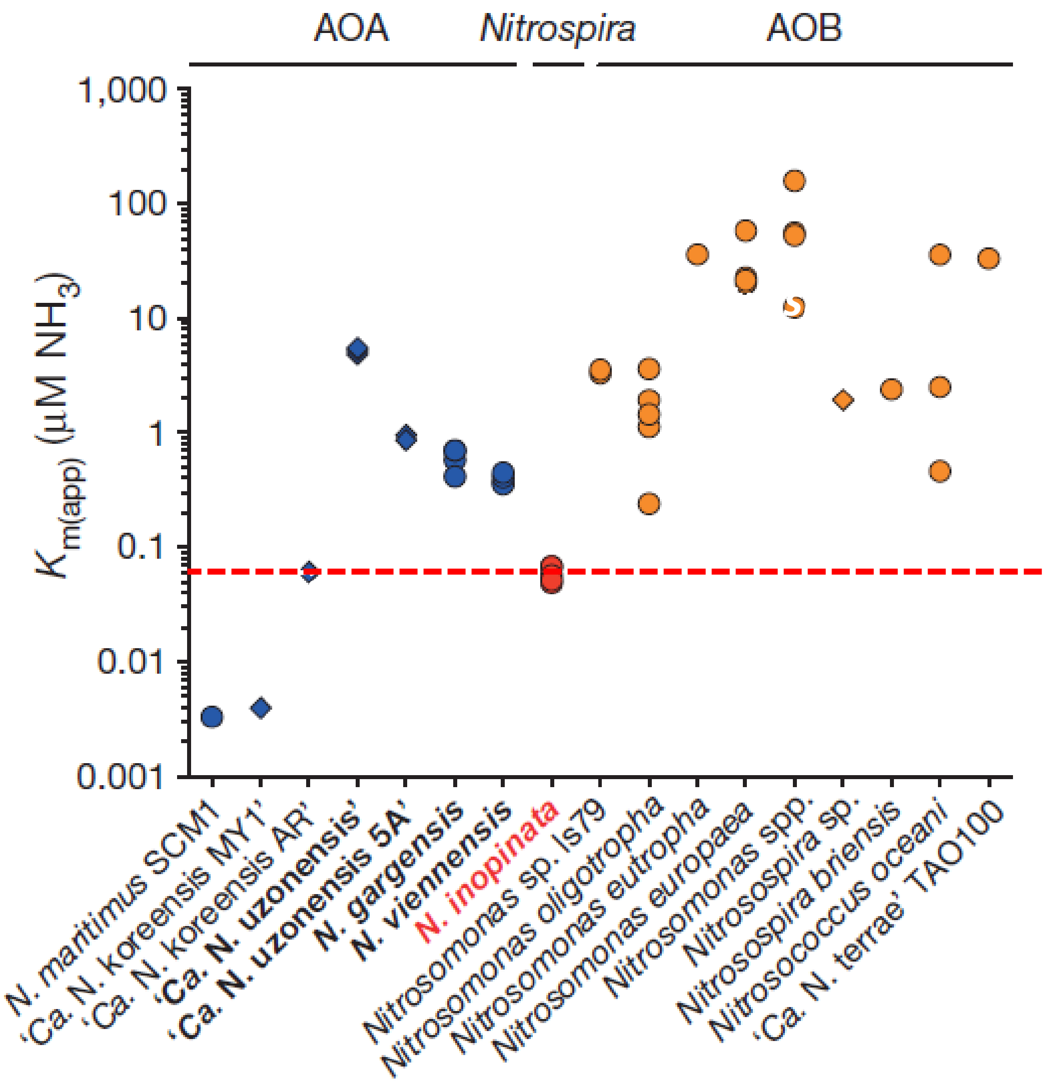
<u>Results from all replicates:</u>

Whole-cell affinity constant:
$K_{m(app)}$ = 63 ± 10 nM NH_3

Maximum oxidation rate:
V_{max} = 14.8 ± 1.2 µmol N (mg protein^{-1}) h^{-1}

Kits et al. Nature 549: 269–272 (2017)

COMAMMOX IS HIGHLY COMPETITIVE AT LOW [NH_3]

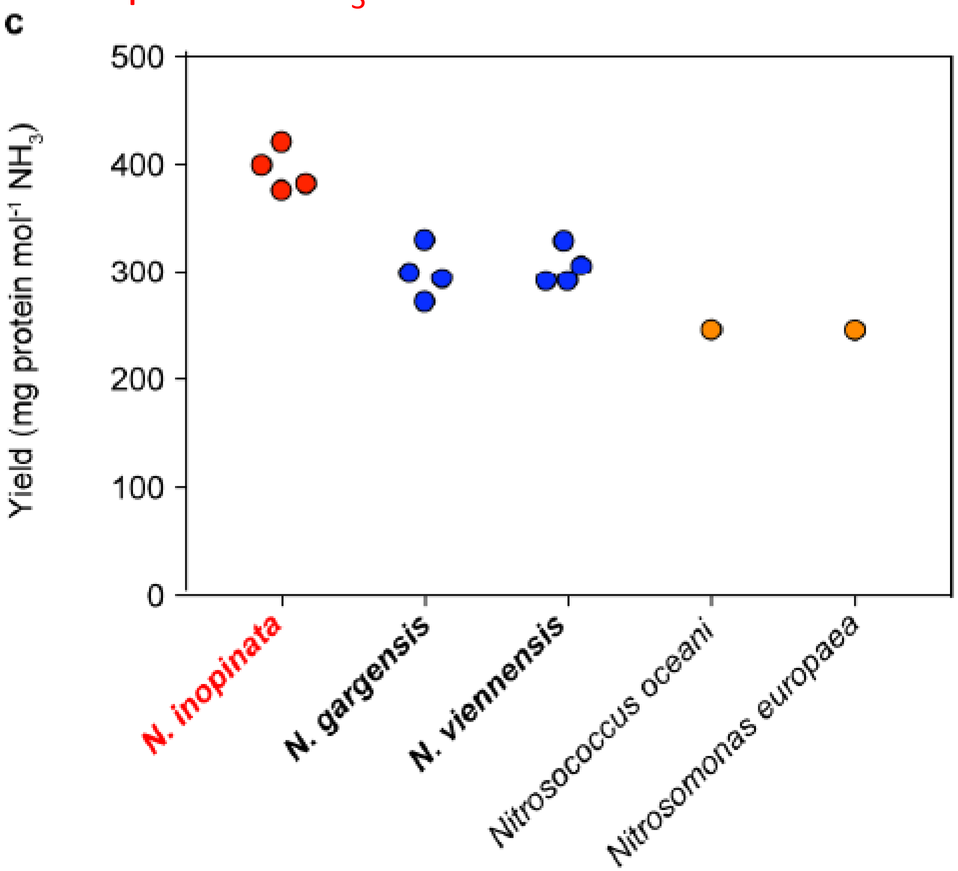


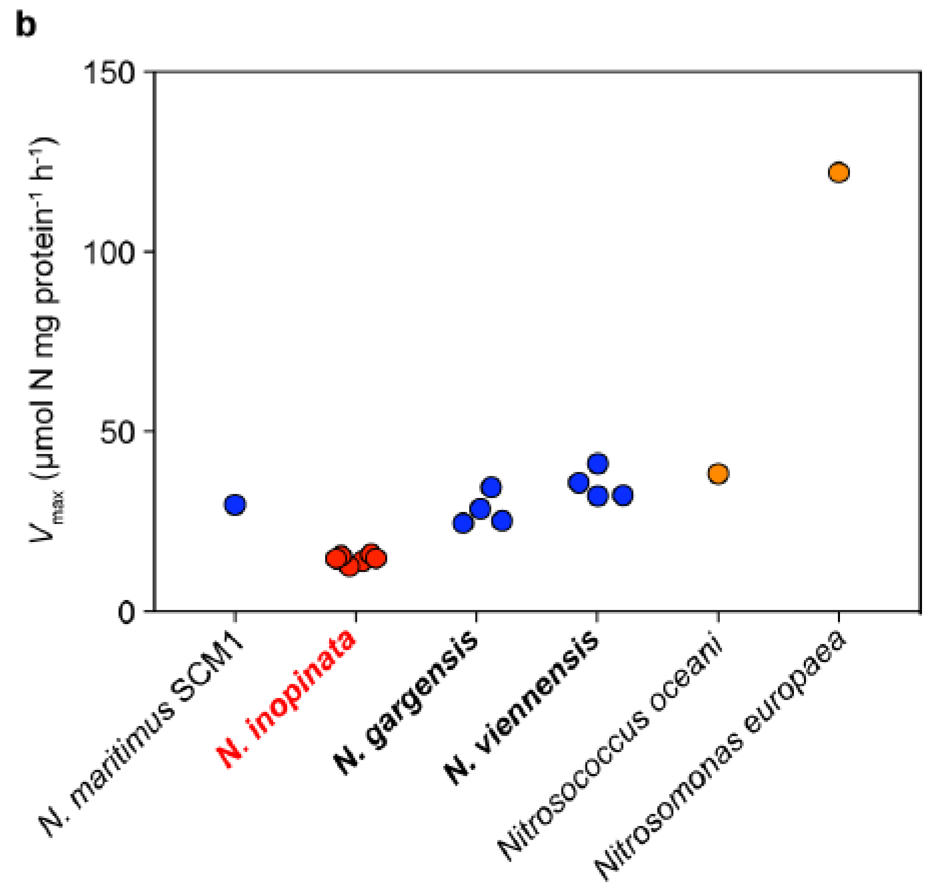
Kits et al.
Nature 549: 269–272 (2017)

COMAMMOX IS A YIELD STRATEGIST

Yield: how much biomass is formed per mol NH_3 oxidized

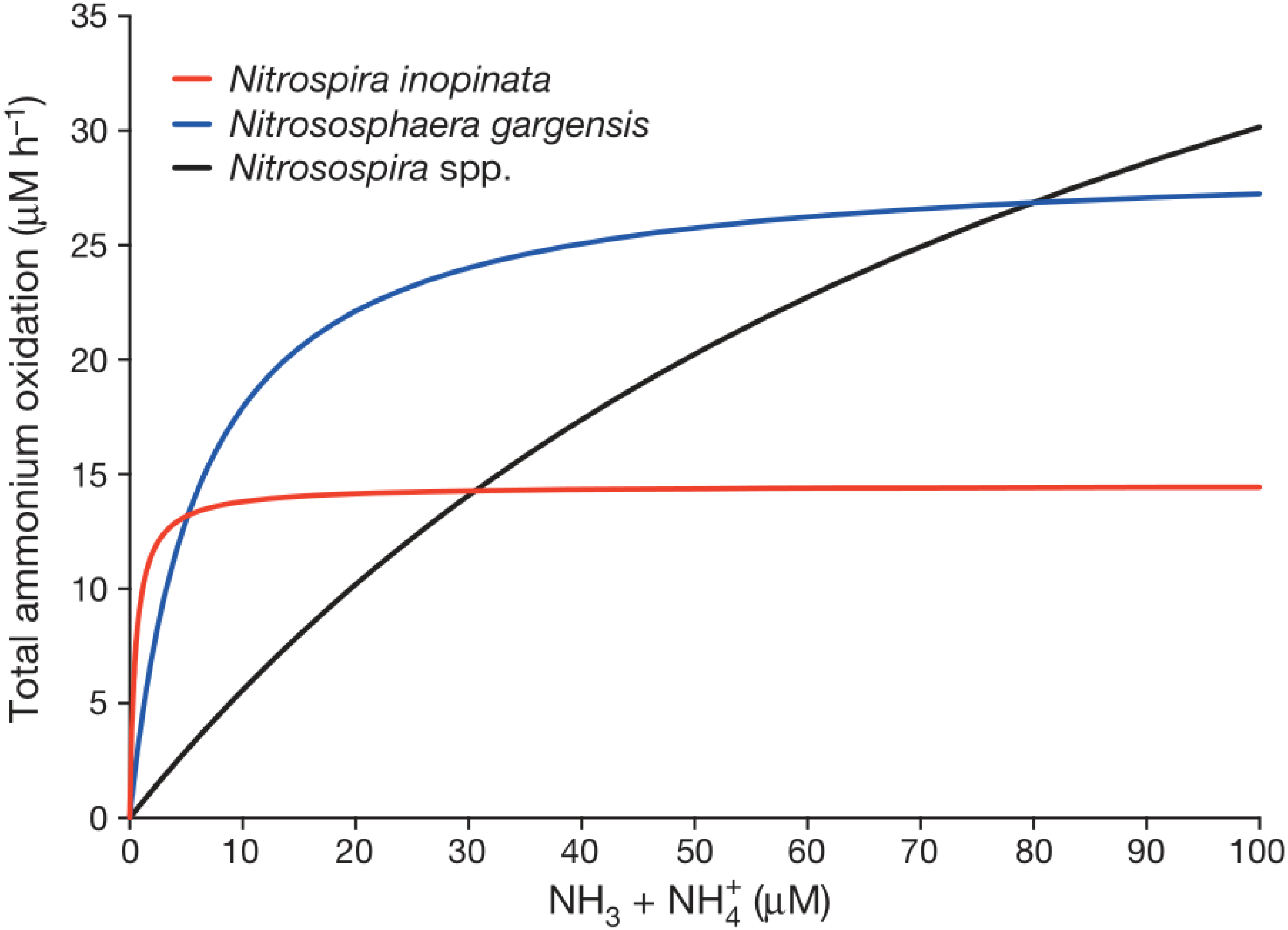
Rate: how fast can the organism grow





Kits et al. Nature 549: 269–272 (2017)

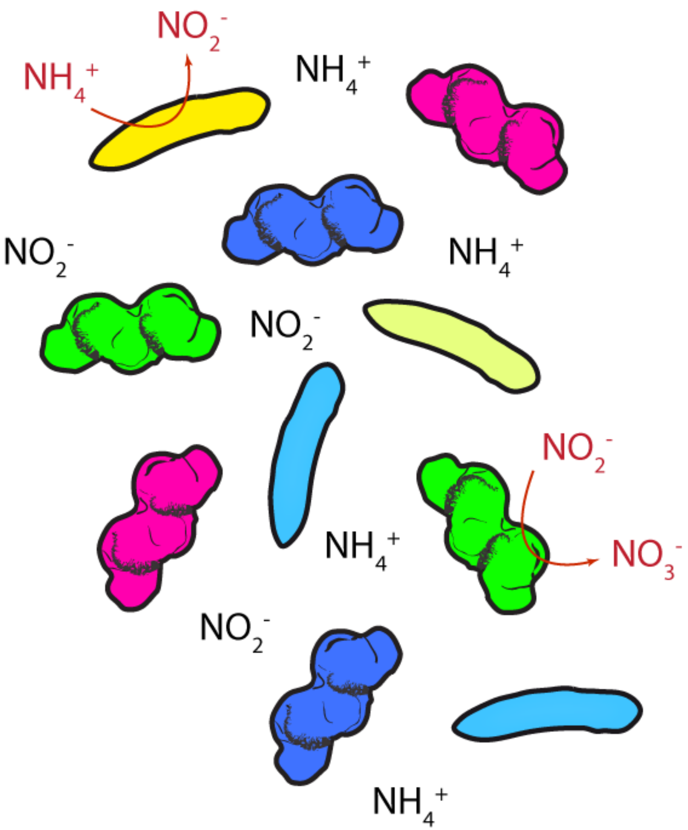
COMAMMOX' WINDOW OF OPPORTUNITY



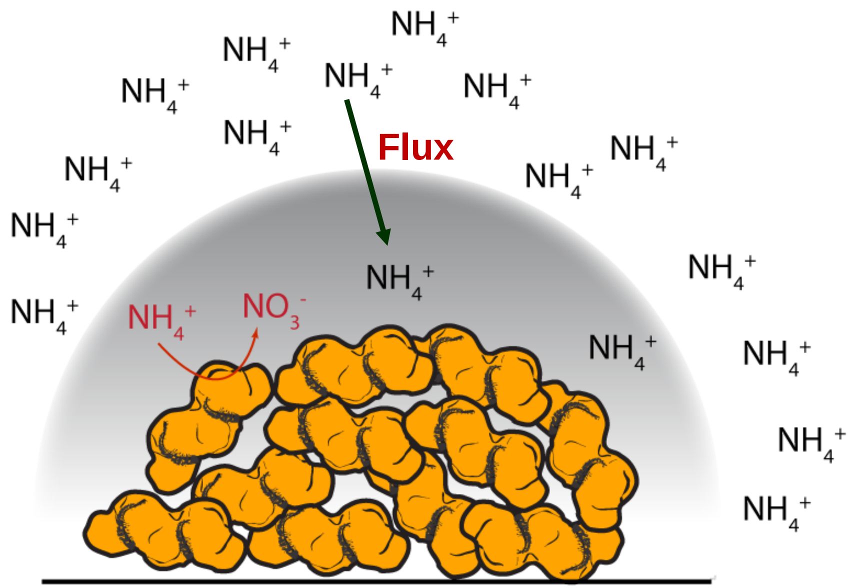
Kits et al. Nature 549: 269–272 (2017)

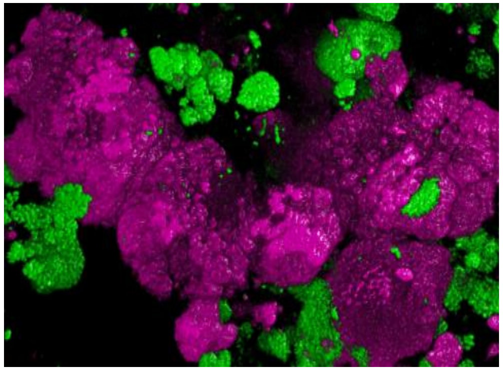
RATE VERSUS YIELD STRATEGIES

Rate strategy



Yield strategy (comammox)





Nitrospira
in biofilm

Kits *et al*. 2017, Nature 549: 269-272
Kreft 2004, Microbiology 150:2751-2760
Costa et al. 2006, Trends. Microbiol. 14:213-219

MORE KINETIC DATA ARE NEEDED !

the international water association

Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle

K. Dimitri Kits[1], Christopher J. Sedlacek[1], Elena V. Lebedeva[2], Ping Han[1], Alexandr Bulaev[2], Petra Pjevac[1], Anne Daebeler[1], Stefano Romano[1], Mads Albertsen[3], Lisa Y. Stein[4], Holger Daims[1] & Michael Wagner[1]

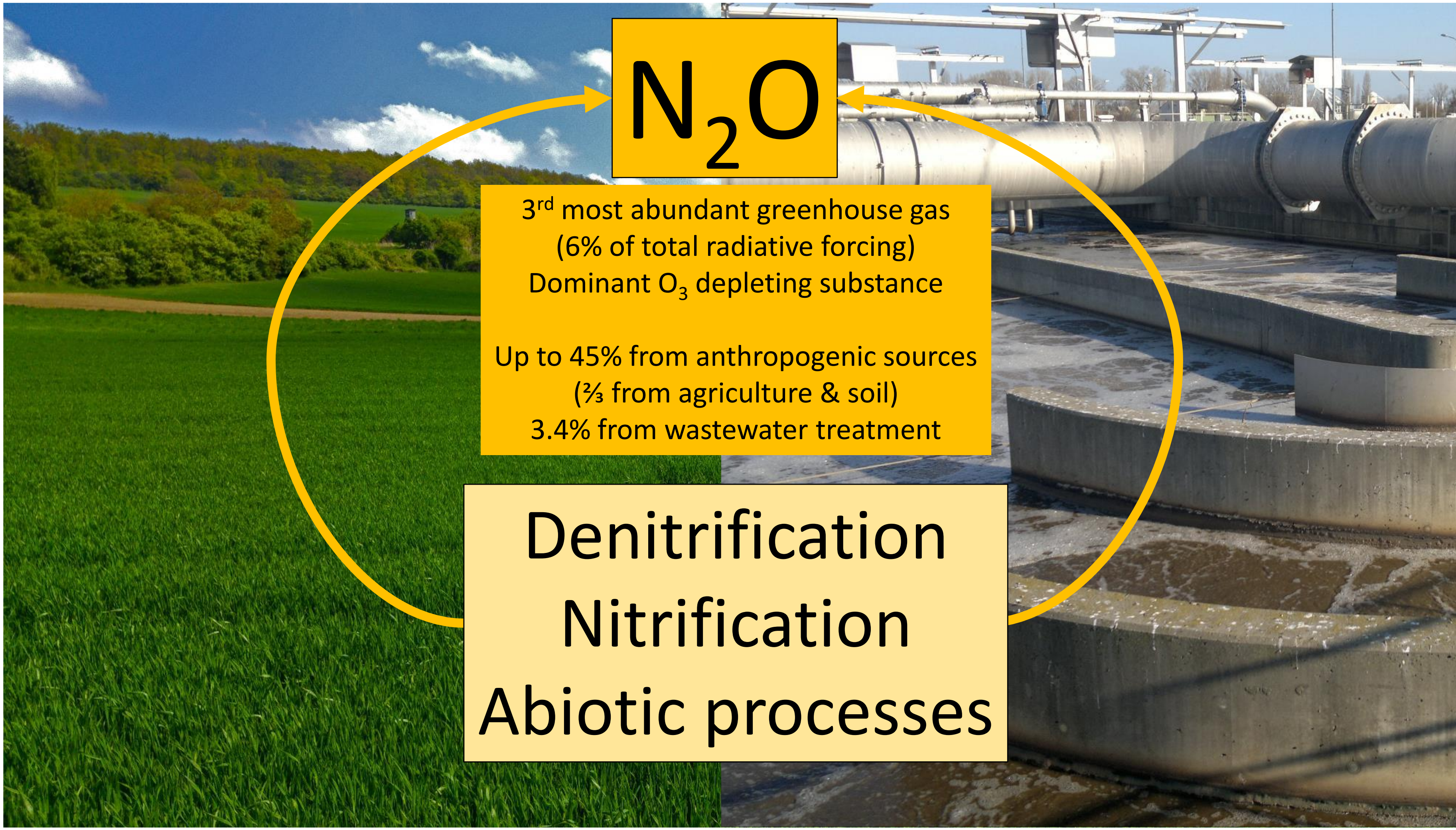
Enrichment and physiological characterization of a novel comammox *Nitrospira* indicates ammonium inhibition of complete nitrification

Dimitra Sakoula[1,3] · Hanna Koch[1] · Jeroen Frank[1,2] · Mike S. M. Jetten[1,2] · Maartje A. H. J. van Kessel[1] · Sebastian Lücker[1]

Organism	$K_{m(app)}$ NH_3	$K_{m(app)}$ NO_2^-	V_{max}
Nitrospira inopinata	63 ± 10 nM	449 ± 66 µM	15 ± 1 µM
Nitrospira kreftii	40 ± 10 nM	13 ± 4 µM	83 ± 15 µM
Nitrospira moscoviensis (*)	---	9 µM	18 µM

* Nowka et al. App. Environ. Microbiol. 81: 745-753 (2015)

N_2O: A MAJOR GREENHOUSE GAS



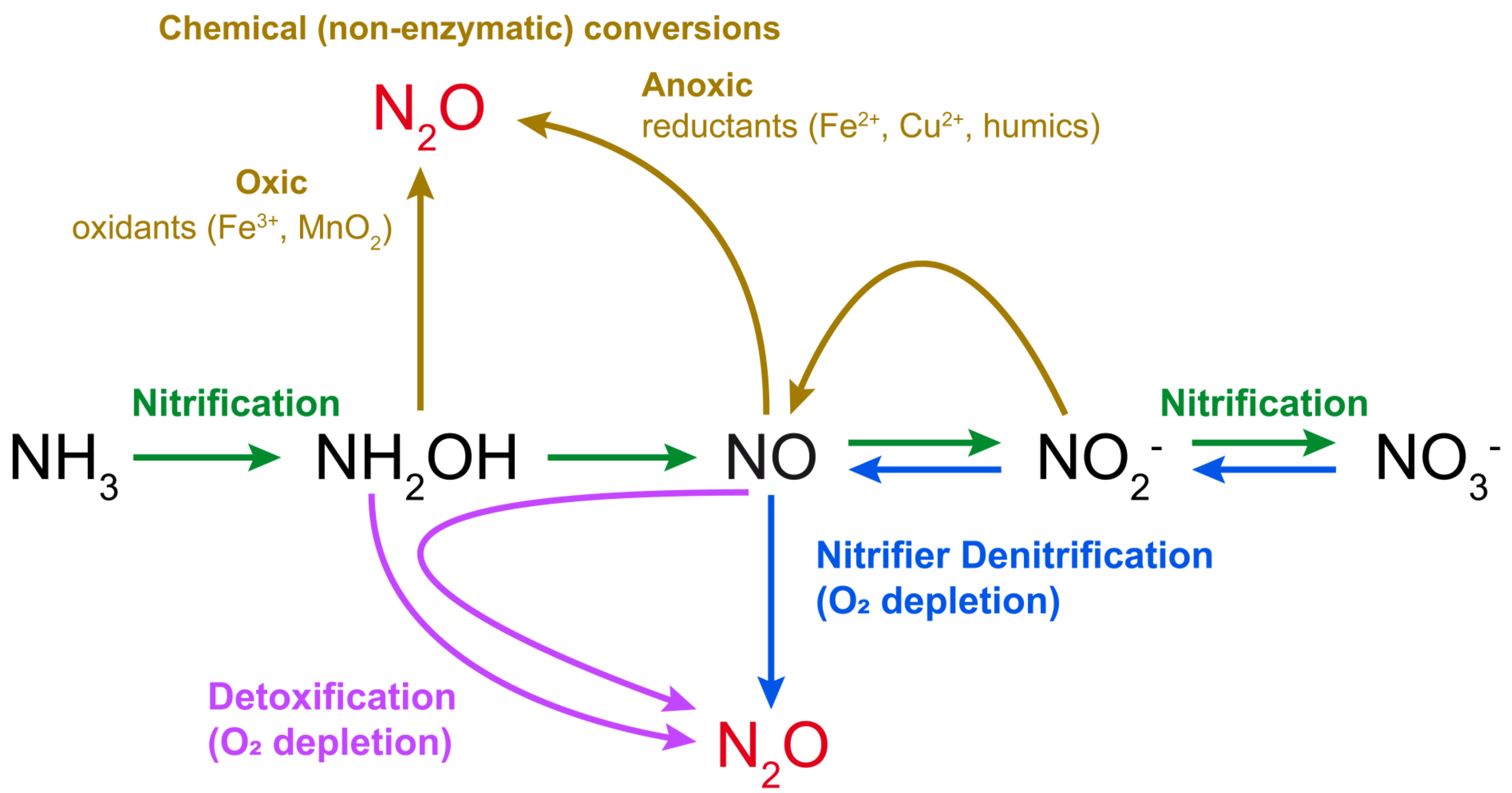
N_2O

3rd most abundant greenhouse gas
(6% of total radiative forcing)
Dominant O_3 depleting substance

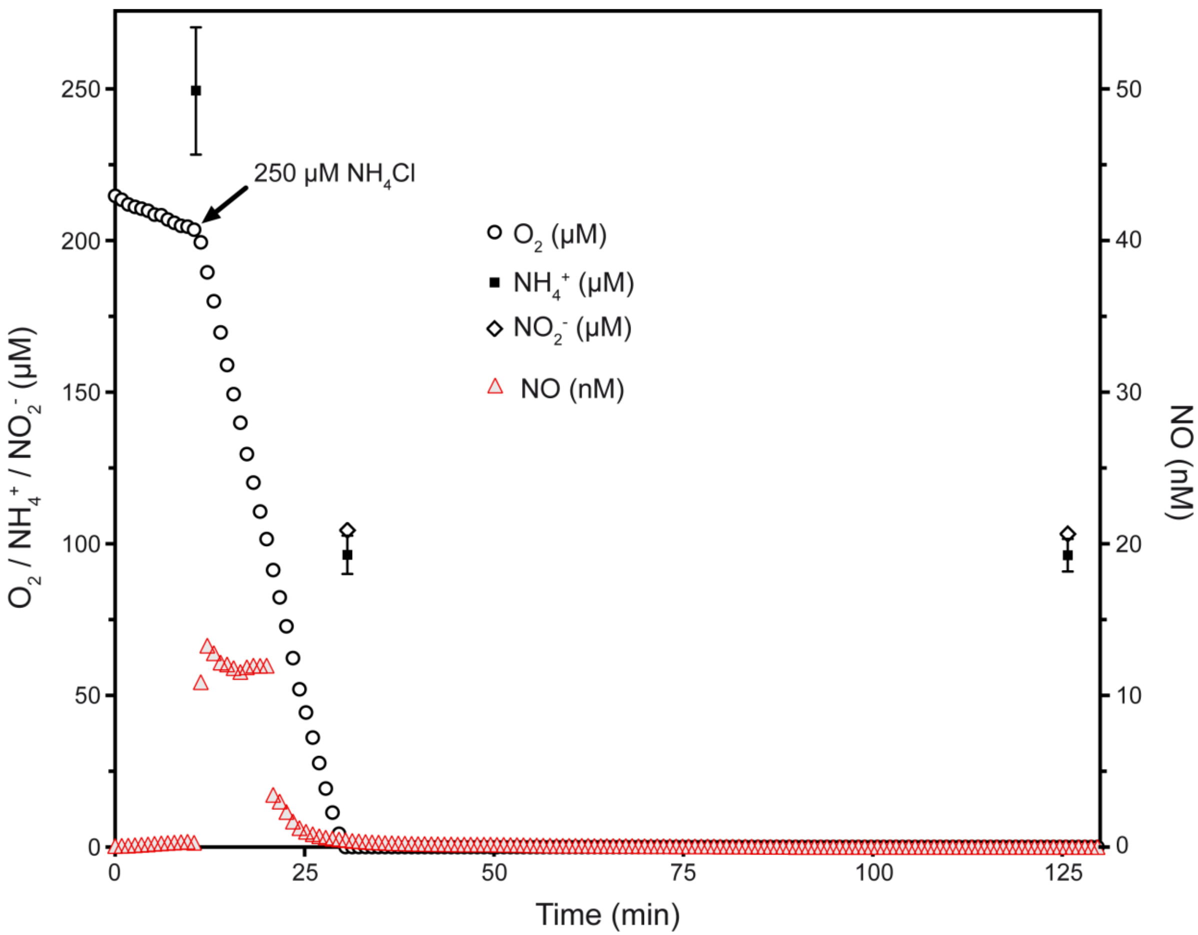
Up to 45% from anthropogenic sources
(⅔ from agriculture & soil)
3.4% from wastewater treatment

Denitrification
Nitrification
Abiotic processes

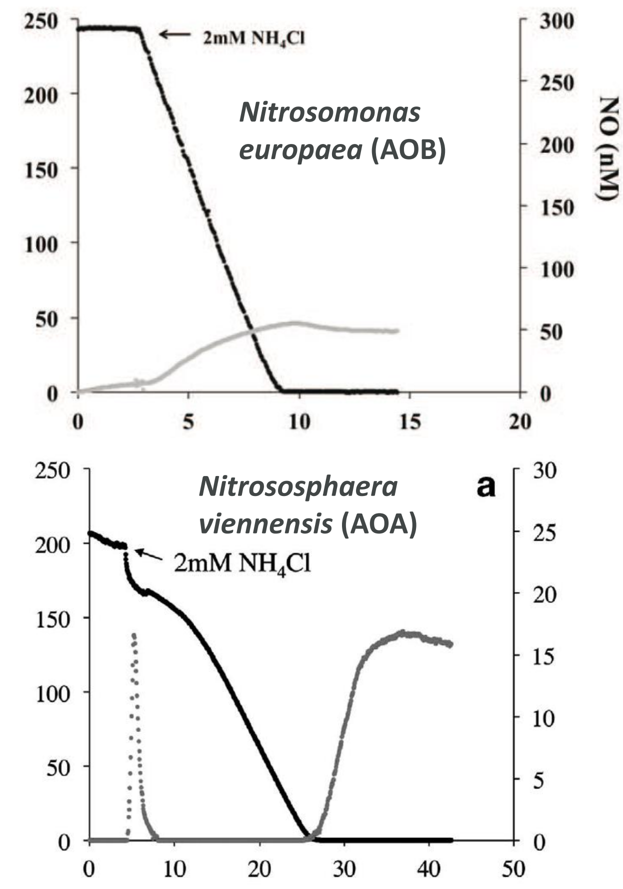
SOURCES OF N_2O IN NITRIFICATION



LOW NO PRODUCTION BY COMAMMOX

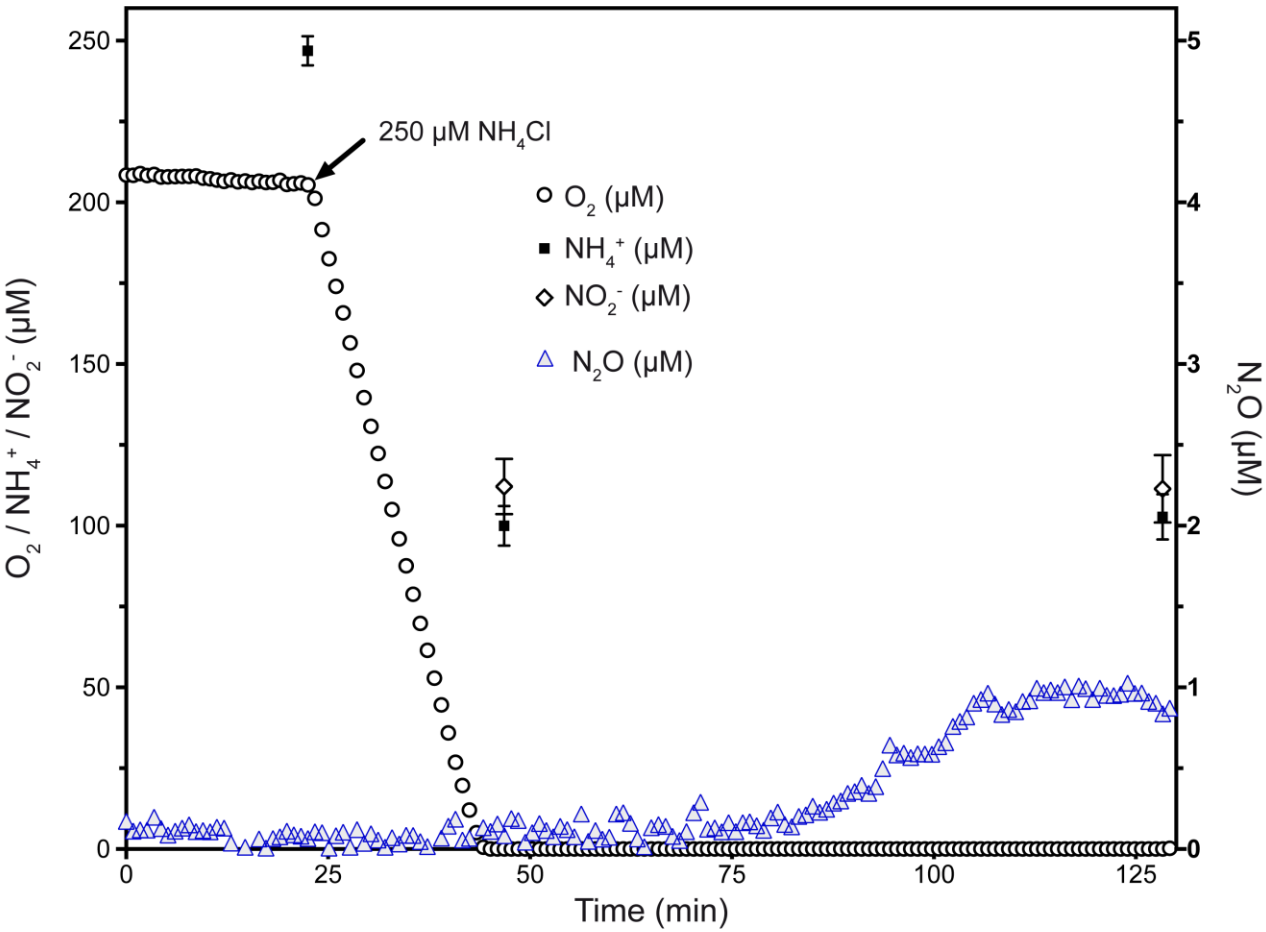


AOB+AOA data from: 2× Kozlowski et al. 2016

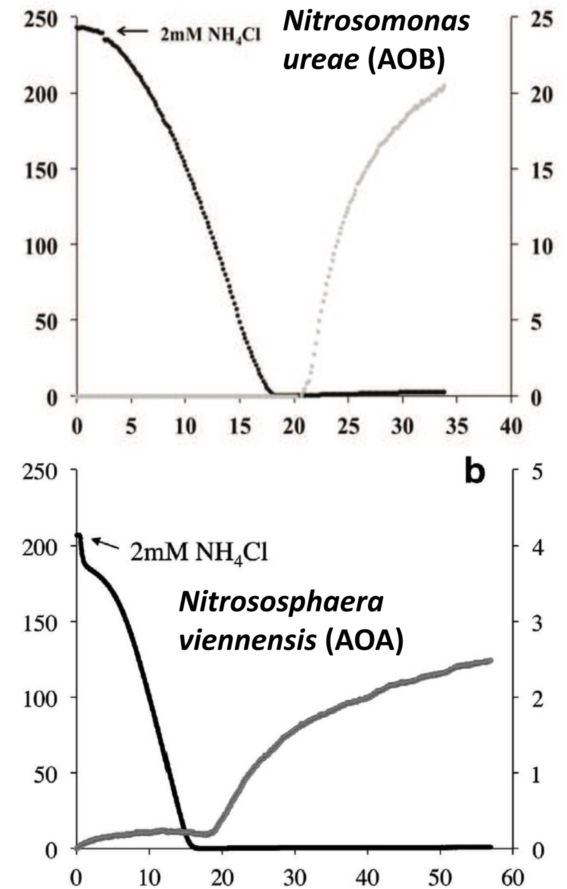


Kits et al. Nature Commun. 10: 1836 (2019)

LOW N_2O PRODUCTION BY COMAMMOX



AOB+AOA data from: 2× Kozlowski et al. 2016



Kits et al. Nature Commun. 10: 1836 (2019)

SOURCE OF N_2O IN COMAMMOX

$$N_\beta — N_\alpha — O$$

$$^{15}N — ^{14}N — ^{16}O$$

$$^{14}N — ^{15}N — ^{16}O$$

Site-preference, SP = $\delta^{15}N_\alpha - \delta^{15}N_\beta$

Process	Known SP
Heterotrophic & nitrifier denitrification	~ 0 ‰
NH_2OH conversion	30.8 – 35.6 ‰ (AOB); 13.1 – 30.8 ‰ (AOA)

***Nitrospira inopinata*, NH_3-limited growth: SP = 33.2 ± 1.2 ‰**

O_2-limited growth: SP = 33.4 ± 1.0 ‰

Kits et al. Nature Commun. 10: 1836 (2019)

N_2O YIELDS OF AMMONIA OXIDIZERS

the international water association

N_2O yields of comammox (*Nitrospira inopinata*)

NH_3-limited growth: 0.072% (N_2O / NH_3 ratio %)
O_2-limited growth: 0.069% (N_2O / NH_3 ratio %)

N_2O yields of other ammonia oxidizers

Soil AOA: 0.07 – 0.09% (not influenced by O_2)
AOB: 0.27% (in hypoxia)

AOA and AOB values from Stieglmeier et al, ISME J. 8: 1135-1146 (2014)

Kits et al. Nature Commun. 10: 1836 (2019)

OUTLOOK: FUTURE COMAMMOX RESEARCH

200 liter bioreactor

Johanna Wiesinger
Chris Sedlacek
and *Nitrospira inopinata*

Physiology, biochemistry, key enzymes of comammox

ACKNOWLEDGMENTS

the international water association

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Ping Han
Anna Mueller
Man-Young Jung
Shurong Liu
Dimitra Sakoula
Chris Sedlacek
Andrew Giguere
Johanna Wiesinger

Aalborg University
Mads Albertsen
Per Nielsen

Winogradsky Institute of Microbiology
Elena Lebedeva
Alexandr Bulaev

Forschungszentrum Jülich
Nicolas Brüggemann

Radboud University Nijmegen
Mike Jetten
Sebastian Lücker

erc
Advanced Grant
NITRICARE (**Michael Wagner**)

FWF
Der Wissenschaftsfonds.

universität wien

the international water association

MODEL DEVELOPMENT FOR THE COMAMMOX PROCESS

JACEK MAKINIA, GDAŃSK UNIVERSITY OF TECHNOLOGY, POLAND

inspiring change

PLAN OF PRESENTATION

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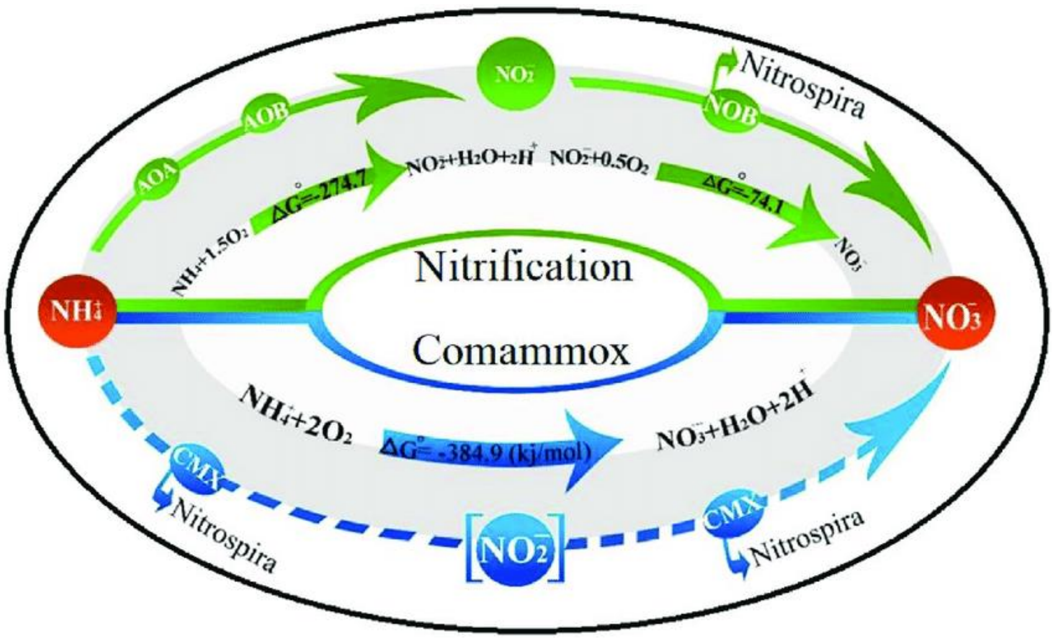
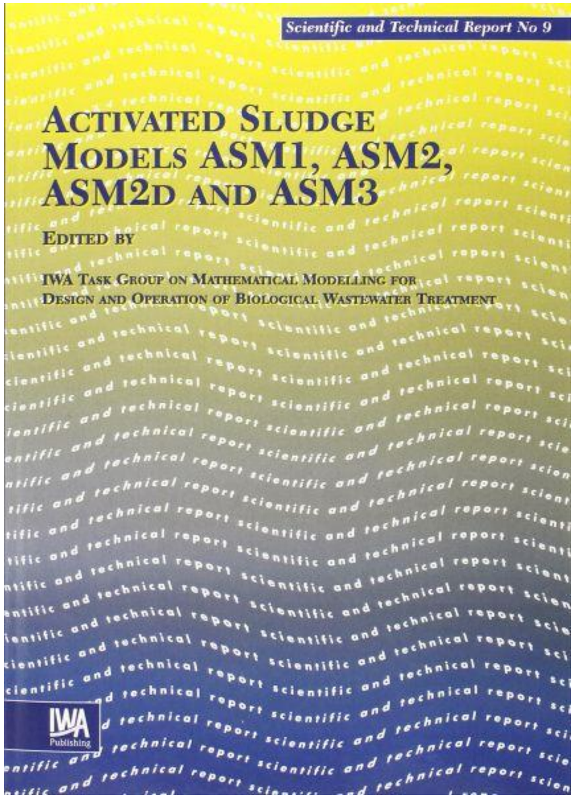
- Conceptualization of the comammox model (three possible scenarios)
- Comparison of kinetic parameters for *Nitrospira* and comammox bacteria
- Integration of comammox into the extended ASM (with two-step nitrification and heterotrophic denitrification)
- Impact of initial biomass concentrations and kinetic parameters on model predictions
- Assessment of comammox contribution to the nitrogen conversions

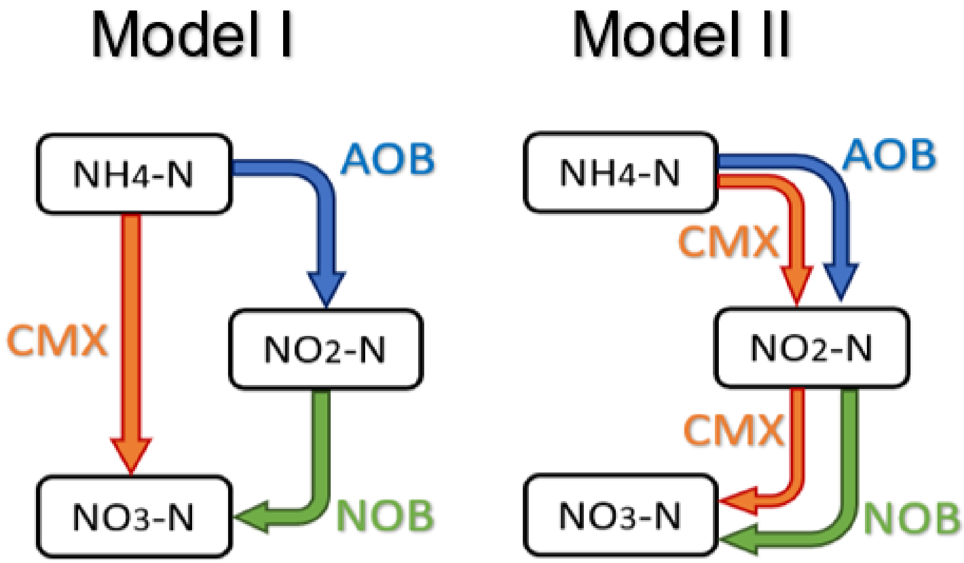
GOLDEN RULES OF MODELING

- *"No model is perfect, some are useful"*
- *"A model should be as simple as possible, and only as complex as needed"*

INTEGRATION OF AMMONIA OXIDATION IN ASMs

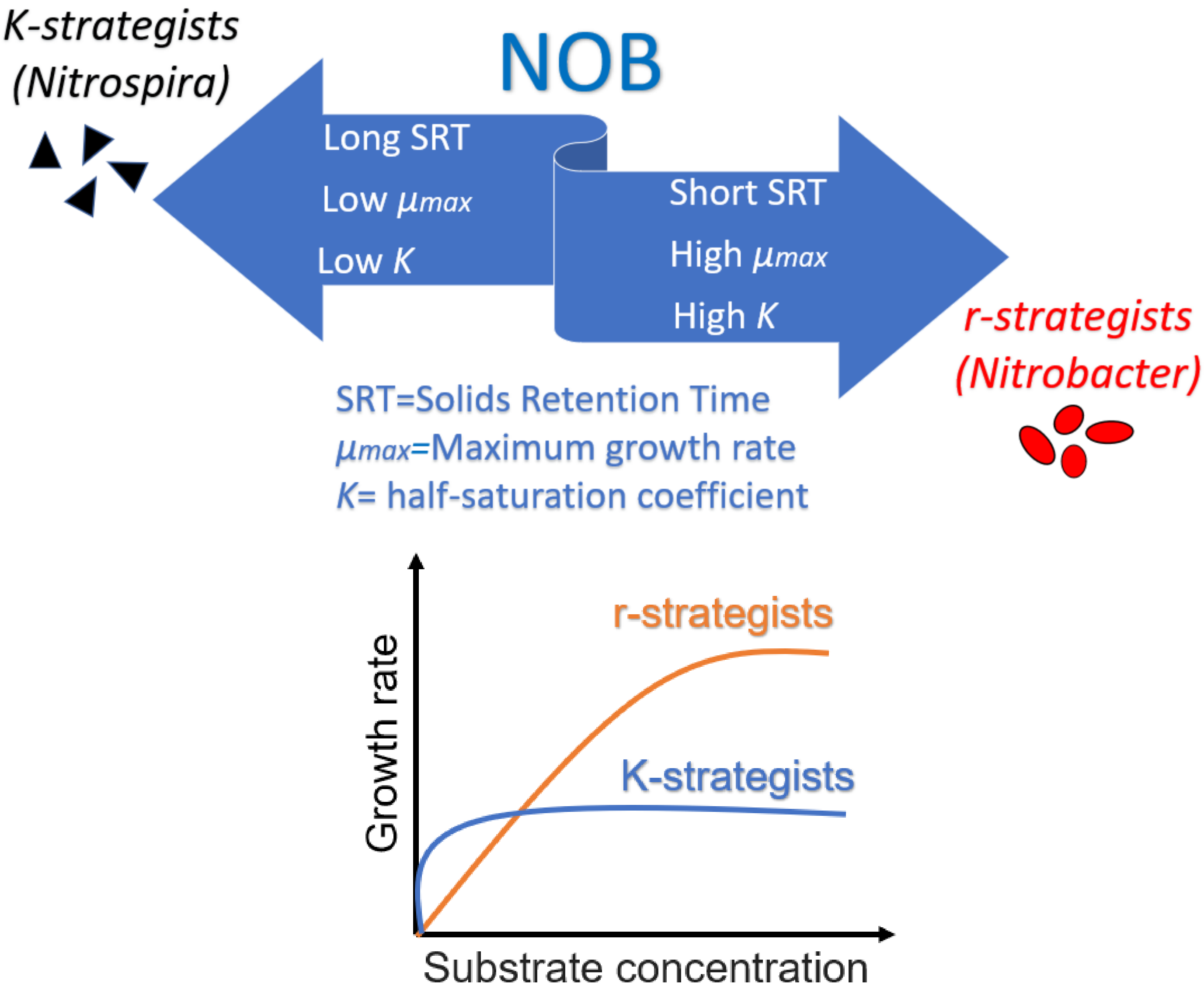
Ammonia oxidation	Activated Sludge Models (ASMs)
	

CONCEPTUAL COMAMMOX MODELS



- **Simulation platform - GPS-X, Hatch (Canada)**
- **Special utility (Model Developer) was used to implement the new model**

KINETIC CHARACTERISTICS OF NOB



REVIEW OF KINETIC PARAMETERS FOR NOB

$$\mu_{NET} = \mu_{max} \cdot \left(\frac{S}{K_S + S}\right) \cdot \left(\frac{S_O}{K_O + S_O}\right) - b$$

Parameters	Unit	*Nitrospira*	Comammox bacteria
μ_{max}	d^{-1}	0.18-0.69	0.15-0.69
$K_{S(NH4)}$	mg N/L	-	0.0005-0.012
$K_{S(NO2)}$	mg N/L	0.08-1.20	0.17-6.29
K_O	mg O_2/L	0.04-0.74	0.33-0.54
b	d^{-1}	0.05-0.27	0.05

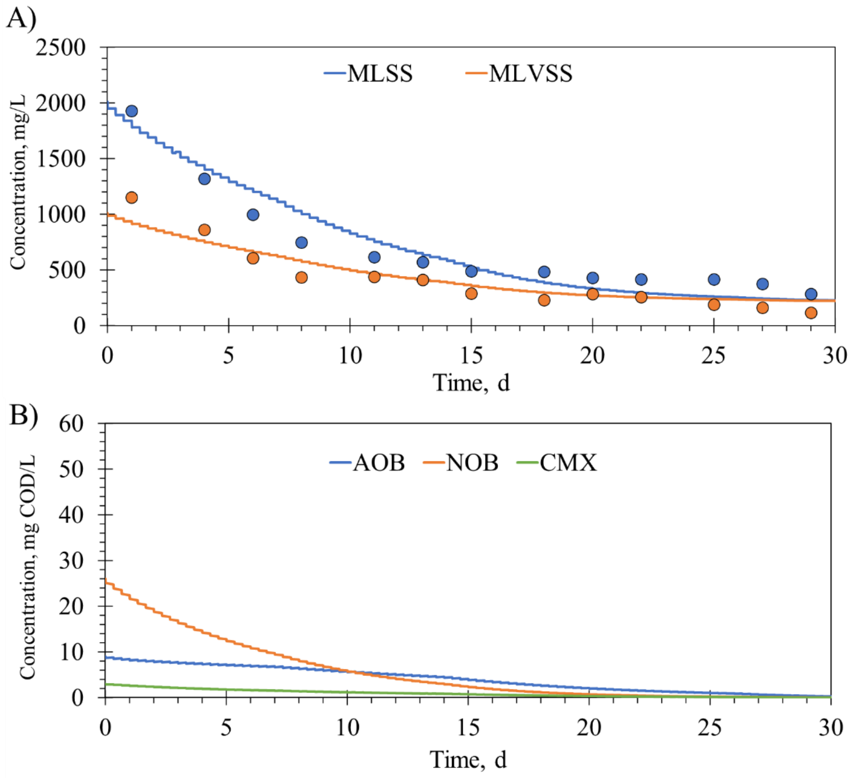
EXPERIMENTAL DATA

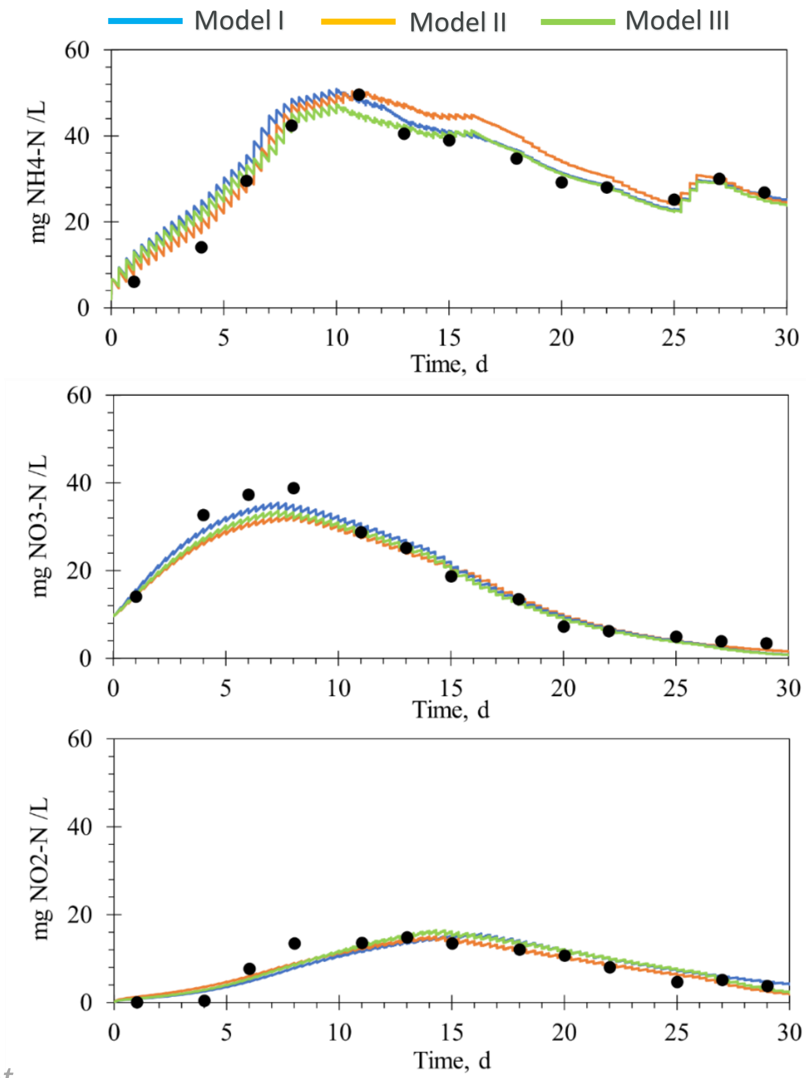
10L sequencing batch reactor

Operating conditions

- Temperature = 12^{o}C vs. 20^{o}C
- SRT decreasing from 4 d to 1 d
- DO = 0.6 ± 0.1 mg/L
- Initial MLSS = ≈ 2000 mg/L
- N source: NH_4-N vs. NO_2-N

SIMULATION RESULTS





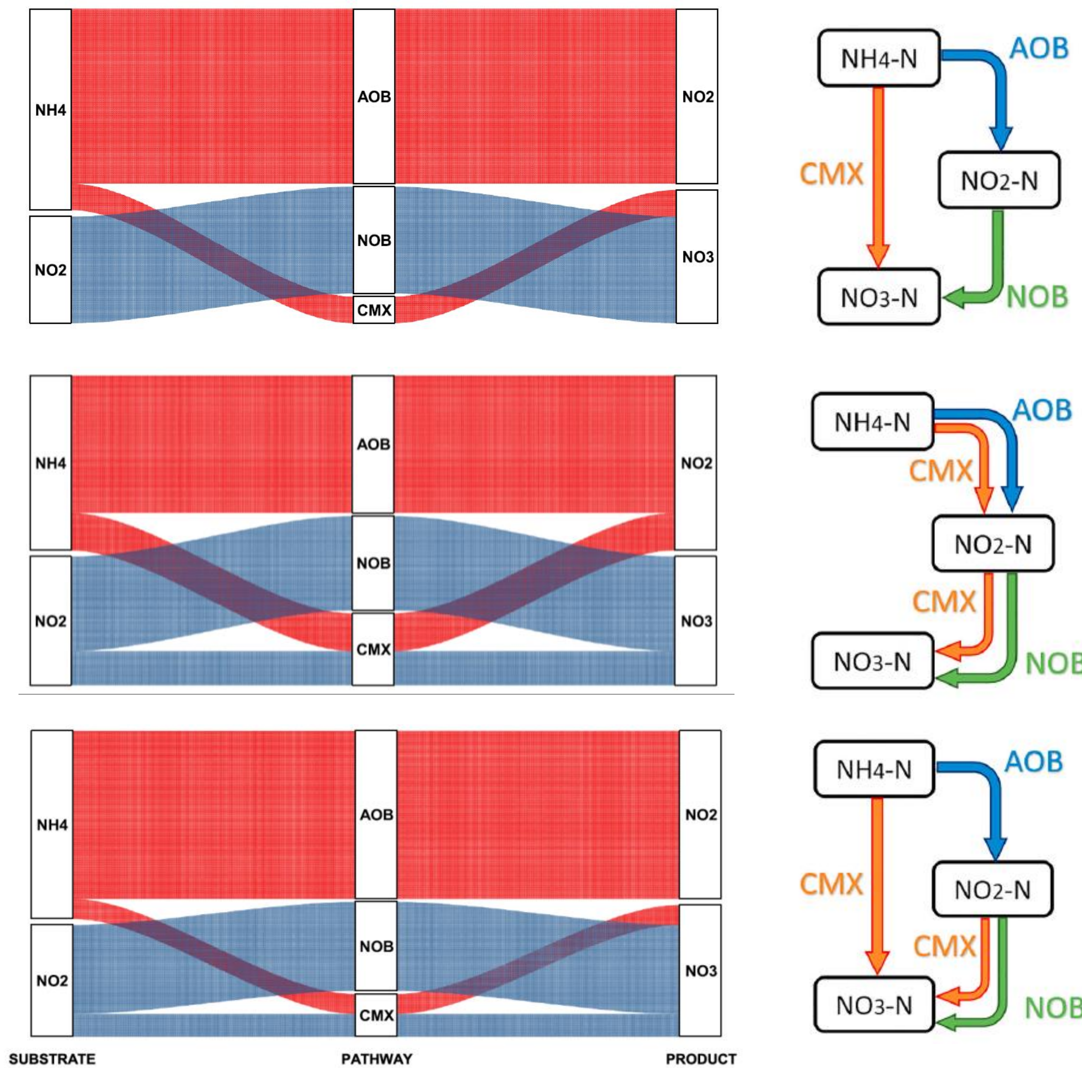
Mehrani et al. (2021). Journal of Environmental Management

NITROGEN CONVERSIONS

Model I

Model II

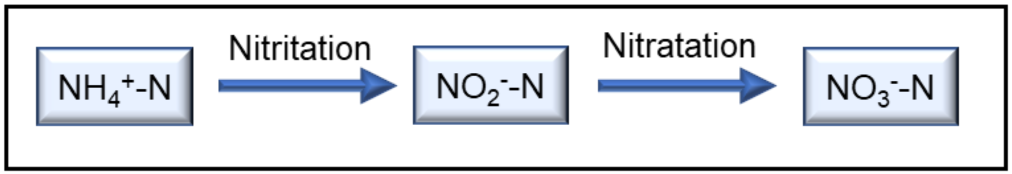
Model III



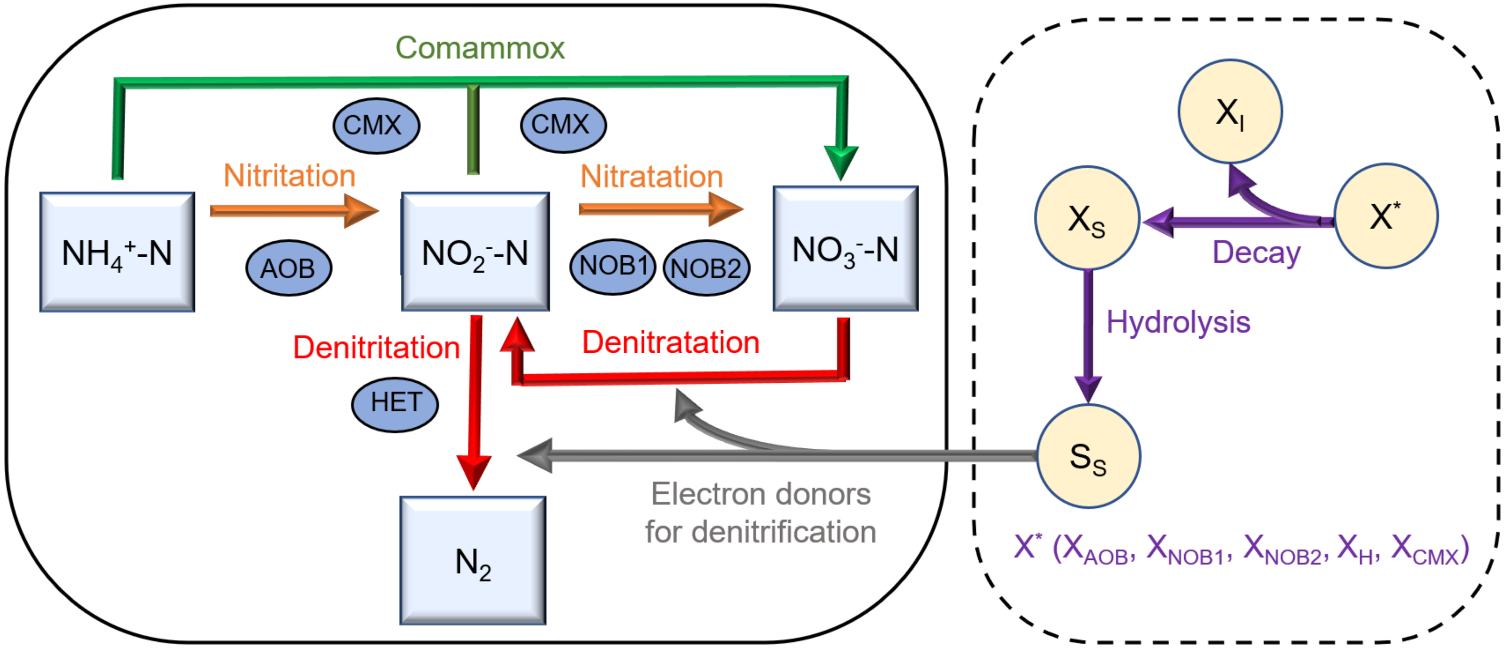
Mehrani et al. (2021). Journal of Environmental Management

EXTENDED TWO-STEP MODEL

A) Conventional two-step model



B) Extended two-step model



MODEL DEVELOPMENT

Step 1:
Mathematical model development and implementation

↓

Step 2:
Setting the initial biomass composition

↓

Step 3:
Sensitivity analysis and correlation matrix

↓

Step 4:
Model calibration and validation

↓

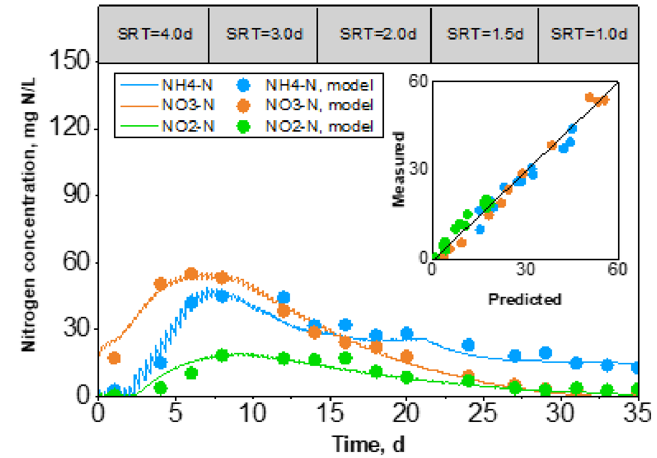
Step 5:
RSM-based impact assessment of the extended model

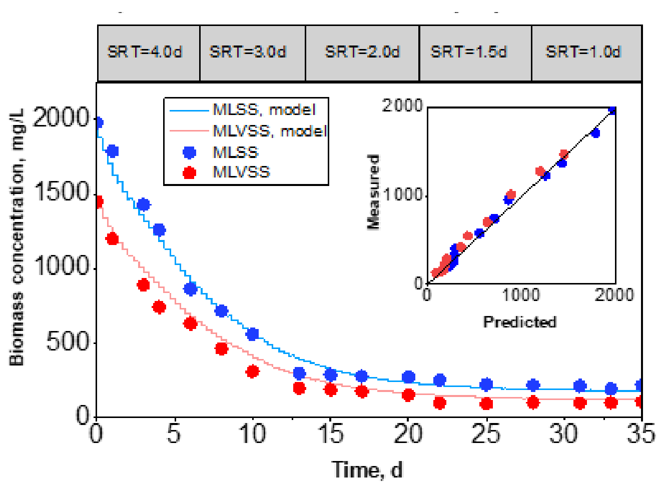
↓

Step 6:
Model evaluation and comparison

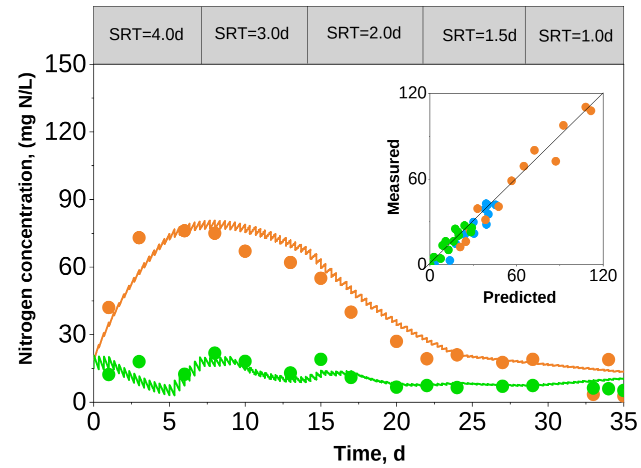
MEASURED DATA VS. PREDICTION

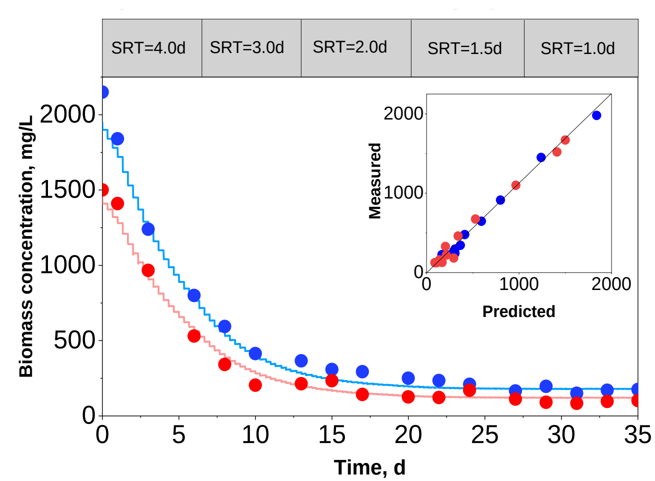
Calibration phase (NH_4-N, 12°C)





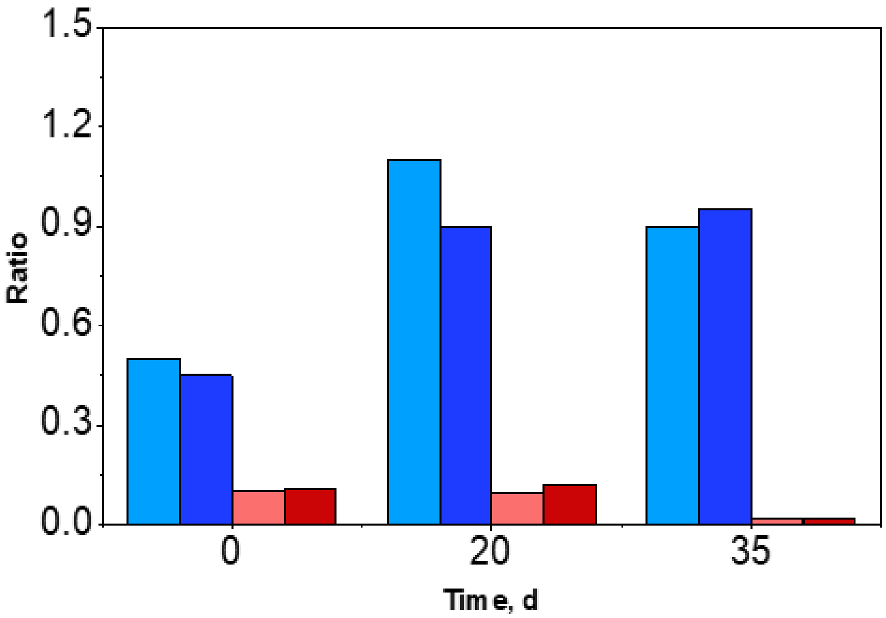
Validation phase (NO_2-N, 20°C)



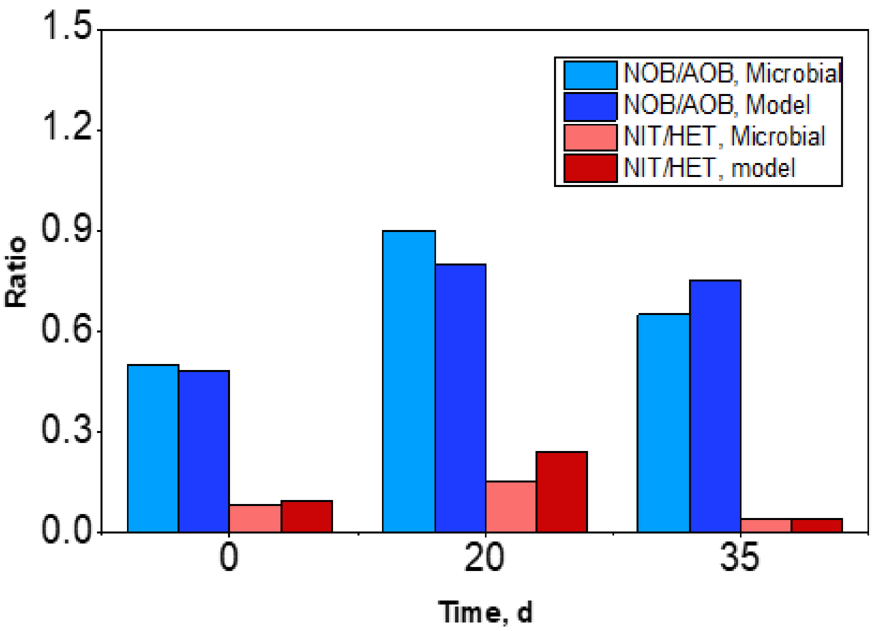


RATIOS OF NOB/AOB AND NIT/HET

Calibration phase (NH_4-N, 12ºC)



Validation phase (NH_4-N, 20ºC)



COMAMMOX RELATIVE ABUNDANCE

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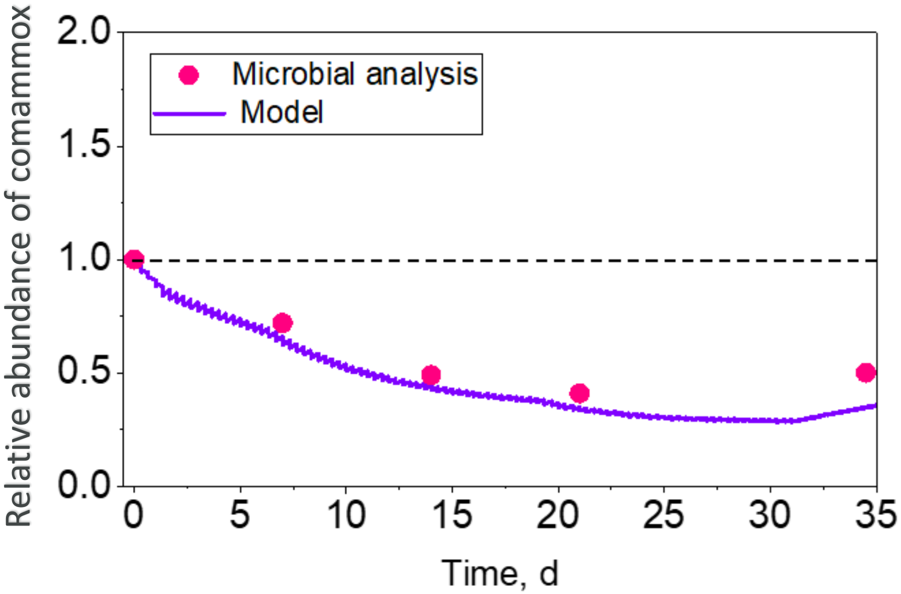
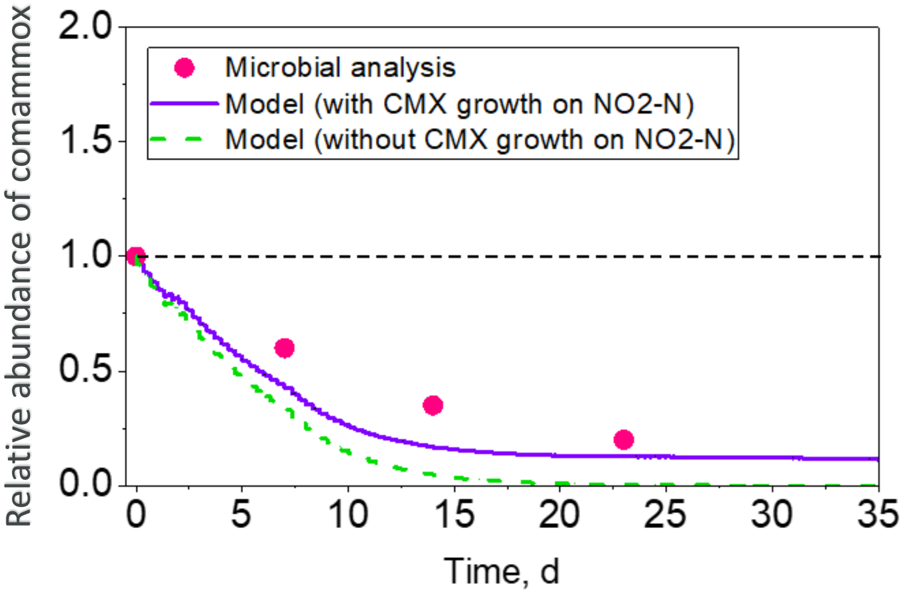
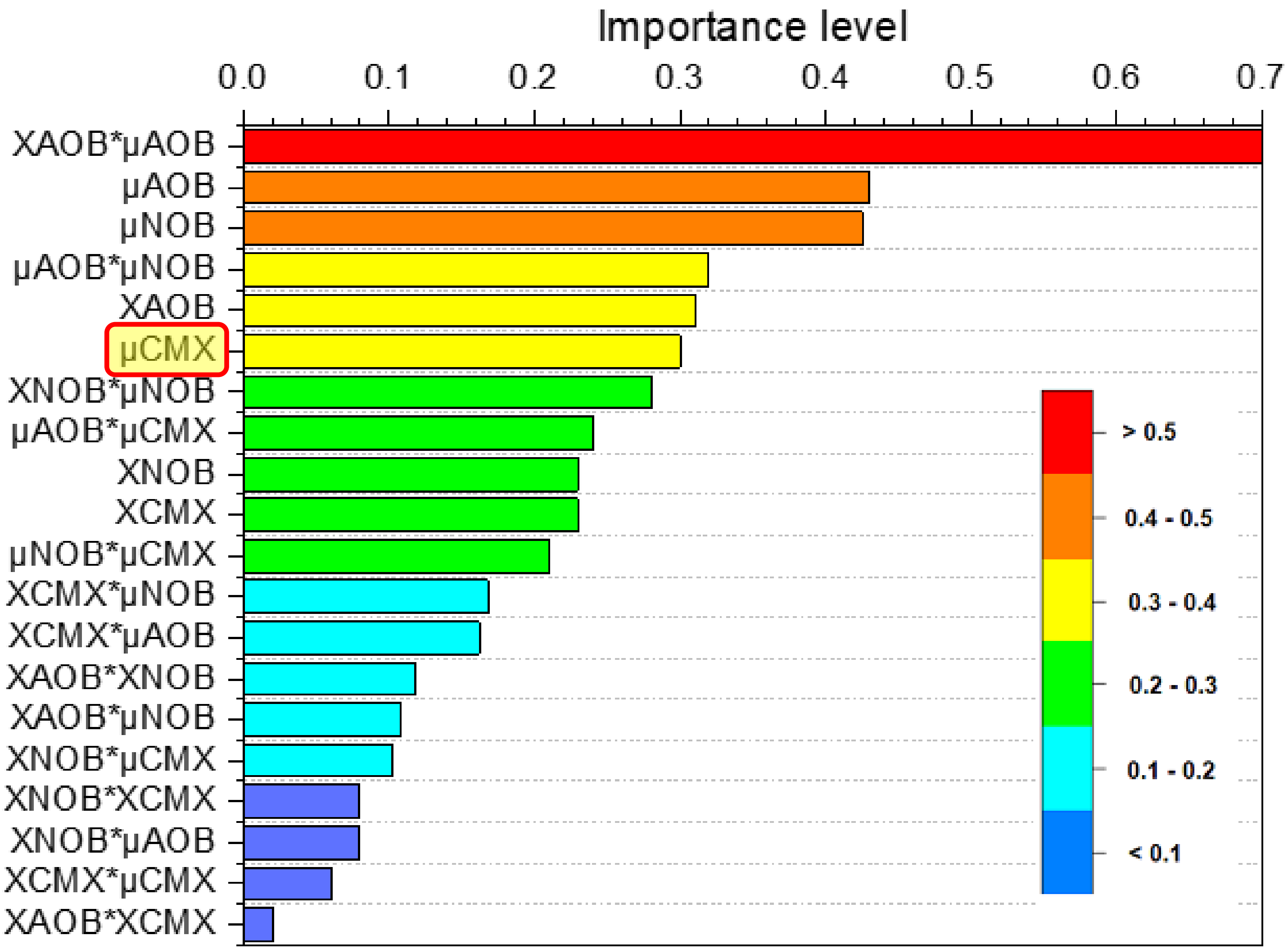
Calibration phase (NH_4-N, 12°C)



Validation phase (NO_2-N, 20°C)



EFFECT OF INPUT PARAMETERS



N CONVERSIONS (EXTENDED MODEL)

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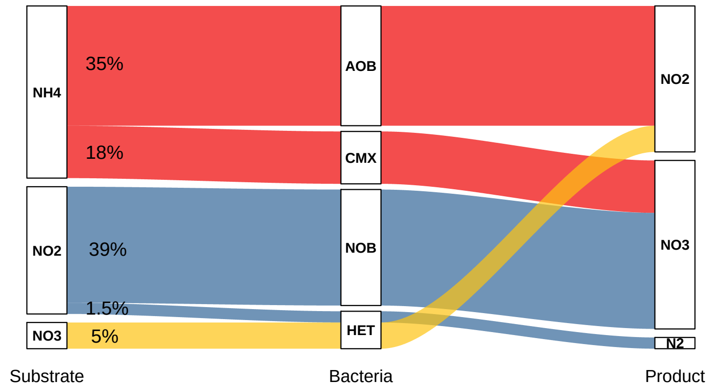
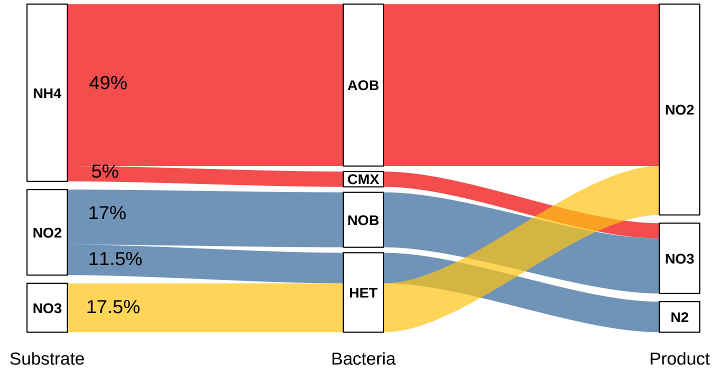
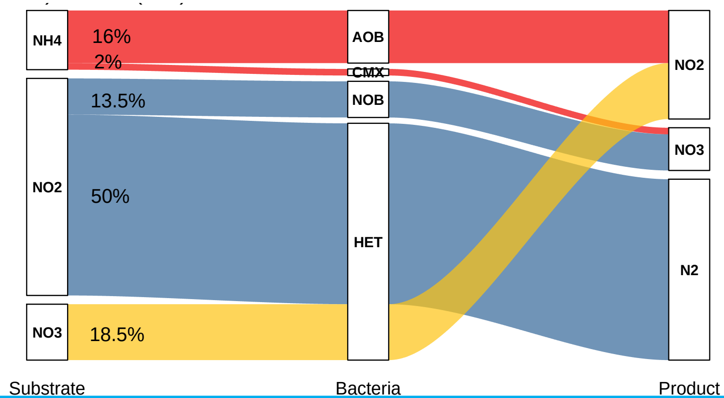
Beginning, t = 0 d



Middle phase, t = 20 d



End, t = 35 d



SUMMARY

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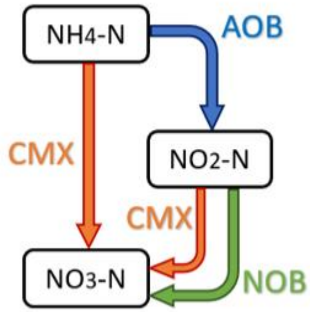
- Integration of commamox in two-step nitrification models is straightforward
- Model III (involving both NH_4-N and NO_2-N conversions) is recommended for further research on comammox modeling
- Challenges: growth of comammox bacteria on NO_2-N and preferable substrate (NH_4-N vs. NO_2-N)
- The initial concentrations of comammox bacteria and their kinetic/stoichiometric parameters are needed for simulations
- The role of commamox in N conversions in activated sludge systems should not be neglected, but requires further investigation



RELATED PUBLICATION

○ **Mehrani et al., 2021. Incorporation of the complete ammonia oxidation (comammox) process for modeling nitrification in suspended growth wastewater treatment systems,** Journal of Environmental Management, 297, 113223. https://doi.org/10.1016/j.jenvman.2021.113223

ACKNOWLEDGEMENTS

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This project was supported by the Polish National Science Center under project no. UMO-2017/27/B/NZ9/01039.

National Science Centre Poland

Identification, characteristics and modeling of the COMAMMOX process - a missing link in the nitrogen cycle in wastewater treatment systems

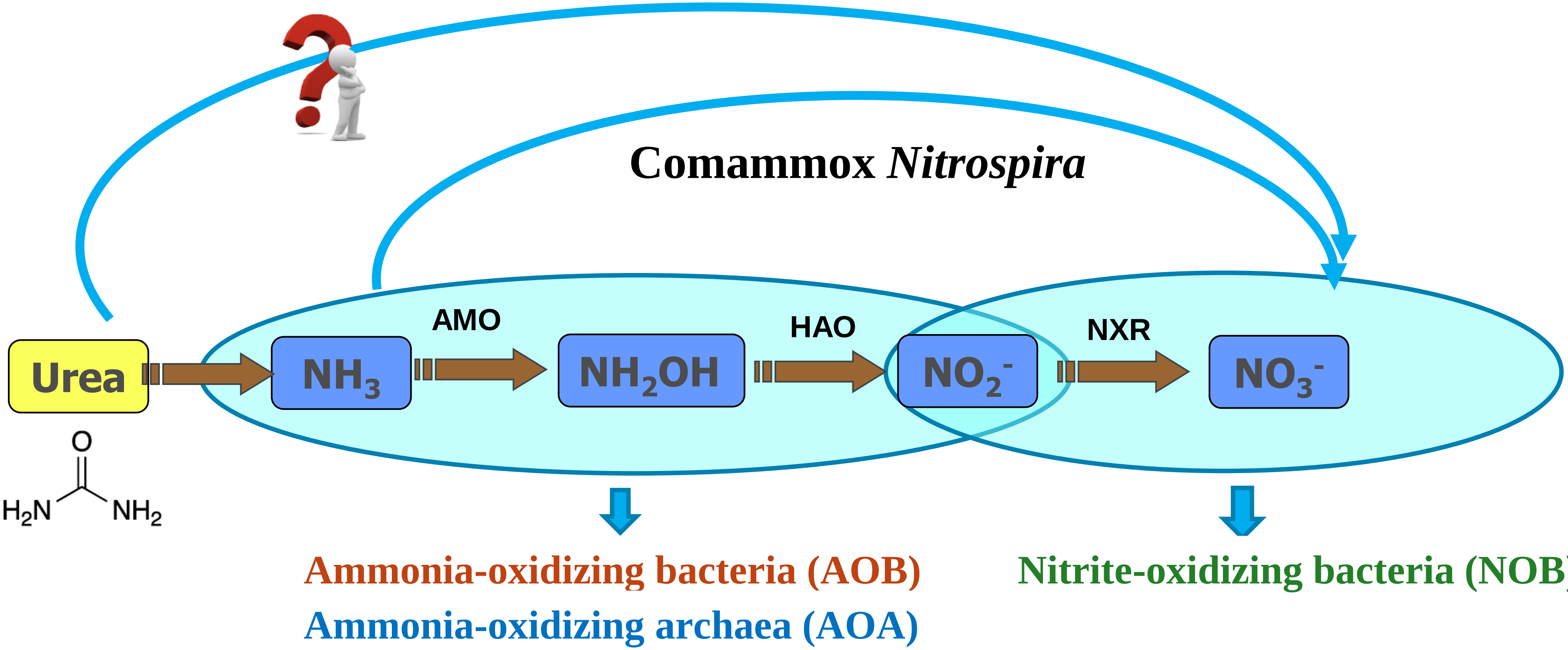
Thank you for your attention

Urea-based comammox *Nitrospira* and their potential applications

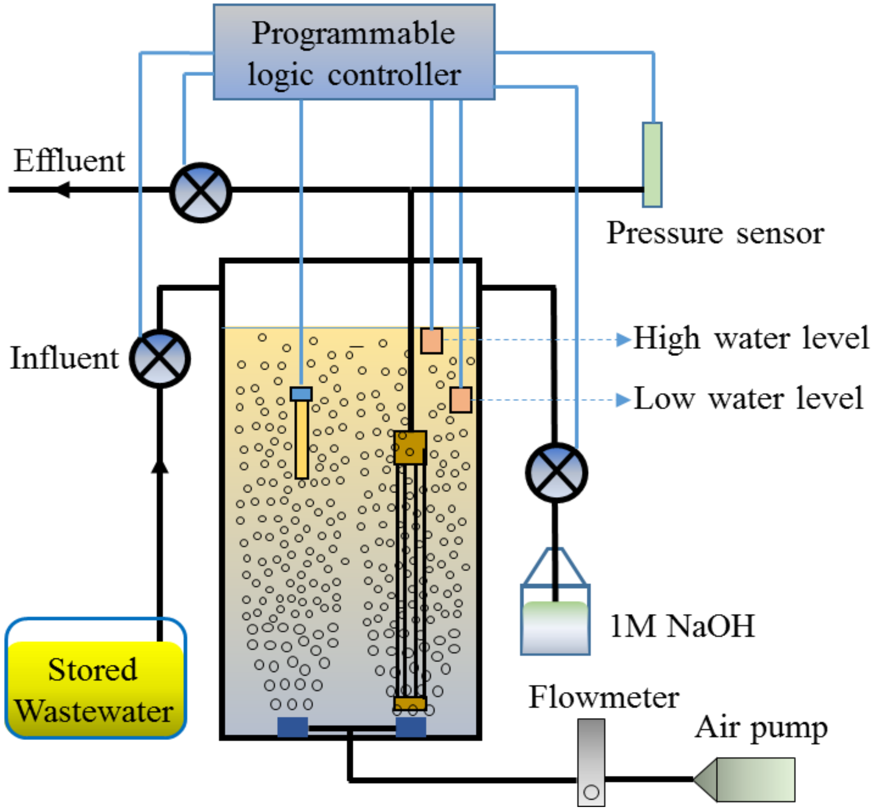
JIANHUA GUO, UNIVERSITY OF QUEENSLAND, AUSTRALIA

inspiring change

NITRIFICATION AND MICROORGANISMS



AN INTERESTING OBSERVATION



Membrane bioreactor (MBR): 12 L

Inoculum: nitrifying activated sludge from a municipal WWTP

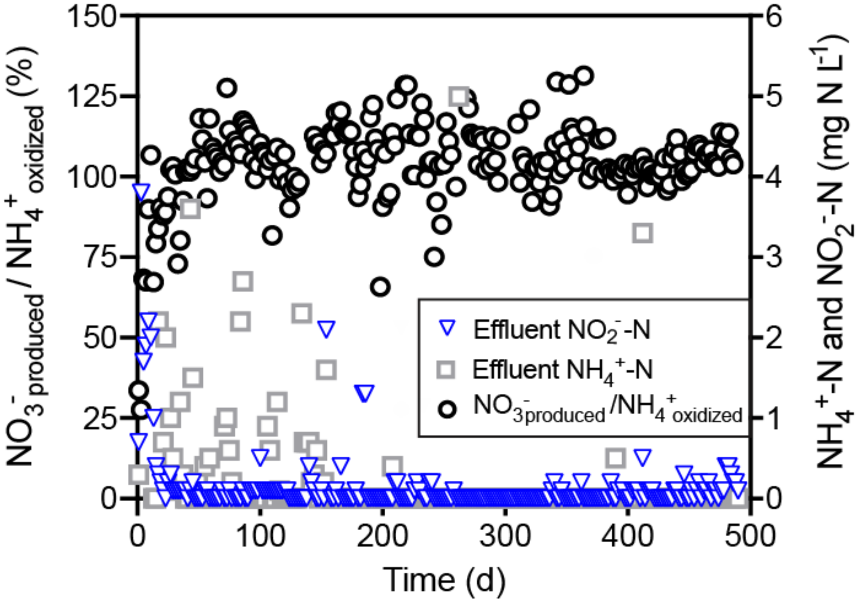
DO: 4 mg O_2/L

Influent: real source-separated urine

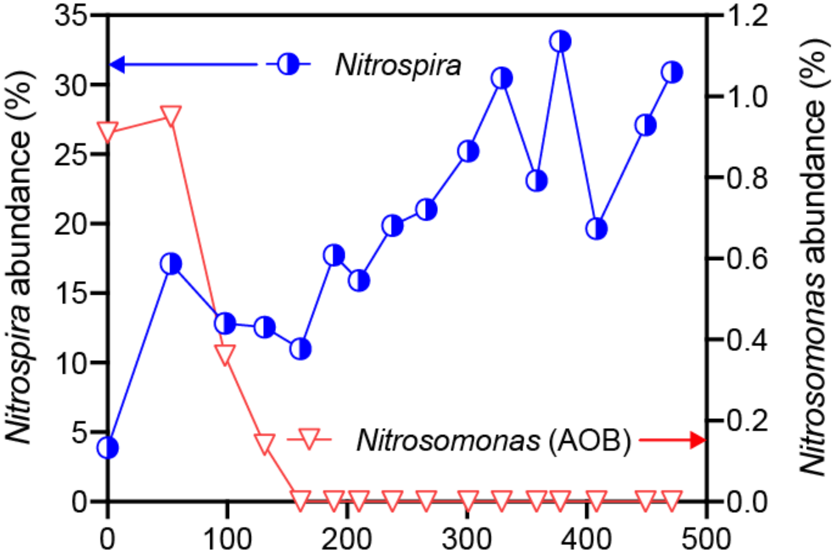
Urea concentration: 10-50 mg N/L

Time (days)	TKN average (mg N/L)[a]	pH	NO_3^- (mg N/L)
1-185	242.4 ± 63.6	9.0 ± 0.1	< 3 mg/L
186-221	176.6 ± 20.4		
222-490	211.0 ± 33.5		

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS



Long term performance



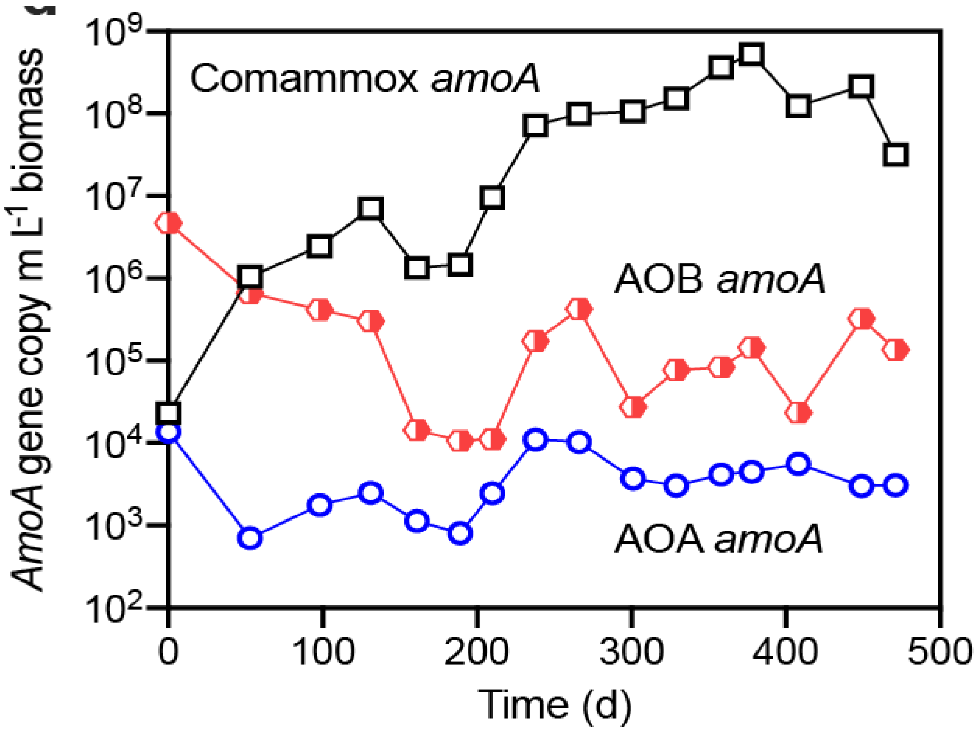
16S rRNA gene sequencing

Are they comammox *Nitrospira*?

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

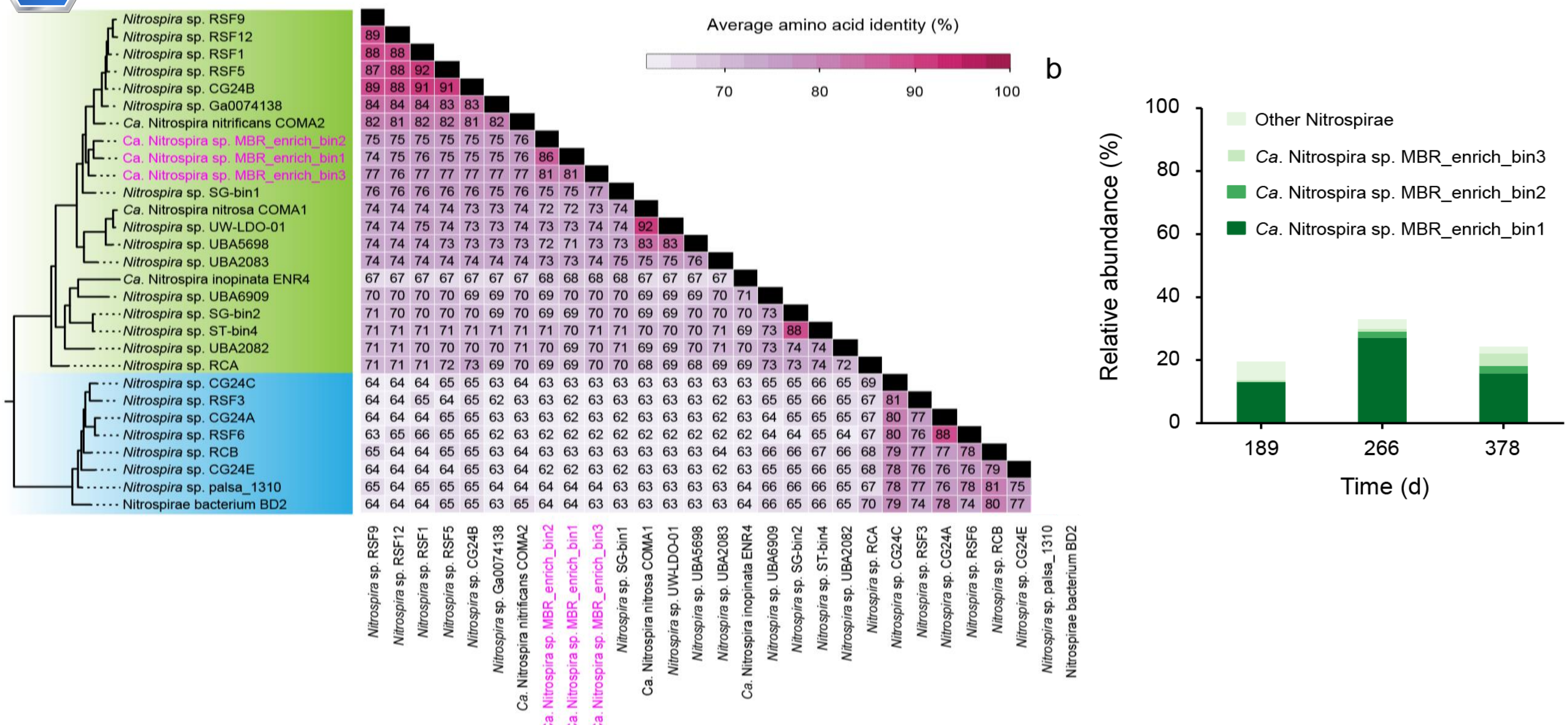
1

Experiment 1: qPCR



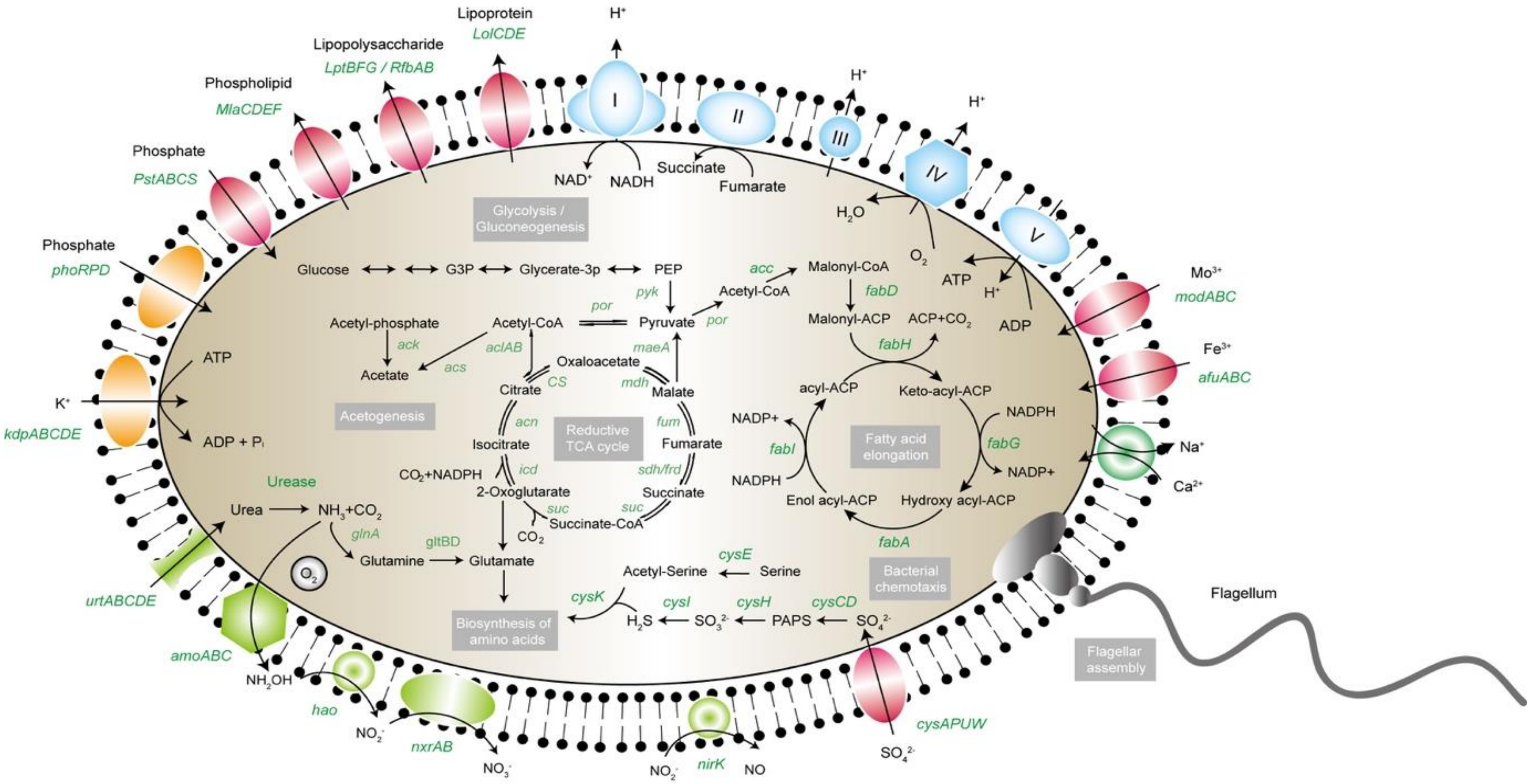
NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

2 Experiment 2: Metagenomic sequencing



Three new clade A comammox *Nitrospira* were recovered by metagenomics

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

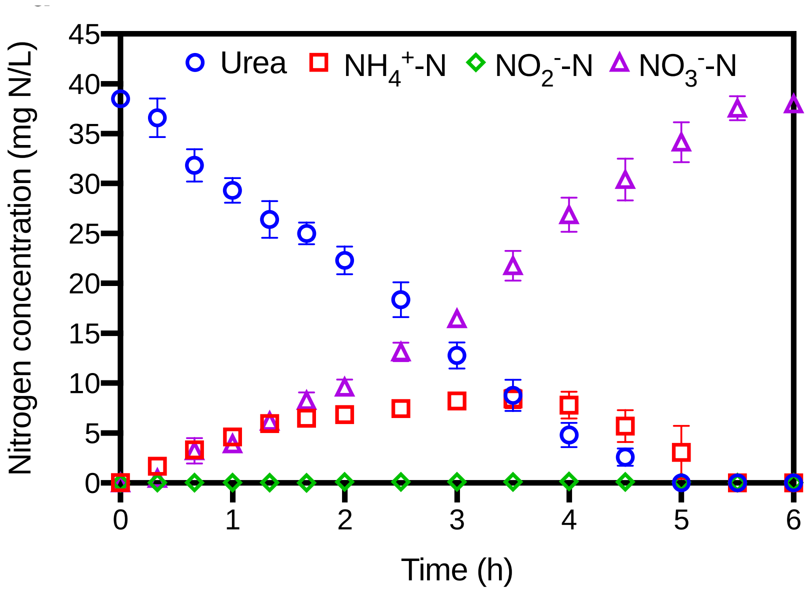


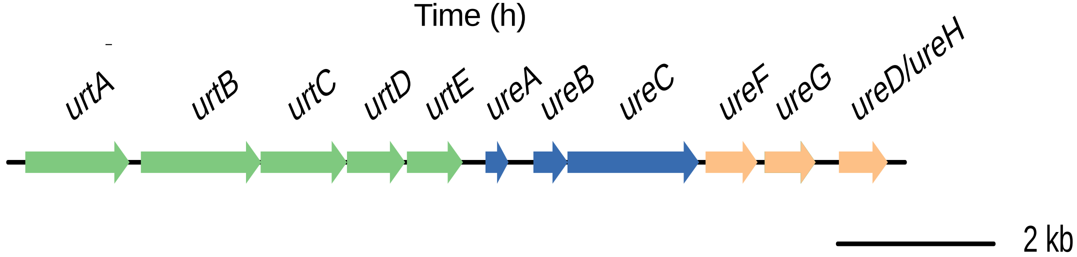
Cell metabolic cartoon constructed from the MBR comammox *Nitrospira* genome annotations

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

the international water association

Can these comammox *Nitrospira* utilise urea?





The three recovered genomes contained a complete urea utilization pathway.

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

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Selective enrichment and metagenomic analysis of three novel comammox *Nitrospira* in a urine-fed membrane bioreactor

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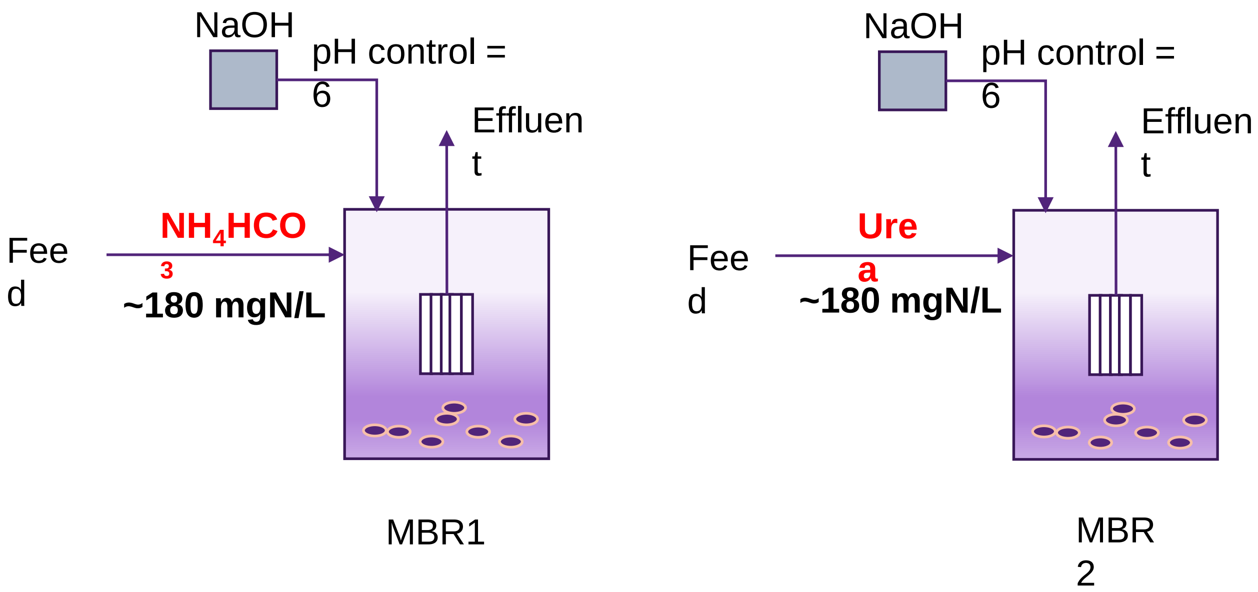
Can this phenomenon be reproduced?



Working volume: 2L
Inoculum: activated sludge from a full-scale WWTP in Brisbane
HRT = 3d
DO = 4~5 mg/L

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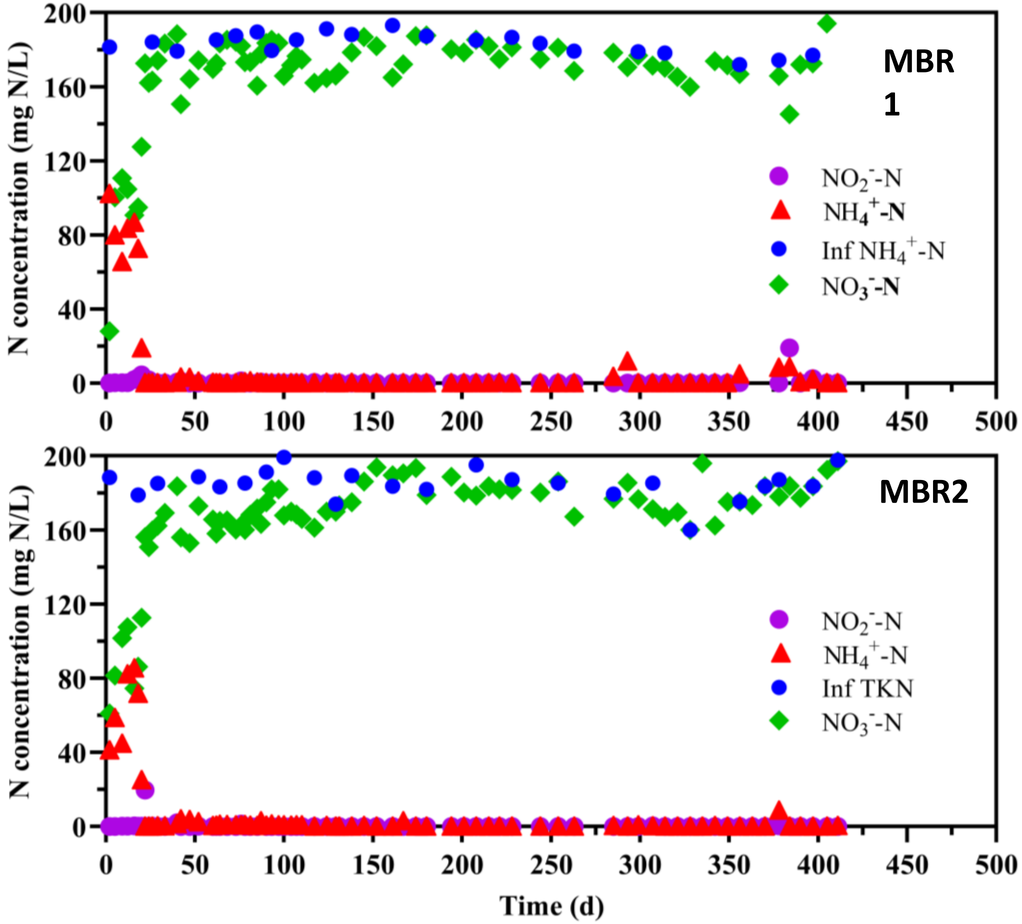
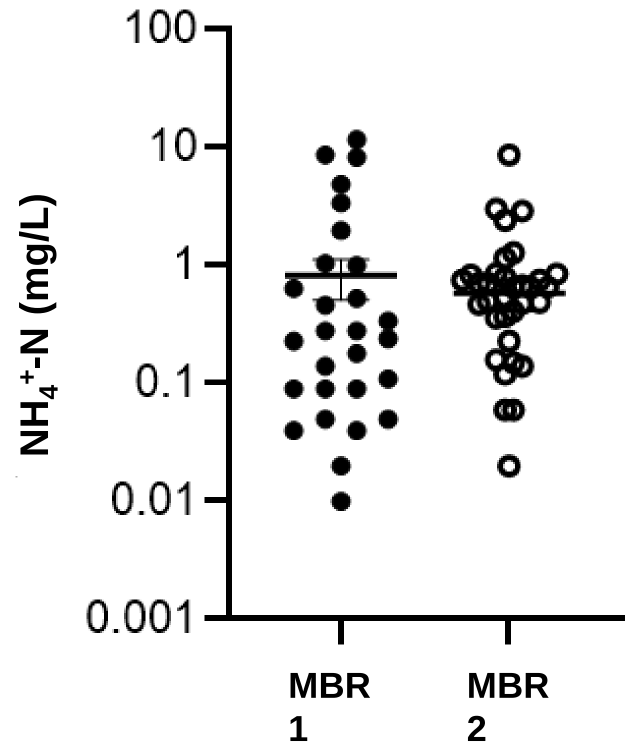
Nitrification performance





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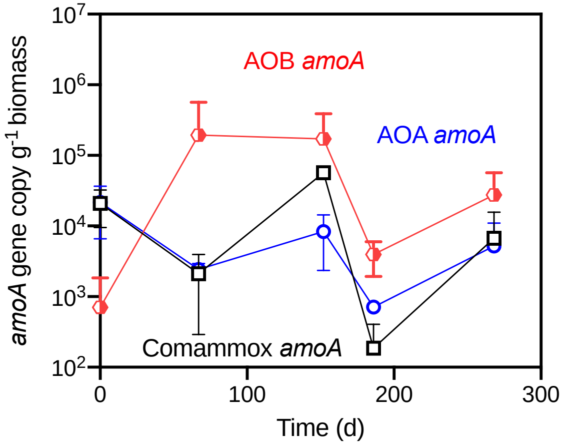
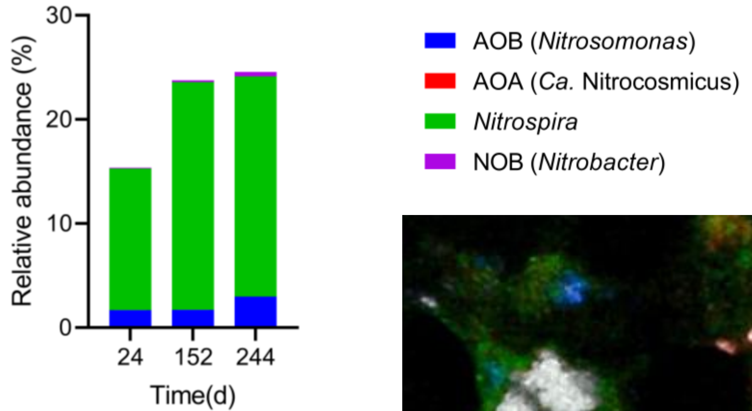
the international water association

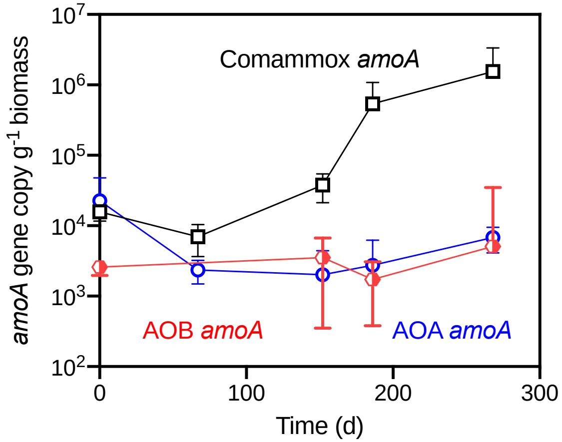
Comammox bacteria dominate in urea-fed bioreactor

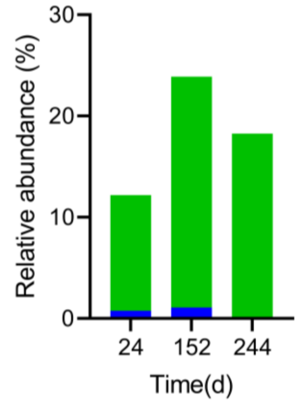
MBR1

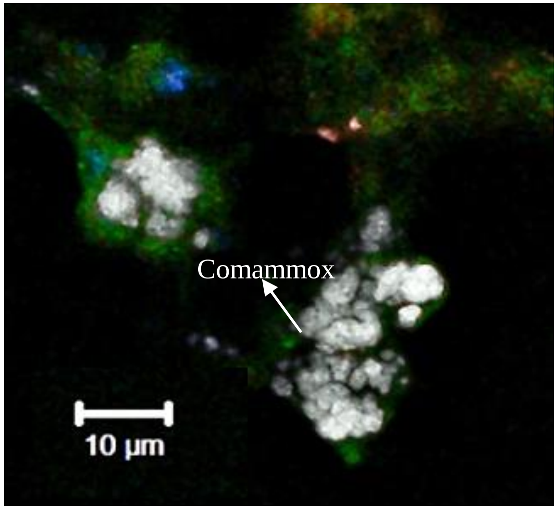




MBR2







MBR2

Green: Total bacteria
Blue: *Nitrospira*
White (Blue+Red+Green): Comammox Nitrospira

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

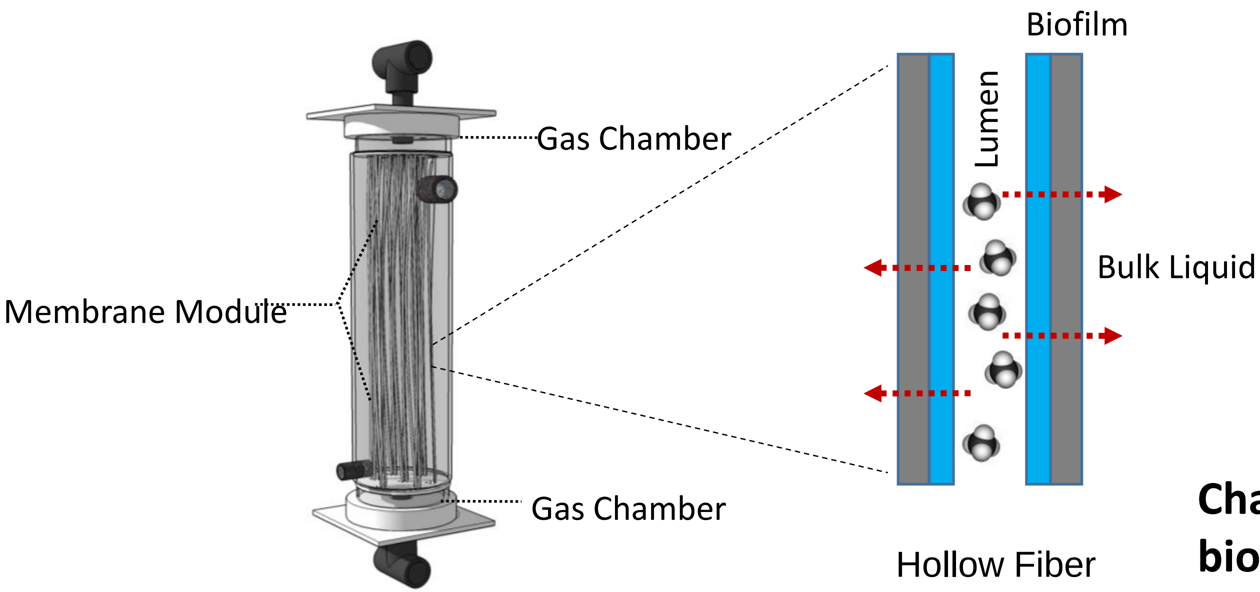
How could we apply comammox bacteria in water/wastewater treatment?

1. Produce less N_2O
2. Enable a complete ammonia removal due to a very low *Ks*
3. Enable to degrade micropollutants via the co-metabolic pathway of AMO

1. Slow-growing rate
2. Difficult to enrich
3. How to apply them under real-world conditions?

NITRIFICATION PERFORMANCE AND NITRIFYING COMMUNITY COMPOSITIONS

Membrane-aerated biofilm bioreactor (MABR)



Advantages:

- Separate and prolong SRT
- Enhance gas transfer efficiency

Challenge 1 related to Insufficient biomass will be solved!

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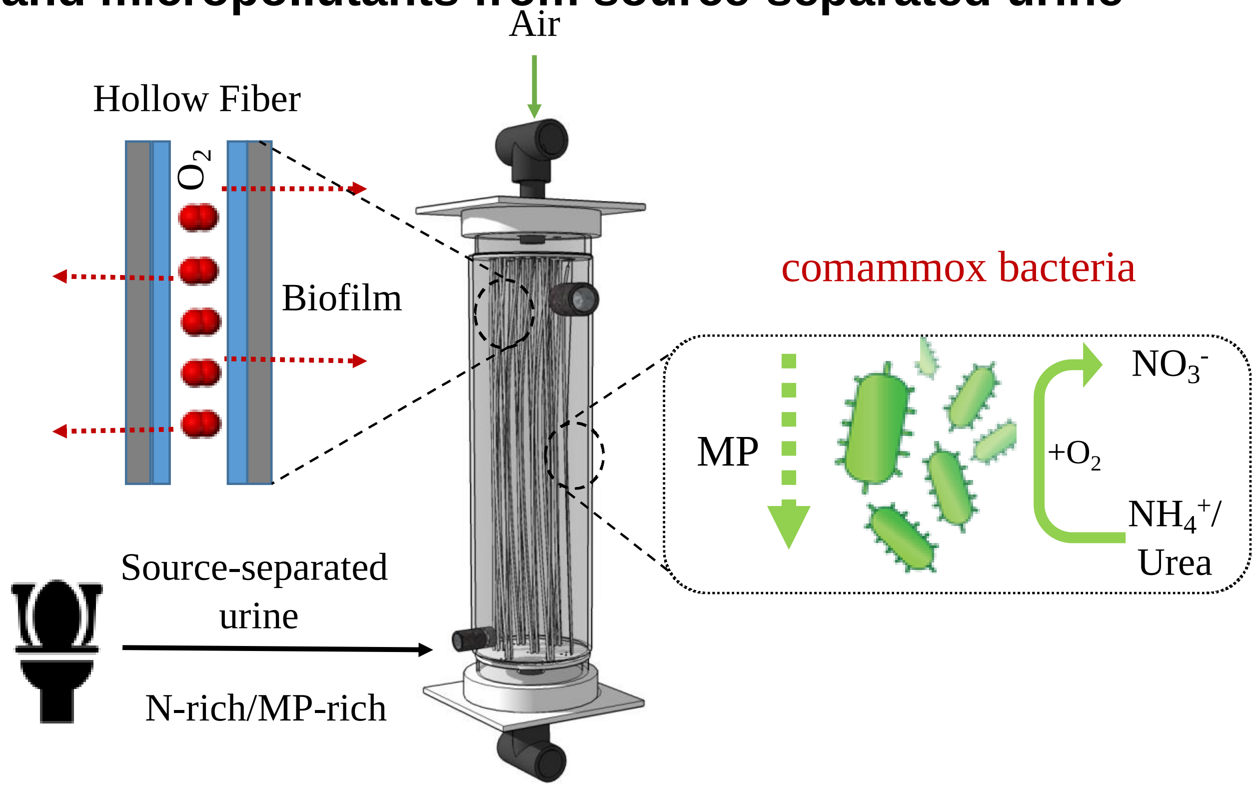
A potential application: simultaneously remove ammonium and micropollutants from source-separated urine



Challenges 2&3 will be sorted out!

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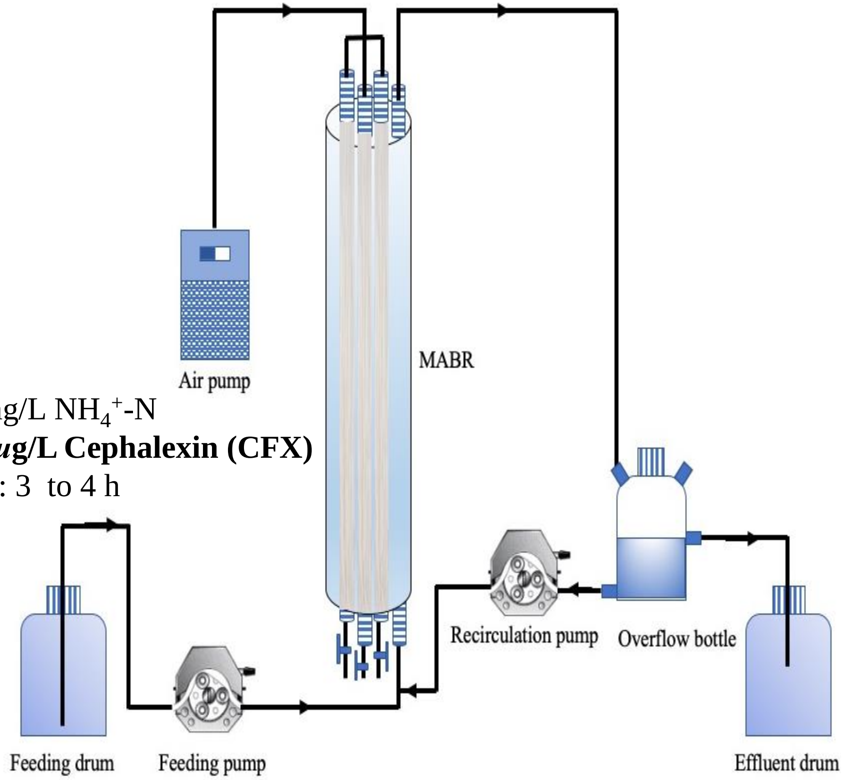
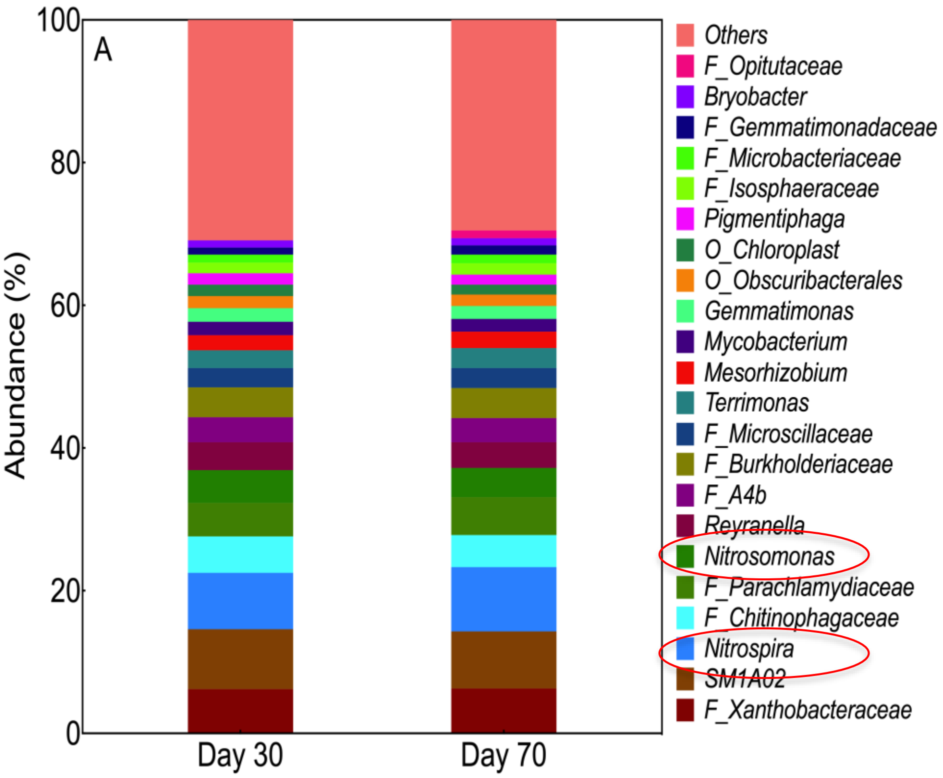
Preliminary results



50 mg/L NH_4^+-N
100 μg/L Cephalexin (CFX)
HRT: 3 to 4 h



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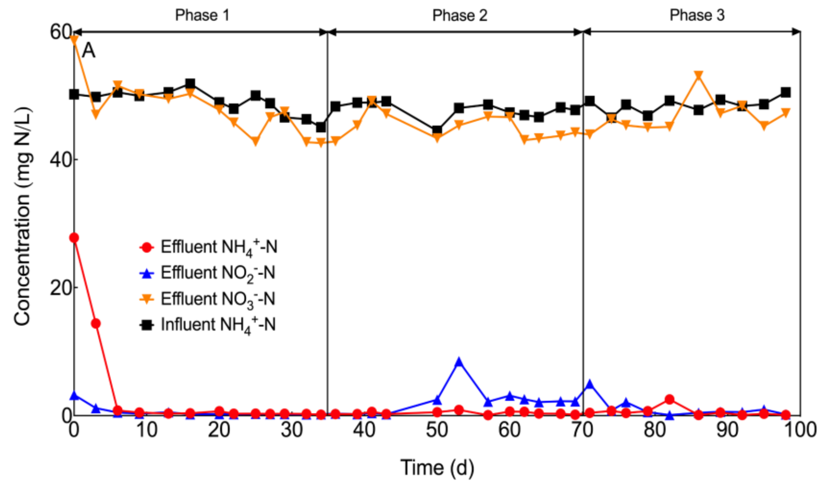

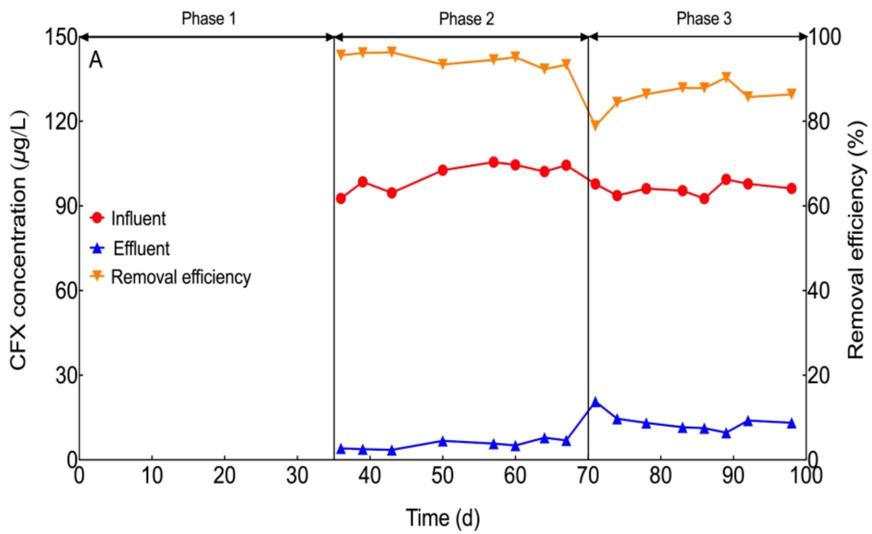

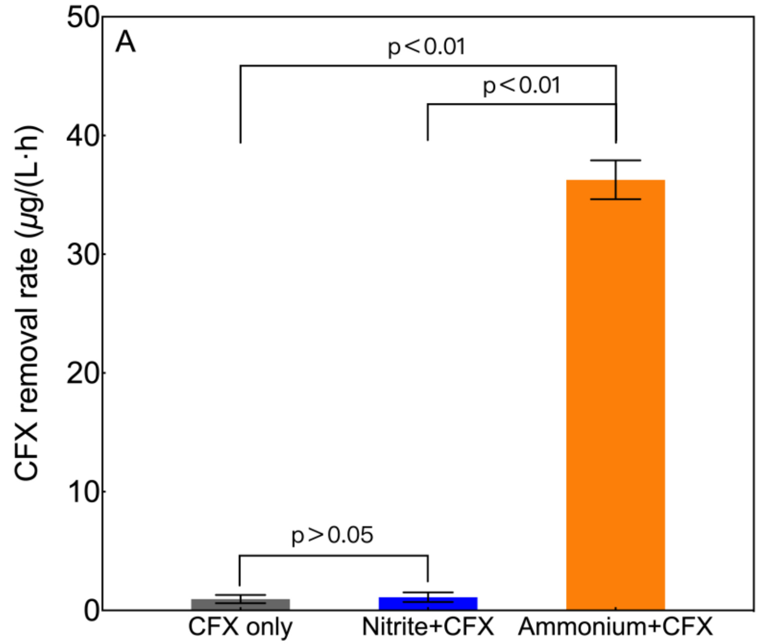

This project is still on-going!

ACKNOWLEDGEMENTS

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