What is wrong with the Nitrogen Cycle– and how can we fix it ?

Christa Schleper

Archaea Biology and Ecogenomics Unit
Department of Functional and Evolutionary Ecology
University of Vienna

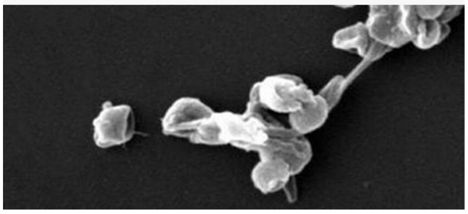
universität wien

rchaea Biology and Ecogenomics VIENNA

1

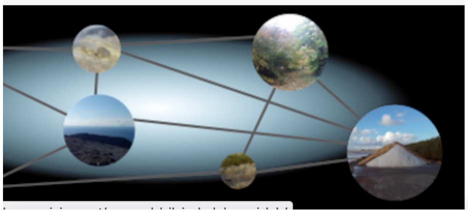
Archaea Unit @ Uni Wien

Archaea Ecology and Evolution
Christa Schleper

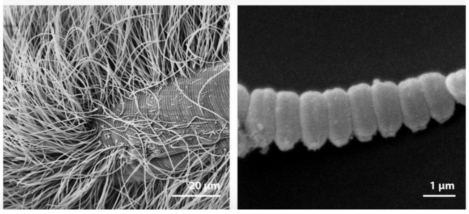


Archaea Physiology & Biotechnology
Simon Rittmann

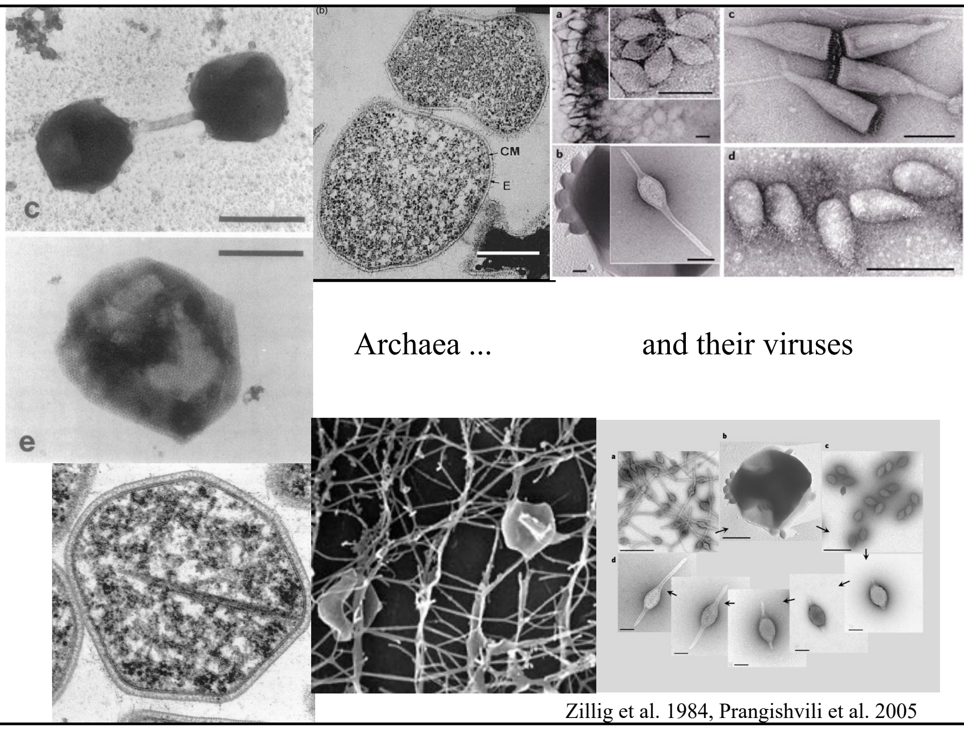
Genome Evolution and Ecology
Filipa Sousa



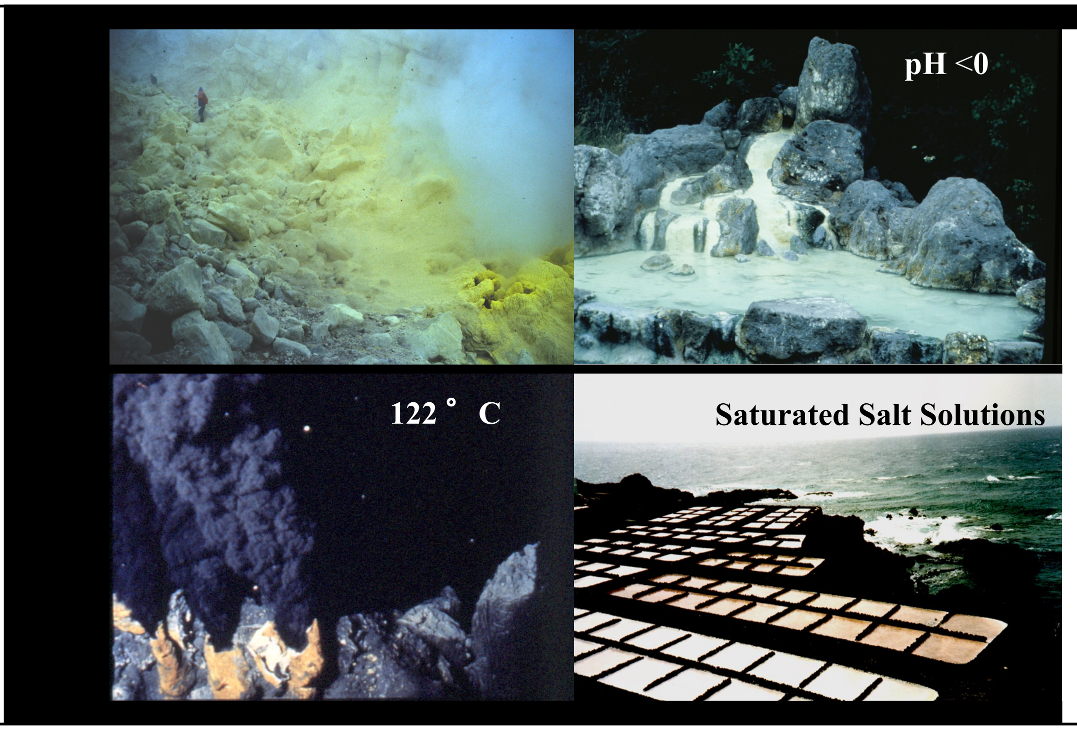
Environmental Cell Biology
Silvia Bulgheresi



2

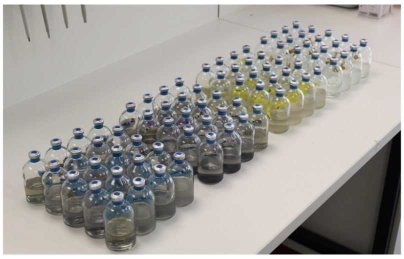

Archaea ...
and their viruses
Zillig et al. 1984, Prangishvili et al. 2005

3


pH <0
122 ° C
Saturated Salt Solutions

4

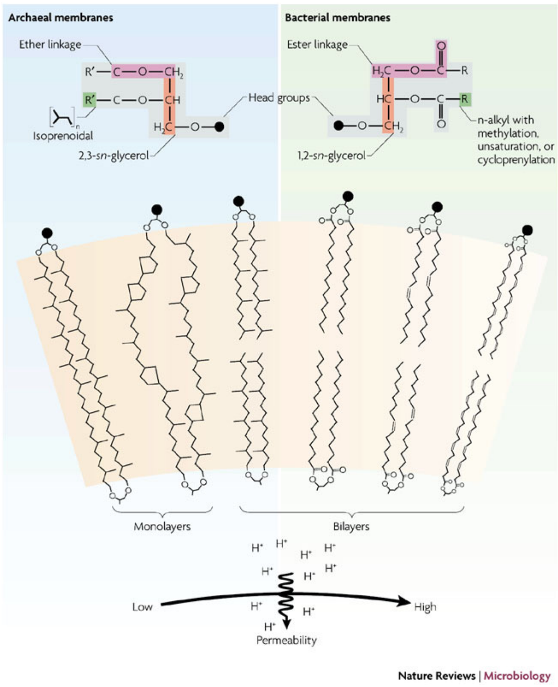
Archaea Biotechnikum
University of Vienna



5

Lipids of Archaea

Very rigid:
Ether link
Isoprenoid side chains
Mono- and bilayer



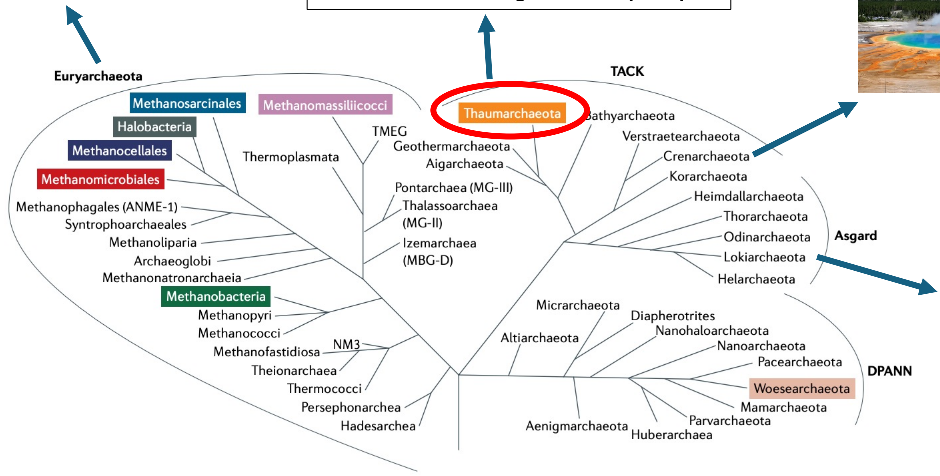
6

Archaea @ Uni Wien

Methanogens
-methane production

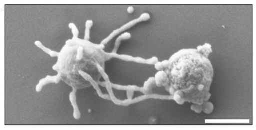
Ammonia Oxidizing Archaea (AOA)

Extreme Environments/Viruses



Moissl-Eichinger *et al.*, 2021

Eukaryogenesis



Actin cytoskeleton and complex cell architecture in an Asgard archaeon
Thiago Rodrigues-Oliveira, Florian Wollweber, Rafael I. Ponce-Toledo, Jingwei Xu, Simon K.-M. R. Rittmann, Andreas Klingl, Martin Pilhofer & Christa Schleper

7

Biological nitrification inhibition - the consortium

Archaea Biology and Ecogenomics VIENNA

universität wien

MoSys

UNIVERSITY OF THESSALY

ÉCOLE CENTRALE LYON

UNIVERSITY OF ALBERTA

JIRCAS 国際農研

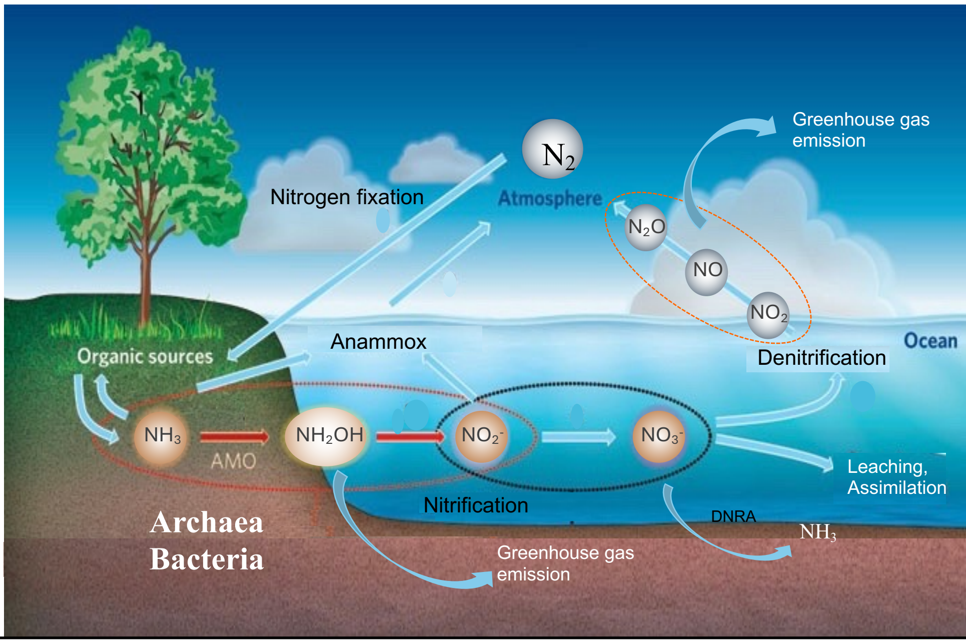
ACTIONr

HORIZON-WIDERA-2021-ACCESS-03

The Grantham Foundation for the Protection of the Environment

8

Microorganisms turn the Nitrogen Cycle



Schleper N&V 2008

9

Industrial Fixation of Atmospheric Nitrogen

1909: Fritz Haber discovers the process of ammonia synthesis from atmospheric nitrogen and hydrogen

$$N_2 + 3\,H_2 \rightarrow 2\,NH_3$$

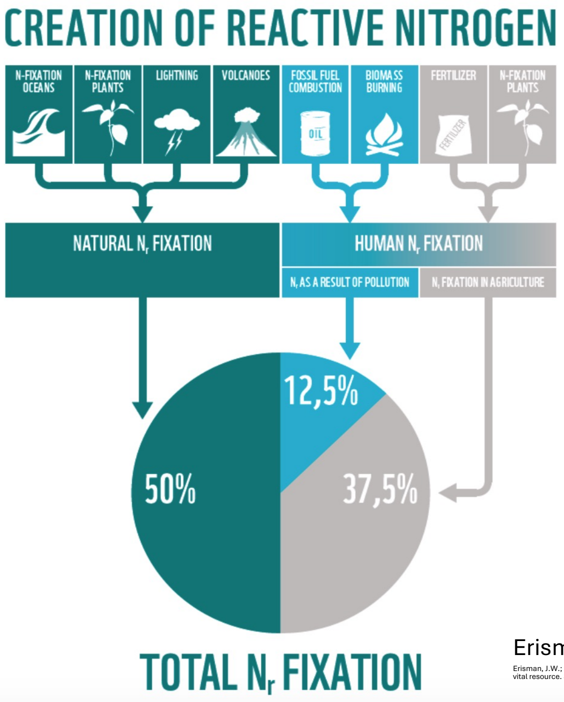
High temperature
High pressure
catalysis

1913: Carl Bosch develops the process to industrial size

https://www.bild.bundesarchiv.de/dba/de/search/?query=Bild+183-S13651

Das Bundesarchiv Bild 183-S13651

10

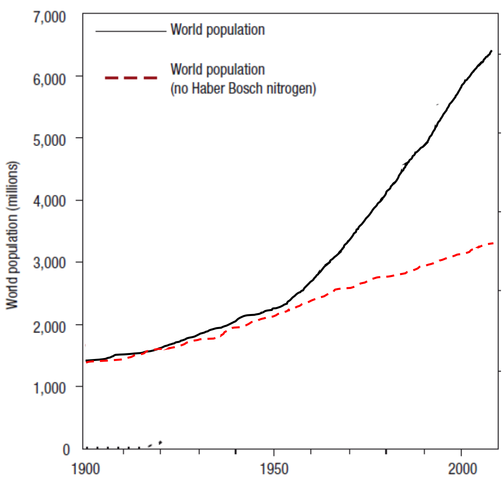


Erisman et al. 2015.

Erisman, J.W.; J.N. Galloway; N.B. Dice; M.A. Sutton; A. Bleeker; B. Grizzetti; A.M. Leach & W. de Vries. 2015. Nitrogen: too much of a vital resource. Science Brief. WWF Netherlands, Zeist, The Netherlands.

11

Around 50% of the world's population is fed by Haber-Bosch nitrogen



modified from Erisman *et al.*, 2008 Nature Geosciences

12

The green revolution:

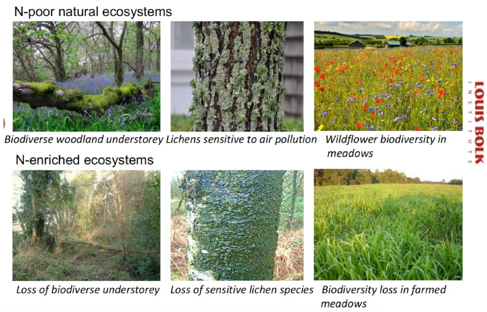
Use of mineral fertilizer in India (1000 t)

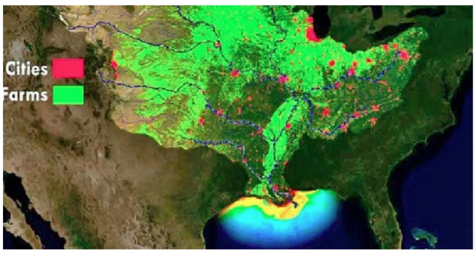
	Nitrogen Stickstoff	Phosphate Phosphat	Potassium Kali
1960/61	212	53	29
1965/66	575	133	77
1970/71	1 479	541	236
1975/76	2 149	467	278
1980/81	3 678	1 214	624
1985/86	5 661	2 000	810
1990/91	7 997	3 221	1 328
1995/96	9 823	2 898	1 156
1999/2000	12 475	4 906	1 762

https://www.cfr.org/blog/india-needs-second-green-revolution

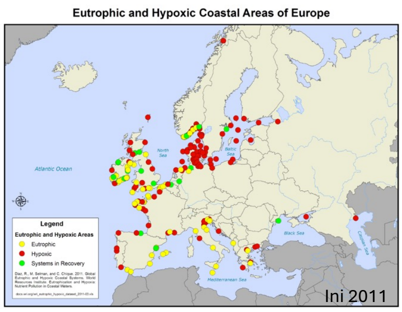
13

An over-fertilized world





https://www.cnet.com/news/gulf-of-mexico-dead-zone-could-



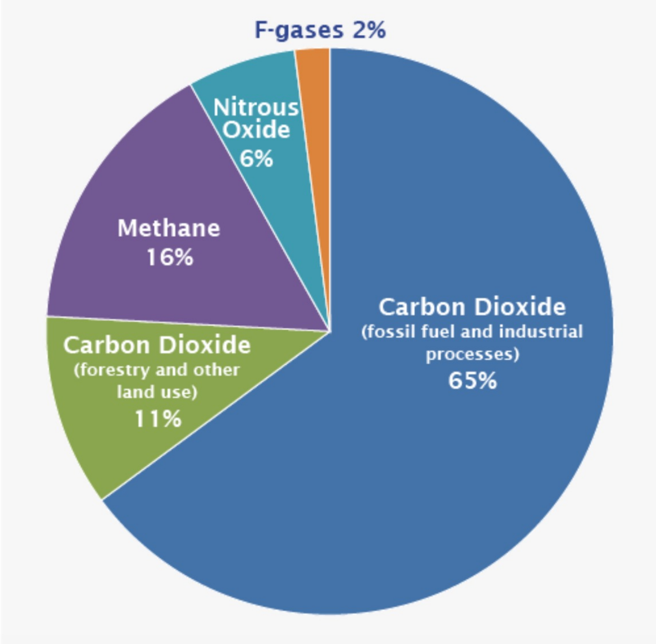
14

Nitrous Oxide : N_2O

Nitrous oxide (N_2O)
- 3rd most important greenhouse gas
- damages ozon layer

298 x more effective than CO_2

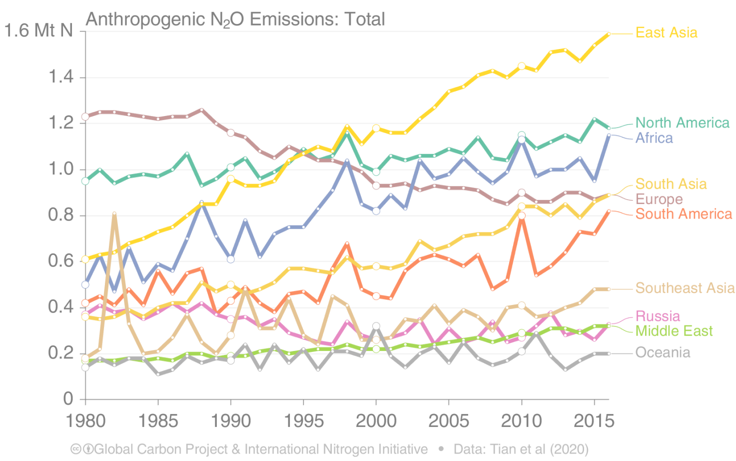
Responsible for **6.5% of global warming**
(global carbon project, 2020)



15

GLOBAL CARBON PROJECT

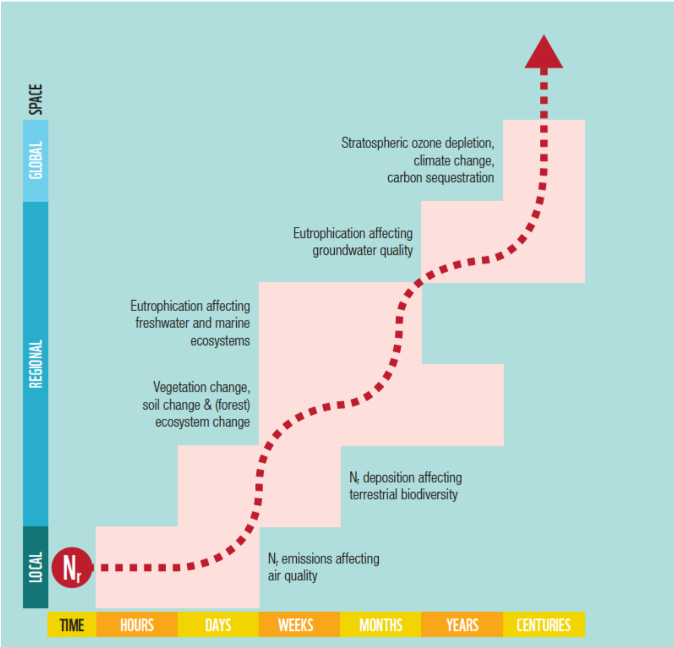
2020 update on N_2O



- anthropogenic emissions responsible for the growth in atmospheric N_2O, increased by 30% since 1980, dominated by **nitrogen fertilization in croplands.**
- The recent increase in global N_2O emissions **exceeds the emission trends** of the least optimistic scenarios developed by the Intergovernmental Panel on Climate Change (IPCC), underscoring the urgent need to mitigate N_2O emissions.

16

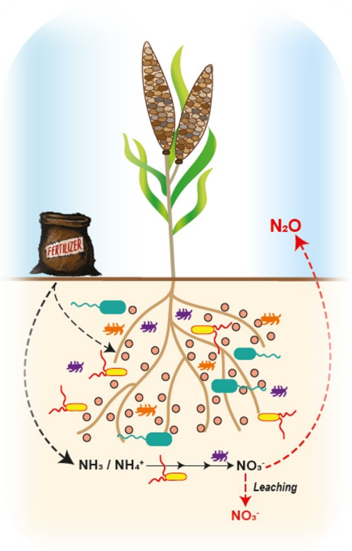
The Nitrogen Cascade



One Nitrogen atom can have multiple impacts on different scales

Erisman et al. 2005
Galloway et al. 2003

17



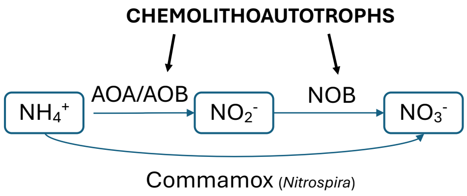
The problem:

Up to 70% of N-fertilizer ends up in the Environment (not in the plant!)

Due to low NUE
(Nitrogen Use Efficiency)

18

Nitrification



Bacteria

Archaea

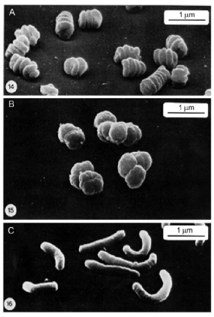
Ammonia oxidation

$NH_3 + O_2 + 2H^+ 2e- \rightarrow NH_2OH + H_2O$

$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$

AOB:
- *Nitrosomonas* (Betaprot.)
- *Nitrosococcus* (Gammaprot.)
- *Nitrosospira* (Betaprot.)

AOA:
- Nitrososphaera
- Nitrosocosmicus



SEM of AOB: Nitrosospira briensis, Nitrosospira multiformis, Nitrosovibrio tenuis (Norton et al. 2008)

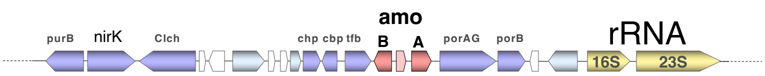
Nitrite oxidation

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e-$

NOB:
- *Nitrobacter* (Alphaprot.)
- *Nitrospina* (Deltaprot.)
- *Nitrococcus* (Gammaprot.)
- *Nitrospira* (Betaprot.)

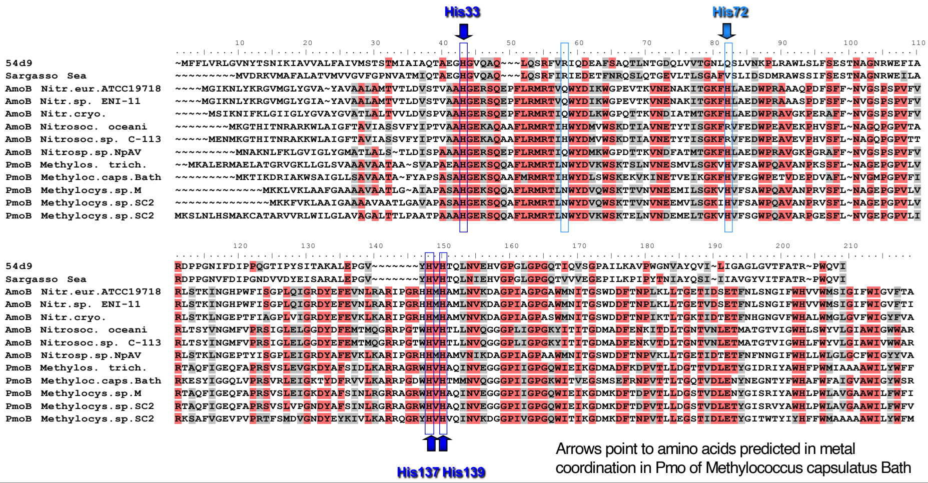
19

Discovery of Ammonia monooxygenase in Archaea and Bacteria



Soil fosmid 54d9

Treusch et al. 2005

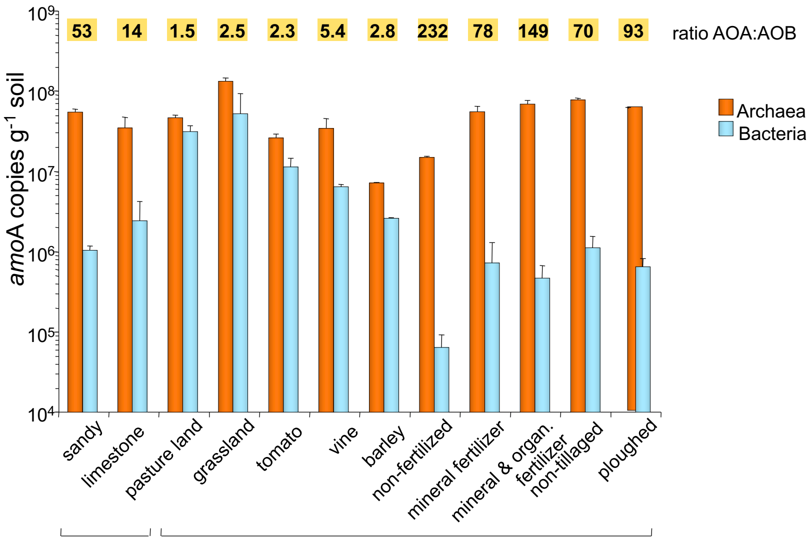


Arrows point to amino acids predicted in metal coordination in Pmo of Methylococcus capsulatus Bath

Treusch et al. 2005

20

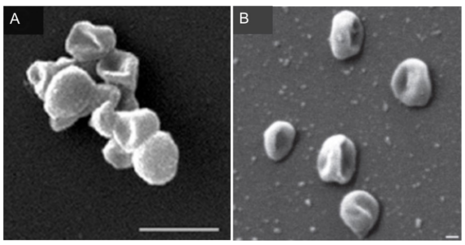
AOA Outnumber AOB in Soils



Leininger *et al*., 2006

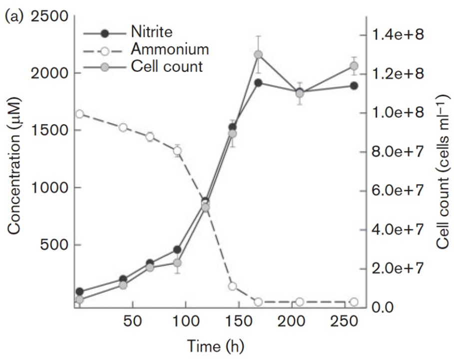
21

Nitrososphaera viennensis



Tourna *et al*. (2011) and Stieglmeier *et al*. (2014).

Nitrososphaera viennensis
(2 mM oxidized ammonia)



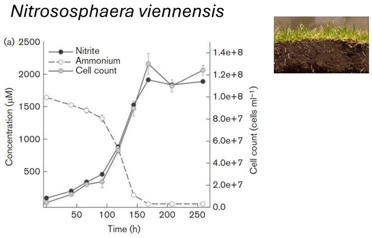
Stieglmeier *et al*. (2014)

NH_4^+ → NO_2^-

22

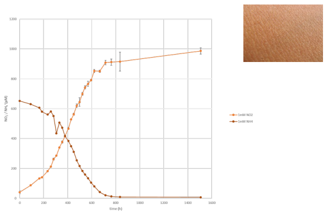
AOA Isolated in Vienna

Nitrososphaera viennensis



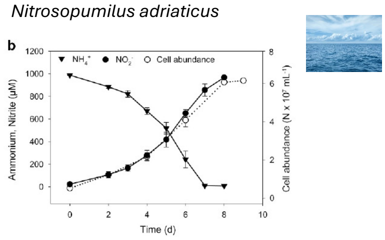
Stieglmeier *et al*,. 2014

Nitrosocosmicus epidermidis

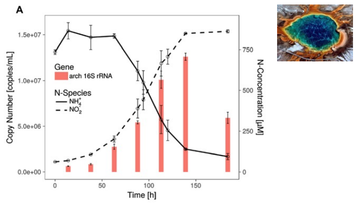


Isolated by Ülkü Perier (in preparation)

Nitrosopumilus adriaticus

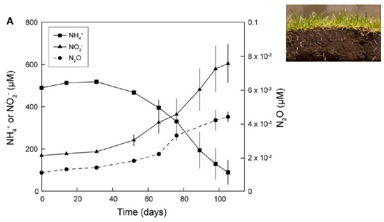


Nitrosocaldus cavascurensis



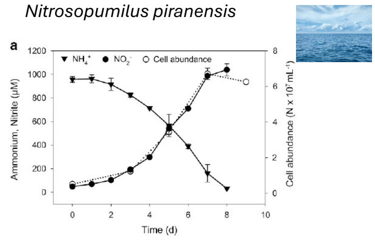
Abby *et al*., 2016
Isolated by Michael Melcher.

Nitrosocosmicus arcticus



Isolated by Max Dreer (in preparation)

Nitrosopumilus piranensis



Bayer *et al*., 2016

23

Ammonia Oxidation in the Two Domains of Life

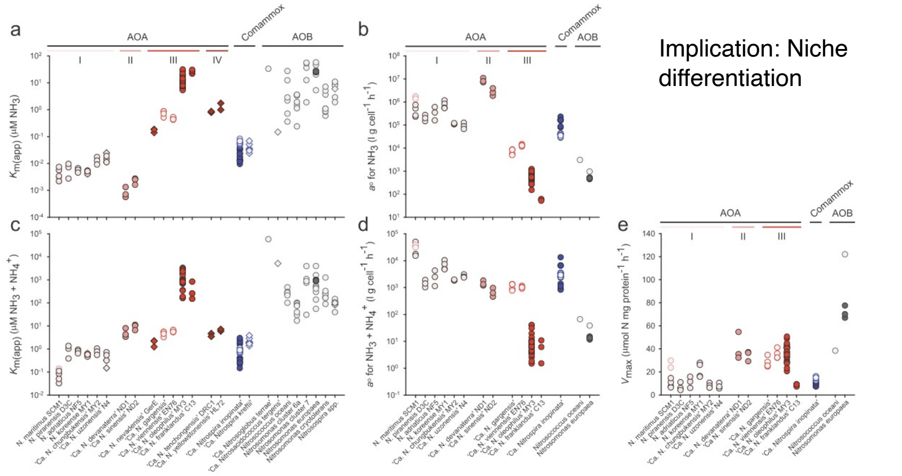
	Ammonia Oxidizers	
	Bacteria	Archaea
First Step	3 subunit AMO	6 subunit AMO
Second Step	HAO	?
Third Step?	?	?
Electron Carriers	cytochrome *c* (Fe)	plastocyanin (Cu)
Quinones	Ubiquione	Menaquinone
Carbon Fixation	3-HP/4-HB	CBB
Membrane	Lipid bi-layer (gram neg.)	S-layer
Lipids	Fatty acids	Isoprenoid lipids
Informational Machinery	Bacterial	Eukaryotic-like

AOA

AOB/Comammox

24

1. Niche differentiation: Most AOA lineages have a higher affinity for NH_3 than AOB



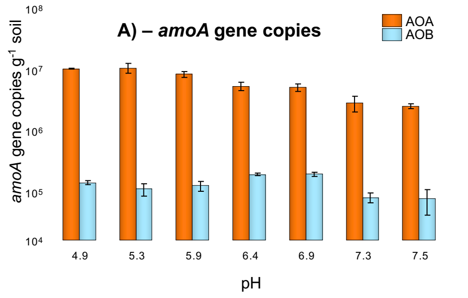
Implication: Niche differentiation

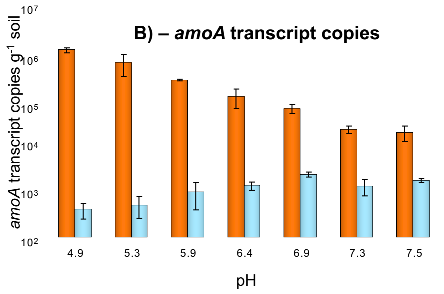
Fig. 2 Substrate-dependent oxidation kinetics of ammonia-oxidizing microorganisms. The (**a**) apparent substrate affinity ($K_{m(app)}$) for NH_3, (**b**) specific substrate affinity (a^o) for NH_3, (**c**) $K_{m(app)}$ for total ammonium, (**d**) a^o for total ammonium, and (**e**) maximum oxidation rate (V_{max}), of AOA (red), comammox (blue), and AOB (black) are provided. Symbols filled with light gray represent previously published values from reference studies (references provided in Materials and Methods). The four different gradations of red differentiate the four AOA phylogenetic lineages: (I) *Nitrosopumilales*, (II) '*Ca.* Nitrosotaleales', (III) *Nitrososphaerales*, and (IV) '*Ca.* Nitrosocaldales'. Measurements were performed with either pure (circles) or enrichment (diamonds) cultures. Multiple symbols per strain represent independent measurements performed in this study and/or in the literature. The individual Michaelis–Menten plots for each AOM determined in this study are presented in Figs. S1, S3–5, and S8. Note the different scales.

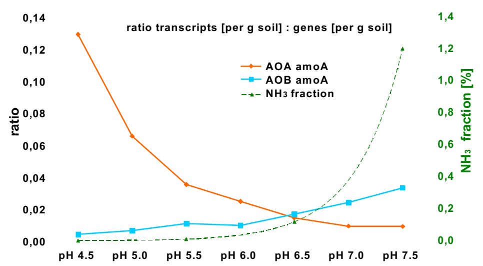
Jung et al. 2021, ISME

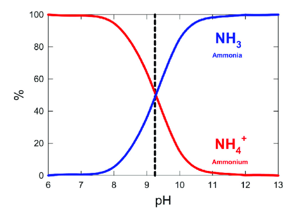
25

2. Niche differentiation: AOA are active at low pH – i.e. in acidic agricultural soils







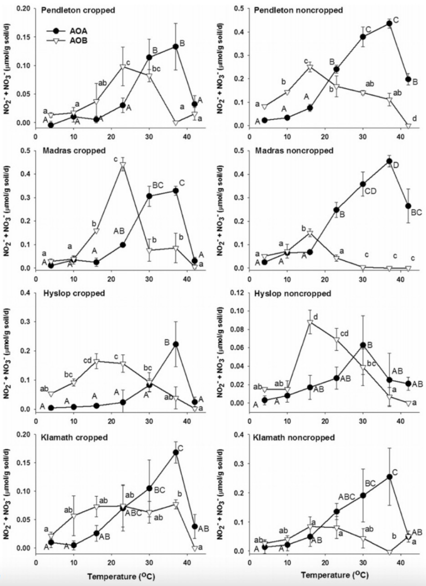


Nicol et al 2008 Env. Microb.

26

3. Niche differentiation: Archaea are more active at higher temperatures

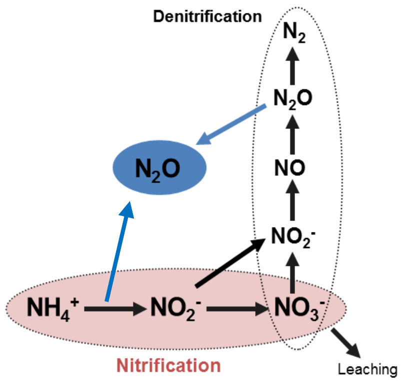
Niche differentiation : Temperature

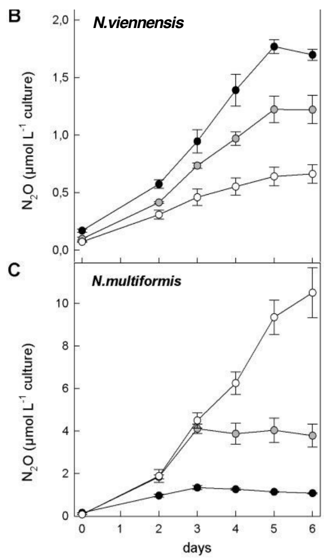


Taylor et al., 2017. *The ISME journal*

27

4. AOB produce N_2O under oxygen depletion





Stieglmeier et al. ISME J. 2016

28

Solution to Nr losses in agriculture: Inhibit Nitrification

Fertilizer

NH_4^+ → NO_2^-

Ammonia Oxidizers

Fertilizer + Nitrification Inhibitor (NI)

NH_4^+ → NO_2^-

Ammonia Oxidizers

29

Solution to Nr losses in agriculture: Inhibit Nitrification

Fertilizer

NH_4^+ → NO_2^-

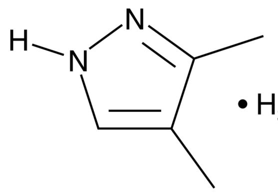
Ammonia Oxidizers

Fertilizer + Nitrification Inhibitor (NI)

NH_4^+ → NO_2^-

Ammonia Oxidizers

3,4-dimethylpyrazole phosphate (DMPP)
*In use since ~2000.



• H_3PO_4

Synthetic Nitrification Inhibitor (SNI)

Slows down nitrification, less nitrogen lost, more nitrogen for plants, less fertilizer needed.

30

Archaea vs. Bacteria: Different sensitivities to nitrification inhibitors

EC_{50} in μM (color scale: 10^5, 10^4, 10^3, 10^2, 10^1, 1)

	NP	EQ	QI	DMPP	EQNL	DCD	
AOB	2.1 (±0.4,aA)	181.4 (±23.3,bB)	199.8 (±10.8,bC)	2.1 (±0.7,aA)	543.4 (±111.5,cC)	221.9 (±29,bA)	*N. europaea*
	0.8 (±0.3,aA)	214.8 (±39.6,bB)	65.1 (±6,aB)	0.6 (±0.1,aA)	360.5 (±105.7,cB)	248.7 (±7.4,bcA)	*N. multiformis*
AOA	1 (±0.3,aA)	1.4 (±0.3,aA)	0.7 (±0.4,aA)	1773.7 (±439.9,bB)	129.5 (±25,aA)	1568.5 (±237.1,bB)	*Ca.* N. franklandus
	6.7 (±1.8,aA)	1 (±0.4,aA)	0.3 (±0,aA)	359.5 (±43.1,bA)	26.6 (±5.7,aA)	477.8 (±56.6,cA)	*Ca.* N. sinensis
NOB	167.8 (±41.2,aB)	166.7 (±53.5,aB)	247.2 (±65.7,aD)	12581.3 (±1979.2,bC)	562 (±38.5,aC)	>100000*	*Nitrobacter* sp.

Nitrapyrin (NP)
Ethoxyquin (EQ)
3,4-dimethylpyrazole-phosphate (DMPP)
dicyandiamide (DCD)

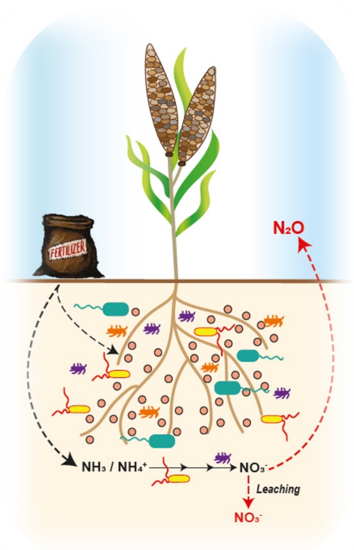
Comparison of Novel and Established Nitrification Inhibitors Relevant to Agriculture on Soil Ammonia- and Nitrite-Oxidizing Isolates

Evangelia S. Papadopoulou[1*] Eleftheria Bachtsevani[1] Eleni Lampronikou[1] Eleni Adamou[1] Afroditi Katsaouni[1] Sotirios Vasileiadis[1] Cécile Thion[2] Urania Menkissoglu-Spiroudi[3] Graeme W. Nicol[2] Dimitrios G. Karpouzas[1]

Front.Microb. 2020

31

What is an ideal nitrification inhibitor ?

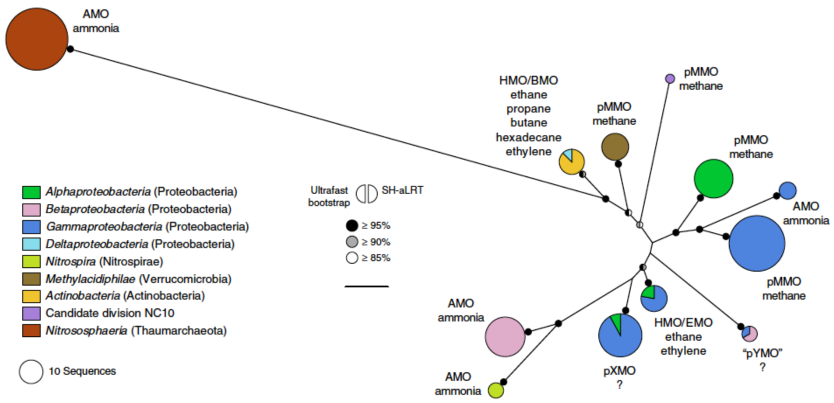


- Inhibits both AOA and AOB
- No ‘off target‘ effects

32

The AMO of Archaea is very distantly related to the bacterial AMO and pMMO

Archaea Bacteria



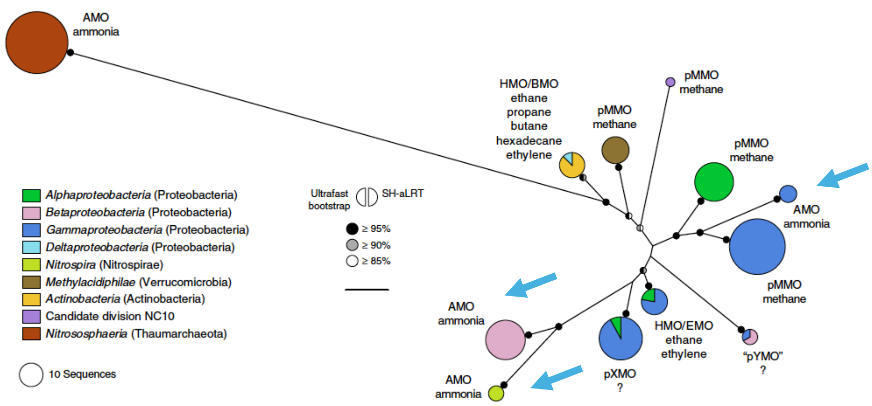
Phylogenetic placement of the AMO within the CuMMO superfamily, (A + B subunit)

Alves et al. Nature Comm. 2018

33

1. The AMO of Archaea is very distantly related to the bacterial AMO and pPMO

Archaea Bacteria

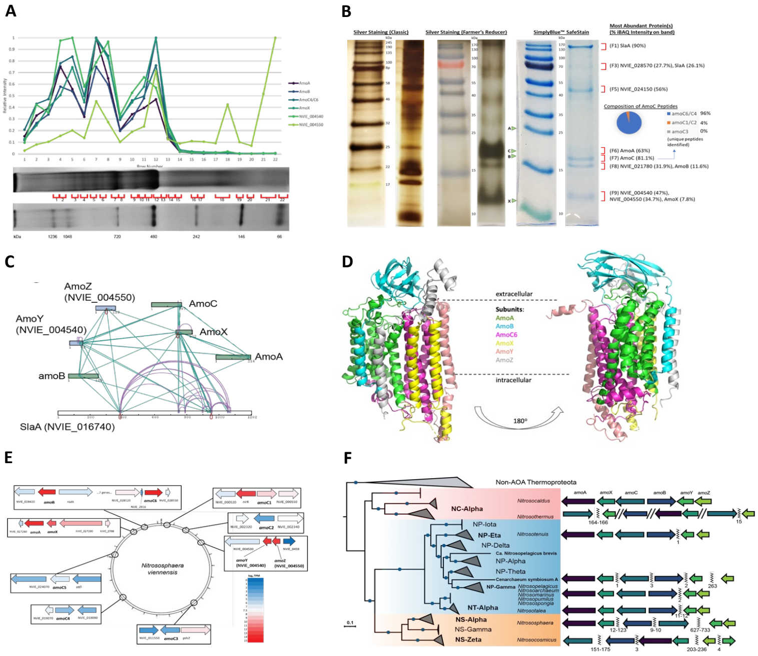


Phylogenetic placement of the AMO within the CuMMO superfamily, (A + B subunit)

Alves et al. Nature Comm. 2018

34

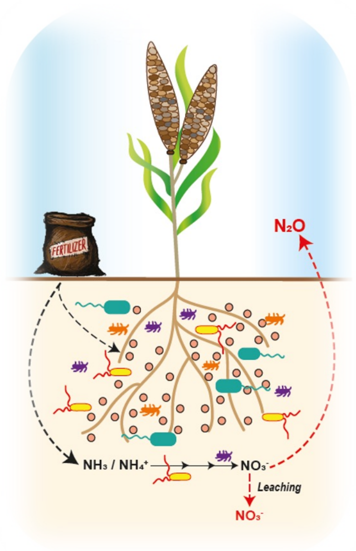
The composition of the archaeal AMO complex is different from bacterial enzymes



Hodgskiss et al. , ISME J 2023

35

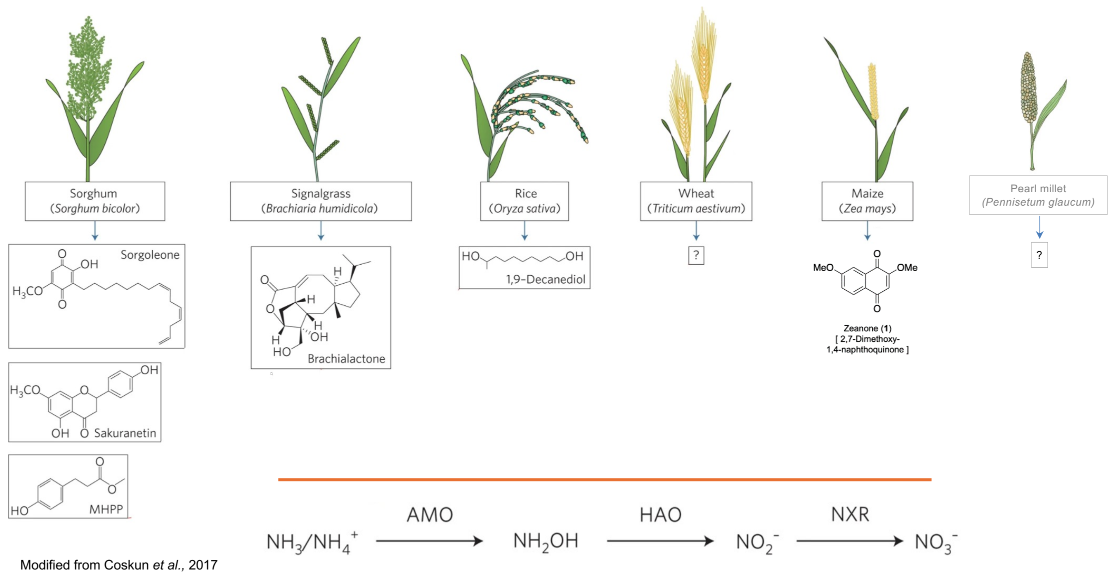
What is an ideal nitrification inhibitor ?



- Inhibits both AOA and AOB
- No ‘off target‘ effects
- Known mode of action
- High performance in different soils
- Short halflife / high biodegradability
- Cheap enough
- Even better: produced by the crop itself

36

Biological Nitrification Inhibitors



Modified from Coskun *et al.*, 2017
Ghatak *et al.*, 2022
Otaka *et al.*, 2022

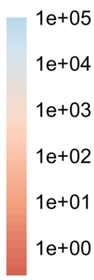
9

37

Archaea vs. Bacteria: differential sensitivities to BNIs

EC_{50} in µM

	N. europaea	Nitrobacter	N. multiformis	N. sinensis	N. franklandianus
Quinic acid	1596.6 (±123.1,aA)	> 3000 (**)	< 3 (*)	84.3 (±14,bB)	>300 (**)
MHPP	339.3 (±32.4,cB)	7.4 (±1.3,fC)	104 (±12.8,bcdC)	268.2 (±3.8,aB)	877.7 (±165.3,bA)
Shikimic acid	> 5000 (**)	238.99 (±25.9,bcB)	191.2 (±42,bcB)	334.9 (±21,aB)	1420.4 (±167.8,aA)
Chlorogenic acid	777.5 (±49.9,bA)	71.9 (±8.9,efB)	81.2 (±9.4,bcdB)	65.5 (±6.9,bB)	148.9 (±80.3,dB)
1-Butyl-4-propyl-triazole	>500 (**)	377.7 (±19.8,aA)	407.3 (±63,aA)	237.4 (±44.8,aA)	460 (±86.1,cA)
1.4-Dibutyltriazole	> 500 (**)	290.6 (±35,abA)	> 500 (**)	283.6 (±71.7,aA)	284.8 (±46,cdA)
Zeanone	> 46 (**)	24.0 (±4.7,fA)	< 0.2 (*)	4.5 (±0.6,bB)	0.4 (±0.1,eB)
Caffeic acid	203.3 (±15.8,cA)	< 3 (*)	140.2 (±12.8,bcdB)	1.8 (±0.2,bC)	19.5 (±6,eC)
2-Methoxy-1.4-naphthoquinone	> 218 (**)	27.8 (±2.2,fA)	> 218 (**)	5.2 (±0.1,bB)	2.9 (±0.3,eB)
Sakuranetin	> 250	155.7 (±20.6,cdeA)	144.3 (±19,bcdA)	< 10 (*)	< 10 (*)
Simvastatin	> 100 (**)	>100 (**)	> 100 (**)	< 5 (*)	25.7 (±5.8,e)



Bacteria (AOB, NOB): N. europaea, Nitrobacter, N. multiformis

Archaea (AOA): N. sinensis, N. franklandianus

Assessing the activity of different plant-derived molecules and potential biological nitrification inhibitors on a range of soil ammonia- and nitrite-oxidizing strains

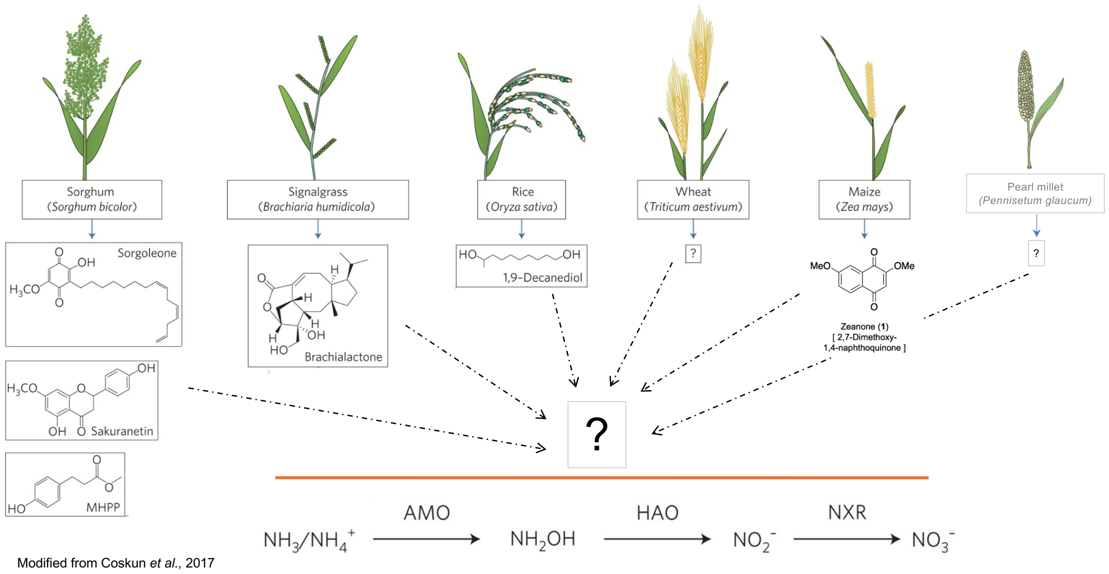
Authors: Maria Kolovou, Dimitra Panagiotou, Lars Süße, Olivier Loiseleur, Simon Williams, Dimitrios G. Karpouzas, Evangelia S. Papadopoulou

AUTHORS INFO & AFFILIATIONS

Env. Microb. 2023

38

Few BNIs and Unknown Mode of Action

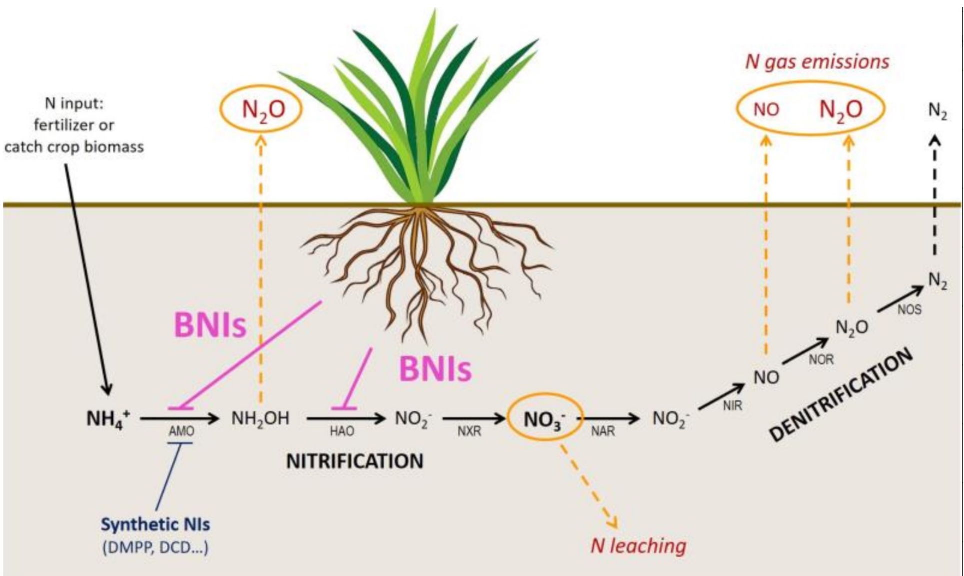


Modified from Coskun *et al.*, 2017
Ghatak *et al.*, 2022
Otaka *et al.*, 2022

9

39

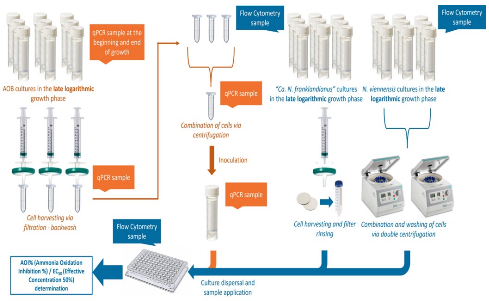
Can we find new inhibitors?

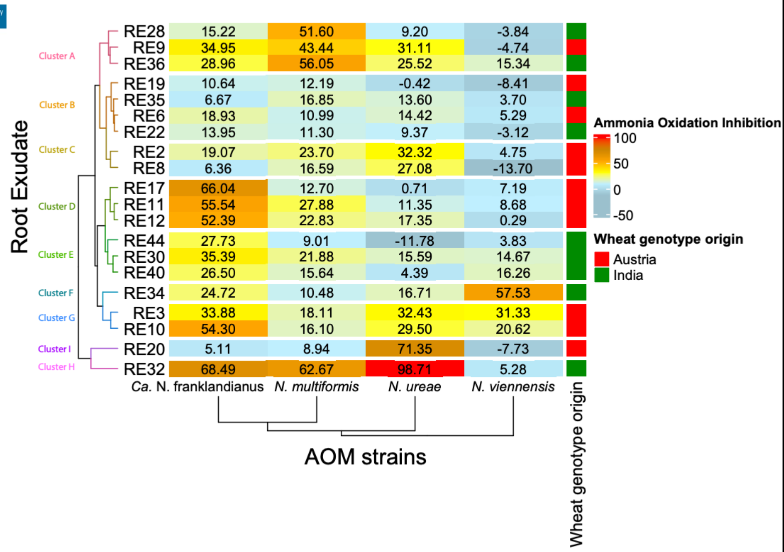


BNI: biological nitrification inhibitor

40

High throughput screening of root exudates from wheat varieties for BNI



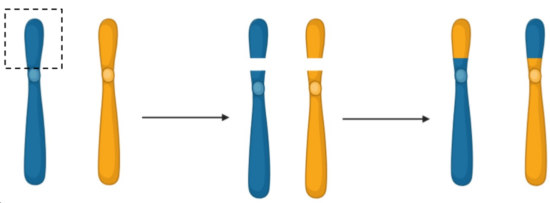


Alexandros Kanellopoulos
Hugo Ribeiro
Andrea Malits
Palak Chaturveda

41

A case study in BNI producing plants: BNI-enabled wheat

BNI-trait:
Lr#n-SA=T3BL.3NsbS



Lr#n= 3Nsb chrom. (Leymus racemosus)

3B chrom. (Triticum aestivum)


PNAS RESEARCH ARTICLE | AGRICULTURAL SCIENCES OPEN ACCESS

Enlisting wild grass genes to combat nitrification in wheat farming: A nature-based solution

Guntur V. Subbarao[a,2,1], Masahiro Kishii[b,1], Adrian Bozal-Leorri[c], Ivan Ortiz-Monasterio[b], Xiang Gao[a], Maria Itria Ibba[b], Hannes Karwat[b], M. B. Gonzalez-Moro[c], Carmen Gonzalez-Murua[c], Tadashi Yoshihashi[a], Satoshi Tobita[d], Victor Kommerell[b], Hans-Joachim Braun[b], and Masa Iwanaga[a]

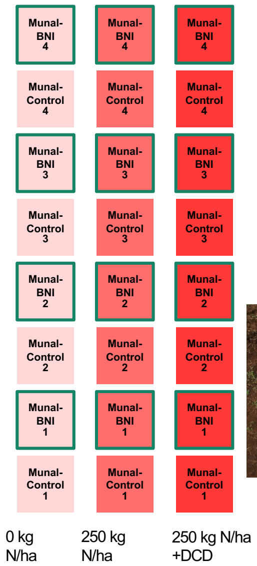
➢ Development of a wheat variety with **nitrification inhibition capacity**.

11

42

Experimental design for the BNI-wheat field experiment

Split-plot design



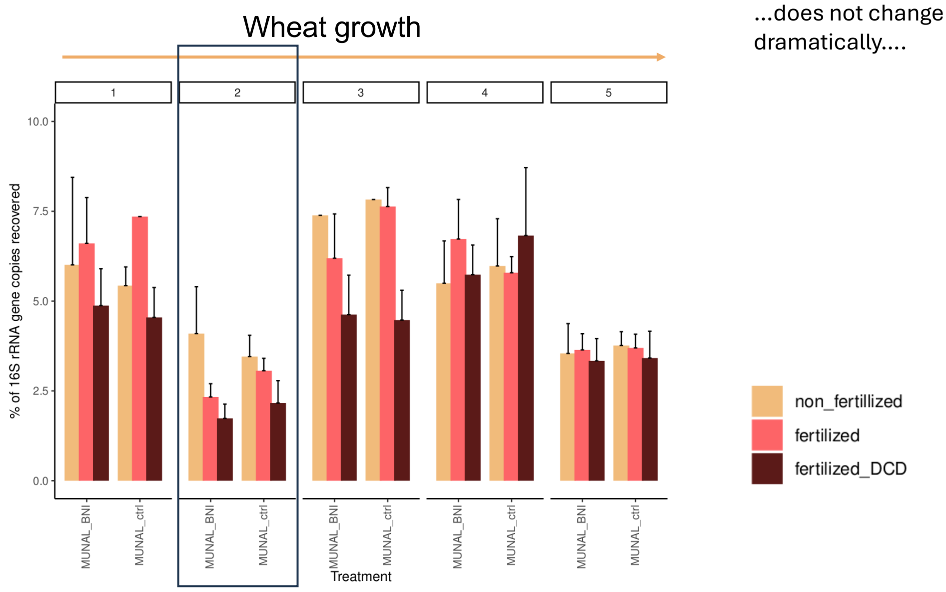
March 19th

May 27th

14

43

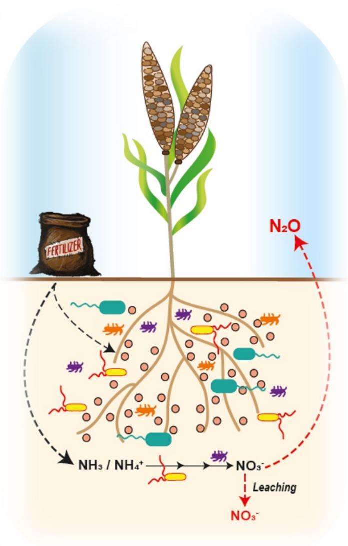
Relative abundance of ammonia oxidizing archaea



...does not change dramatically....

44

Summary



What is an ideal nitrification inhibitor ?

- Inhibits both AOA and AOB
- No ‘off target‘ effects
- Known mode of action
- High performance in different soils
- Short halflife / high biodegradability
- Cheap enough
- Even better: produced by the crop itself

45

BNI consortium Vienna and Collaborators

Archaea Biology and Ecogenomics Vienna

universität wien

MoSys

University of Thessaly

École Centrale Lyon

University of Alberta

JIRCAS 国際農研

ACTIONr

HORIZON-WIDERA-2021-ACCESS-03

The Grantham Foundation for the Protection of the Environment

46