Soil virus ecology and potential of ‘soil phage therapy’ for controlling nitrification

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ACTIONr

ACTIONr PhD summer school

University of Thessaly

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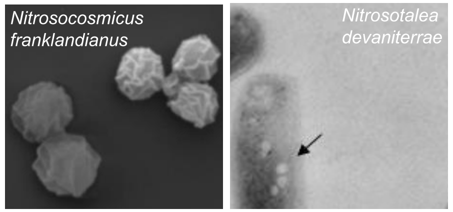
Research themes & experimental approaches

1. Cultivation and ecophysiology of nitrifiers



Canonical AOB
Nitrosomonas europaea
Nitrosomonas nitrosa
Nitrosomonas eutropha
Nitrosomonas ureae
Nitrosomonas oligotropha
Nitrosospira multiformis
Nitrosospira briensis

Comammox
Nitrospira inopinata

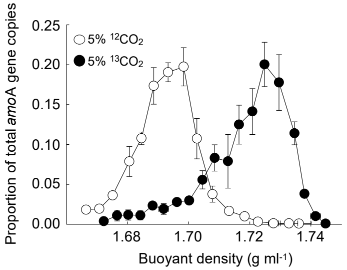
AOA
Nitrosotalea devaniterrae
Nitrosotalea sinensis
Nitrosocosmicus franklandianus
Nitrososphaera viennensis

Canonical NOB
Nitrospira defluvii
Nitrobacter winogradski
Nitrobacter NHB1

2. Molecular ecology and diversity of soil microbes

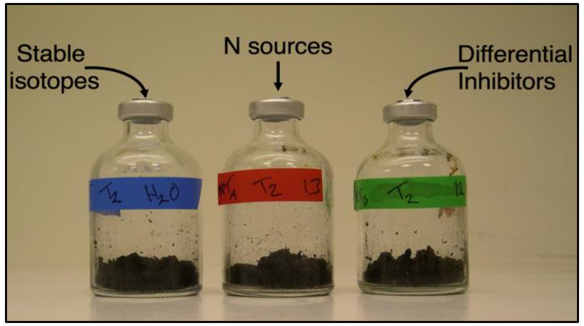
High-throughput sequencing for metagenomics

DNA stable-isotope probing for *in situ* activity



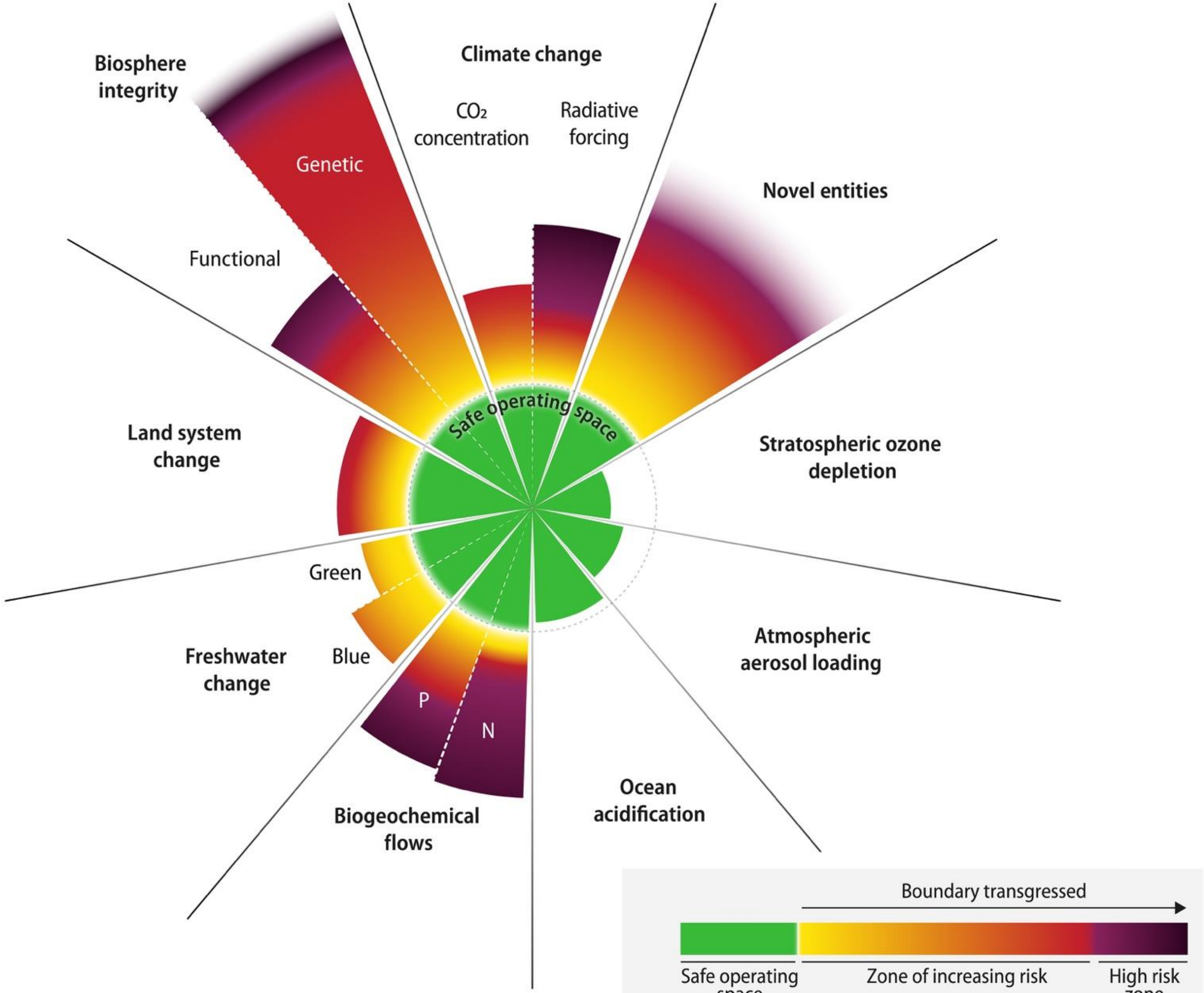
3. Plant-soil interactions and biogeochemical processes

Soil microcosm incubations



Plant mesocosms

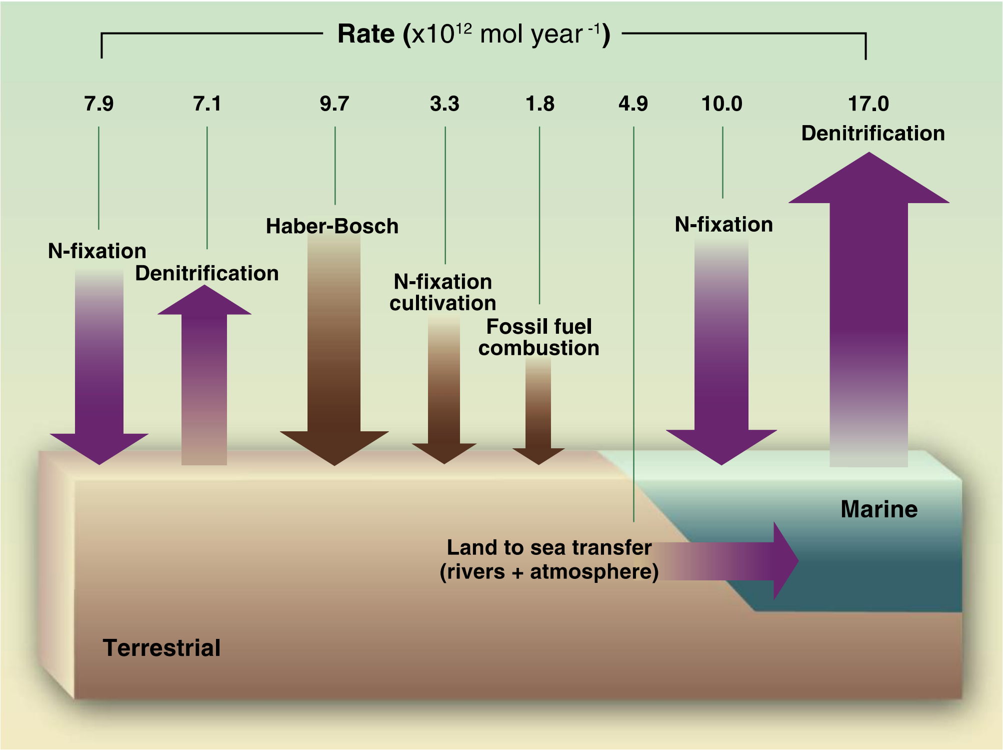
Planetary boundaries: Nitrogen is beyond the safety limit



Richardson et al (2023) Science

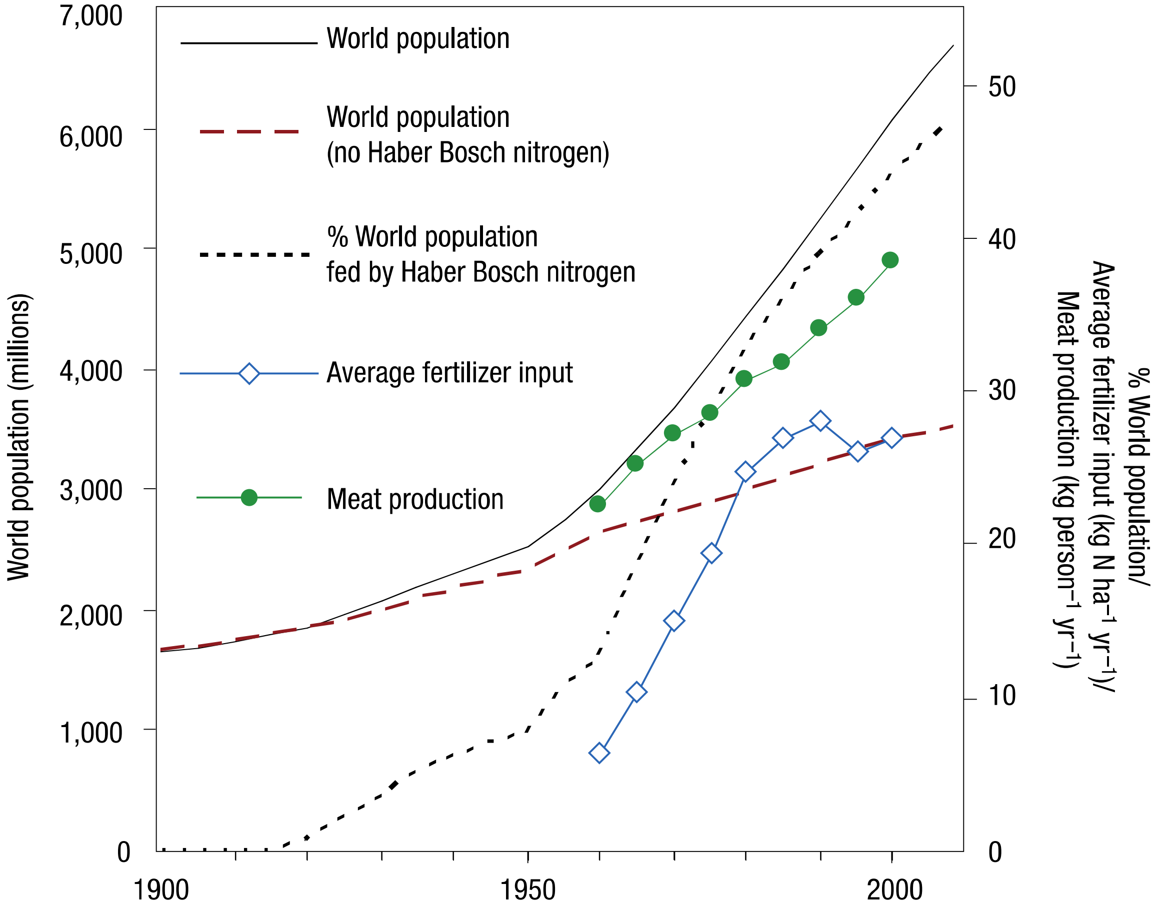
Anthropogenic inputs of reactive N now exceed that of natural processes

Reactive N in terrestrial environments



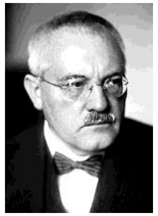
Canfield, Glazer & Falkowski (2010) Science

Haber-Bosch synthetic N fertiliser is responsible for feeding 50% of humanity



Erisman et al. (2008) Nature Geoscience

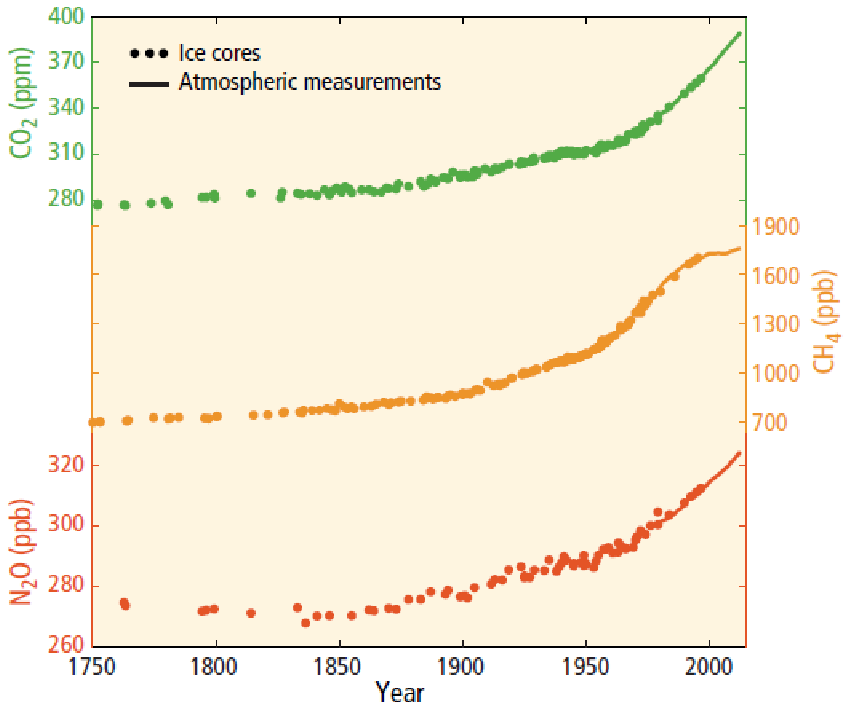
Fritz Haber



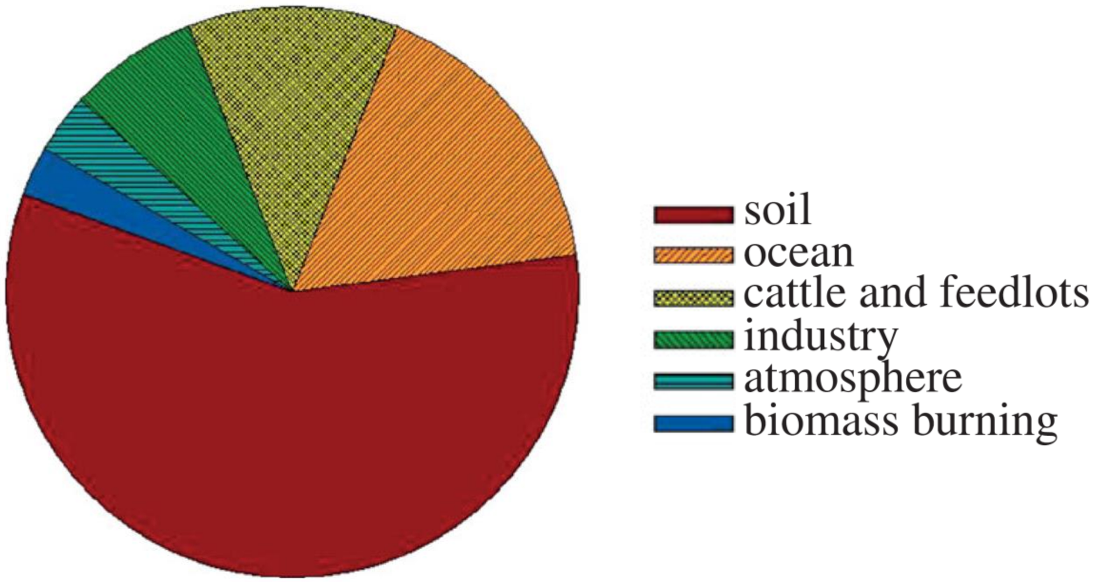
Karl Bosch

Environmental and economic consequences of nitrification in agricultural soils

Greenhouse gas emissions



N_2O sources



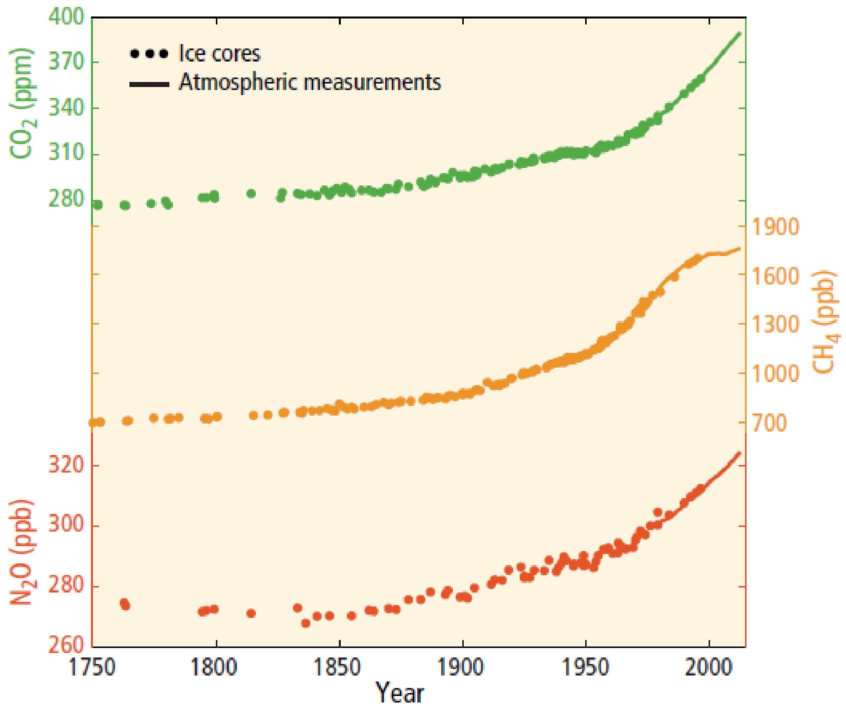
IPCC 2007, 2014

The problem:

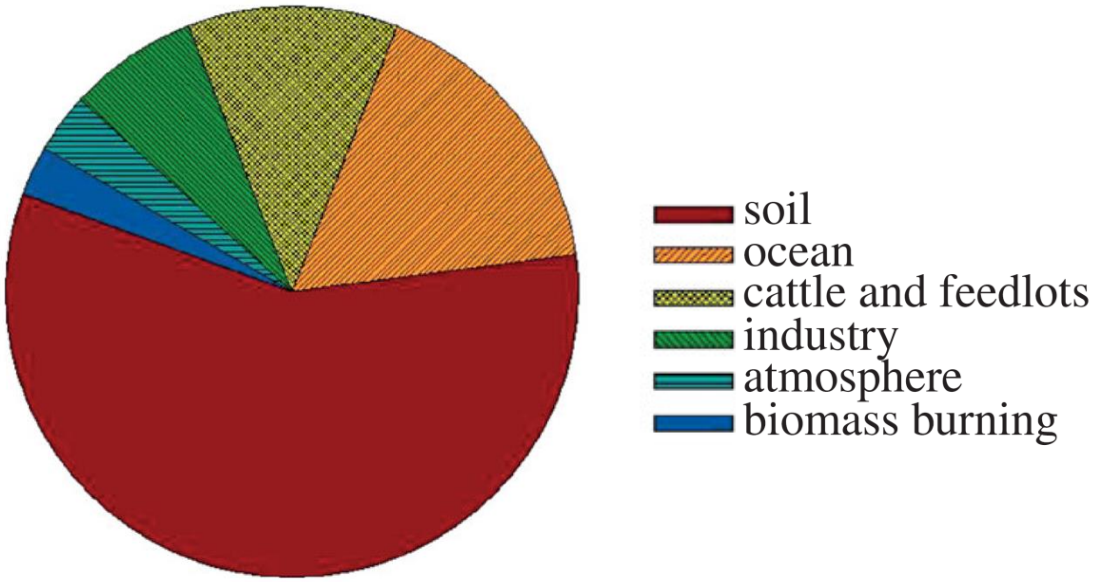
- Global agricultural production expected to increase by 70 - 100% by 2050 to feed 9 billion people
- Agriculture contributes 56% of global anthropogenic emissions of N_2O
- N pollution costs the EU up to €320 billion per year (2x the value that fertilizers add to European farm income)
- Fertiliser production uses ~2% of global energy requirements

Environmental and economic consequences of nitrification in agricultural soils

Greenhouse gas emissions



N_2O sources

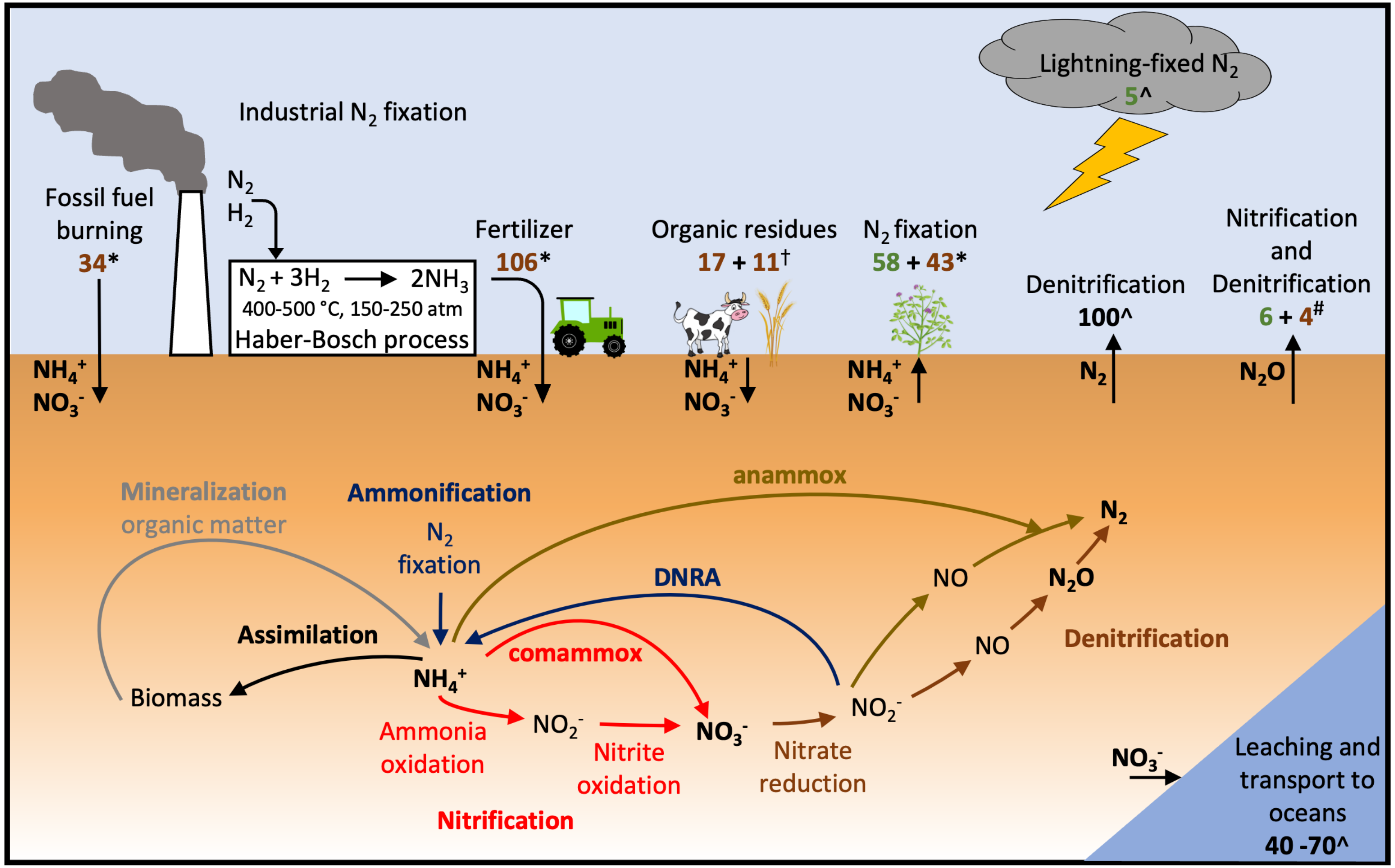


IPCC 2007, 2014

Solutions?

- The EU Green Deal targets a 55% reduction in GHG emissions by 2030
- Biosolutions are encouraged to reduce N_2O emissions (from 7481.08 to 4039.3 Kt CO_2 equivalents)
- After a 10-year usage, 30% of agriculture related GHG emissions could be eliminated (~1.59 Gt CO_2 equiv.)

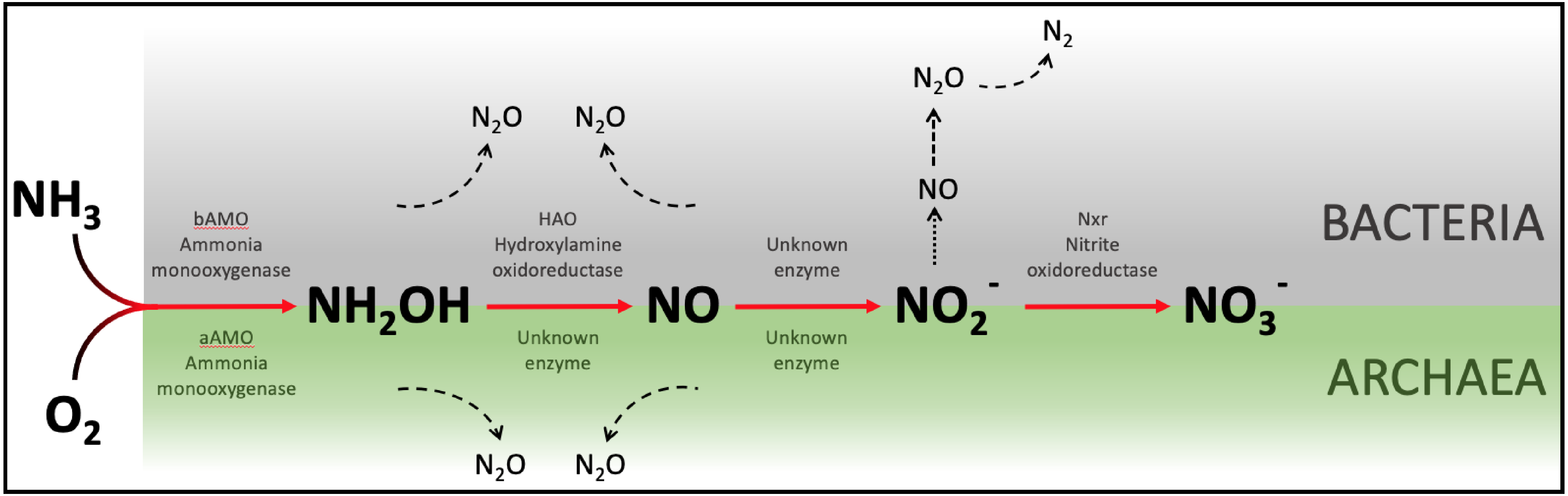
Major processes of the nitrogen cycle



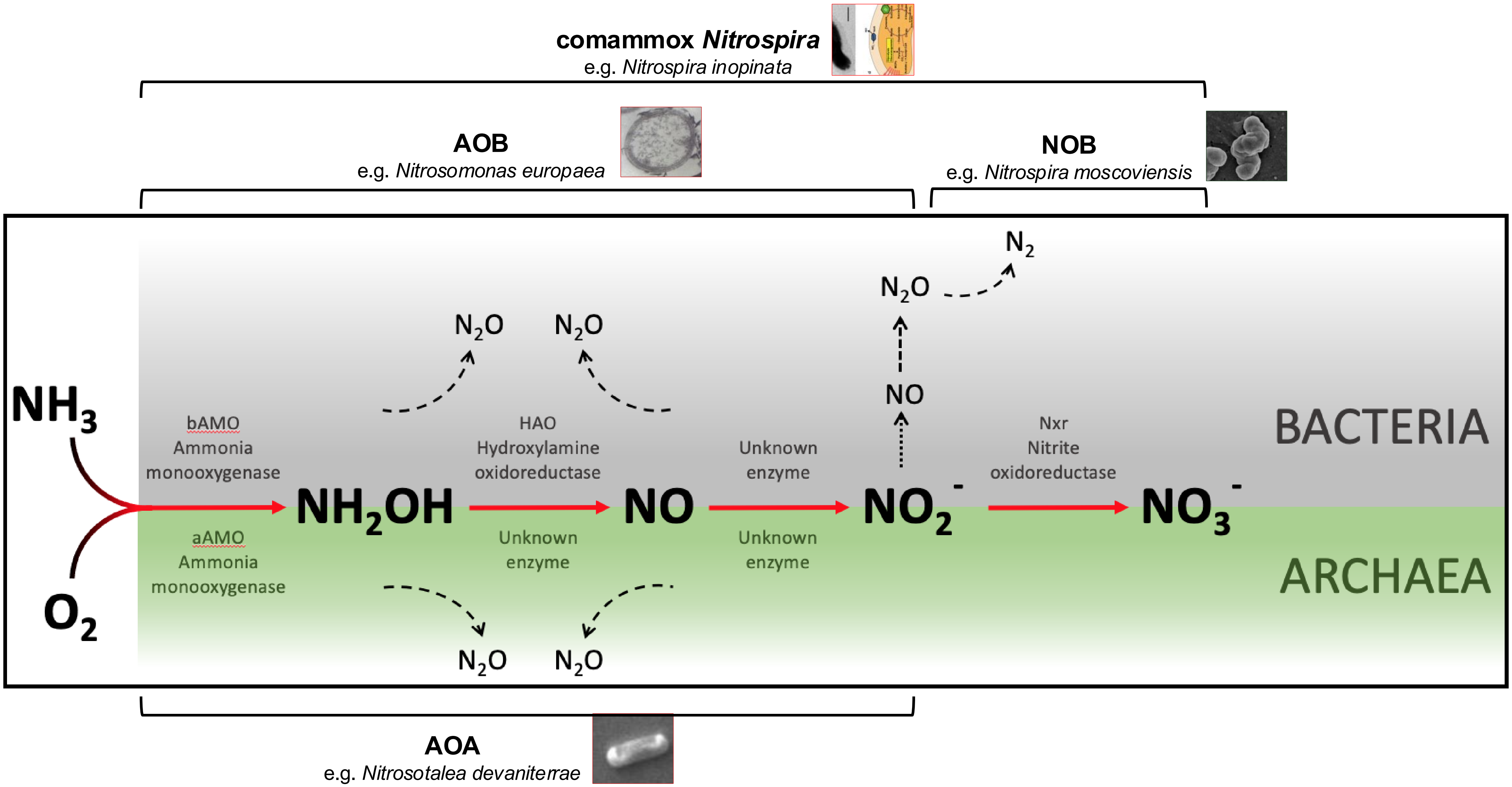
Nitrogen estimates (Tg N per year)
anthropogenic sources
non-agricultural natural sources

anammox = anaerobic ammonia oxidation
DNRA = dissimilatory reduction of nitrate to ammonium
comammox = complete ammonia oxidation

Nitrification



Nitrification



Introduction to viruses

- Most abundant biological entity in biosphere (10^{31})
- Ubiquitous
- Not recognized as true living organisms
- Greatest reservoir of genetic diversity, most of which is uncharacterized
- Play many important roles in ecology, bacterial evolution and diseases

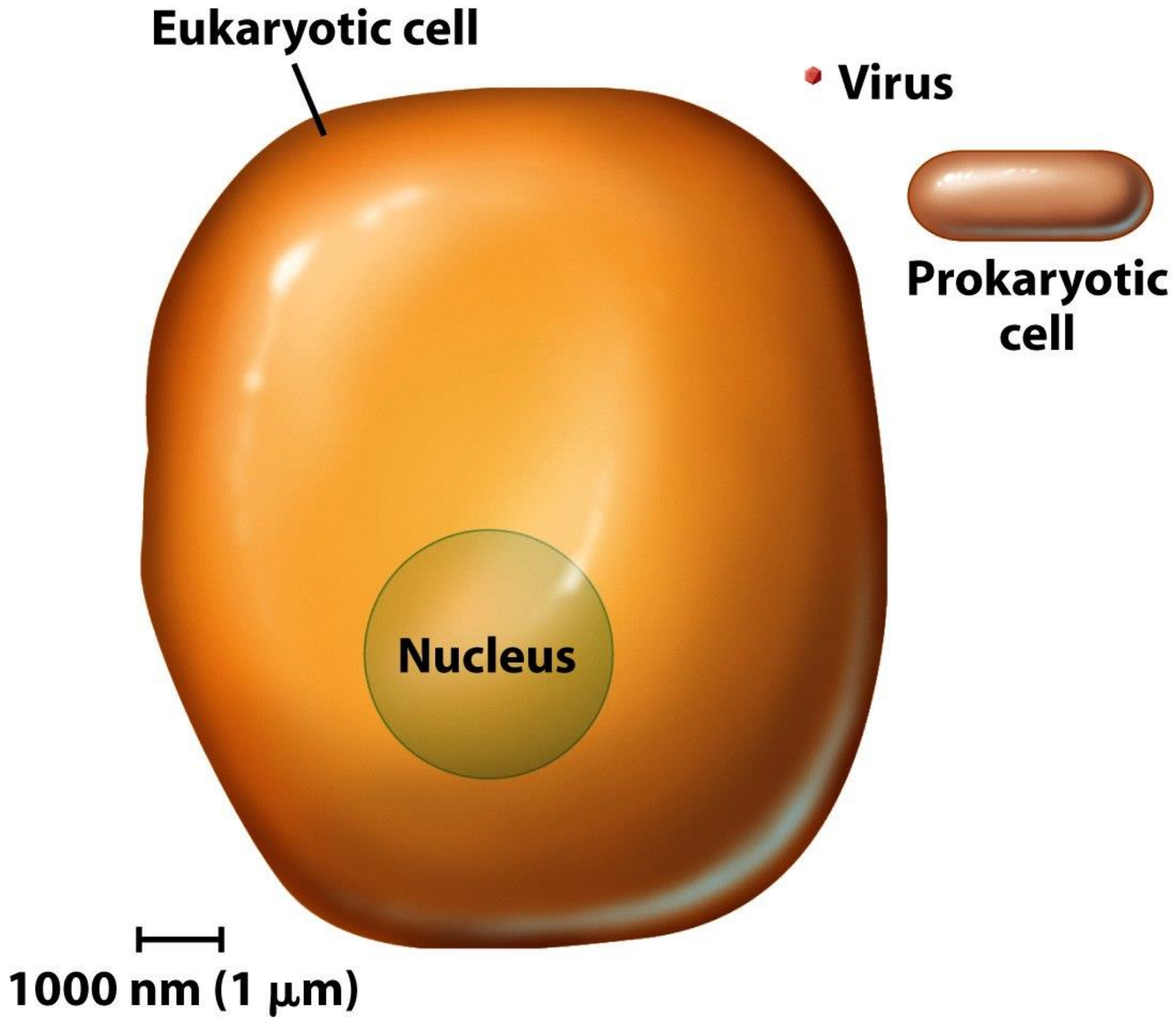
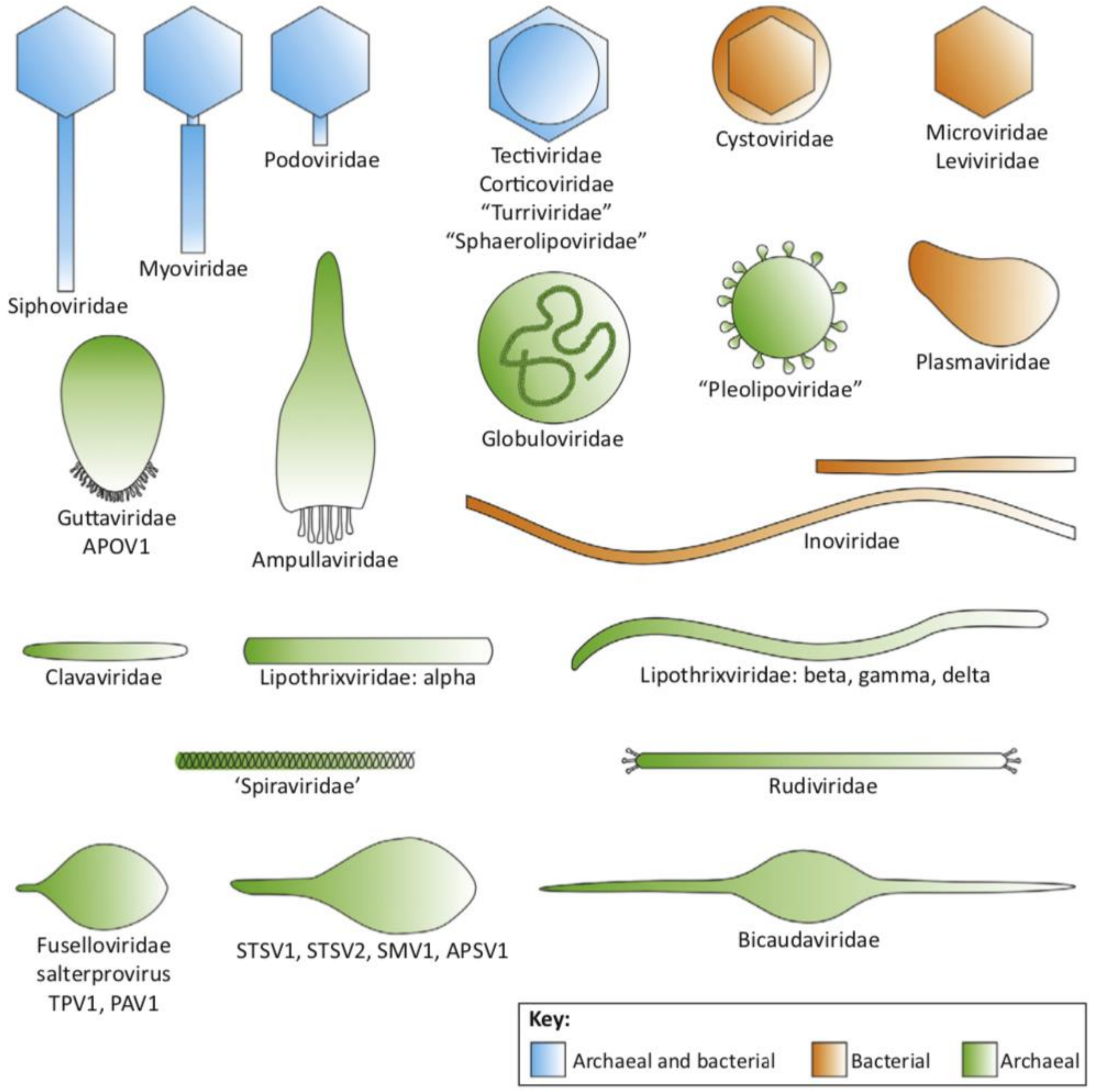


Figure 2-3c Brock Biology of Microorganisms 11/e

Morphological diversity

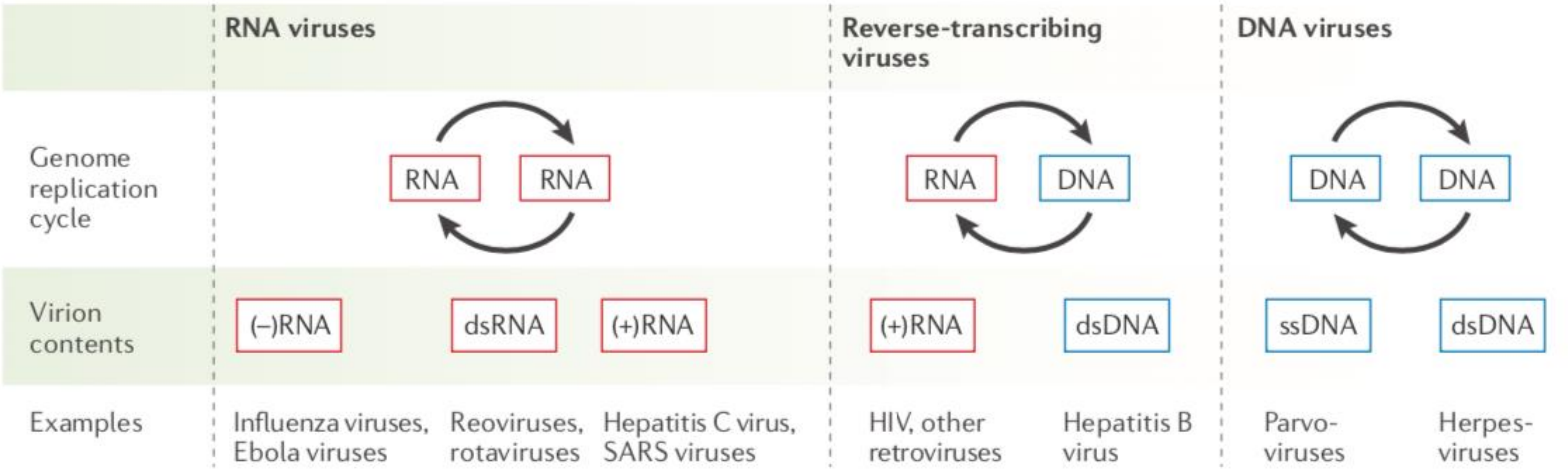
- Traditionally, morphology was the standard approach for classifying viruses at a broad level
- However, morphology does not necessarily reflect evolutionary history
- International Committee on taxonomy of viruses (ICTV)

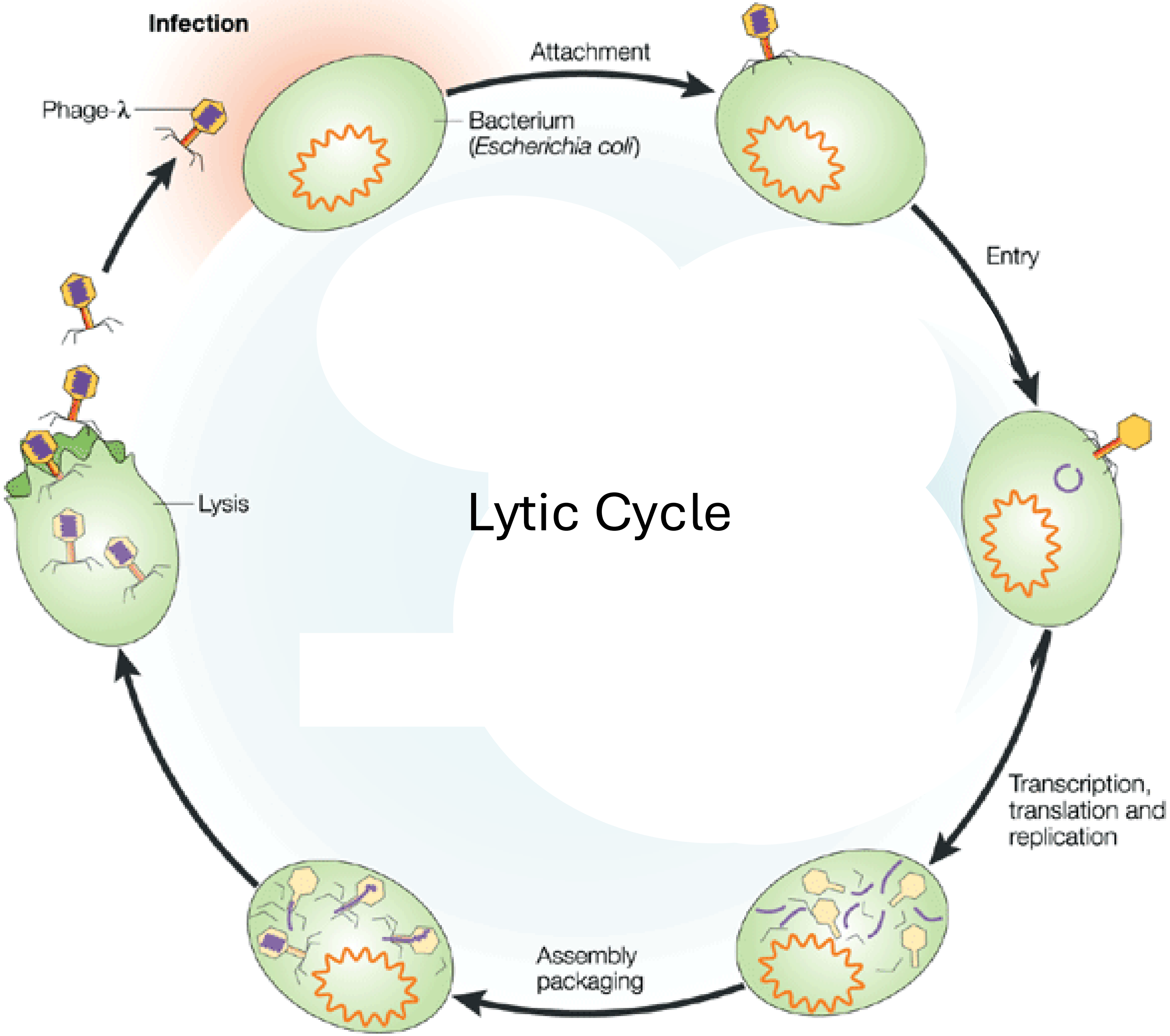


Pietilä et al. (2014) Trends in Microbiology

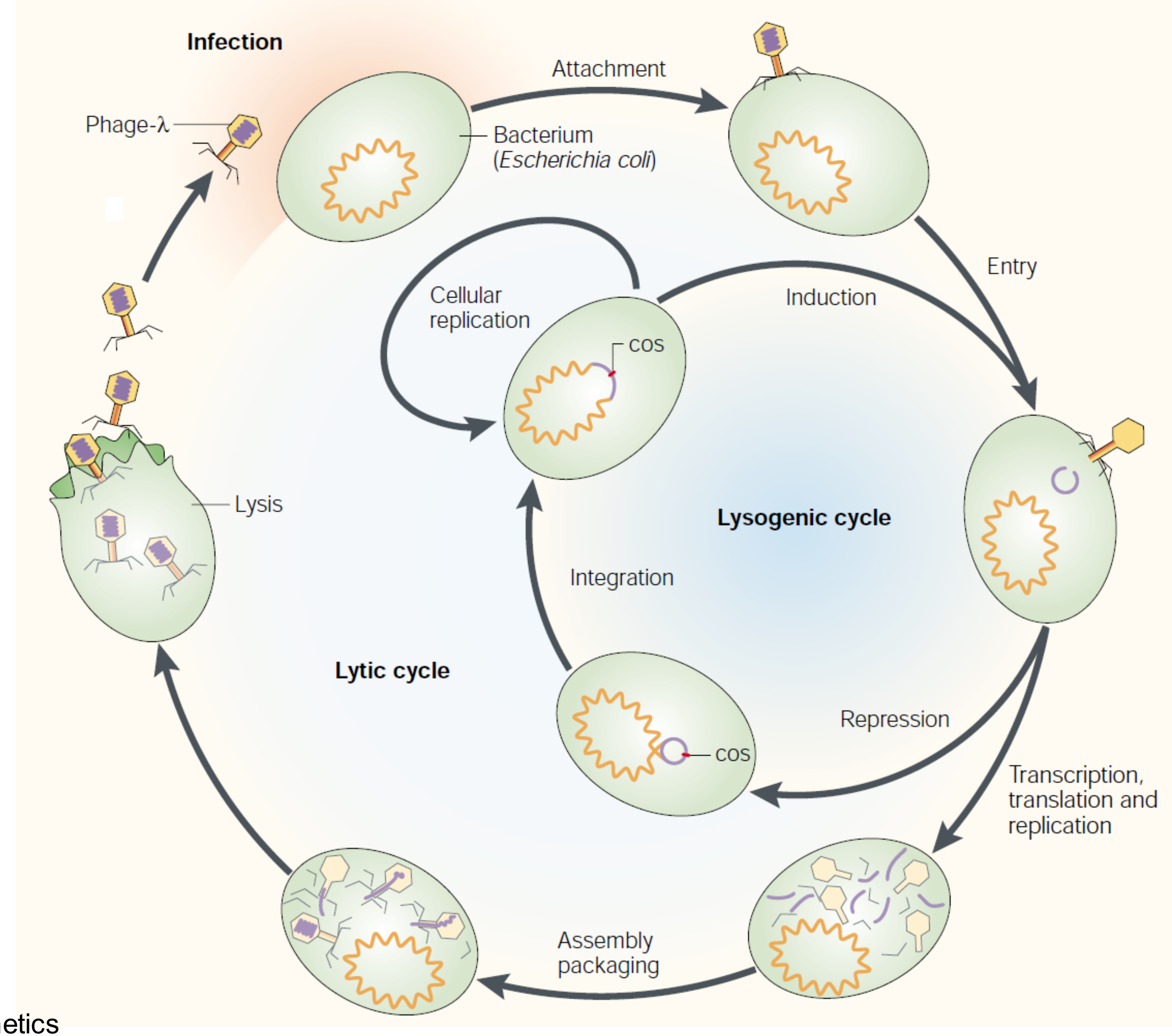
Genomic diversity

- Virus genomes can be double stranded or single stranded DNA or RNA
- Most known bacteriophages have double stranded DNA genomes





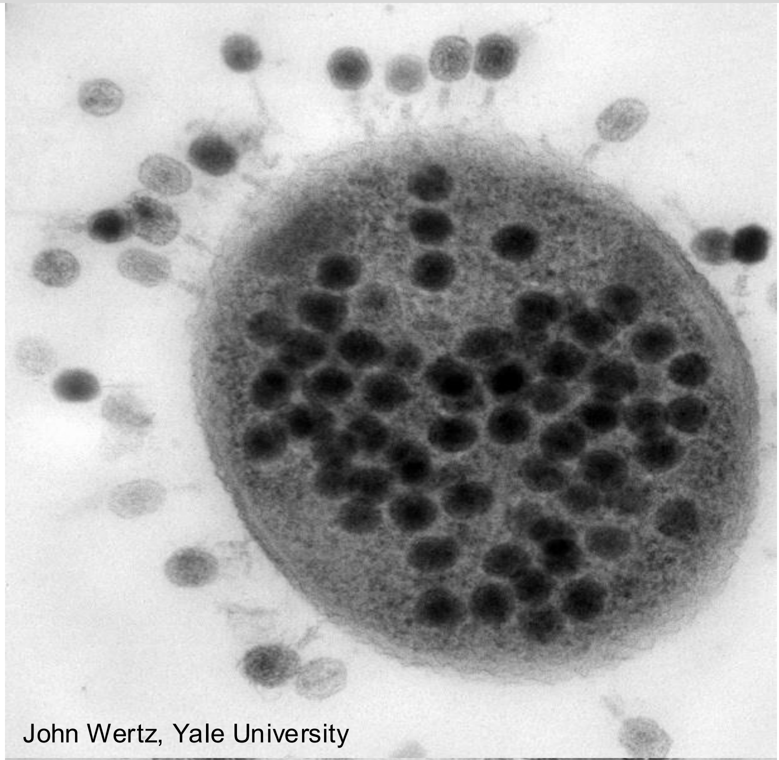
Campbell (2003) Nature Reviews Genetics



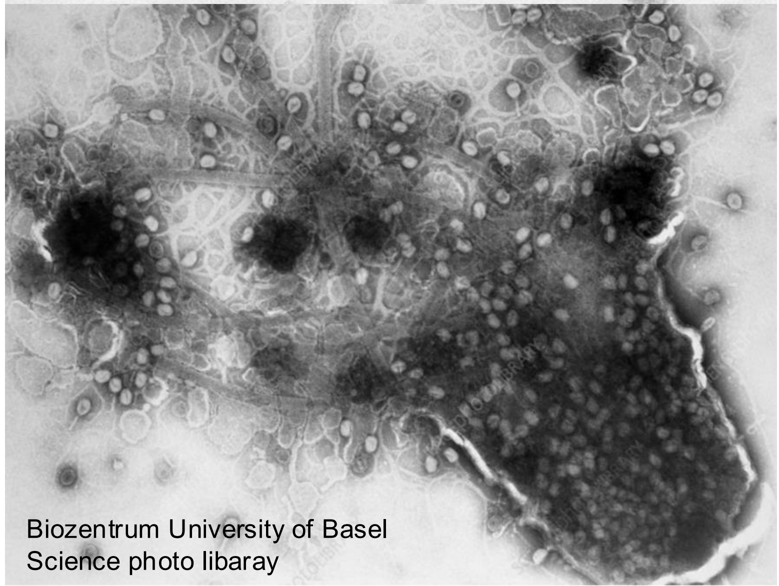
Campbell (2003) Nature Reviews Genetics

Soil virus ecology

- Morphologically and gentically diverse
- Abundant
  - ~10^{10} viral particles per g of soil
  - 10x more abundant than bacteria
- Highly dynamic, infecting most host populations
- Likely influence abundance, structure, and evolution of microbial communities
- Likely influence major biogeochemical processes



John Wertz, Yale University

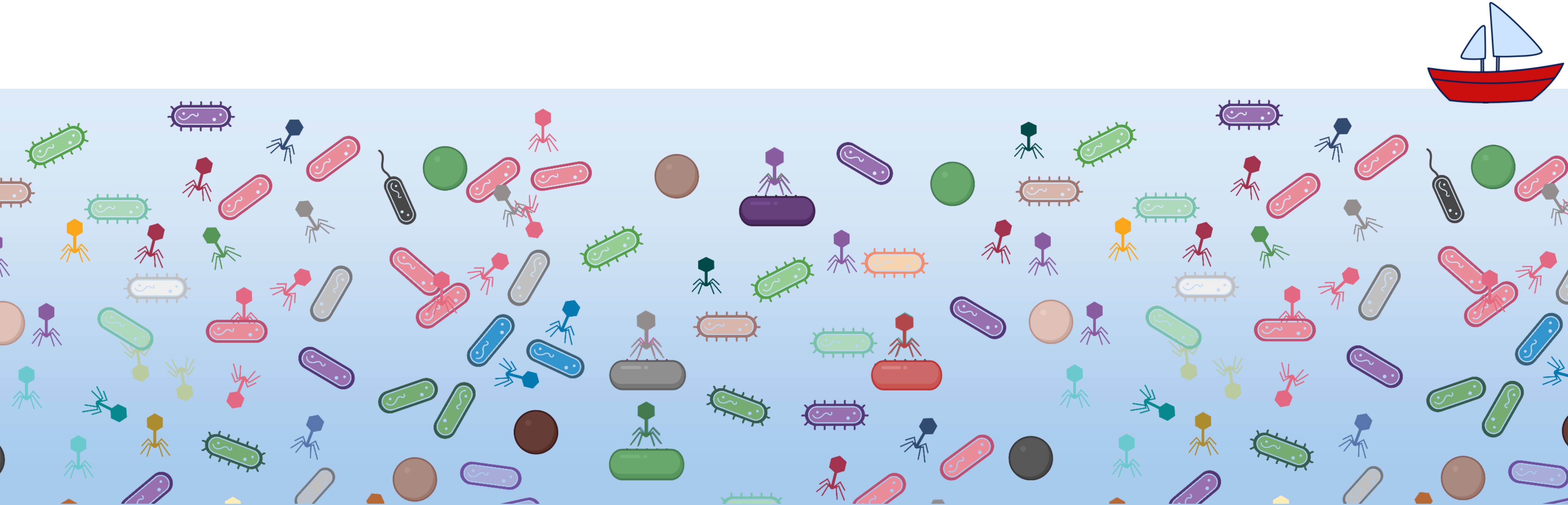


Biozentrum University of Basel
Science photo libaray

Challenges in soil virus ecology

Marine virus activity has global consequences

- 20-40% of all prokaryotes lysed daily
- C release: 150 Gt y^{-1}
- Responsible for 25% of dissolved inorganic C
- Auxiliary metabolic genes confer function after infection



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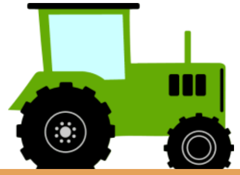
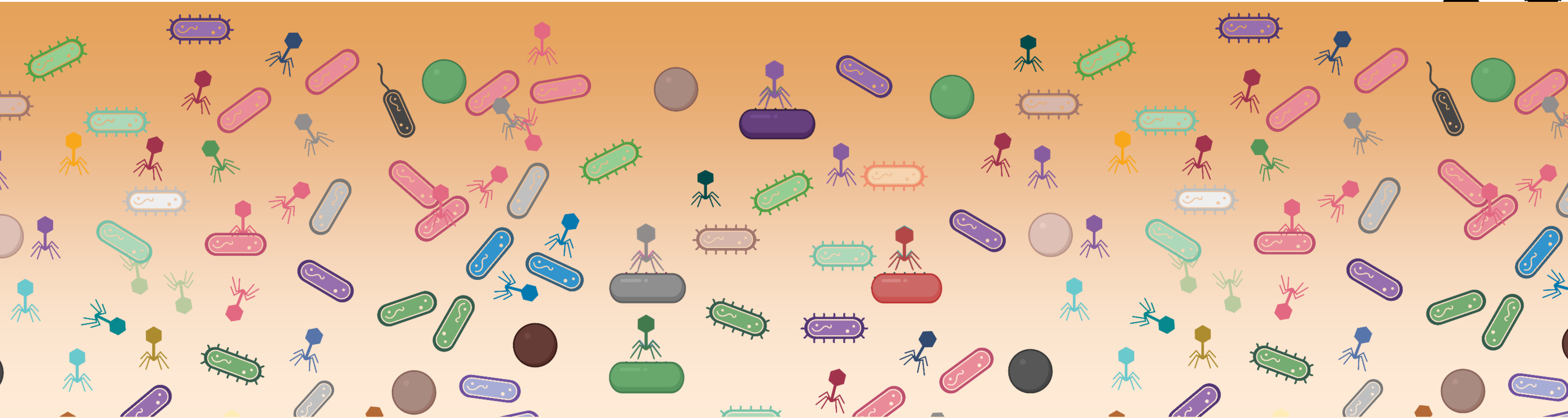
Impact of soil viruses

- Viral lysis rates?
- Fluxes of nutrient release?
- Auxiliary metabolic genes confer function?
- Impact on microbial functional processes?

Hazard et al. (2025) Soil Biology & Biochemistry

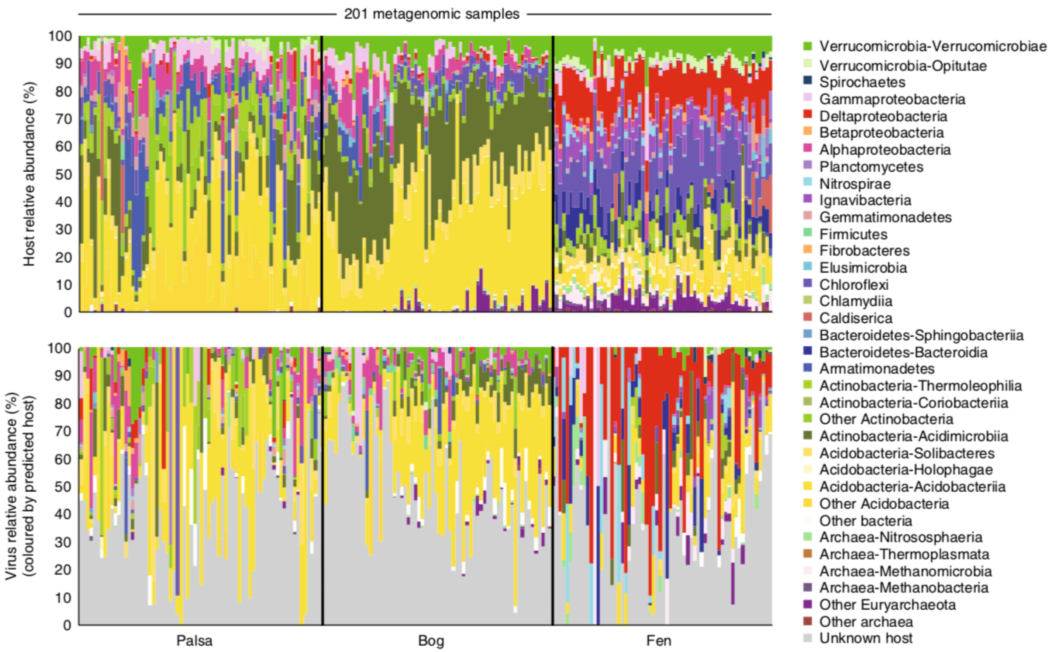




Why don’t we know more about the impact of viruses in soil?

- Vast diversity of hosts and viruses
- Unknown viral genetic diversity
- Physicochemical complexity of soils
- Difficulties in assessing impact of virus-mediated elemental cycling

Why don't we know more about the impact of viruses in soil?



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- Physicochemical complexity of soils
- Difficulties in assessing impact of virus-mediated elemental cycling

- Metagenomic approaches are now enabling characterization of viral communities
- Recent development of bioinformatic tools for predicting viruses, hosts and taxonomy:

    VIBRANT (Kieft et al., 2020)
    VirSorter 2.0 (Guo et al., 2021)
    iPHoP (Roux et al., 2022)
    geNomad (Camargo et al., 2024)
    plus many others…

Emerson et al. (2018) Nature Microbiology

Challenges in soil virus ecology

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Impact of soil viruses

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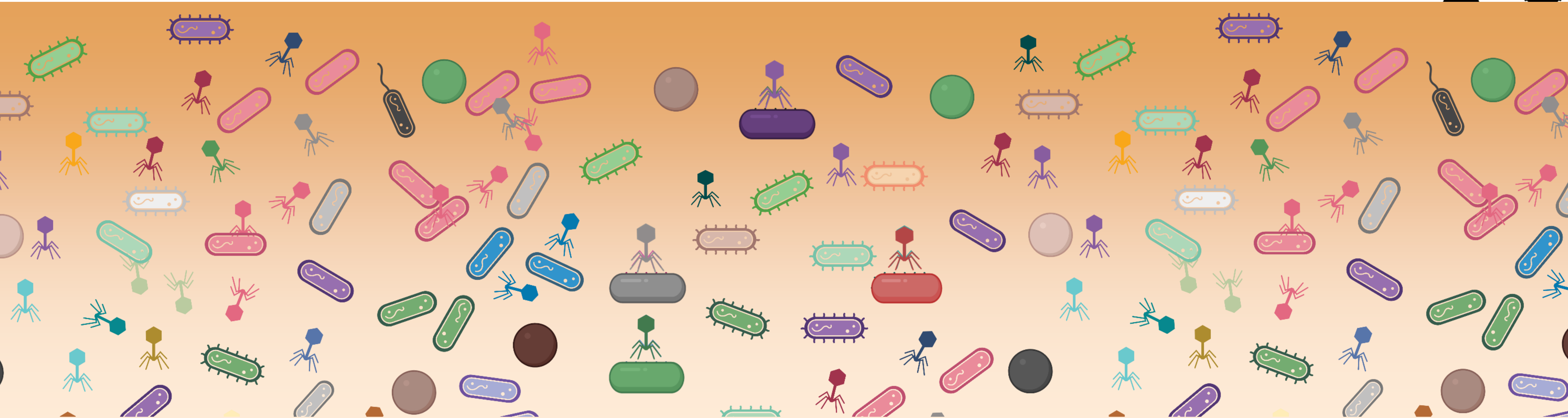
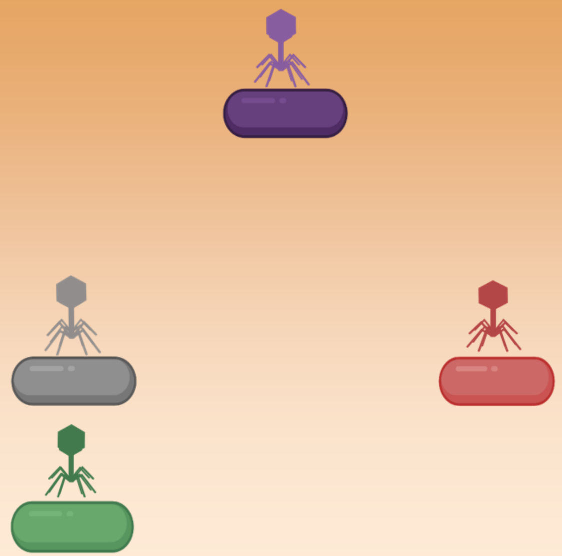
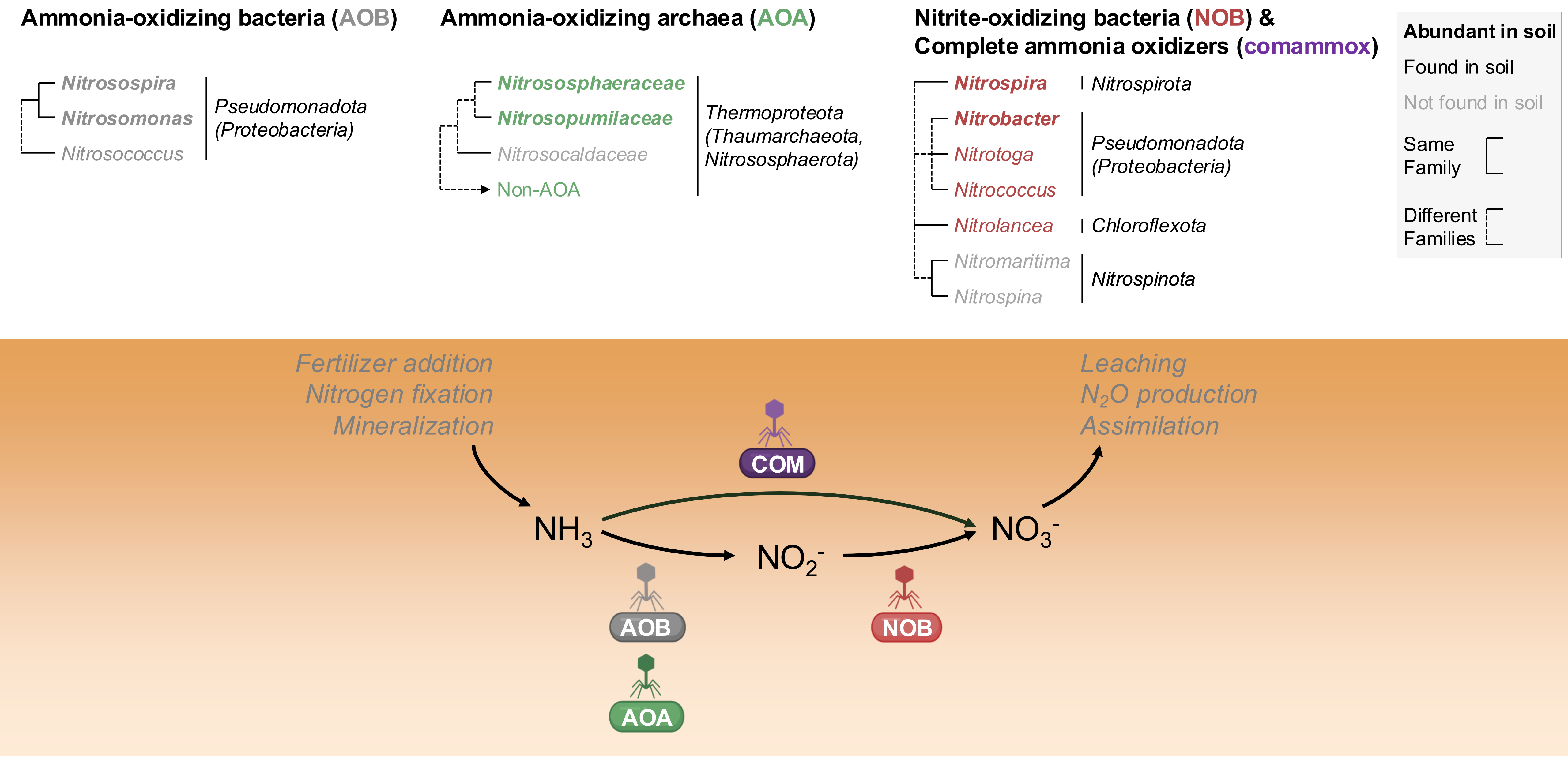
Hazard et al. (2025) Soil Biology & Biochemistry



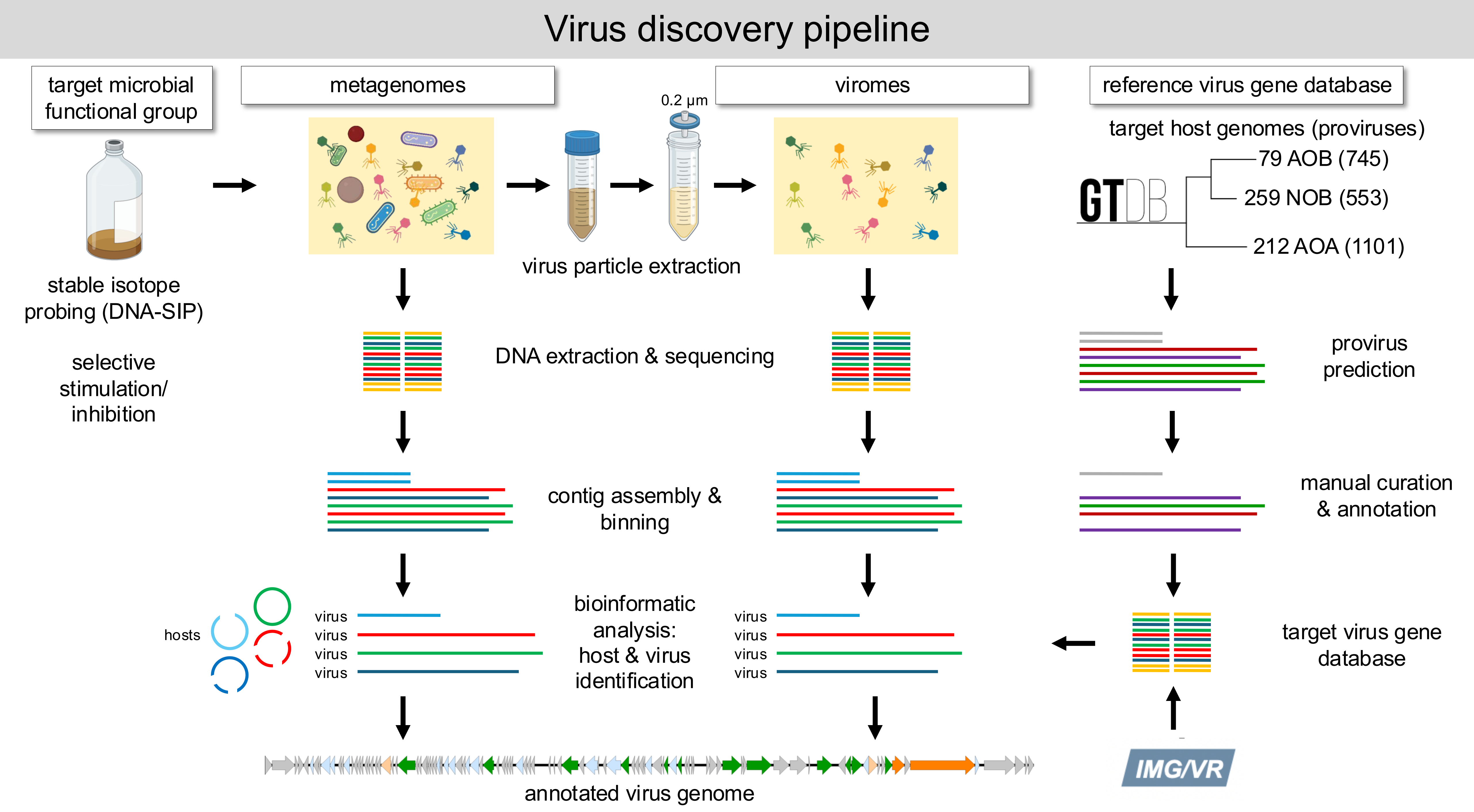
Increasing resolution on host-virus interactions in soil



Nitrifiers



Ammonia-oxidizing bacteria (AOB)
Nitrosospira
Nitrosomonas
Nitrosococcus
Pseudomonadota (Proteobacteria)
Ammonia-oxidizing archaea (AOA)
Nitrososphaeraceae
Nitrosopumilaceae
Nitrosocaldaceae
Non-AOA
Thermoproteota (Thaumarchaeota, Nitrososphaerota)
Nitrite-oxidizing bacteria (NOB) &
Complete ammonia oxidizers (comammox)
Nitrospira
Nitrospirota
Nitrobacter
Nitrotoga
Nitrococcus
Pseudomonadota (Proteobacteria)
Nitrolancea
Chloroflexota
Nitromaritima
Nitrospina
Nitrospinota
Abundant in soil
Found in soil
Not found in soil
Same Family
Different Families
Fertilizer addition
Nitrogen fixation
Mineralization
COM
NH_3
NO_2^-
NO_3^-
AOB
AOA
NOB
Leaching
N_2O production
Assimilation



Virus discovery pipeline
target microbial functional group
metagenomes
0.2 µm
viromes
reference virus gene database
target host genomes (proviruses)
79 AOB (745)
GTDB
259 NOB (553)
212 AOA (1101)
virus particle extraction
stable isotope probing (DNA-SIP)
selective stimulation/ inhibition
DNA extraction & sequencing
provirus prediction
contig assembly & binning
manual curation & annotation
hosts
virus
virus
virus
virus
bioinformatic analysis: host & virus identification
virus
virus
virus
virus
target virus gene database
IMG/VR
annotated virus genome

Enrichment of viruses infecting selected nitrifier groups using differential inhibition

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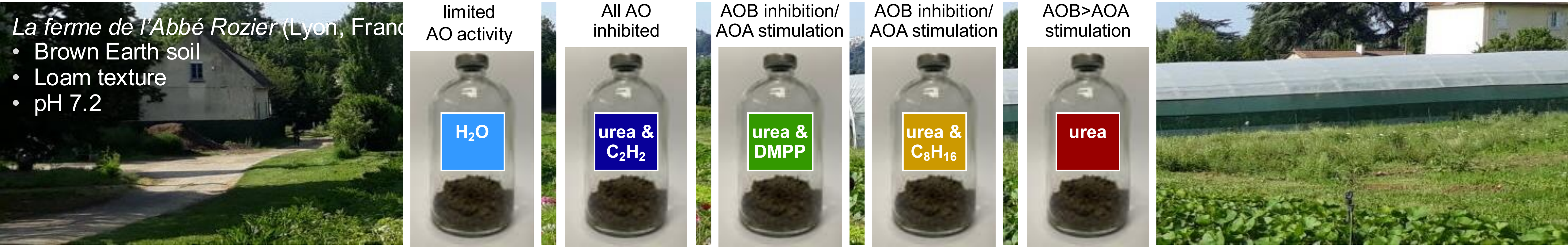
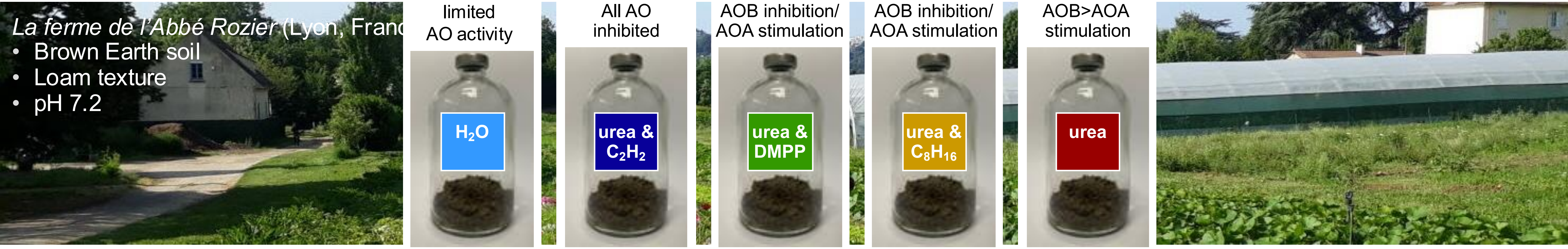
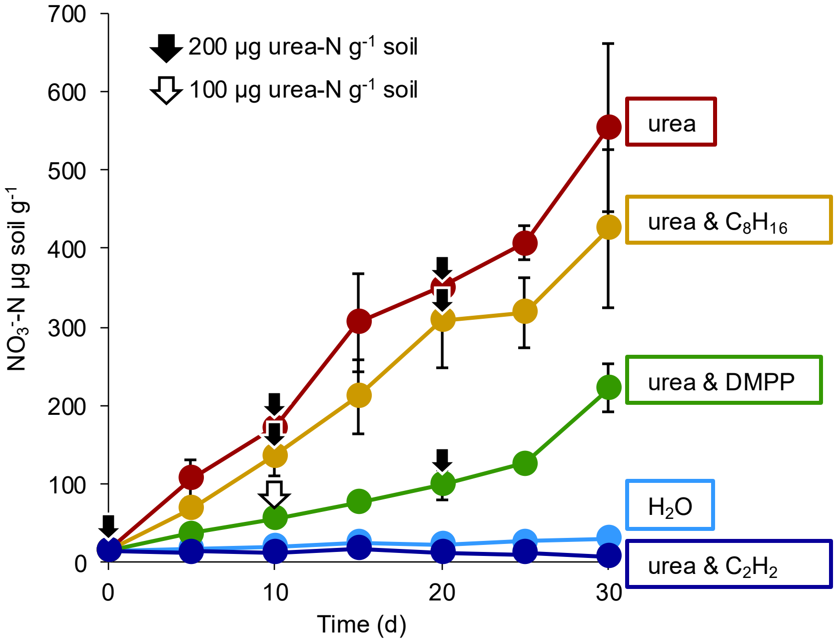


Lee et al. (2024) ISME Journal

Enrichment of viruses infecting selected nitrifier groups using differential inhibition





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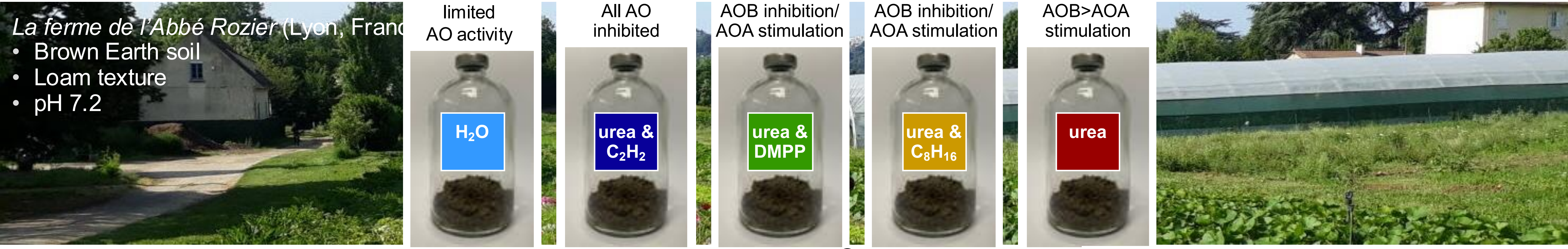

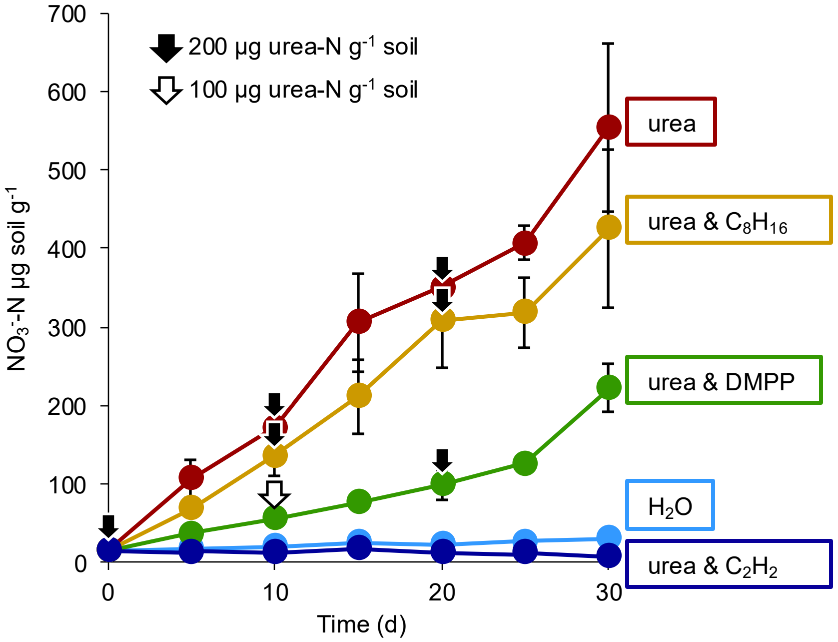

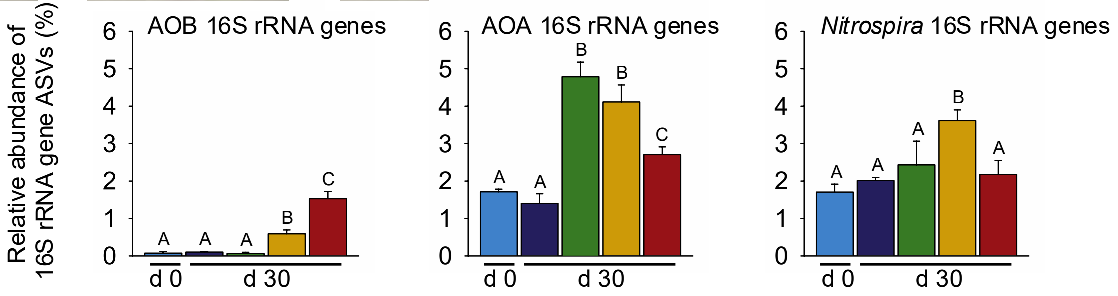

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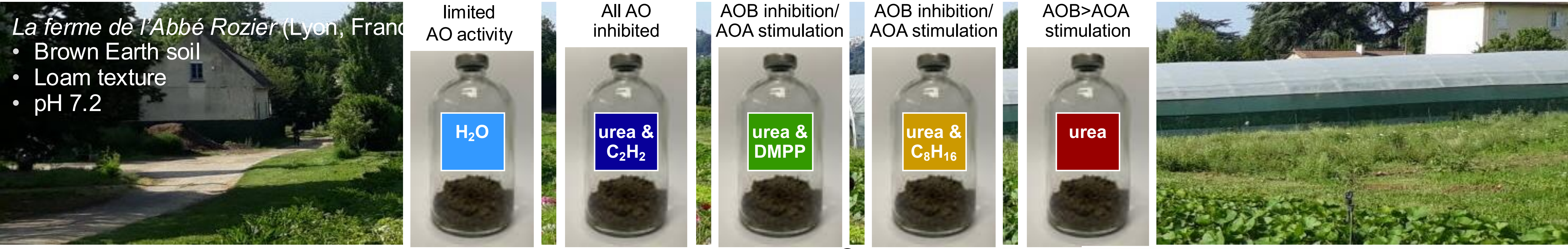
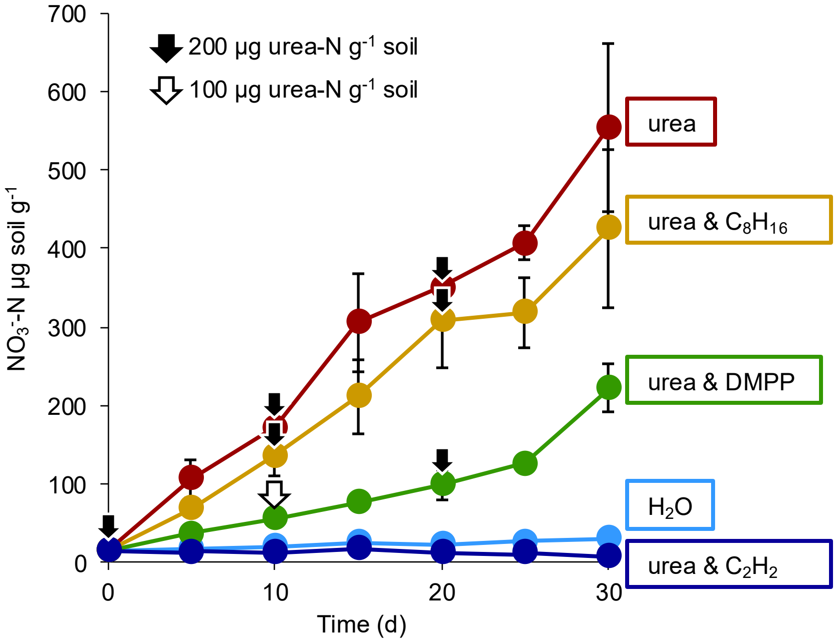
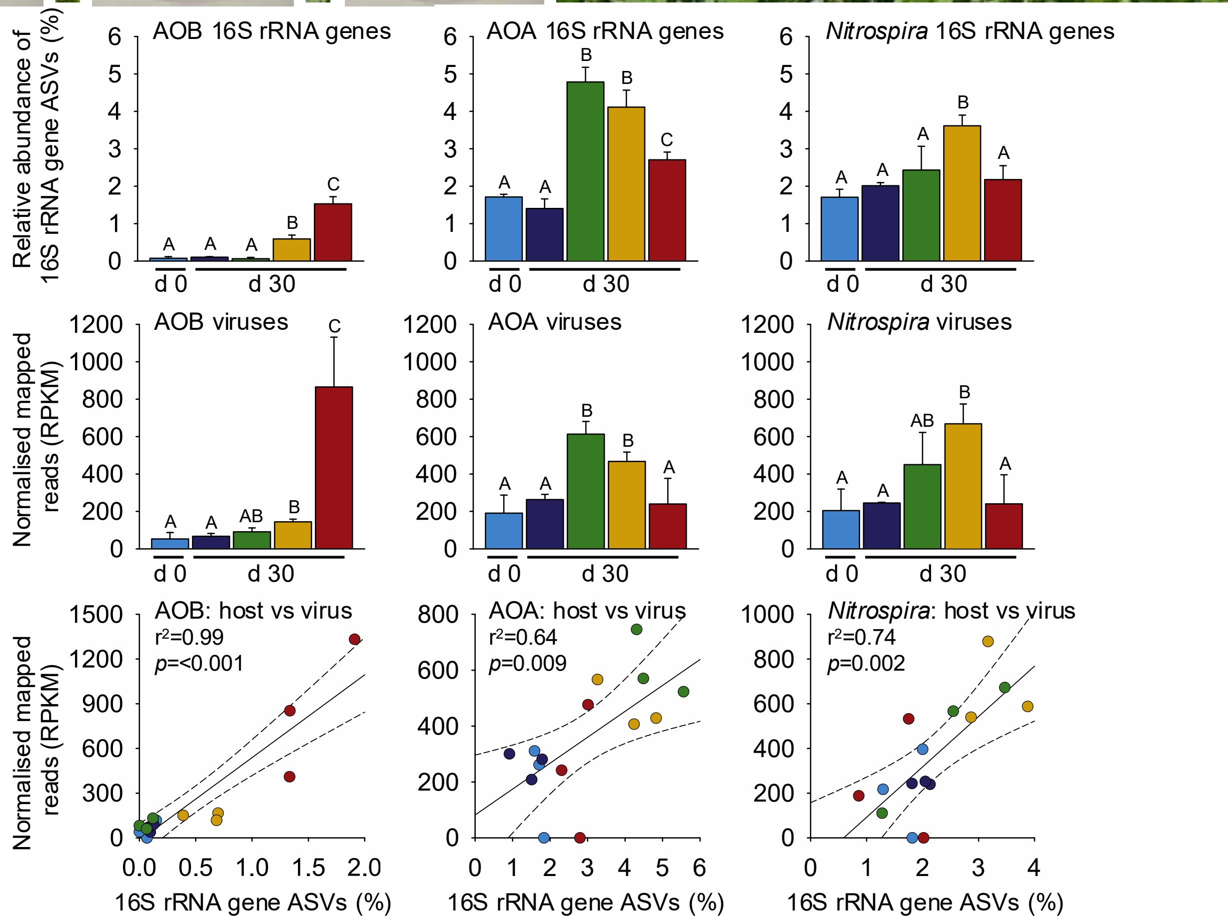


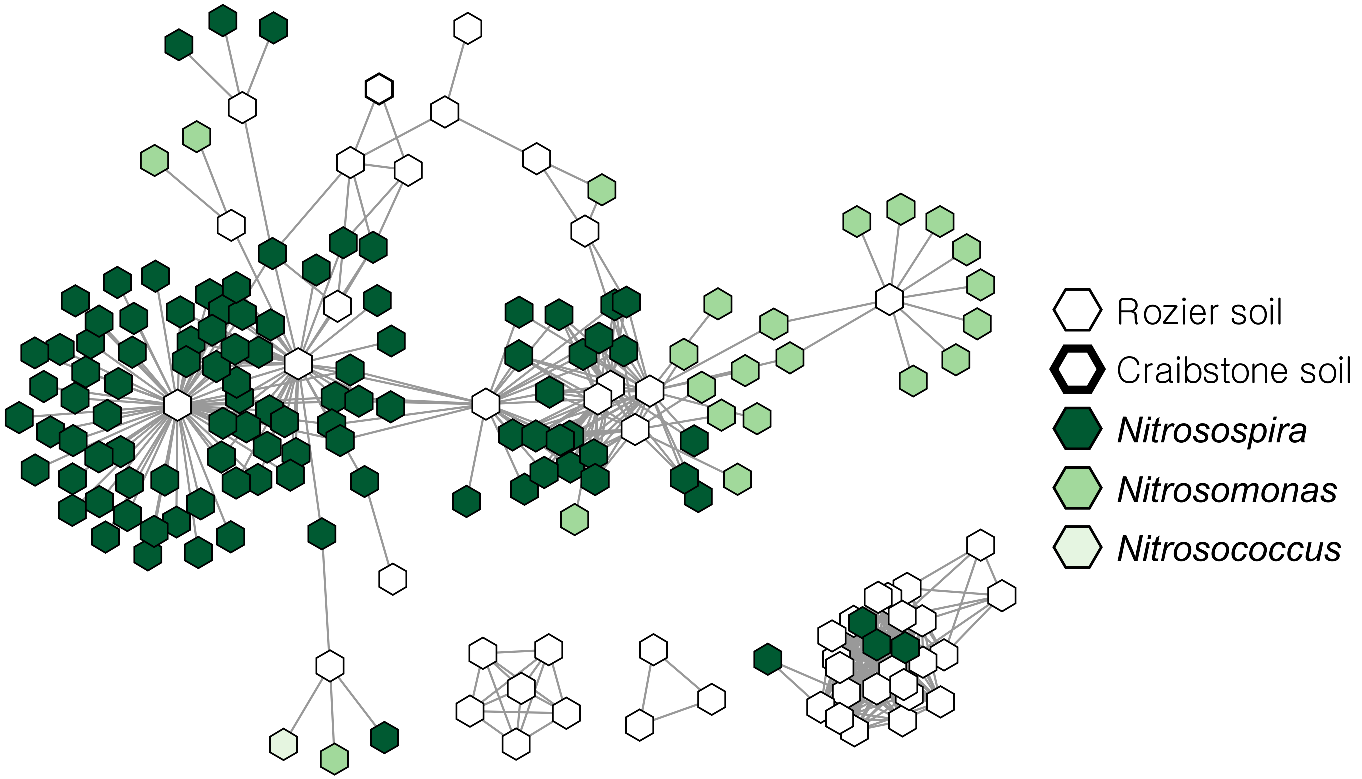




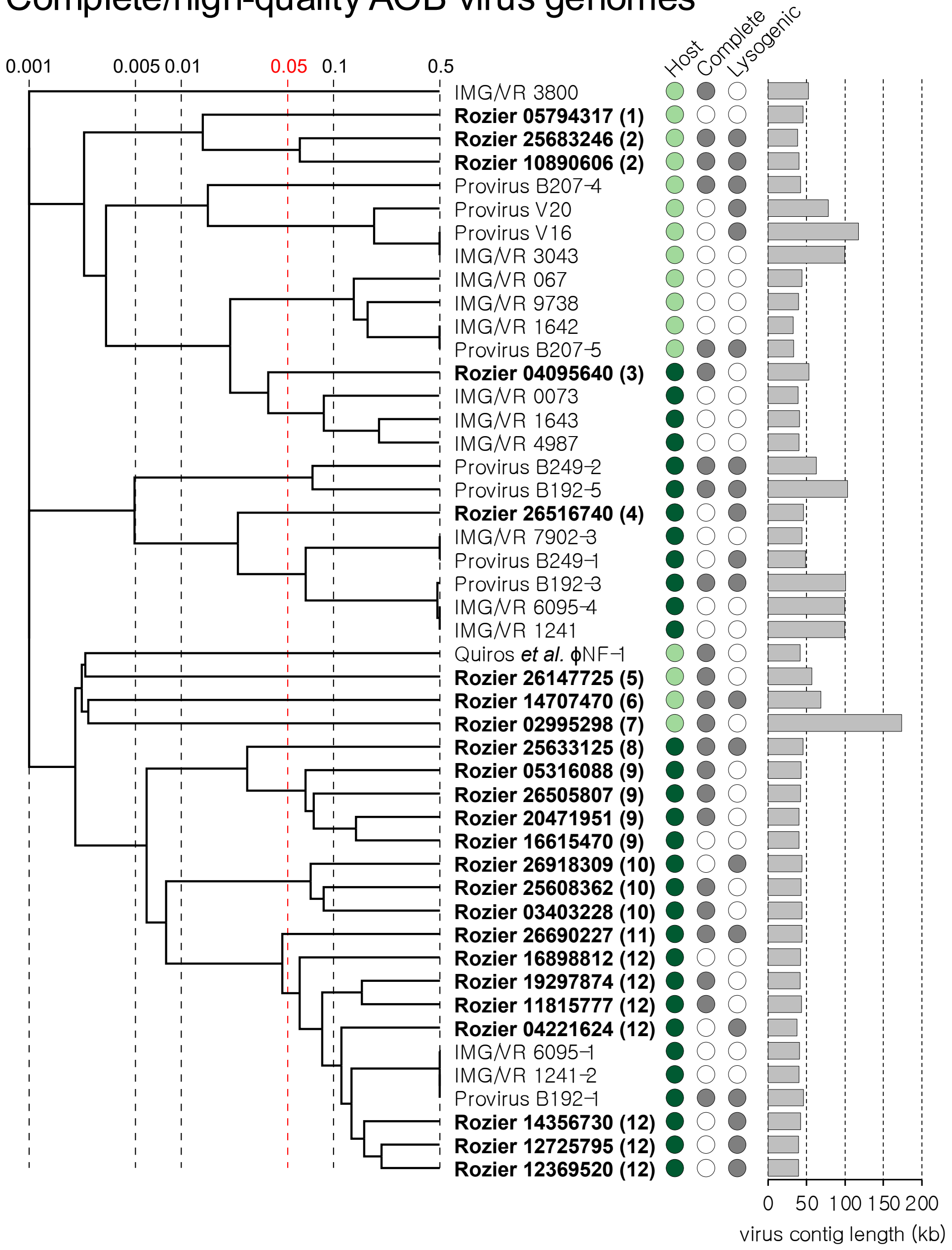
Lee et al. (2024) ISME Journal

Novel AOB soil viruses

Gene-sharing networks with reference (pro)viruses: contigs ≥ 10 kb



Complete/high-quality AOB virus genomes

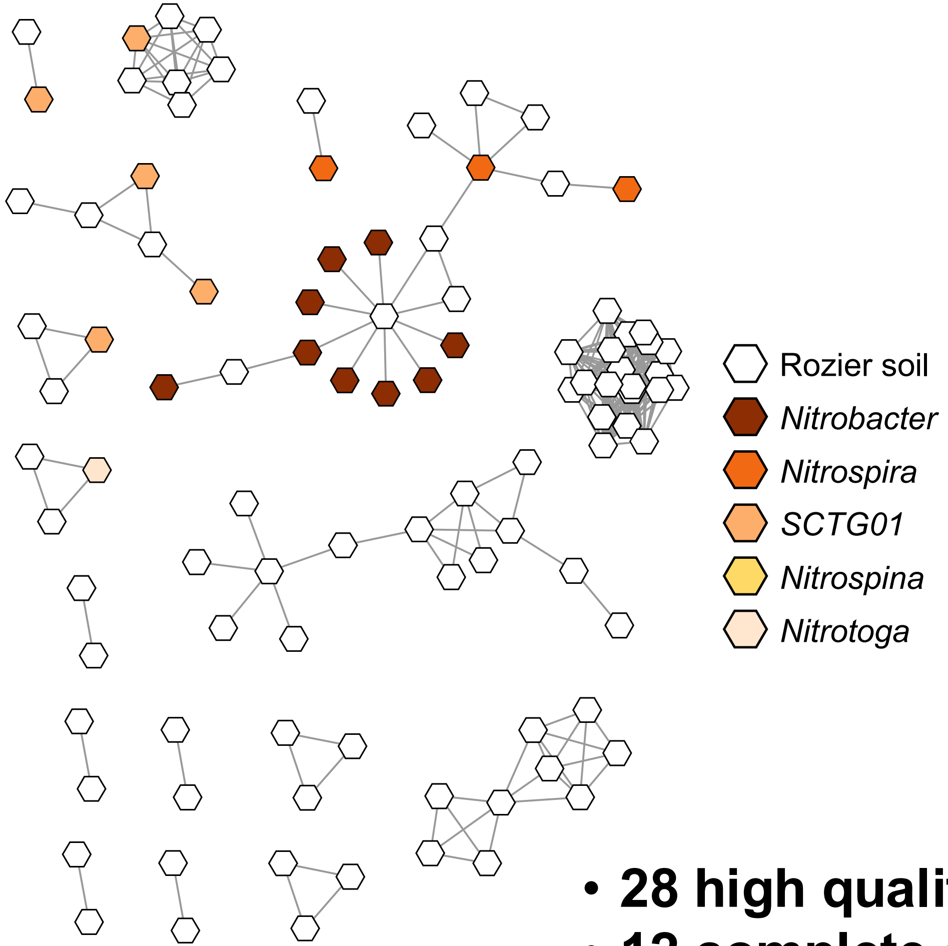


- **47 high quality genomes**
- **23 complete genomes (39 to 173 kb)**
- **12 novel virus families**
- **43% capable of lysogeny**

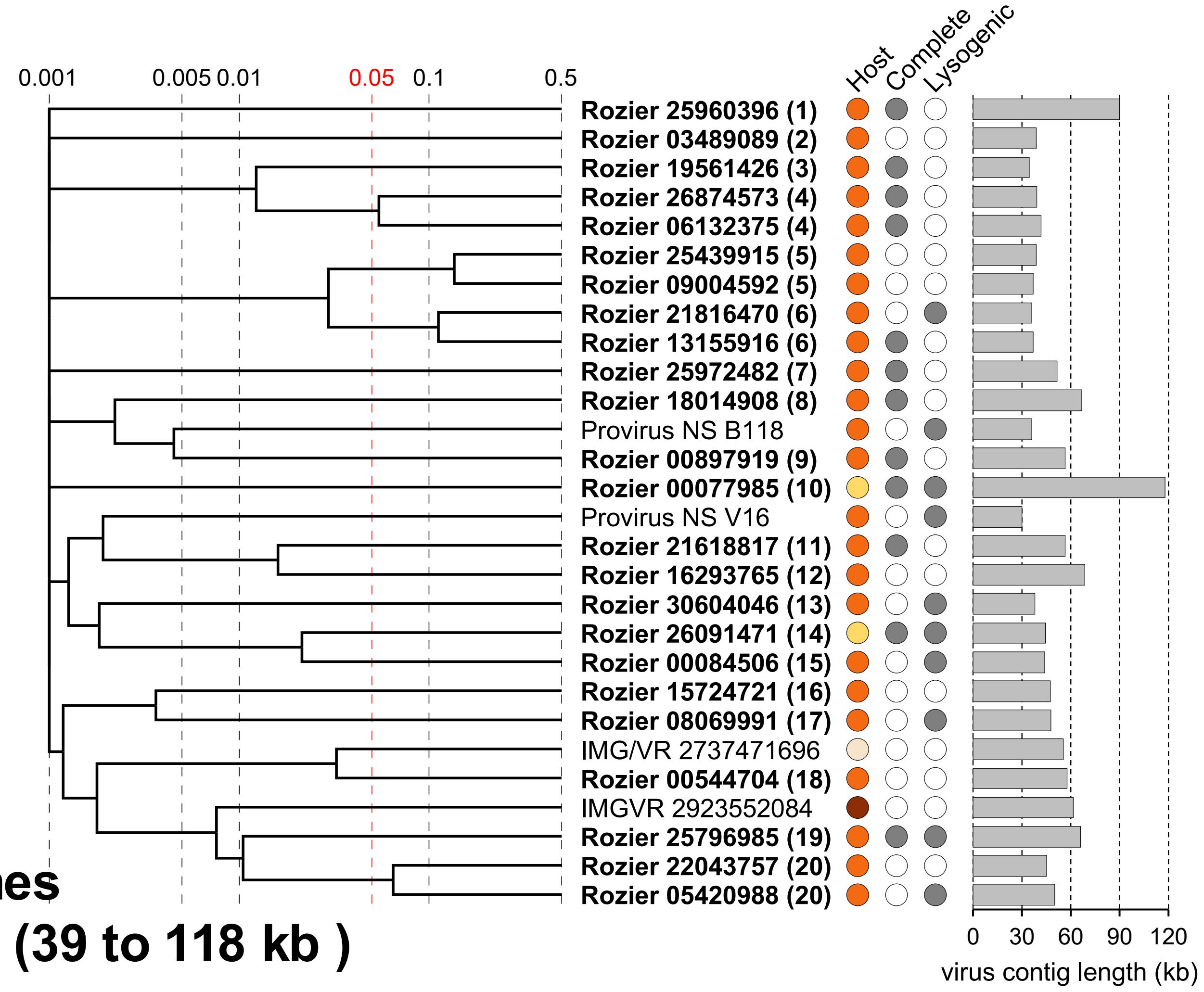
Lee et al. (2024) ISME J

Novel NOB and comammox soil viruses

Gene-sharing networks with reference (pro)viruses: contigs ≥ 10 kb



Complete/high-quality NOB virus genomes

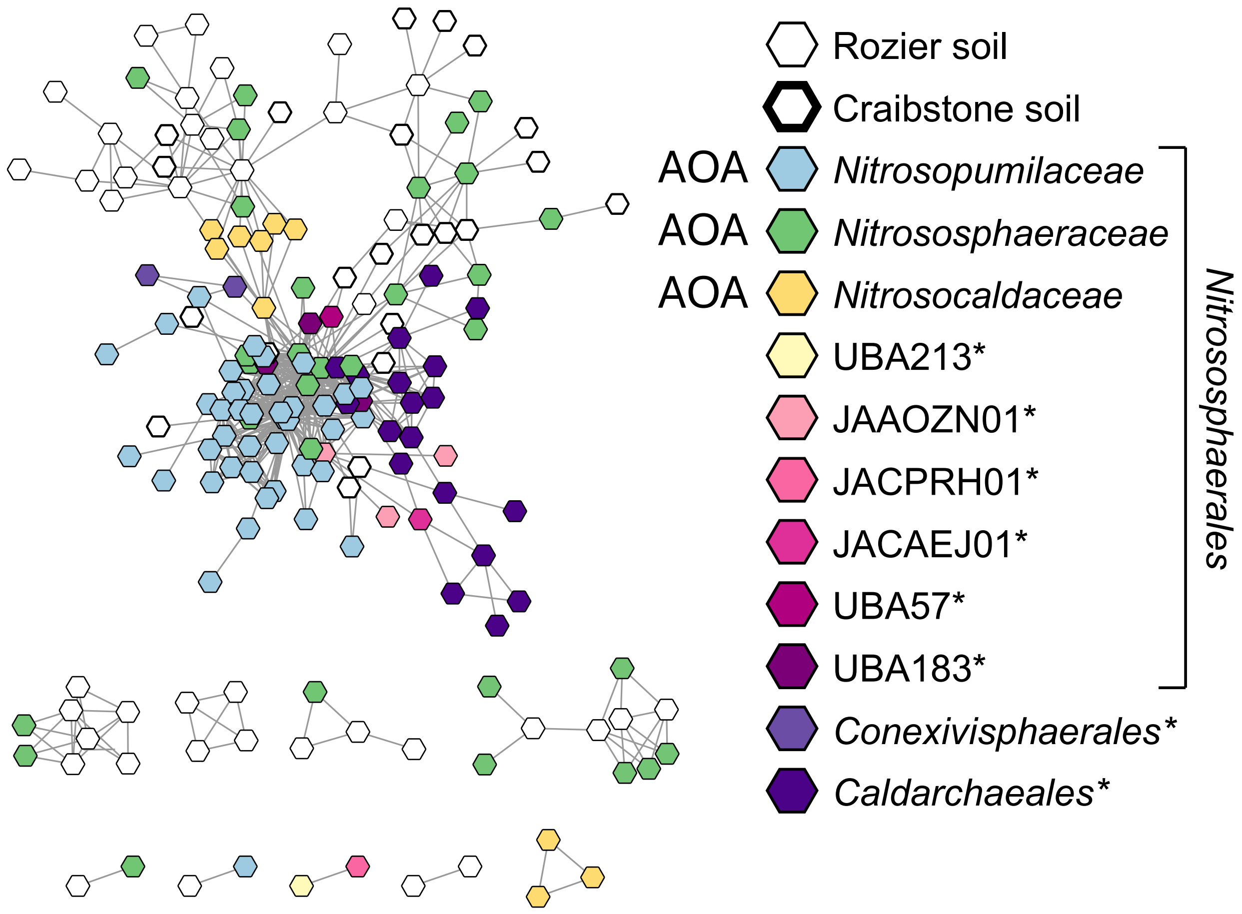


- **28 high quality genomes**
- **12 complete genomes (39 to 118 kb)**
- **20 novel virus families**
- **36% capable of lysogeny**
- **86% infecting *Nitrospira***

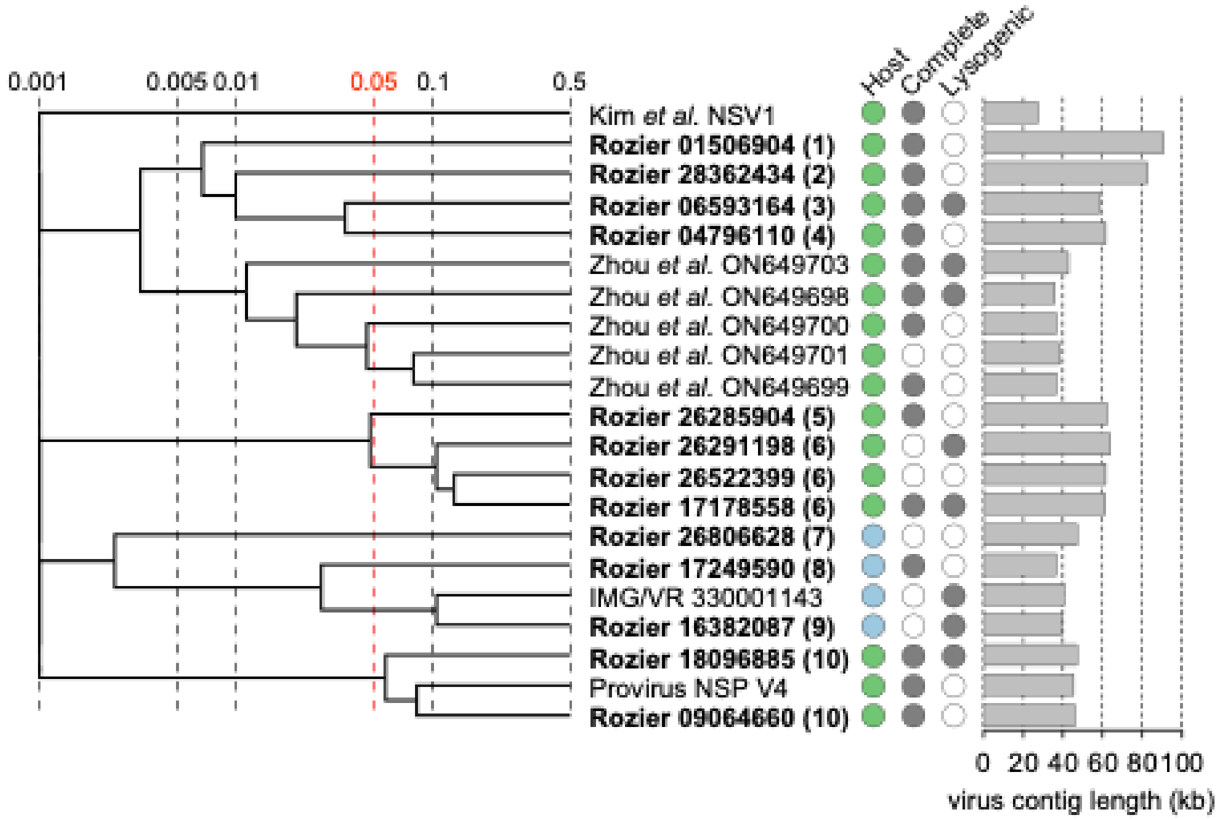
Lee et al. (2024) ISME J

Novel archaea soil viruses of the class *Nitrososphaeria* (including AOA)

Gene-sharing network with reference (pro)viruses: contigs ≥ 10 kb



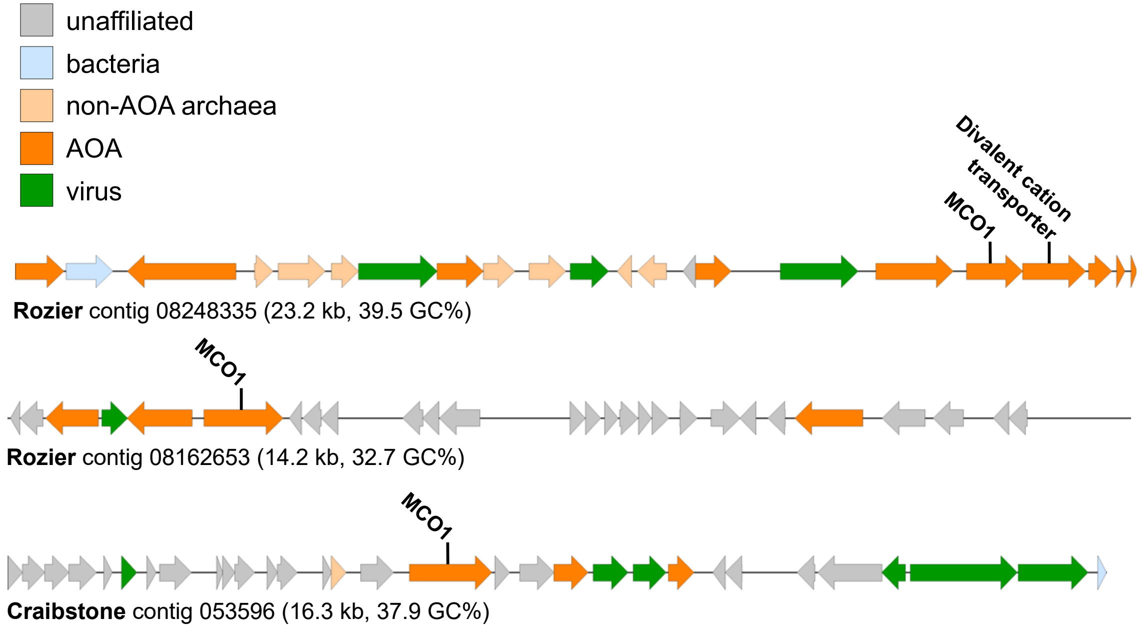
Complete/high-quality AOA virus genomes

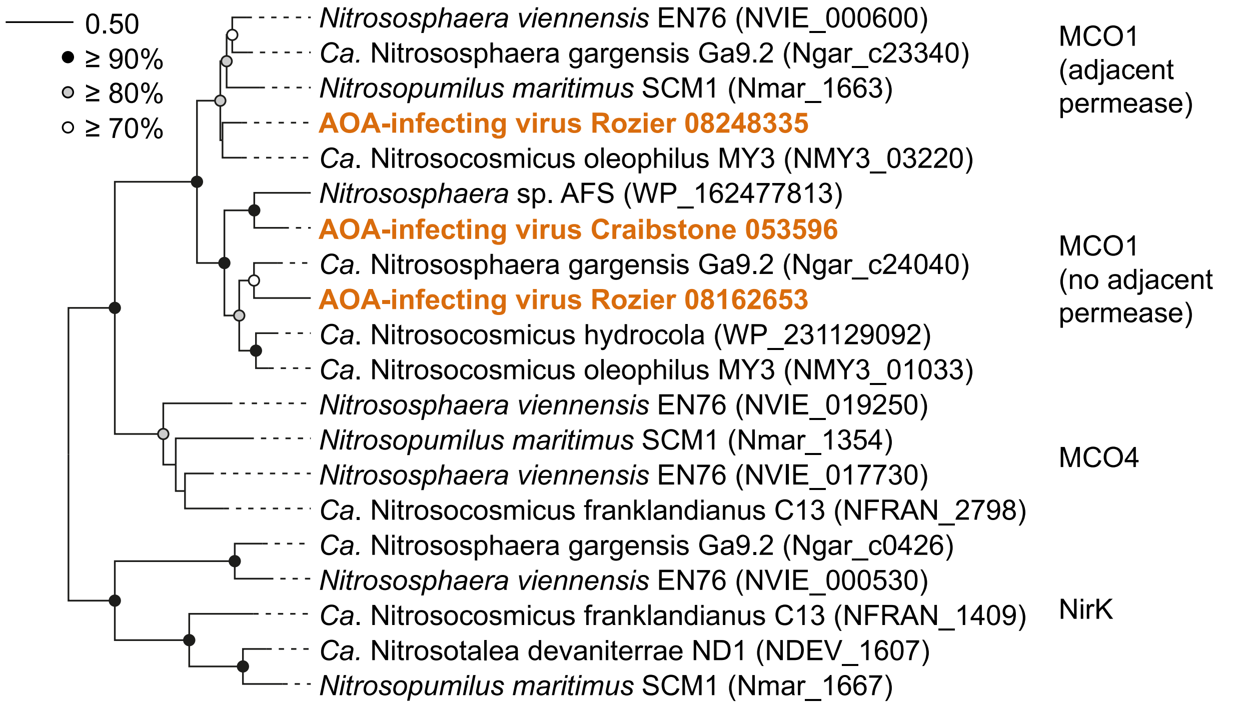


- **21 high quality genomes**
- **15 complete genomes (37 to 91 kb)**
- **10 novel virus families**
- **38% capable of lysogeny**

Lee et al. (2024) ISME J

Type 1 multicopper oxidase (MCO1) genes in AOA soil viruses





MCO lineage designation: Kerou et al. (2016) PNAS

Lee et al. (2024) ISME J

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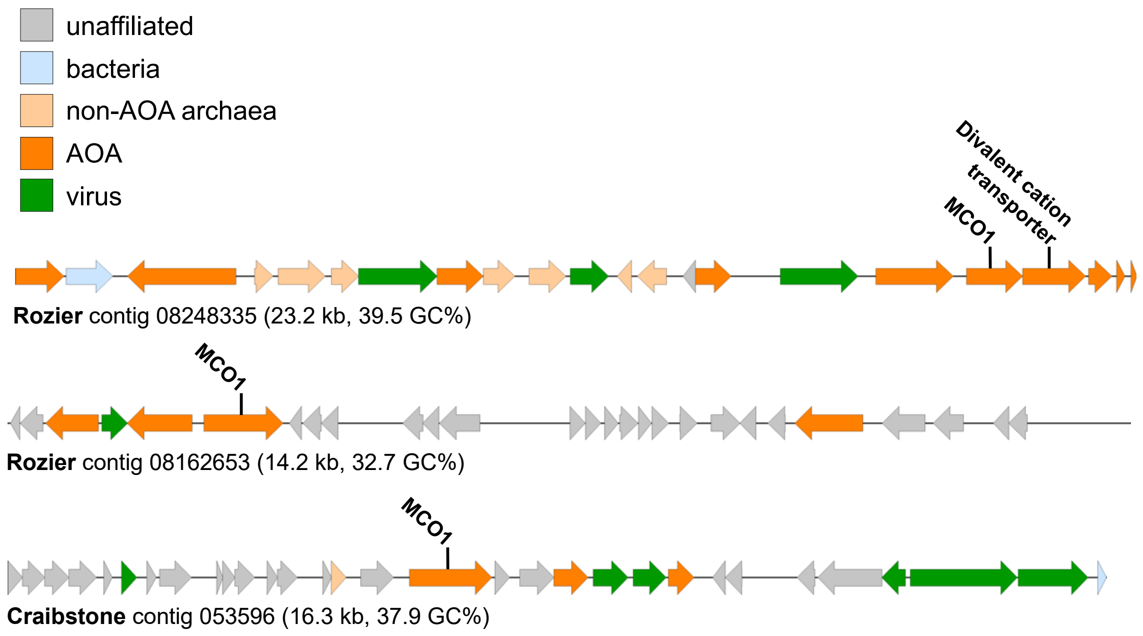
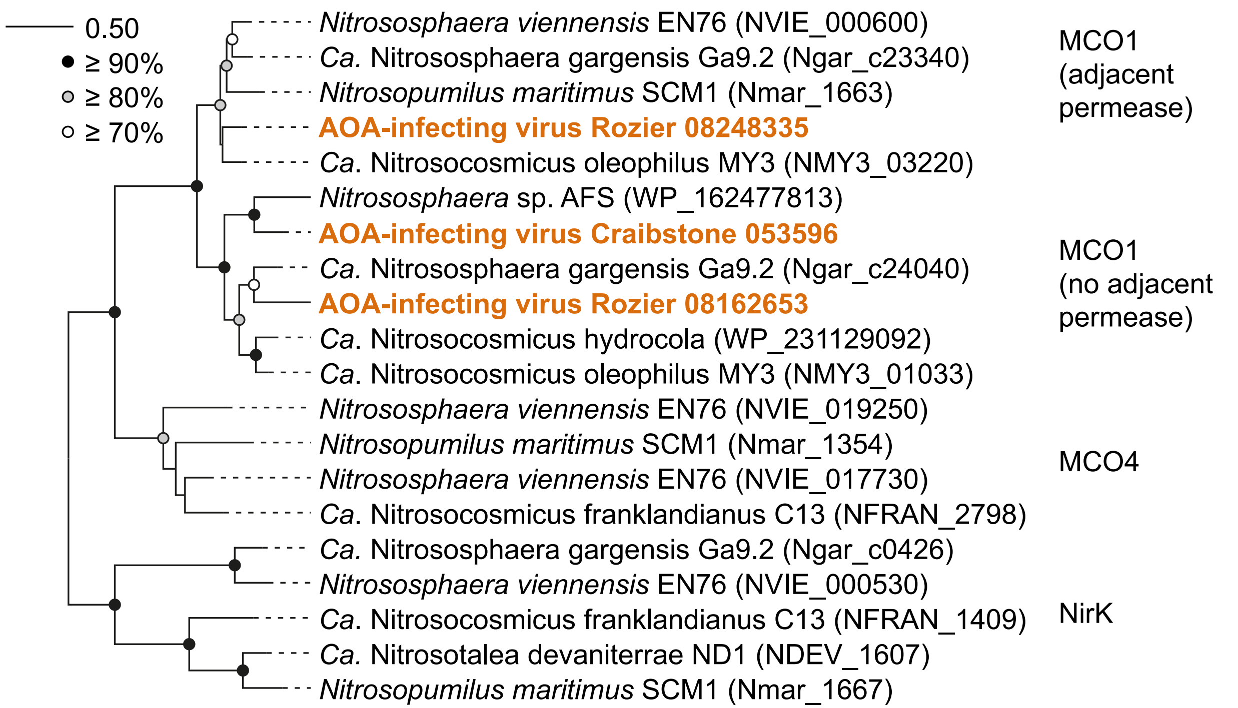


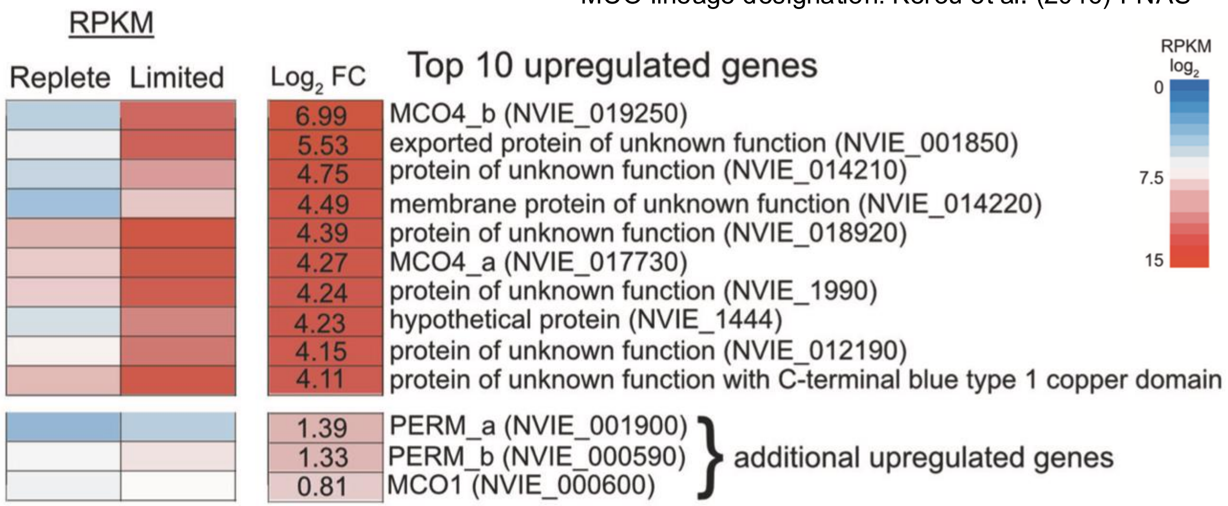


MCO lineage designation: Kerou et al. (2016) PNAS

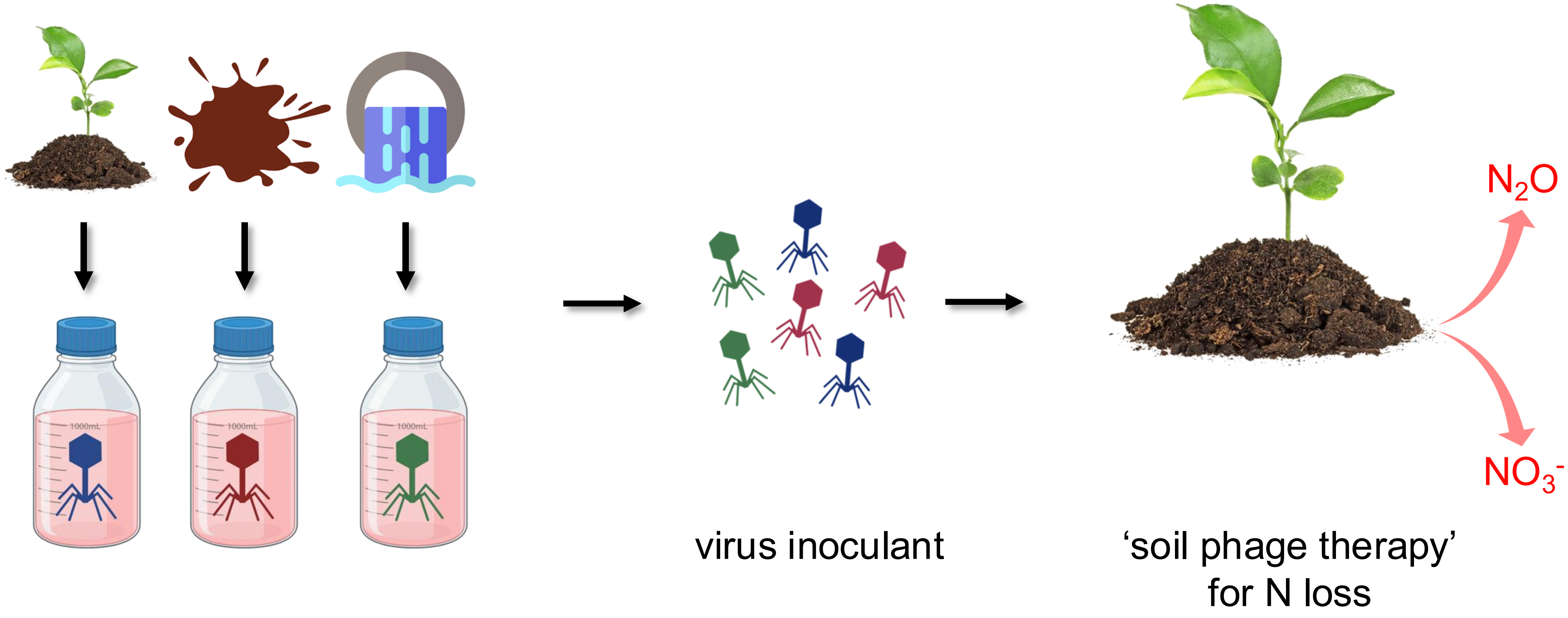
ISME
2020, 14: 2659-2674

Genome wide transcriptomic analysis of the soil ammonia oxidizing archaeon *Nitrososphaera viennensis* upon exposure to copper limitation

Carolina Reyes[1,2,3] · Logan H. Hodgskiss[2,3] · Melina Kerou[2,3] · Thomas Pribasnig[2,3] · Sophie S. Abby[4] · Barbara Bayer[3,5,6] · Stephan M. Kraemer[1,3] · Christa Schleper[2,3]



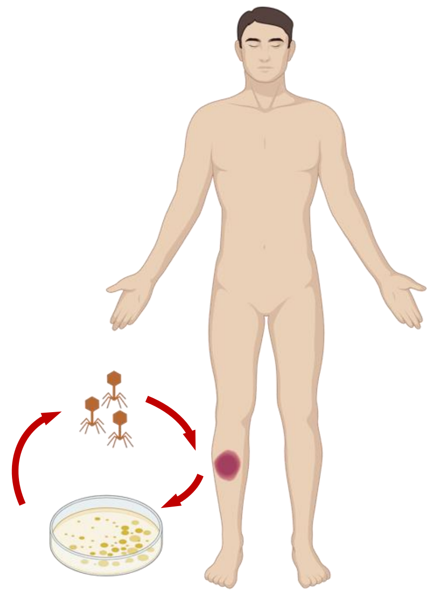
Can viruses of ammonia oxidizers be used to control nitrification?

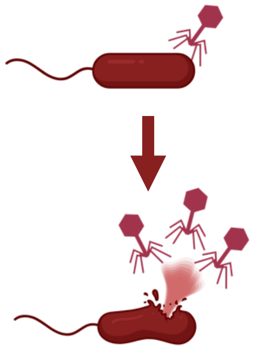


Phage therapy in medicine and biotechnology

- Use of ‘phage’ for treatment of infections first developed in the 1920s
- Global threat of antimicrobial resistance to antibiotics has renewed interest
- Phage therapy is highly-targeted and specific
- **Renaissance for phage-based control**

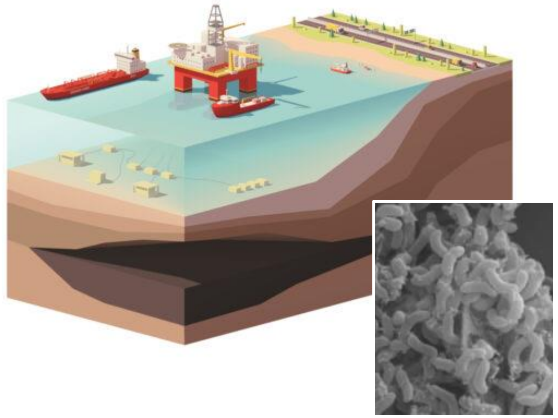
Phage therapy after antibiotic failure





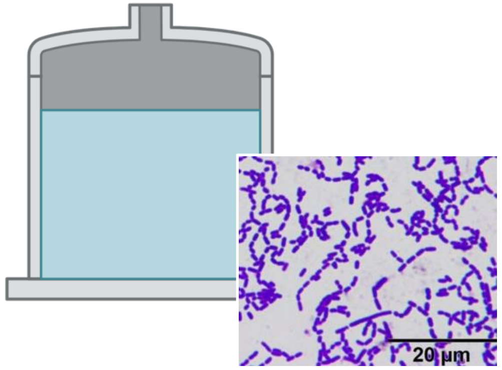
Pseudomonas aeruginosa

Souring of oilfields



Desulfovibrio vulgaris (SRB)

Lactic acid bacteria in bioethanol production



Lactobacillus fermentum

Phage therapy in agriculture

Bacteriophage control of phytopathogens

Erwinia amylovora

Greenphage, France (https://greenphage.com/)

- Melon bacterial blight (*Erwinia tracheiphila*)

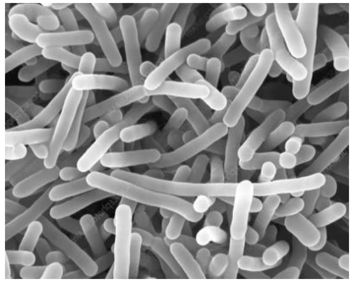
EcoPhage, Israel (https://ecophage.com/)

- Bacterial speck (*Pseudomonas syringae*) and spot (*Xanthomonas vesicatoria*) in tomato and pepper plants
- Fire Blight (*Erwinia amylovora)* in pears and apples
- MOKO disease (*Ralstonia solancearum*) in banana

Certis Biologicals, USA (https://www.certisbio.com)

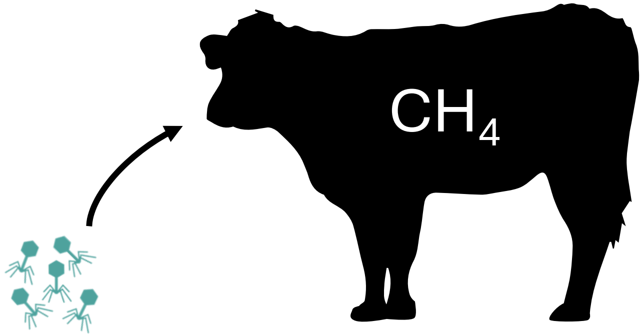
- Fire Blight (*Erwinia amylovora)* in pears and apples
- Citrus Canker (*Xanthomonas Citri)*

Bacteriophage control for food safety



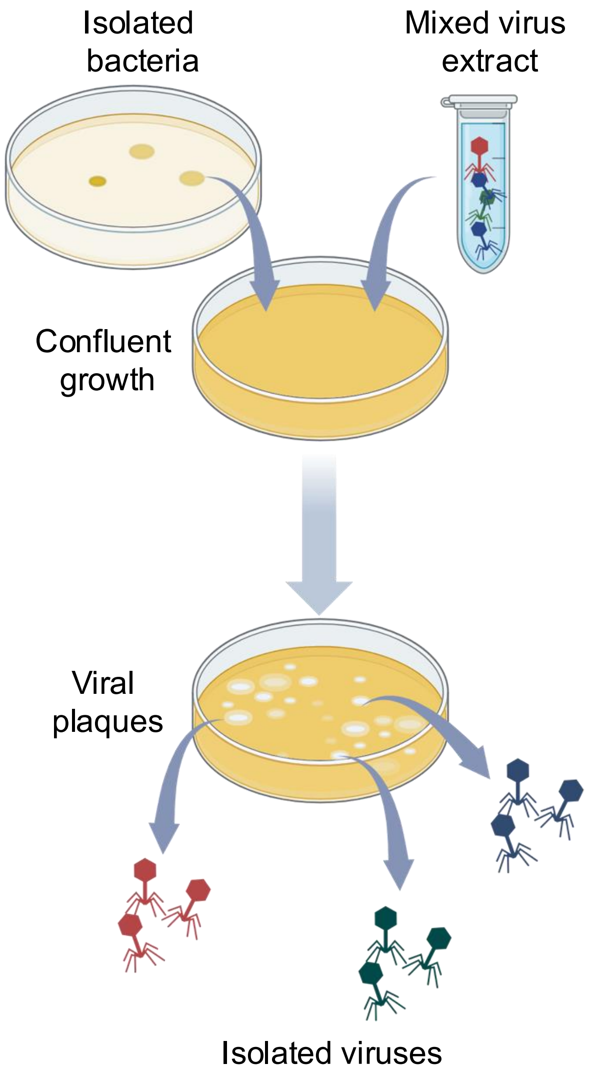
Listeria monocytogenes

Livestock methane amelioration



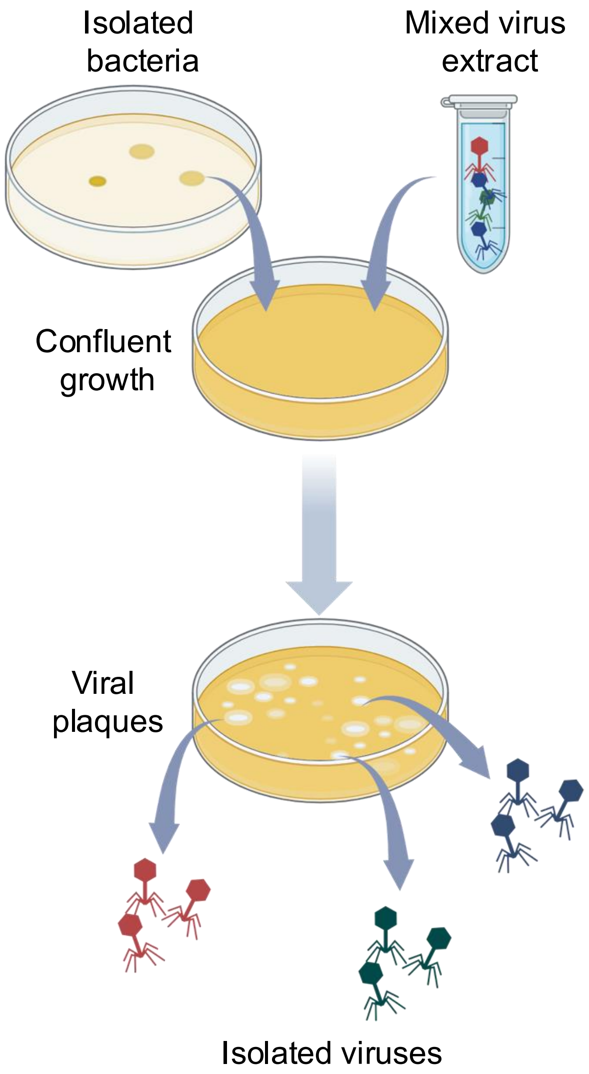
Cultivation of nitrifier viruses

Classical approach for isolating viruses

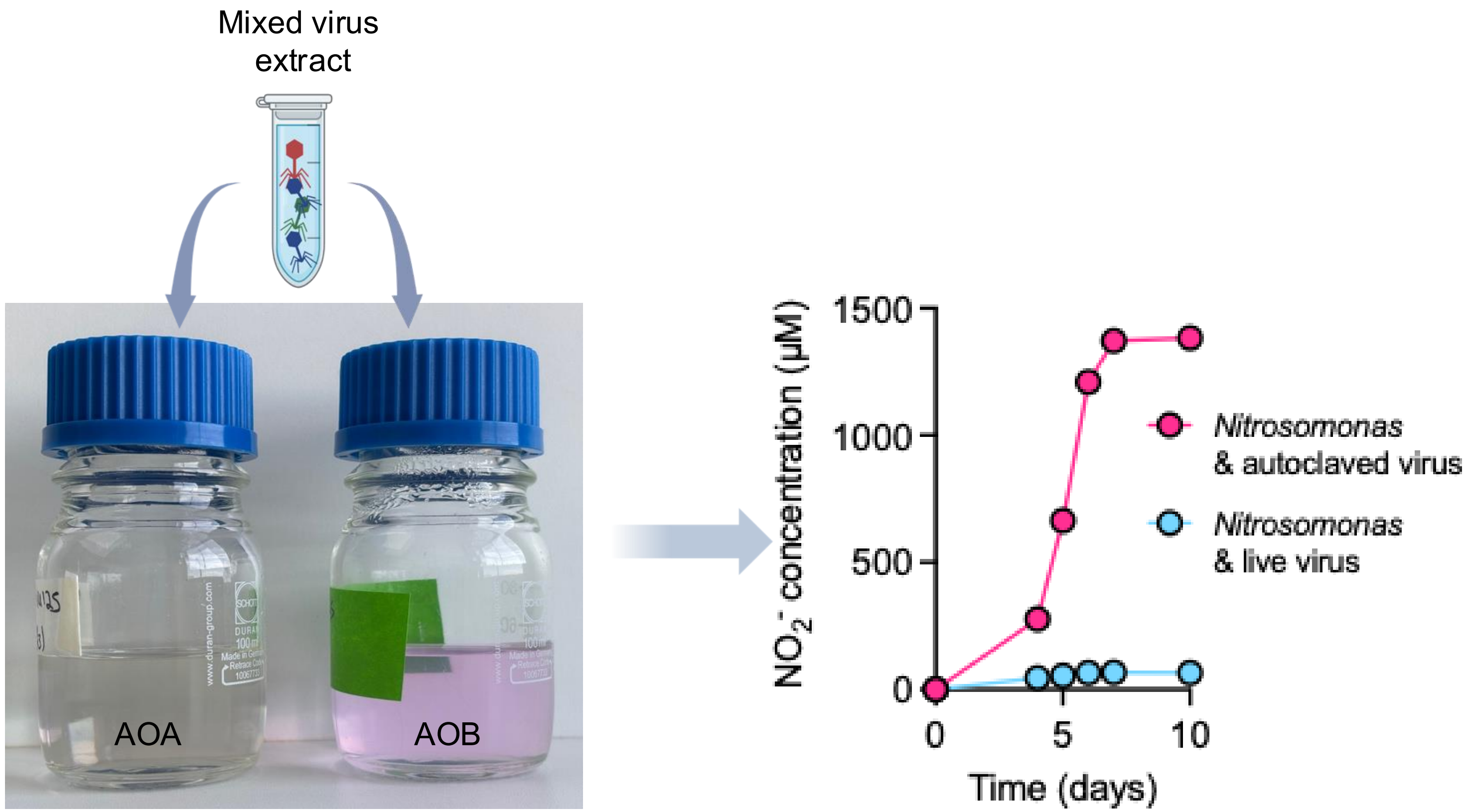


Cultivation of nitrifier viruses

Classical approach for isolating viruses



Novel approach for isolating nitrifier viruses



Considerations for a phage-based approach

- Detailed knowledge of target microbial populations
- Virus host range
- Virus lifestyle (lytic viruses)
- Virus cocktails to prevent phage-resistant strains
- Scaling-up production
- Application approach
- Regulations for products

OECD (2022) Guidance Document for the Regulatory Framework for the Microorganism Group: Bacteriophages. Series on Pesticides No. 108. ENV/CBC/MONO(2022)40
https://www.oecd.org/en/publications/guidance-document-for-the-regulatory-framework-for-the-microorganism-group-bacteriophages_706035c7-en.html

- 1 phage-based microbial pesticides product currently pending approval in the EU
  - *Pectobacterium carotovorum* (potato soft rot)
- 6 products already approved for commercial use in the USA

Acknowledgements

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AXA

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MARIE CURIE ACTIONS

JGI JOINT GENOME INSTITUTE

FRANCE-BERKELEY FUND FONDS FRANCE-BERKELEY

CHINA SCHOLARSHIP COUNCIL

PhD scholarship opportunity

- Interested in pursuing a PhD in soil virus ecology and biogeochemical cycling? 🦠
- Have a Masters degree not from France and ranked in the top 20% of your class? 🎓
- Submission deadline August 20, 2025 (but get in touch now!)

For further details:

https://soilmicrobes.fr/join-the-team/