SCIENCE MEETS LIFE

Op zoek naar de volgende generatie nitrificatie-inhibitoren: sleutels tot duurzaam nutriëntengebruik

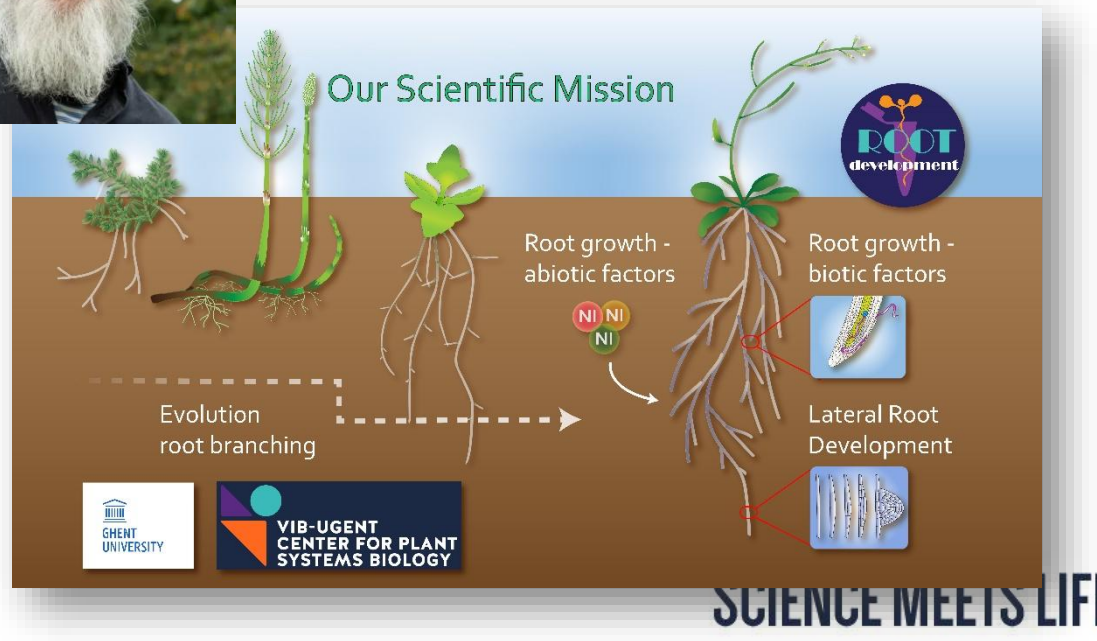
Dr. ir. Hans Motte

VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY

GHENT UNIVERSITY

VIB – who are we?

- Interuniversity life sciences **research institute**
- Mission to **pioneer in strategic basic research** and **translate into real-world applications**
- Partnership with the **5 Flemish universities**
- 10 thematic centers, including the VIB-UGent Center for **Plant Systems Biology** with 20 research groups
- **Root Development** group (Beeckman lab)
  - Focus on:
    - Root development
    - Interaction with environment
    - **Nutrient uptake and use**
    - **High-throughput agrochemical screening**

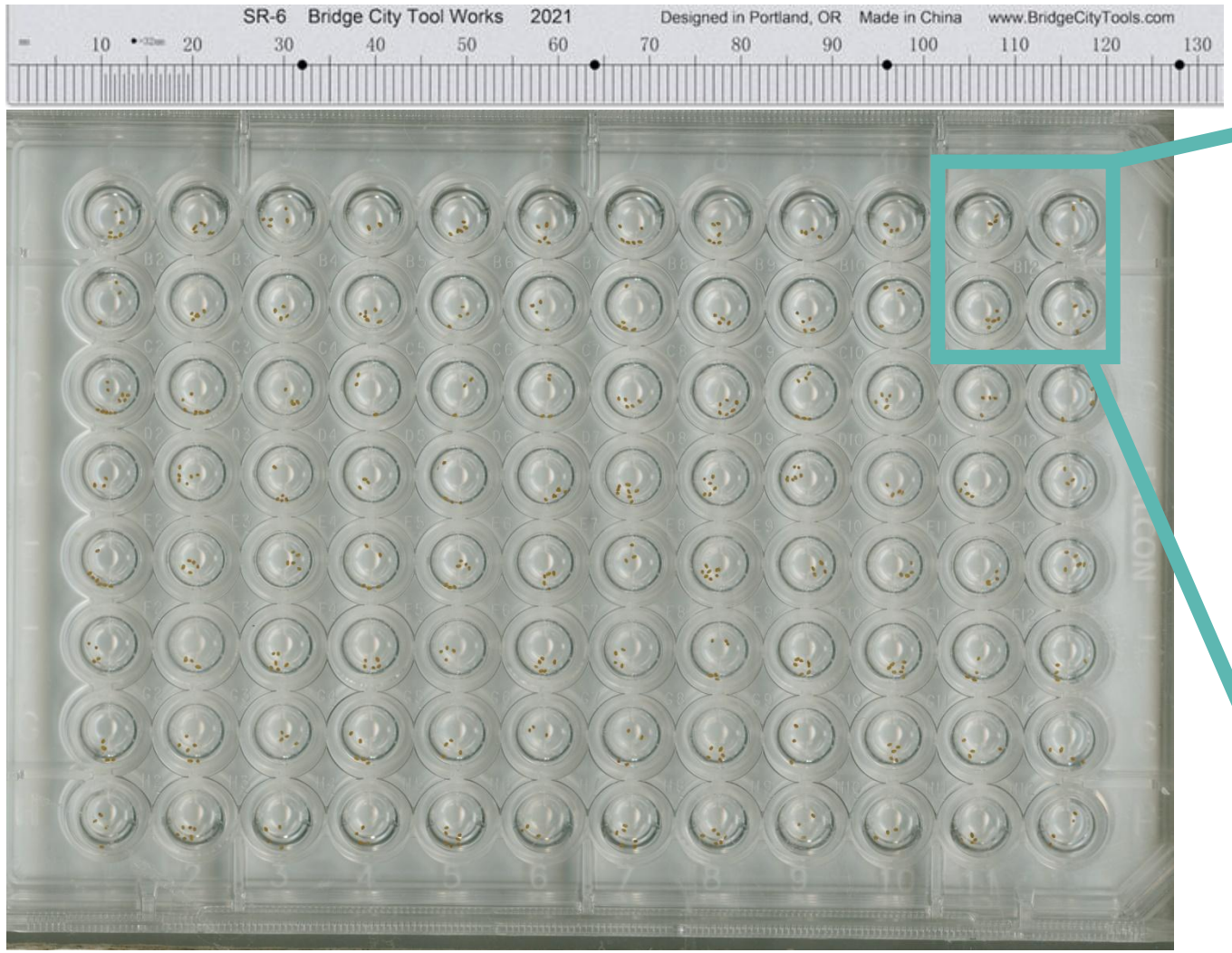


VIB

SCIENCE MEETS LIFE

VIB – high-throughput agrochemical screening

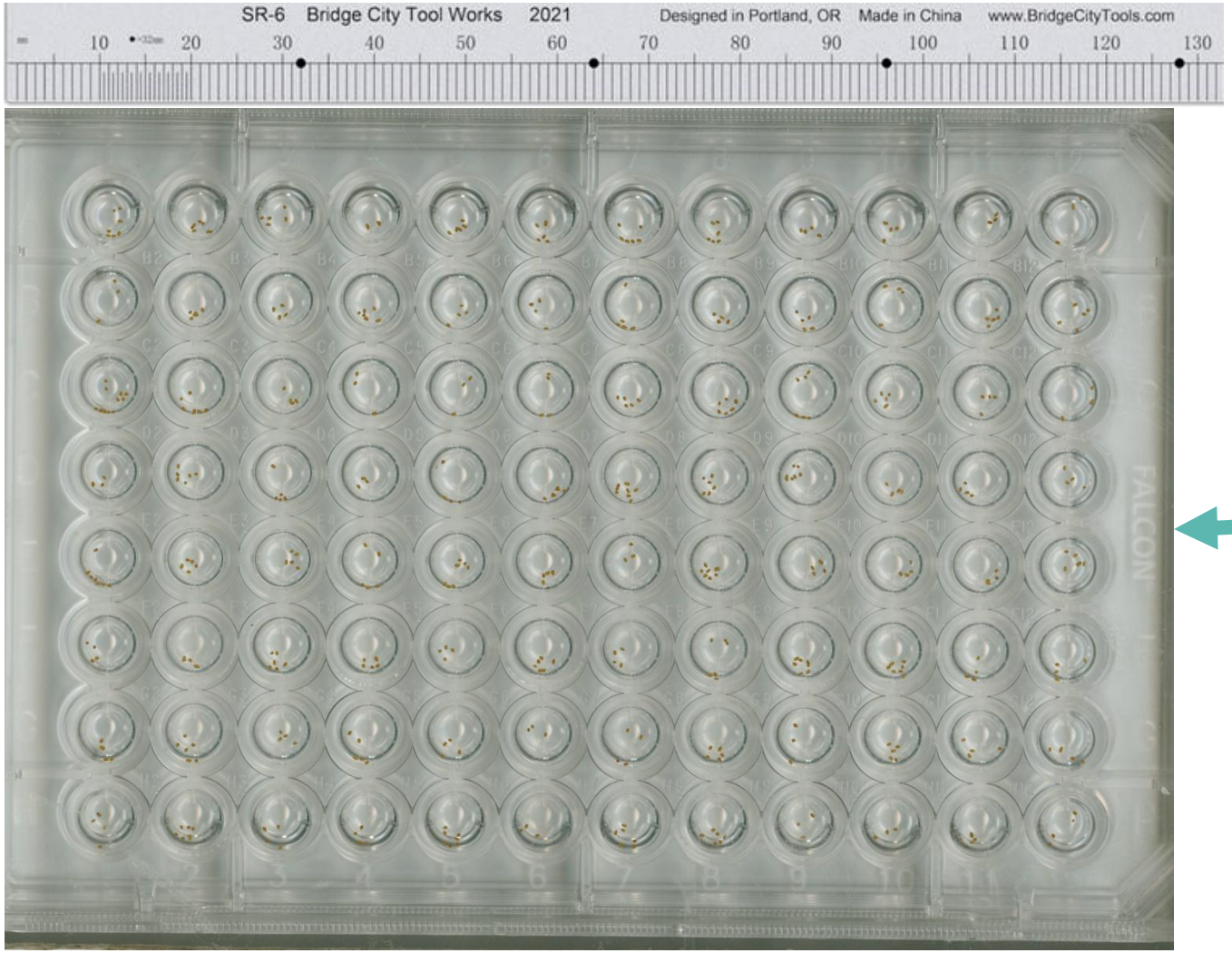
- Miniaturization



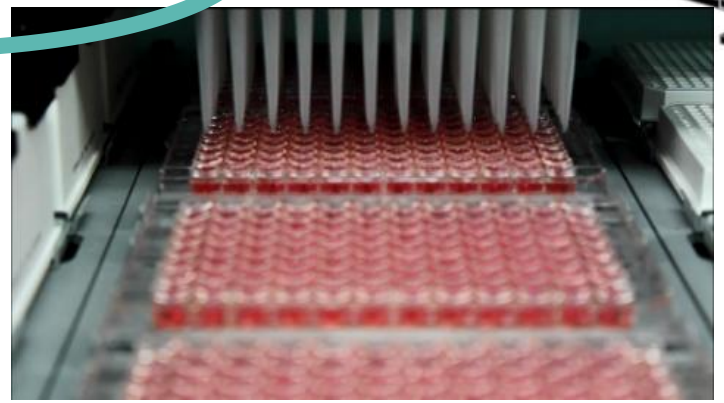
ETS LIFE

VIB – high-throughput agrochemical screening

- **Miniaturization**
- Test **large set** of conditions



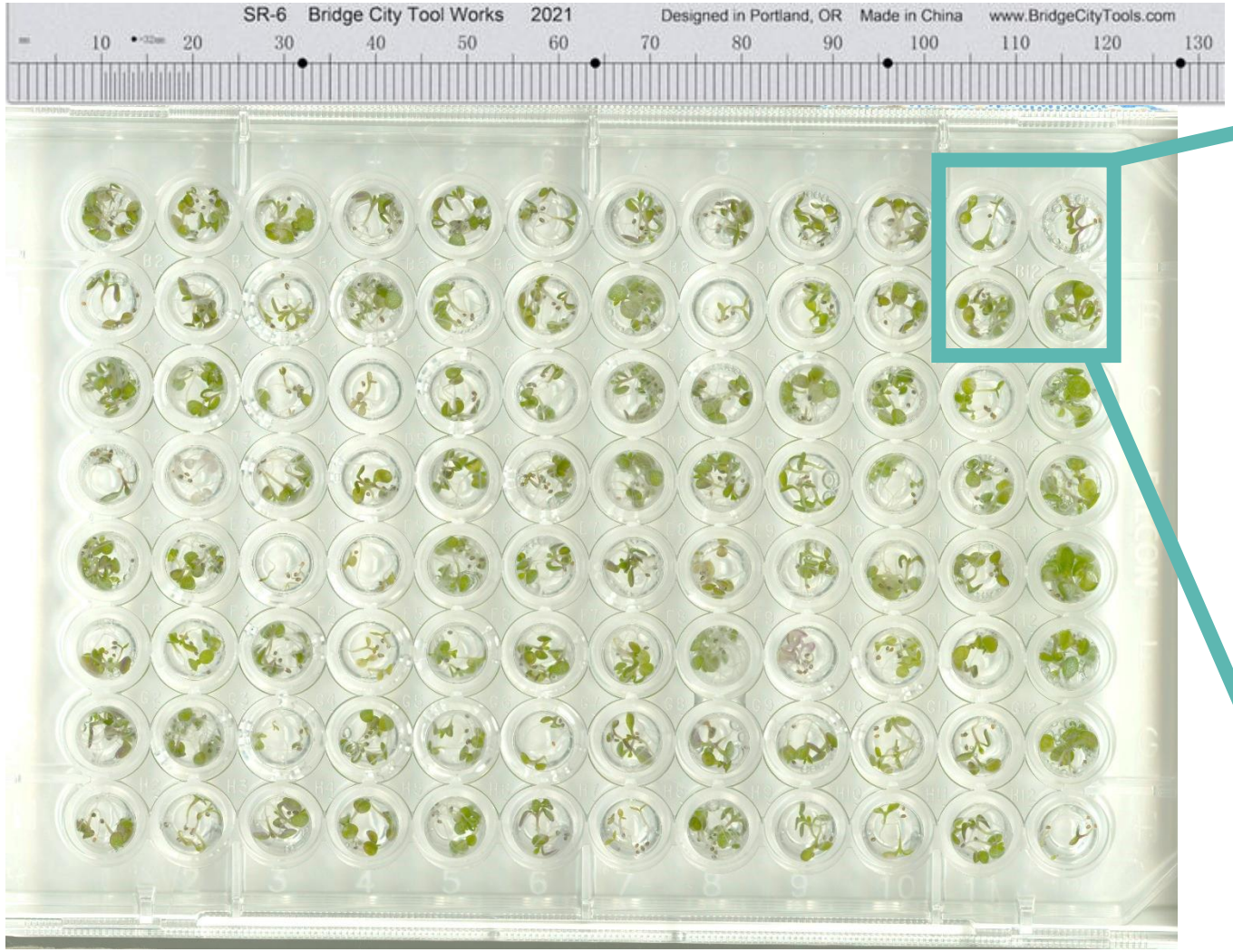
Small molecule collection (10.000s of diverse small molecules)



SCIENCE MEETS LIFE

VIB – high-throughput agrochemical screening

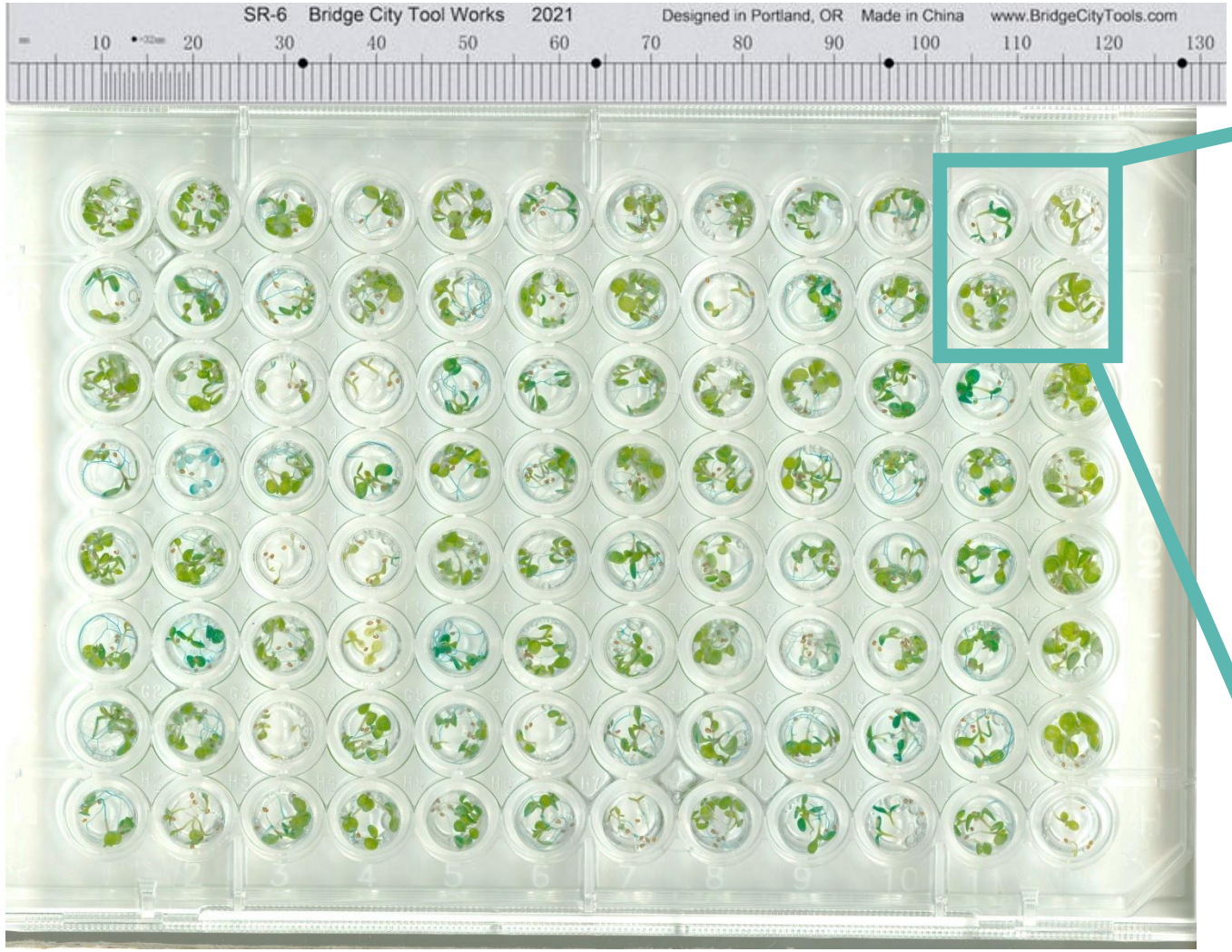
- **Miniaturization**
- Test **large set** of conditions
- Use of **model systems**



ETS LIFE

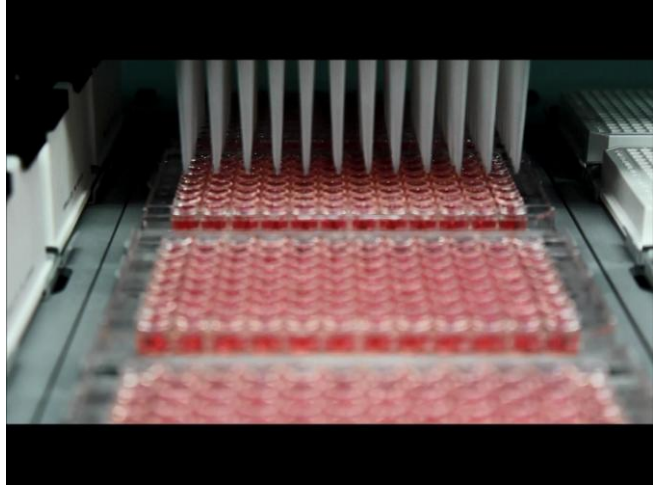
VIB – high-throughput agrochemical screening

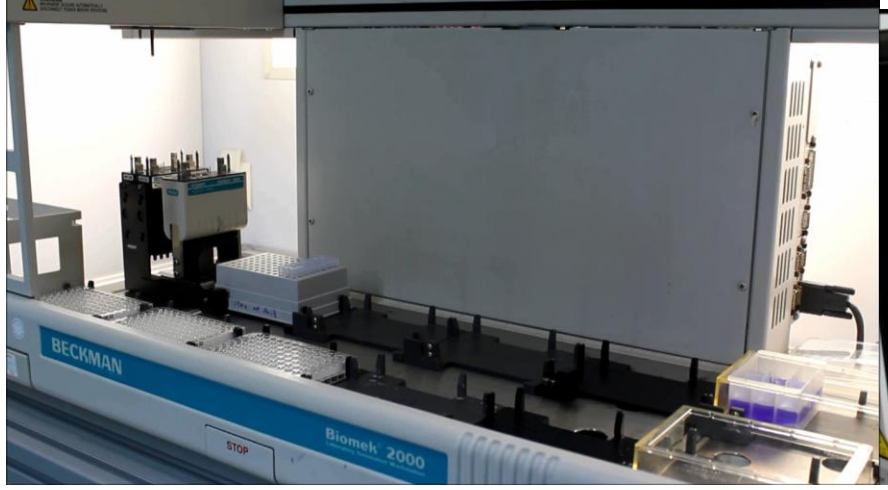
- **Miniaturization**
- Test **large set** of conditions
- Use of **model systems**
- Certain **signal as readout**



Agrochemical screening

- Less trivial than it sounds!
  - Assays need to be very robust to avoid false positives/negatives
- Preferably time- and labor-effective
  - Automation and robotics help

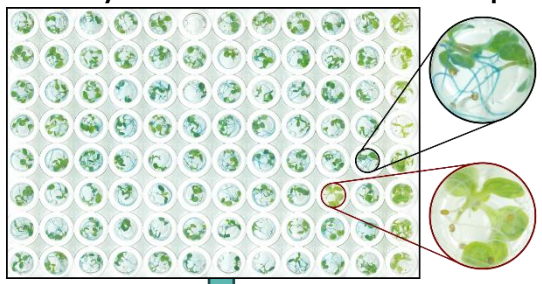


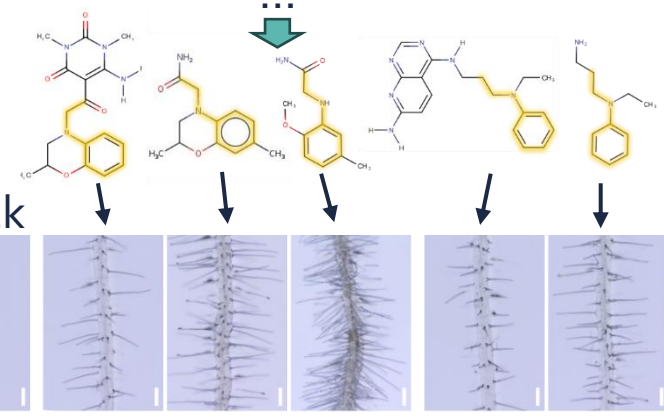

BECKMAN
Biomek 2000
STOP


AUTOMATISCH
STAITENDE
MACHINE

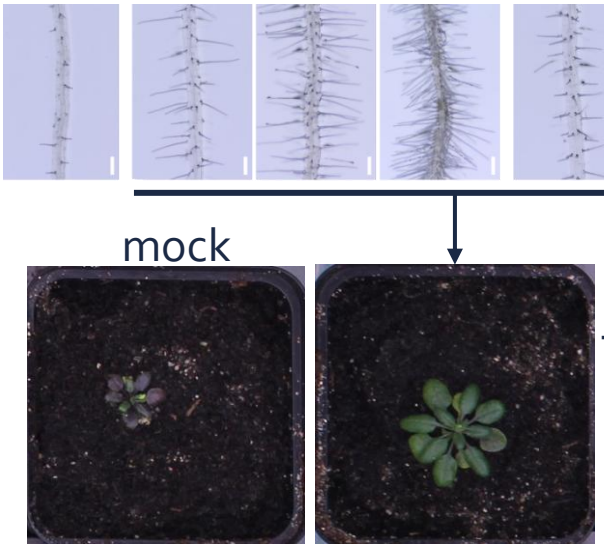
Agrochemical screening – concrete examples

P-deficiency marker in Arabidopsis



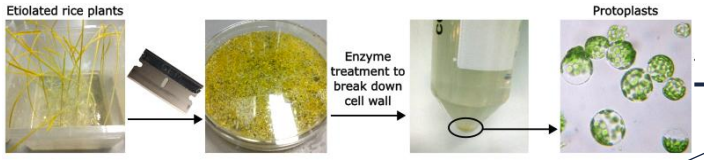


mock

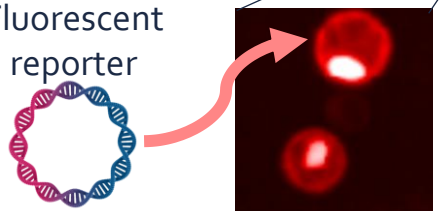


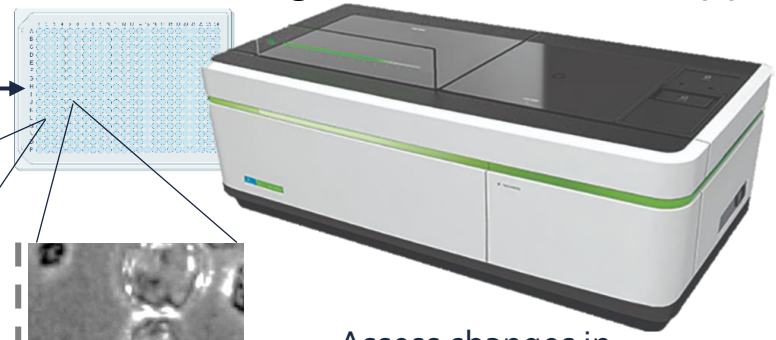
mock

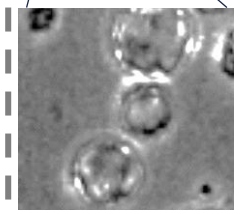
→ Higher PUE by increase of P-uptake area



Fluorescent reporter

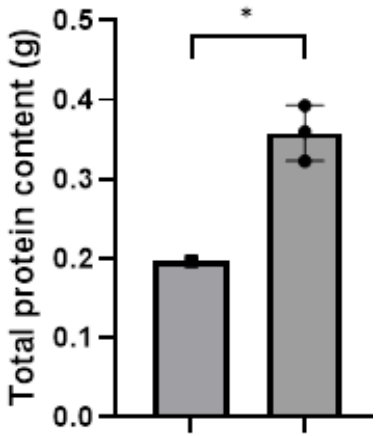






Assess changes in vacuole/cell morphologies
→ Agrochemicals with effect on cell ionic balance

Inducer of AA-metabolism gene
→ Increase in grain protein

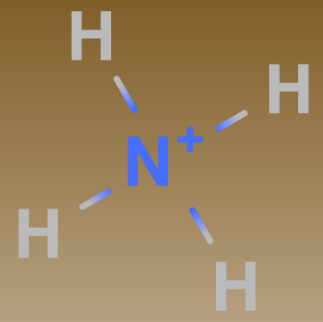


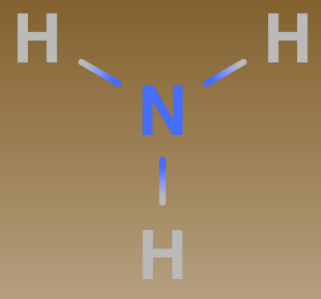
Salt stress

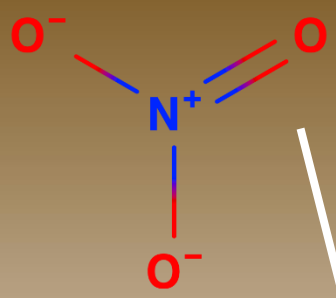
tolerance

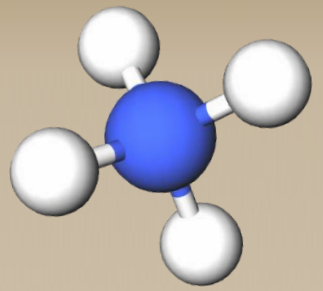
Nitrogen use inefficiency: 60% loss!!

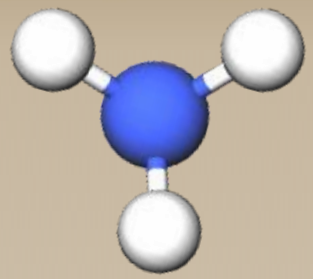
Ammonium NH_4^+ ⇌ **Ammonia** $NH_3 + H^+$ → **Nitrate** NO_3^-

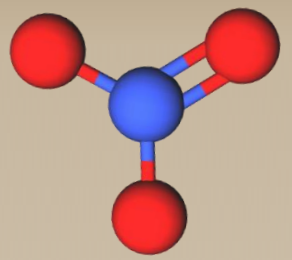




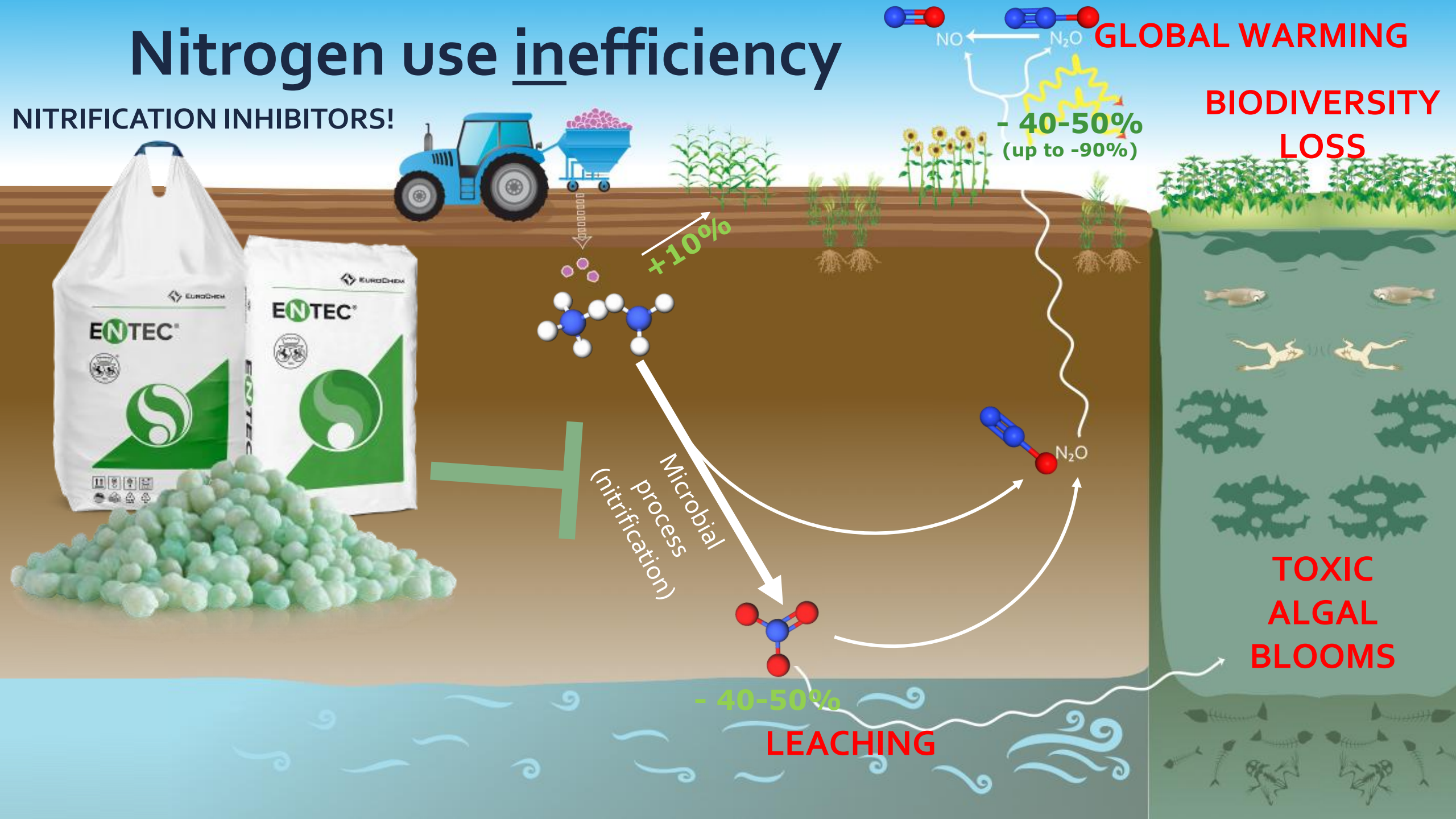






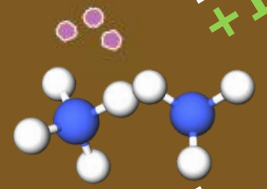


Nitrogen use inefficiency



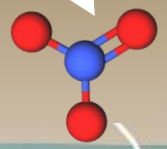
Nitrogen use inefficiency

NITRIFICATION INHIBITORS!



+10%

Microbial process (nitrification)

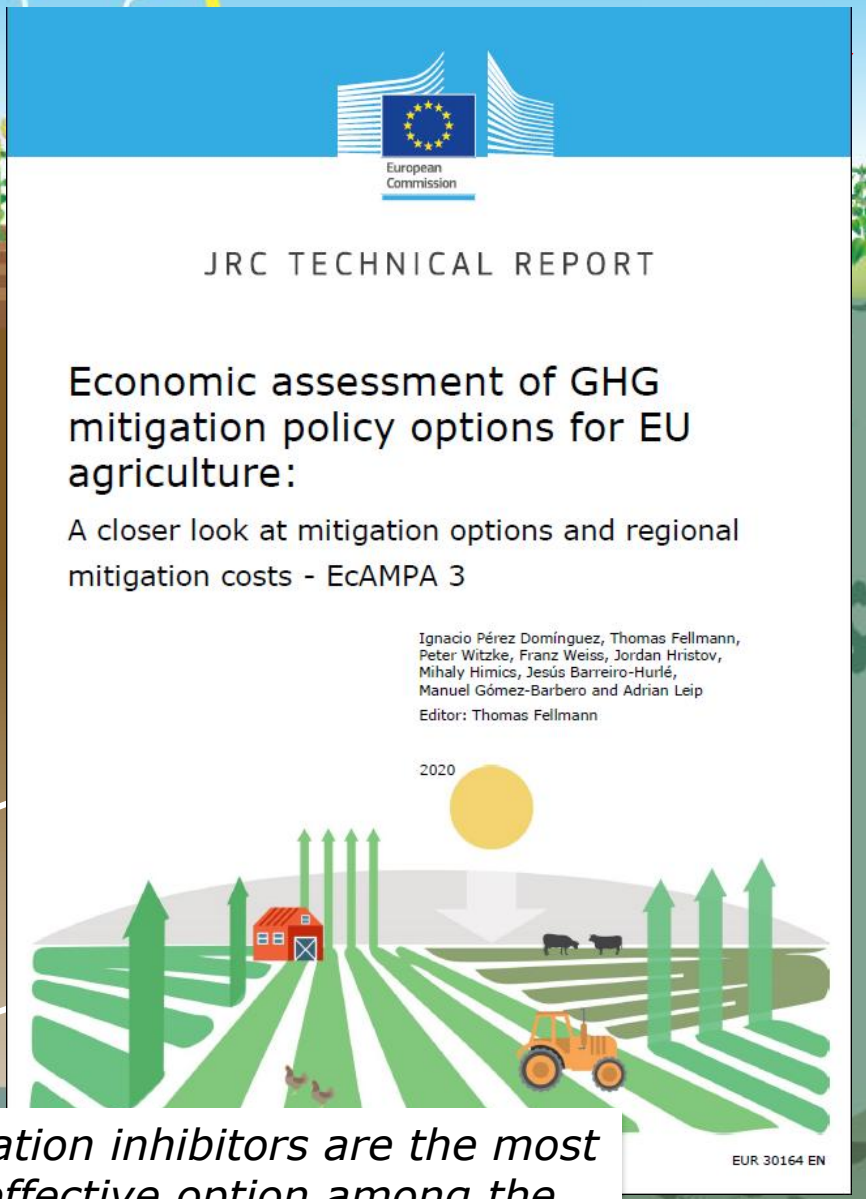


- 40-50%

LEAC

NO ← N_2O

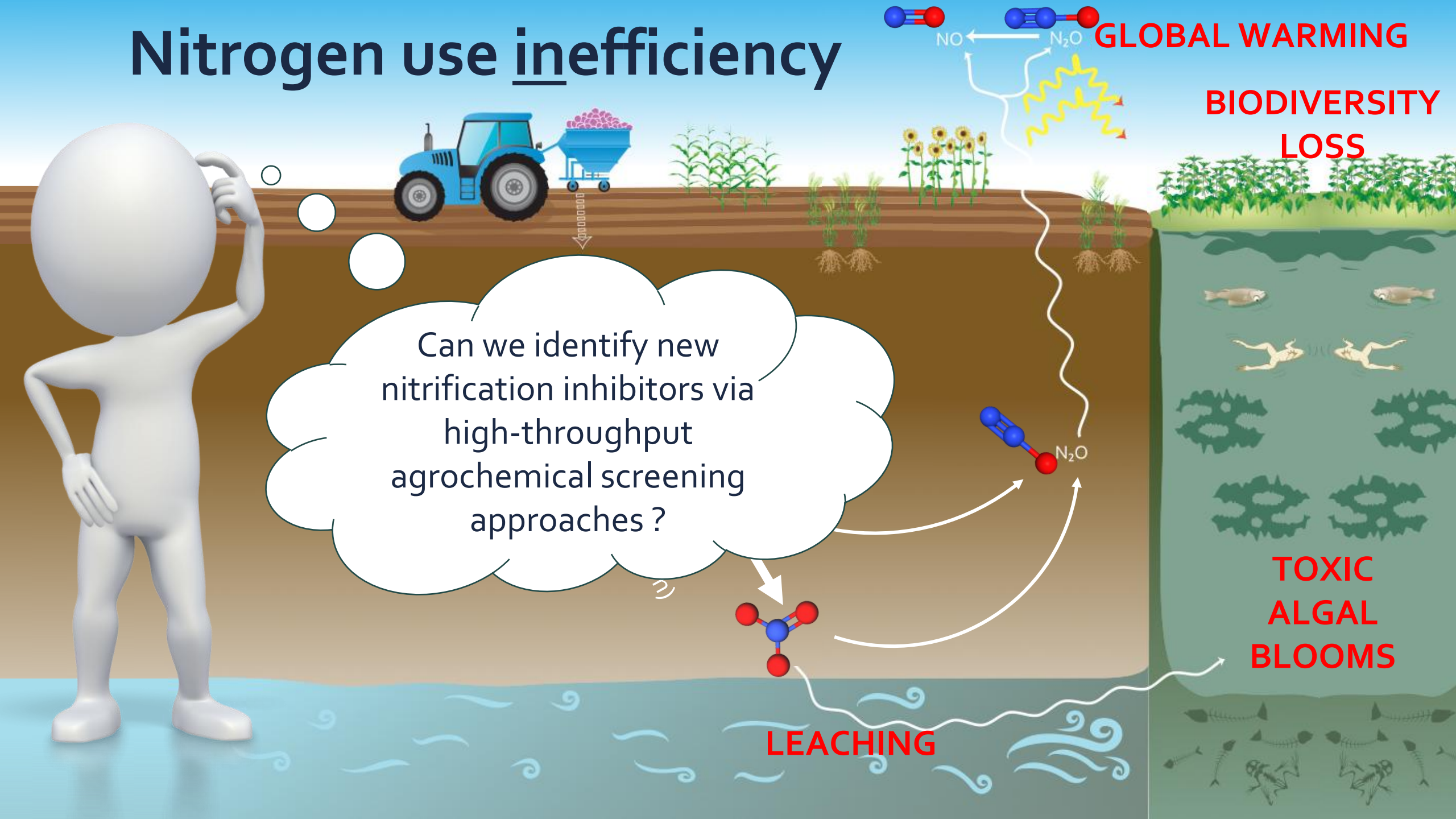
GLOBAL WARMING



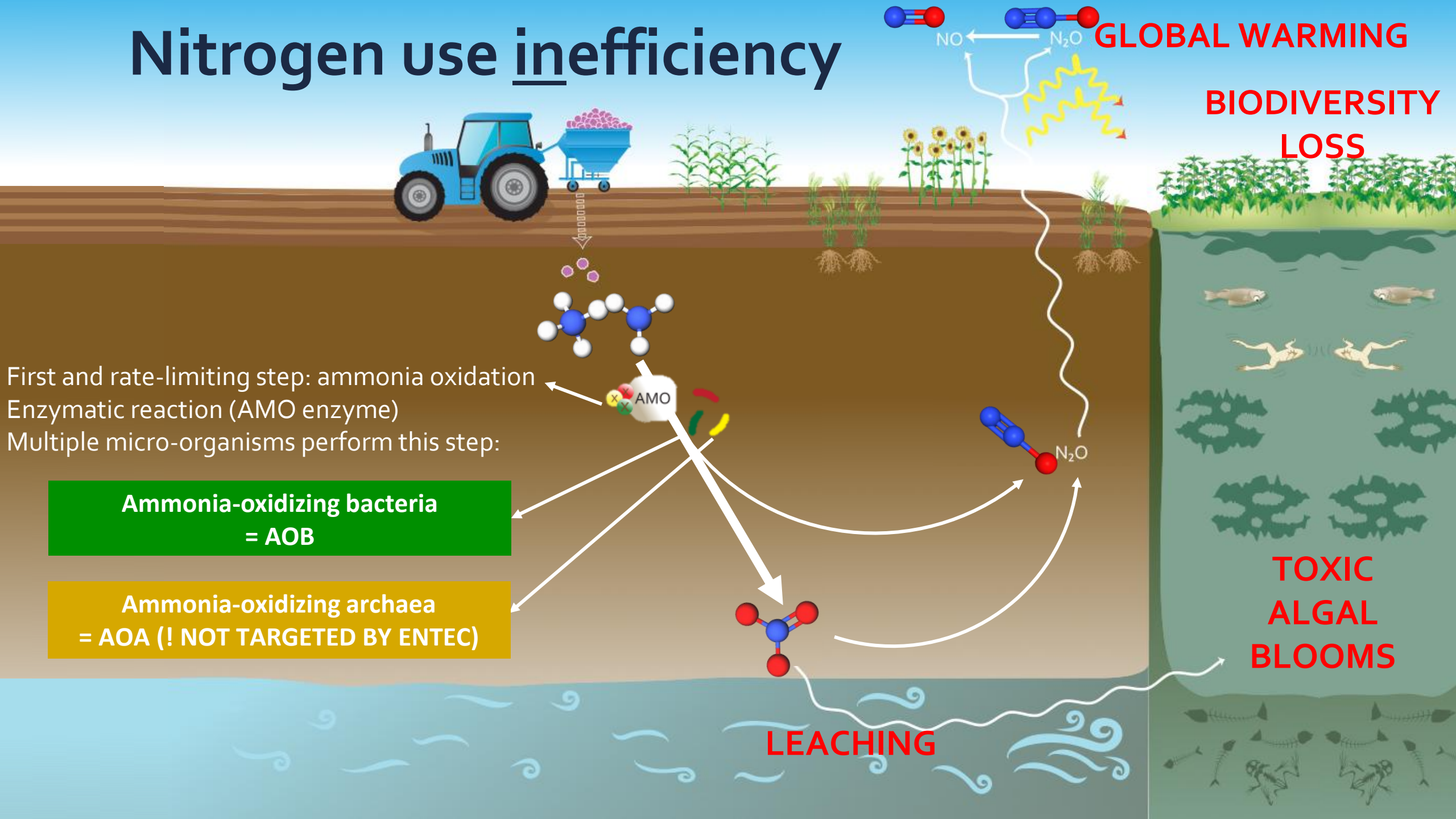
- Limited portfolio
- Efficiency depends on soil/conditions
- Cost

"Nitrification inhibitors are the most cost-effective option among the fertilizer-related measures."

Nitrogen use inefficiency



Nitrogen use inefficiency



First and rate-limiting step: ammonia oxidation
Enzymatic reaction (AMO enzyme)
Multiple micro-organisms perform this step:

Ammonia-oxidizing bacteria = AOB

Ammonia-oxidizing archaea = AOA (! NOT TARGETED BY ENTEC)

High-throughput agrochemical screen to discover new nitrification inhibitors

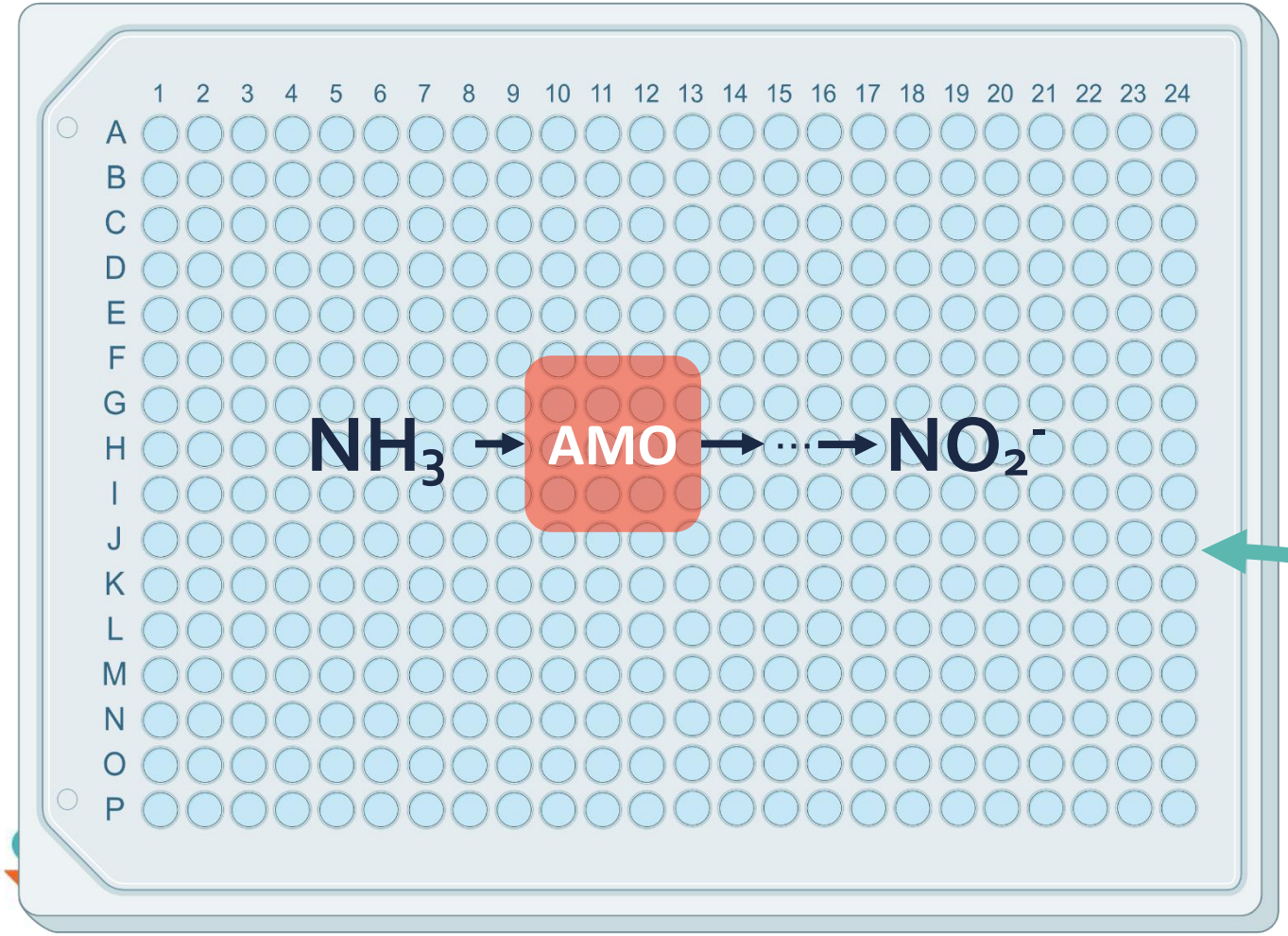
Miniaturization in 384-well plates

VIB

SCIENCE MEETS LIFE

High-throughput agrochemical screen to discover new nitrification inhibitors

Model systems



Ammonia-oxidizing bacteria = AOB

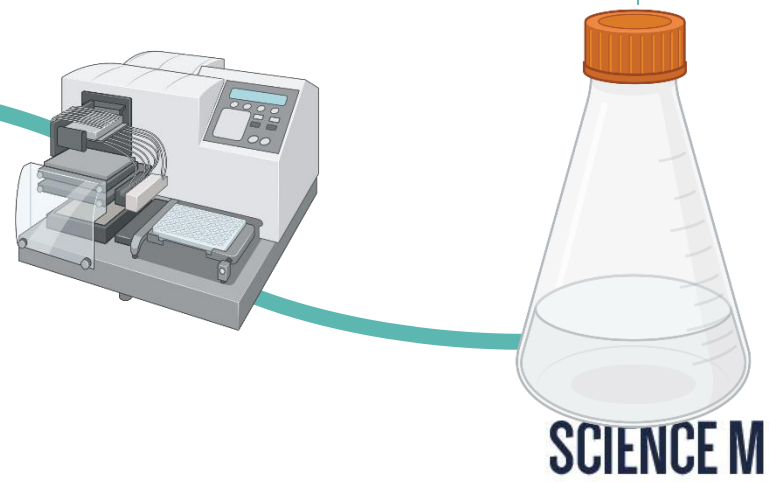
Ammonia-oxidizing archaea = AOA

Nitrosomonas europaea

Nitrososphaera viennensis

Nitrosospira multiformis

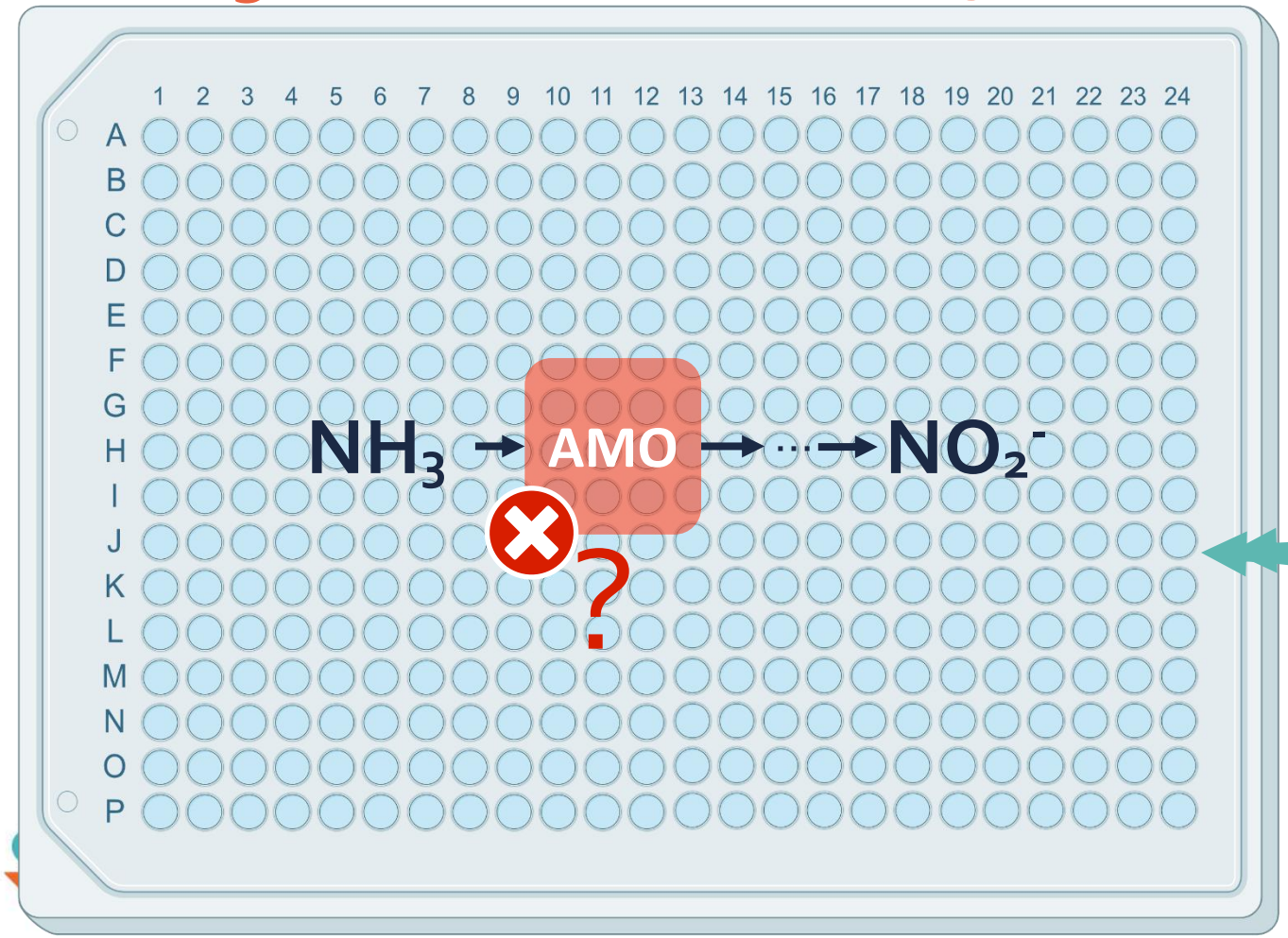
community



SCIENCE MEETS LIFE

High-throughput agrochemical screen to discover new nitrification inhibitors

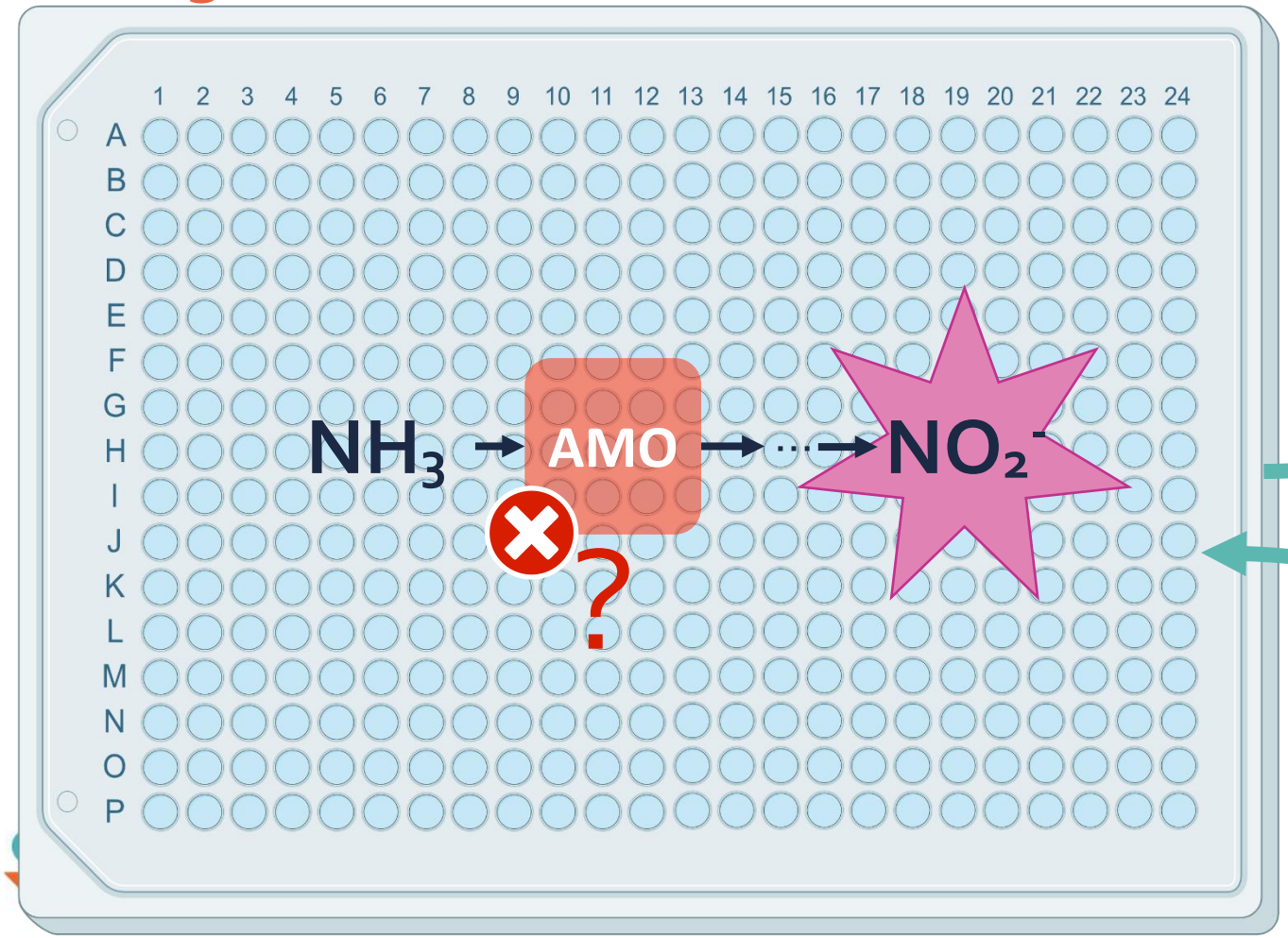
Large set of conditions: ~50.000 small molecules

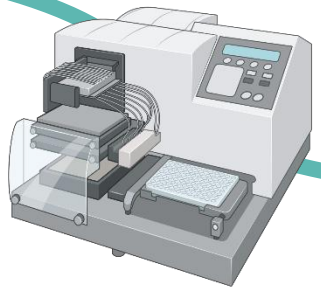


SCIENCE MEETS LIFE

High-throughput agrochemical screen to discover new nitrification inhibitors

Signal and read-out: color reaction with spectrophotometer

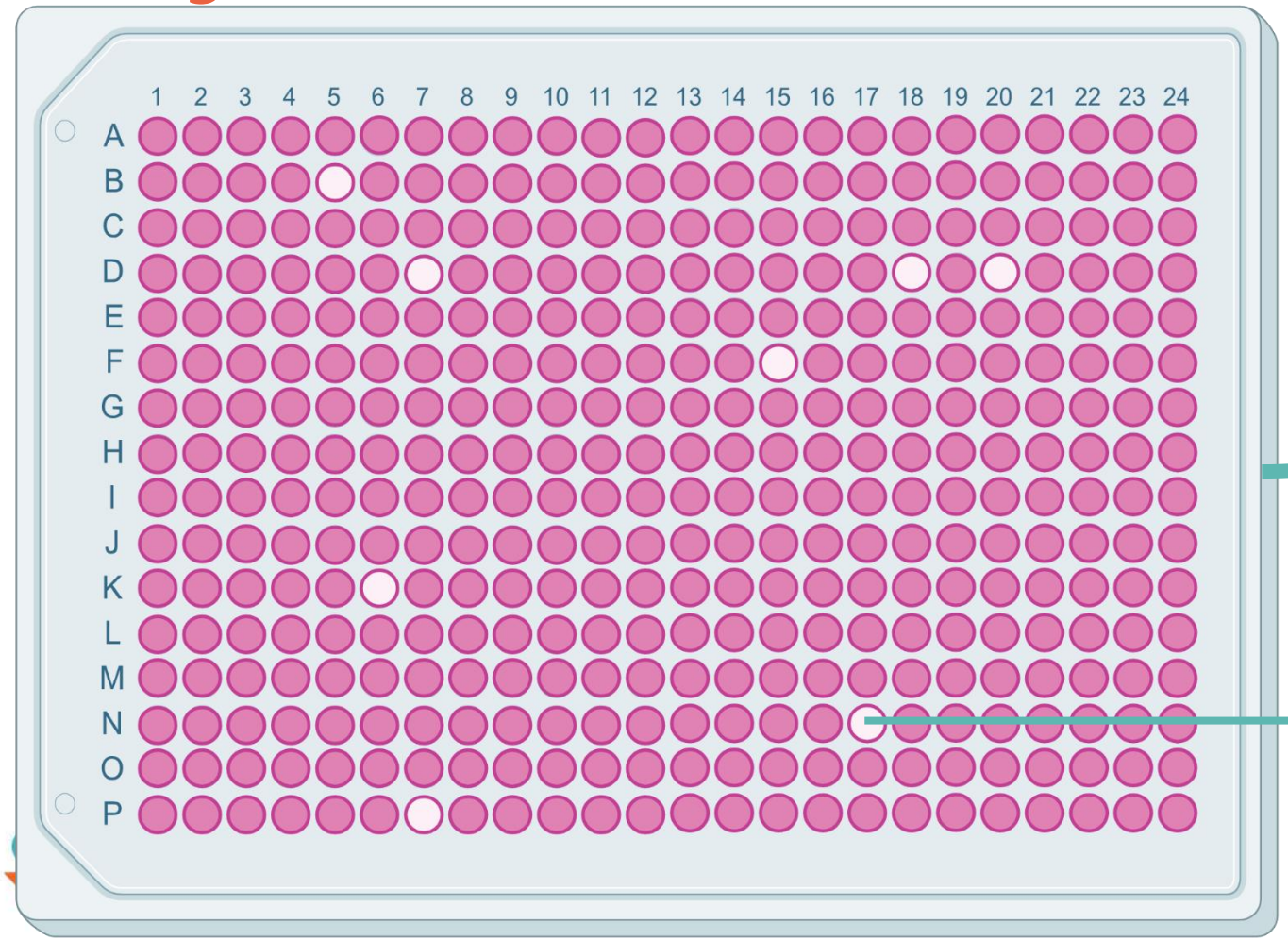




SCIENCE MEETS LIFE

High-throughput agrochemical screen to discover new nitrification inhibitors

Signal and read-out: color reaction with spectrophotometer



Nitrification inhibitor!

SCIENCE MEETS LIFE

From candidate to validated NI

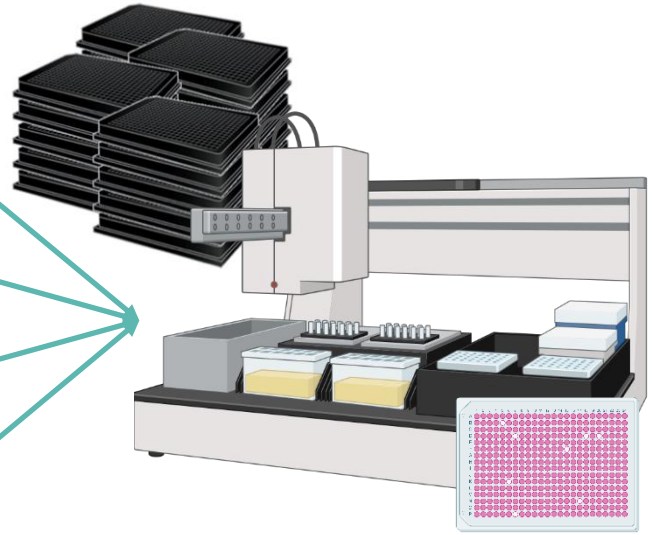
~50k small molecules

Nitrosomonas europaea

Nitrosospira multiformis

Nitrososphaera viennensis

community



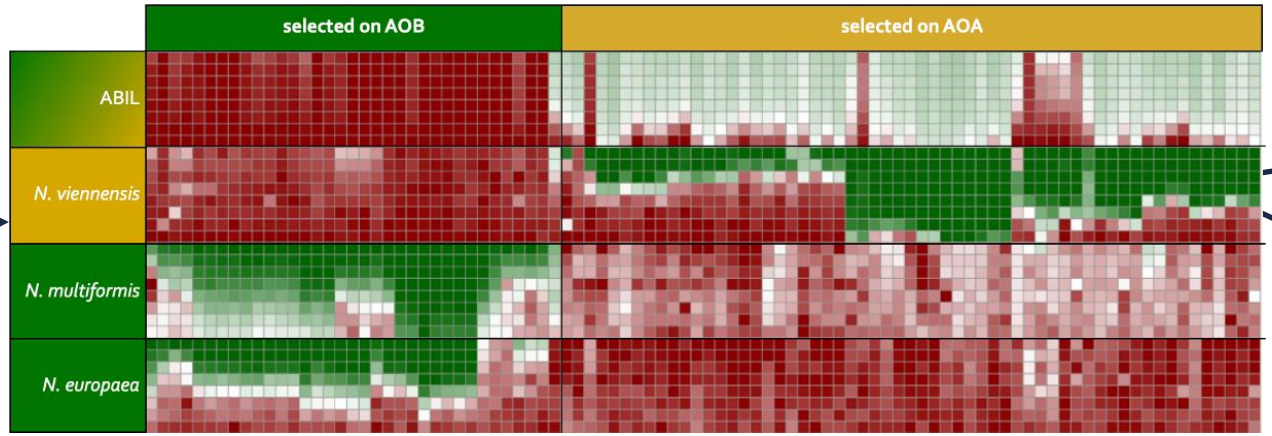
4 x ±320
Candidate NIs

- Confirmation screen
- Toxicity assays
- Efficacy tests
- …

94
compounds

From candidate to validated NI

94 compounds



AOB inhibitors

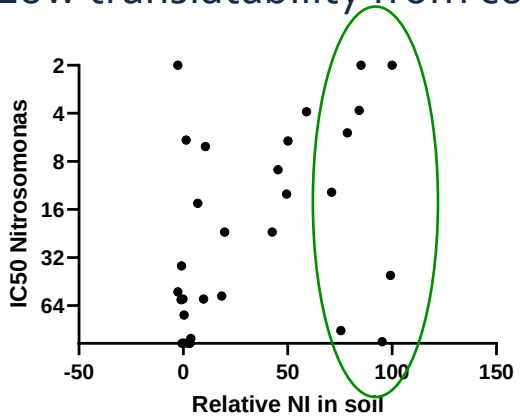
AOA inhibitors

Structural optimization
(testing a lot of chemical variants)

From candidate to validated NI

➔ validation

! Low translatability from culture to soil

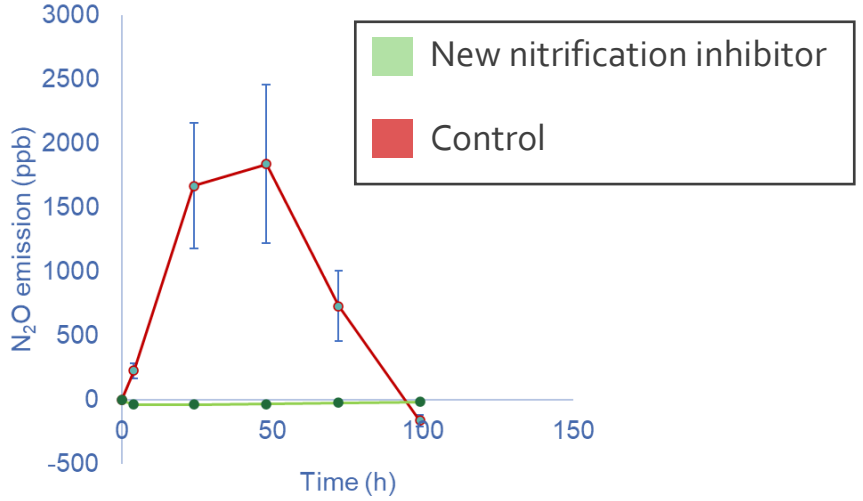


From candidate to validated NI

validation

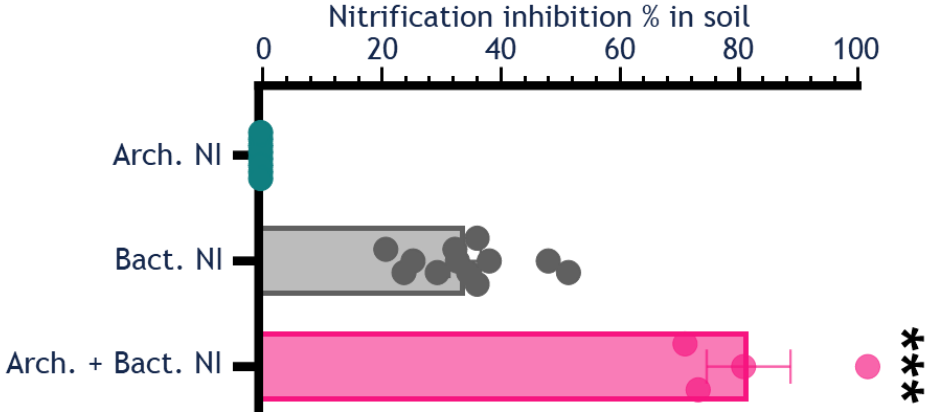
Multiple new AOB NIs

completely block emission of greenhouse gas N2O

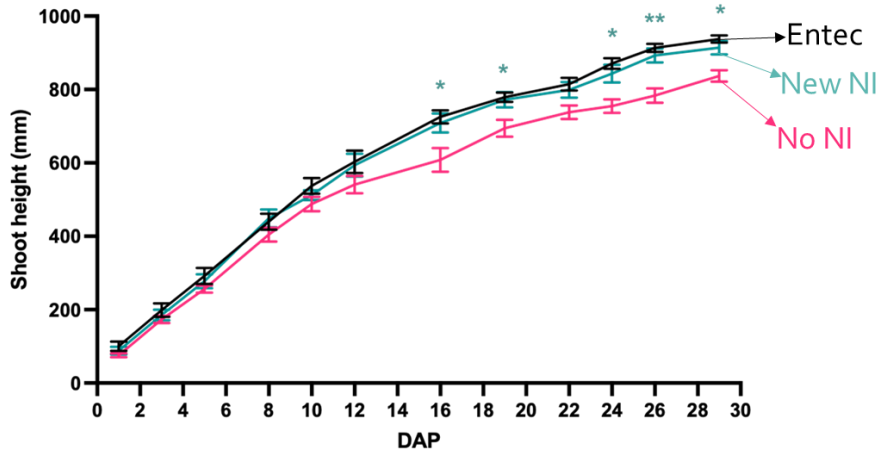


SIAS (Specific Inhibitor of AOA in Soil)

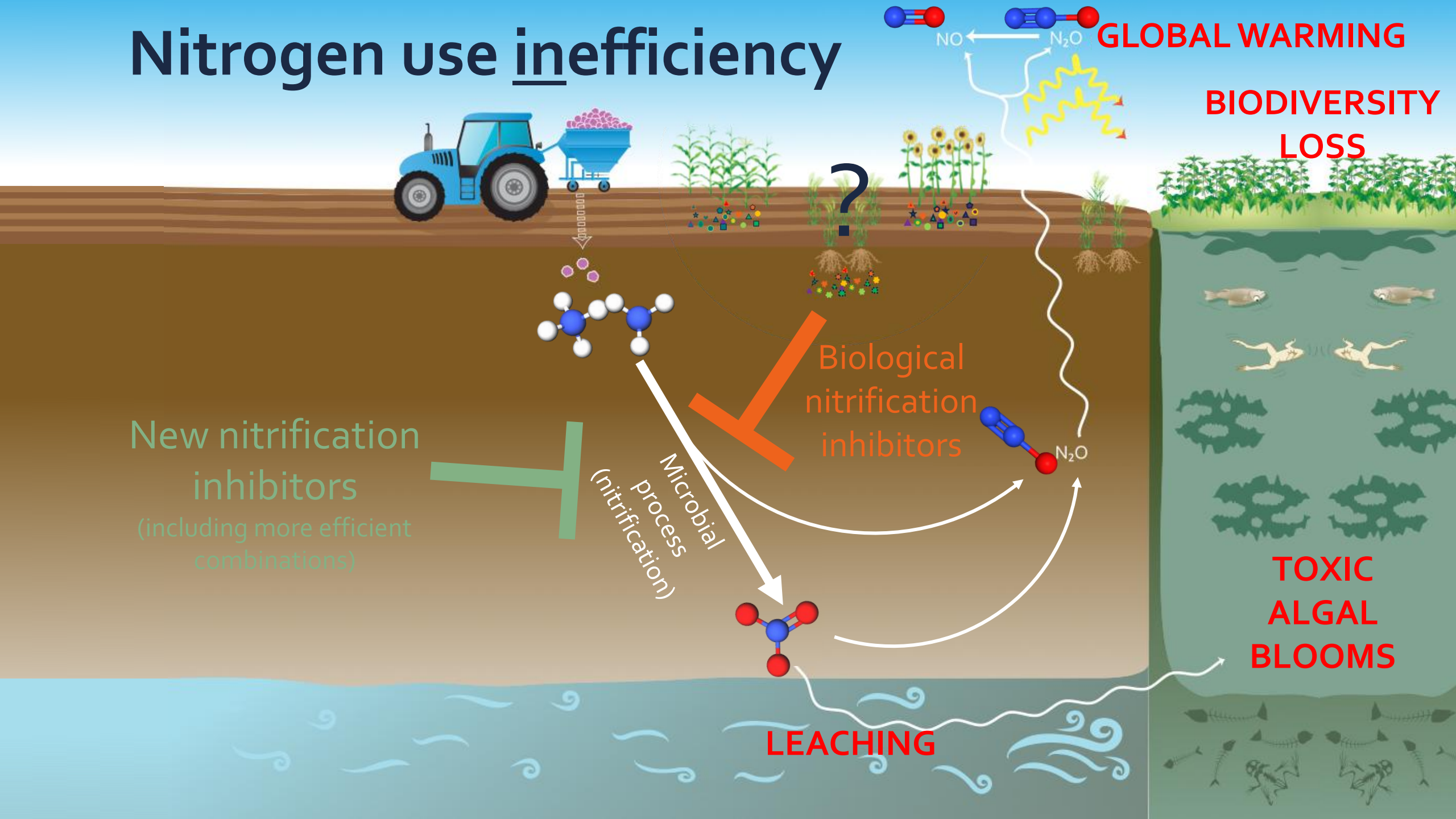
Strongly improve nitrification inhibition



positively affect crop growth



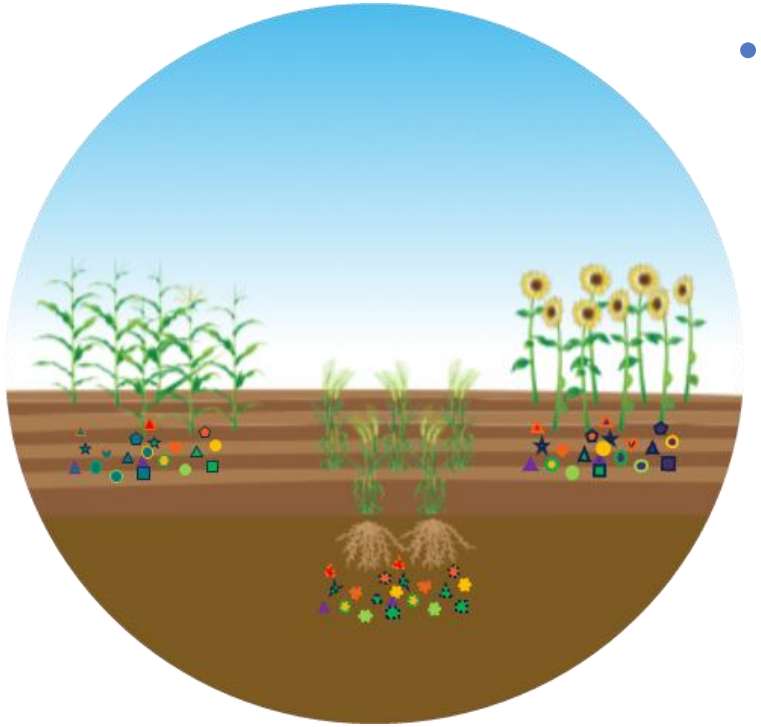
Nitrogen use inefficiency



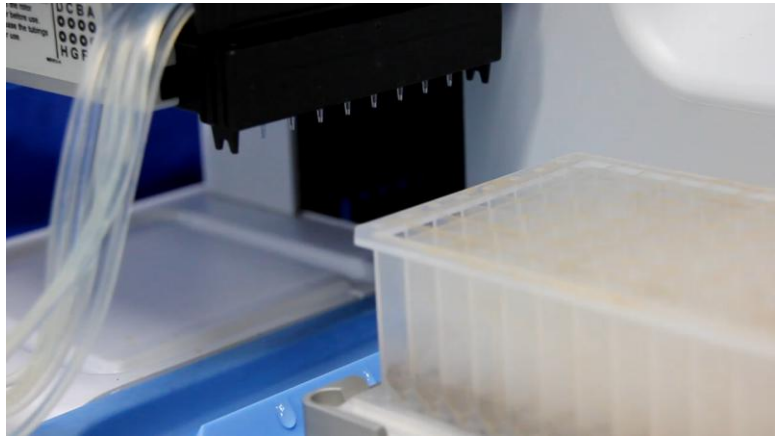
Biological nitrification inhibitors

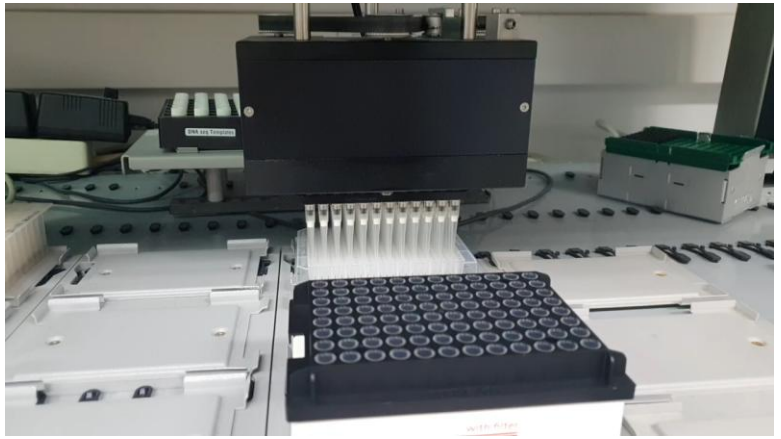
- Produced and secreted by the plants themselves
  - Release of BNIs in the field by a crop OR via other plants
  - BNI release as a breeding trait/ for biotechnological breeding
  - Bioproduction (synthetic biology)
- May further expand the nitrification inhibitor portfolio
- Opportunities for organic agriculture

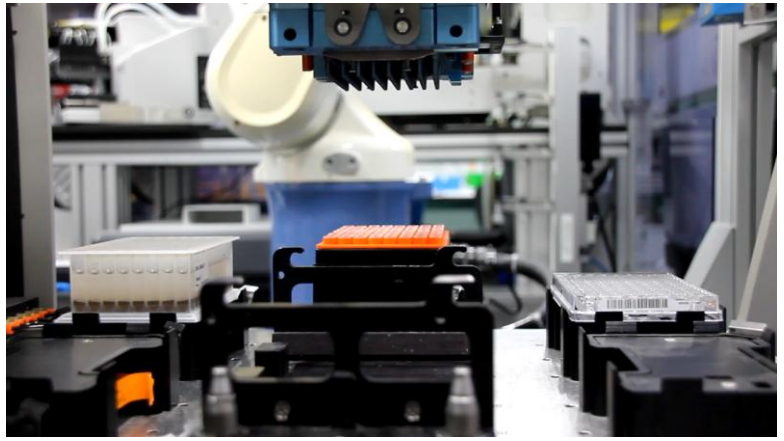
High-throughput biological screen to discover new nitrification inhibitors



- Can we perform a screen directly in soil? Yes we can!







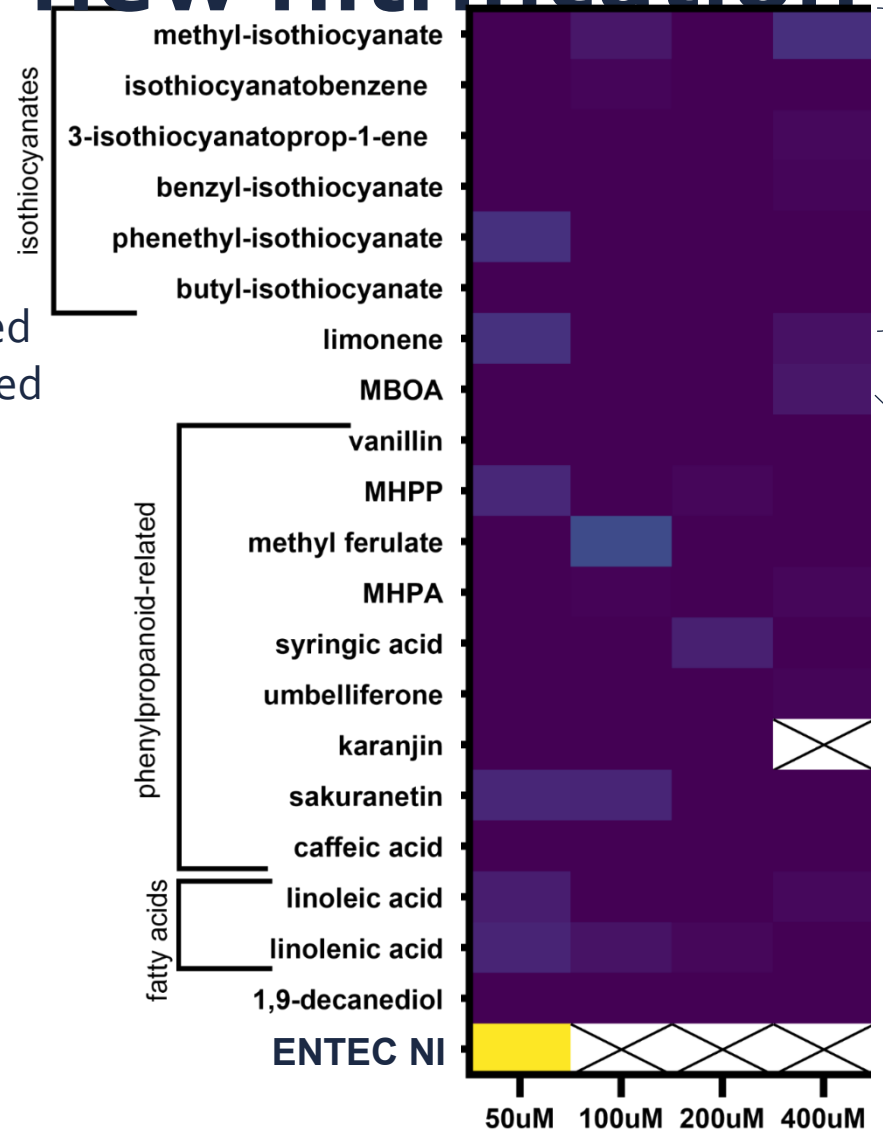
VIB

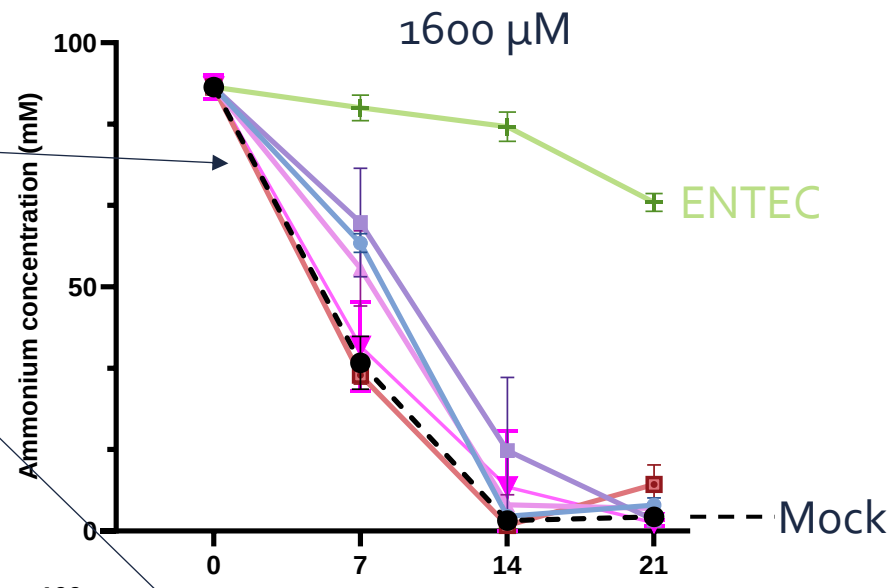
LIFE

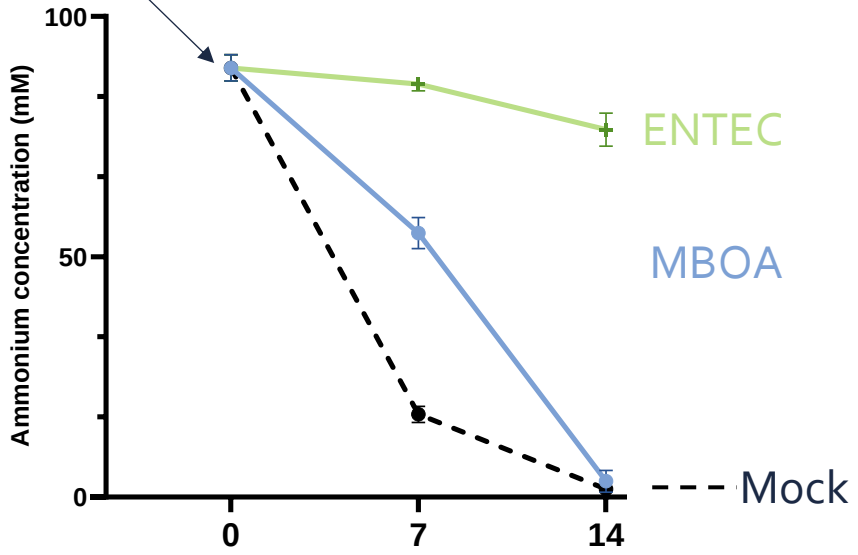
High-throughput biological screen to discover new nitrification inhibitors

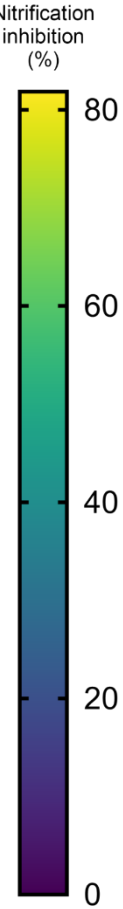
- What is the state of the art?
- What is a good benchmark

20 described BNIs acquired

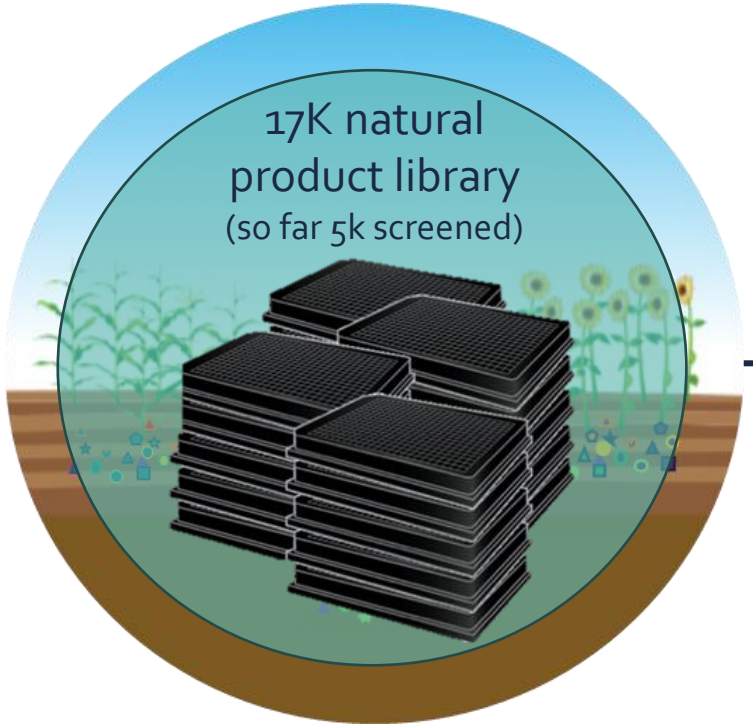


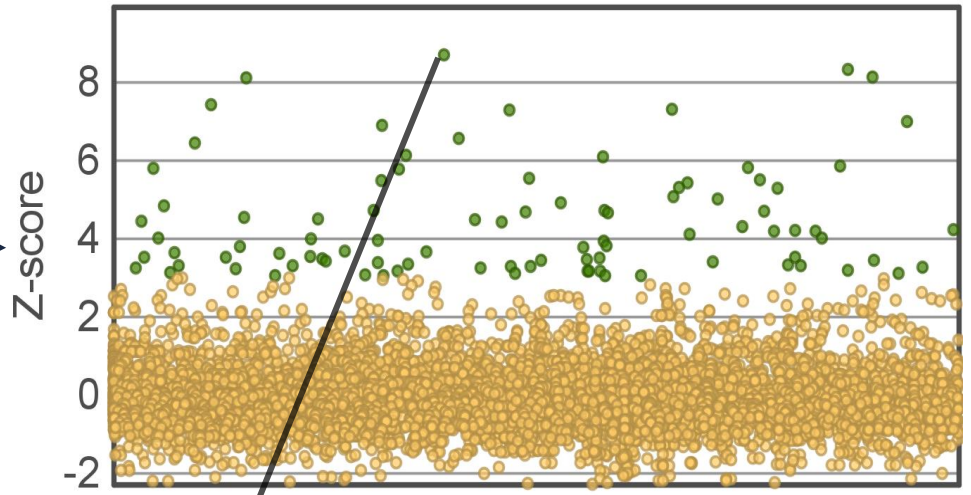


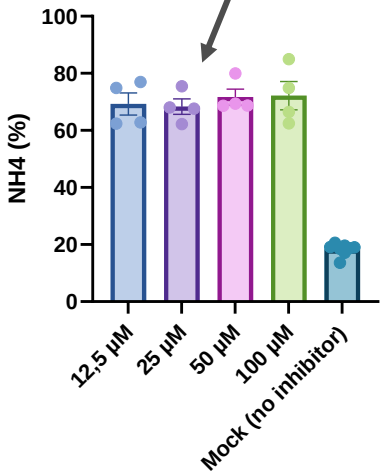




High-throughput biological screen to discover new nitrification inhibitors





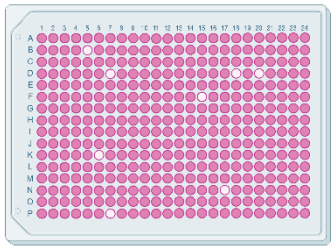


New natural products that strongly inhibit nitrification in soil

VIB

SCIENCE MEETS LIFE

Take home messages



- High-throughput agrochemical screening yields **new nitrification inhibitors and efficient combinations**

- New assay enables **screening directly in soil**

- **Reported biological nitrification inhibitors** produced by plants **do not inhibit nitrification in soil**…

  … but new soil assay enabled **discovery of new one with high performance in soil**

  ⇒ Promising candidates to be used as inhibitors or in breeding traits

VIB

SCIENCE MEETS LIFE

Acknowledgements

EuroChem Antwerpen

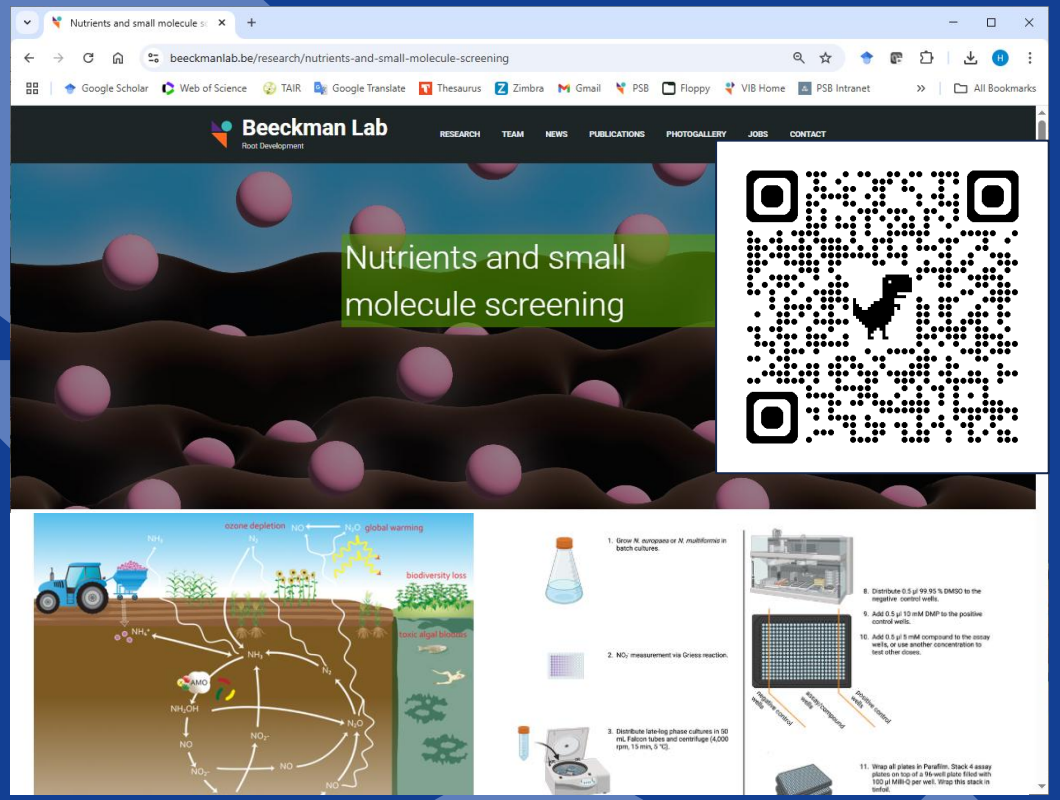
FFAR®

Ghent University

VIB Screening Core

More information

https://www.beeckmanlab.be/research/nutrients-and-small-molecule-screening



Hans Motte (hans-motte-a88b5463)

Hans.Motte@vib.be
Hans.Motte@UGent.be


VIB
science meets life