

# Engineering nitrogen-fixing microbial communities associated with maize and sorghum roots

Jean-Michel Ané

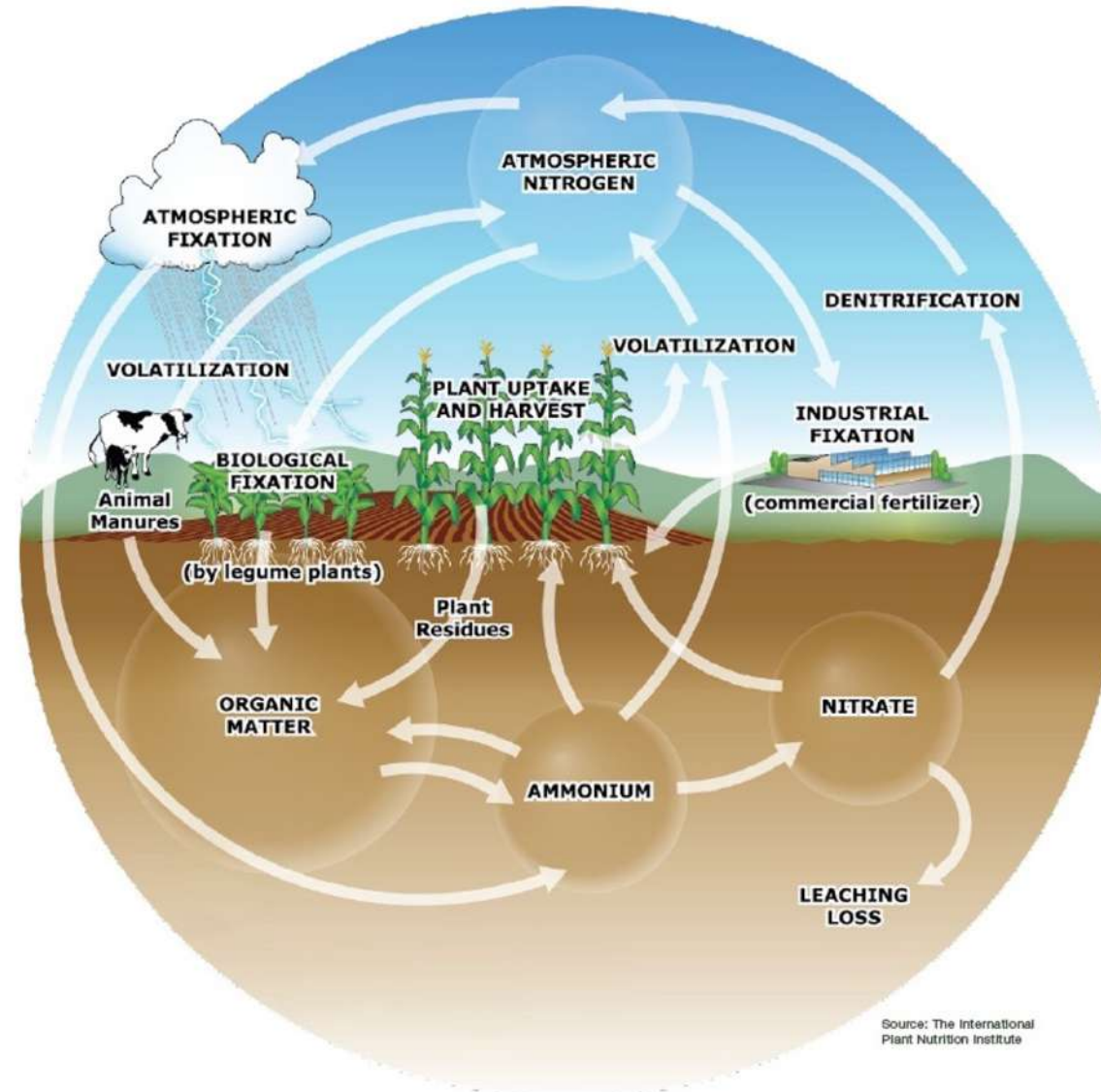
Department of Bacteriology

Department of Plant and Agroecosystem Sciences

University of Wisconsin - Madison



# The nitrogen cycle and biological nitrogen fixation



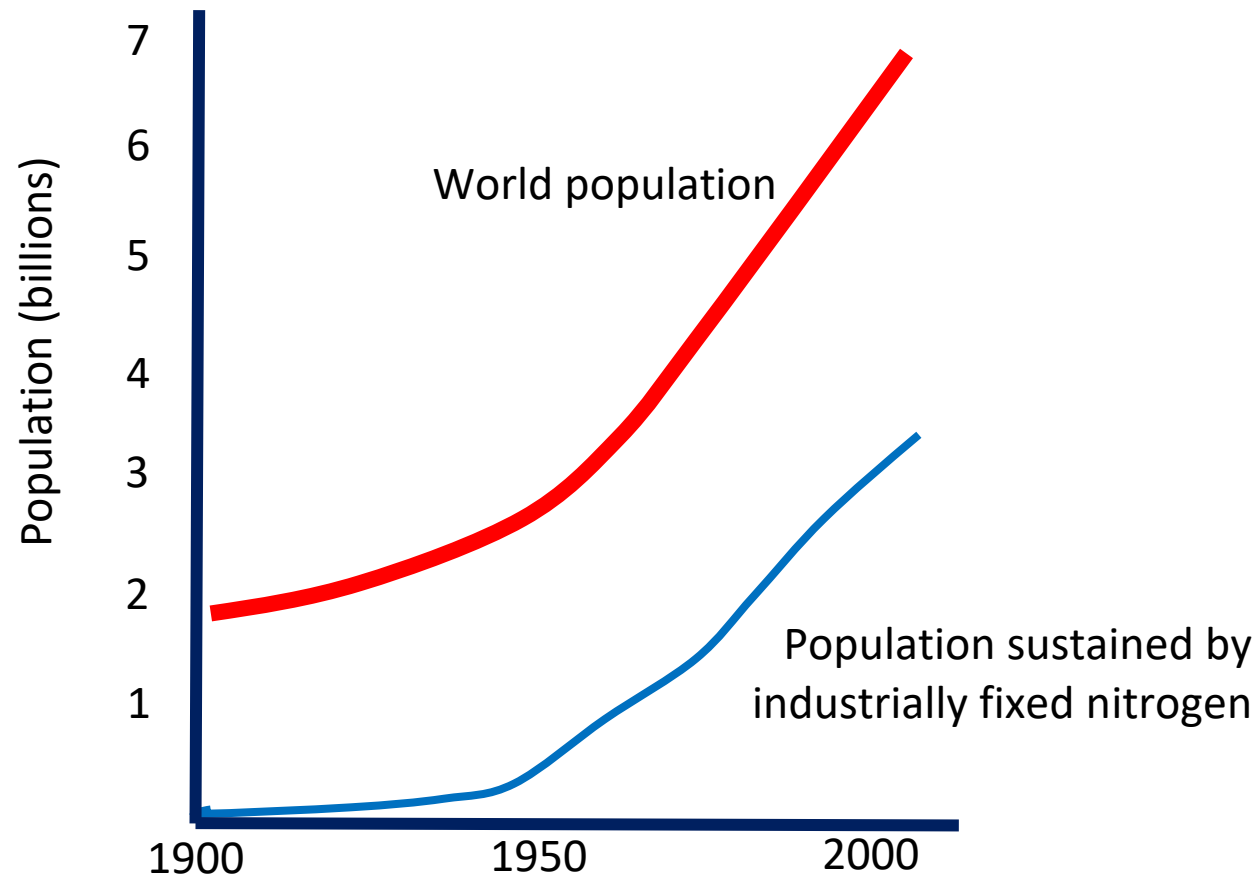
# Nitrogen fixation occurs through biological and non-biological processes

Source of nitrogen fixation	Amount of N fixed (Mt = Million tons)
Atmospheric	<10 Mt/year
Biological (on terrestrial systems)	90-140 Mt/year
Industrial (Haber-Bosch process)	170 Mt/year

# Nitrogen fixation occurs through biological and non-biological processes

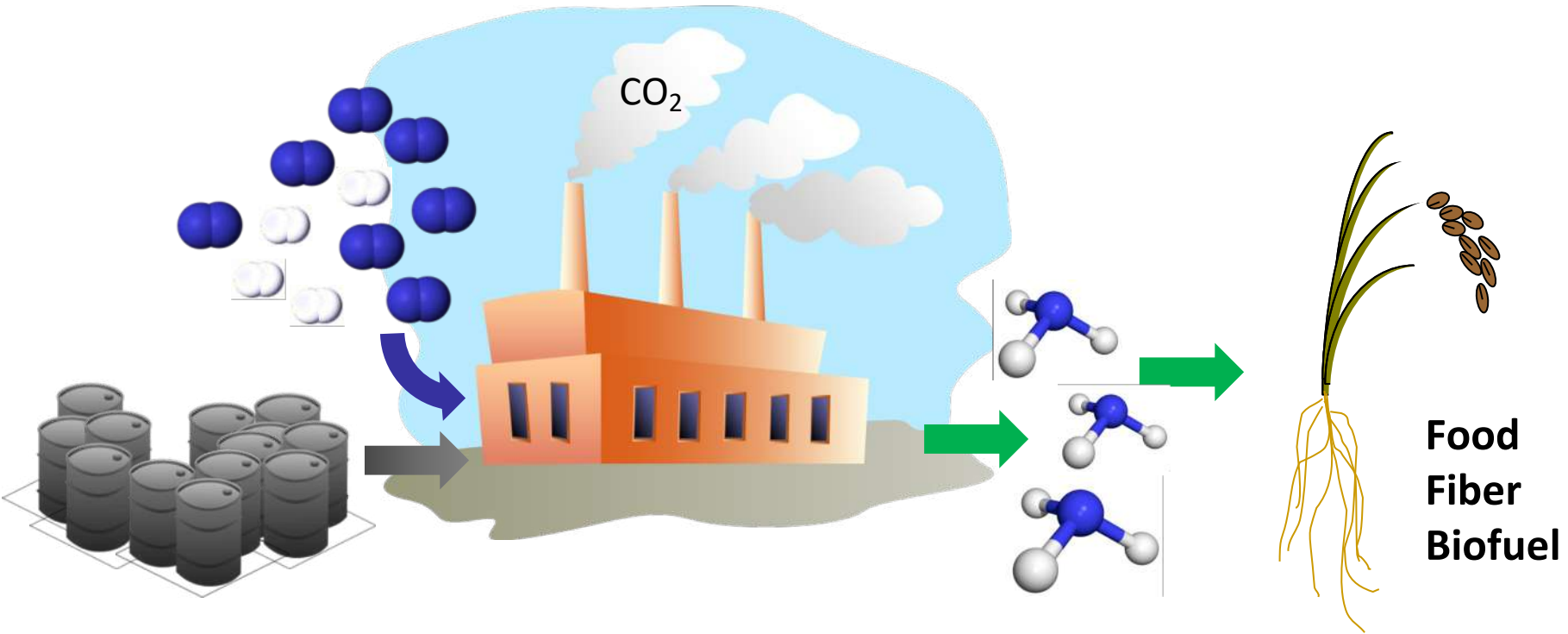
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# Nitrogen fixed by the Haber-Bosch process sustains about half the world population



Source: Erismann, J.W., Sutton, M.A., Galloway, J., Klimont, Z., and Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geosci* 1: 636-639.

# The Haber-Bosch process relies on fossil fuel and requires a lot of it



Natural gas  
(non-renewable resource)

Nitrogen fertilizers

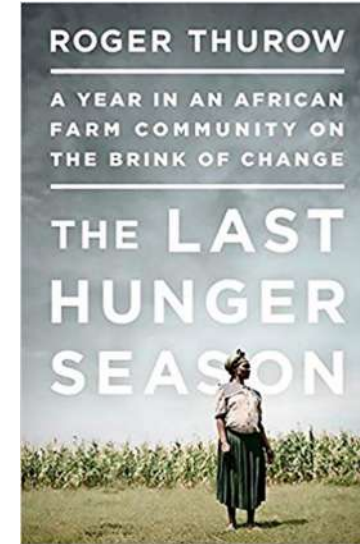
Food  
Fiber  
Biofuel

Credit: Mary Williams

# The nitrogen-related problems are different for developing and developed countries

## Developing countries: food

- Cost of fertilizers
- Logistical access to fertilizers

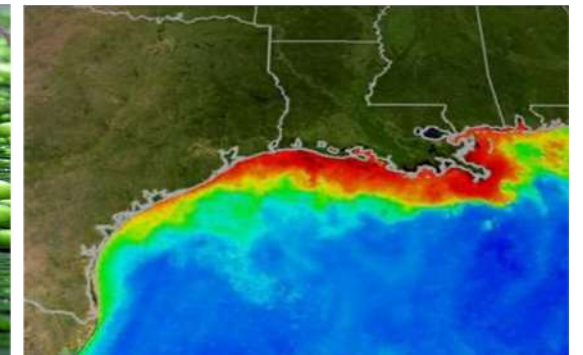


## Developed countries: environment

- Eutrophication of streams, degradation of coastal zones...
- Global warming



Algal blooms and eutrophication



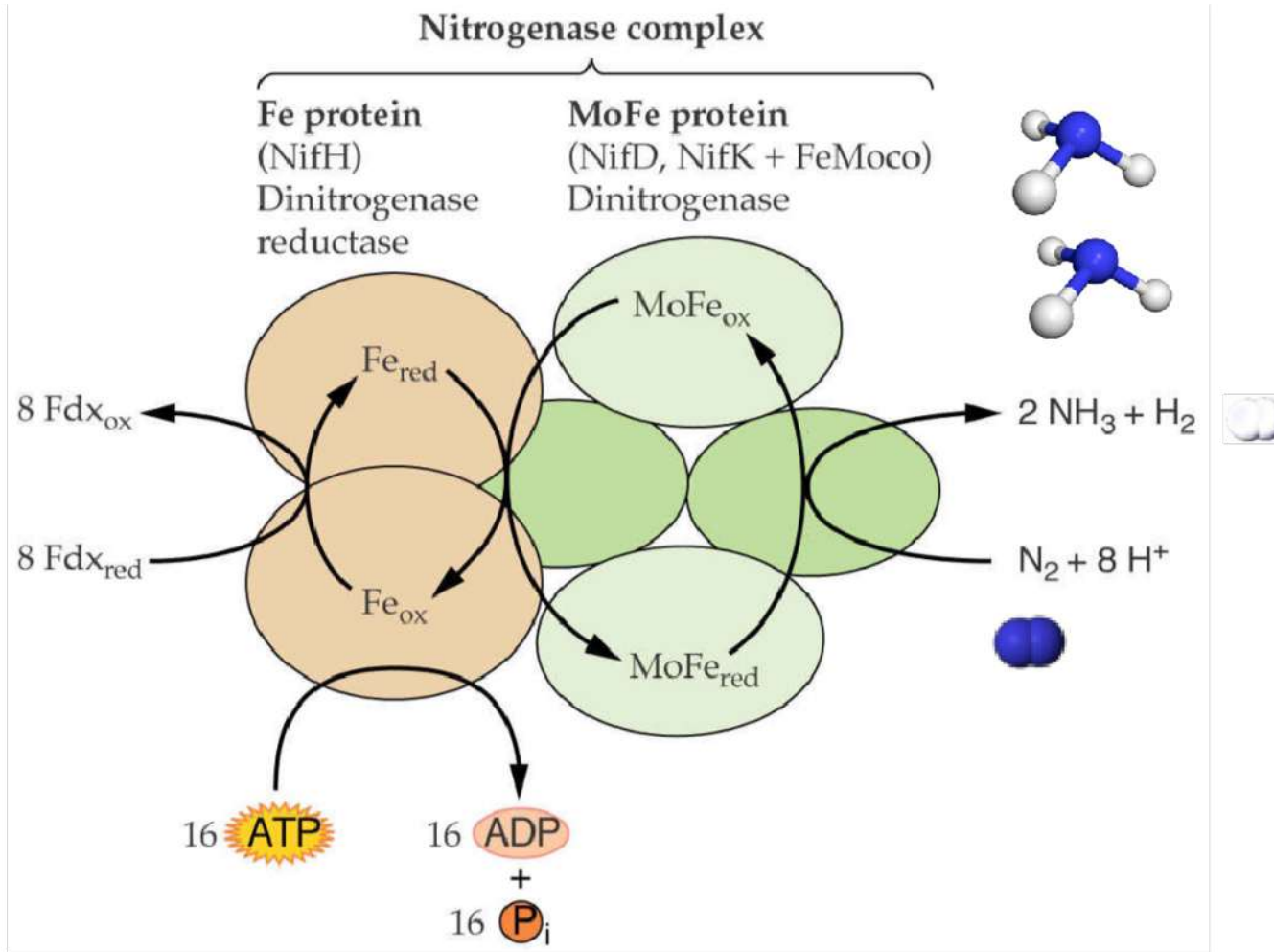
Dead zone in the Gulf of Mexico

# Nitrogen fixation occurs through biological and non-biological processes

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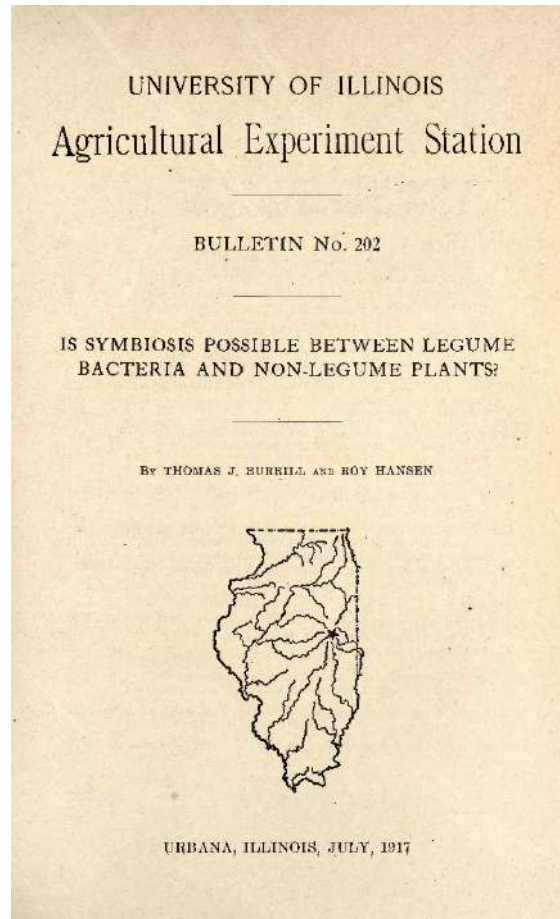
# Biological Nitrogen Fixation (BNF) and the Nitrogenase Complex



3 main problems to solve:

- High energy (ATP) demand
- Protection of the nitrogenase against oxygen
- Transfer of fixed nitrogen to the plant

# Current approaches to improve biological nitrogen-fixation in cereals



Engineering  
root nodules

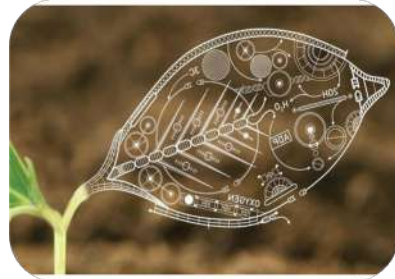
Plant



Microbe

# Current approaches to improve biological nitrogen-fixation in cereals

Engineering nitrogen-fixing plants



Engineering root nodules

Exploring plant natural diversity



Engineering diazotrophs

Plant



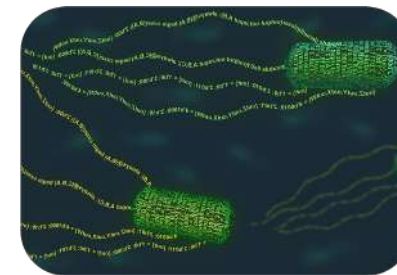
Microbe

# Current approaches to improve biological nitrogen-fixation in cereals



ACS Publications  
Most Trusted. Most Cited. Most Read.

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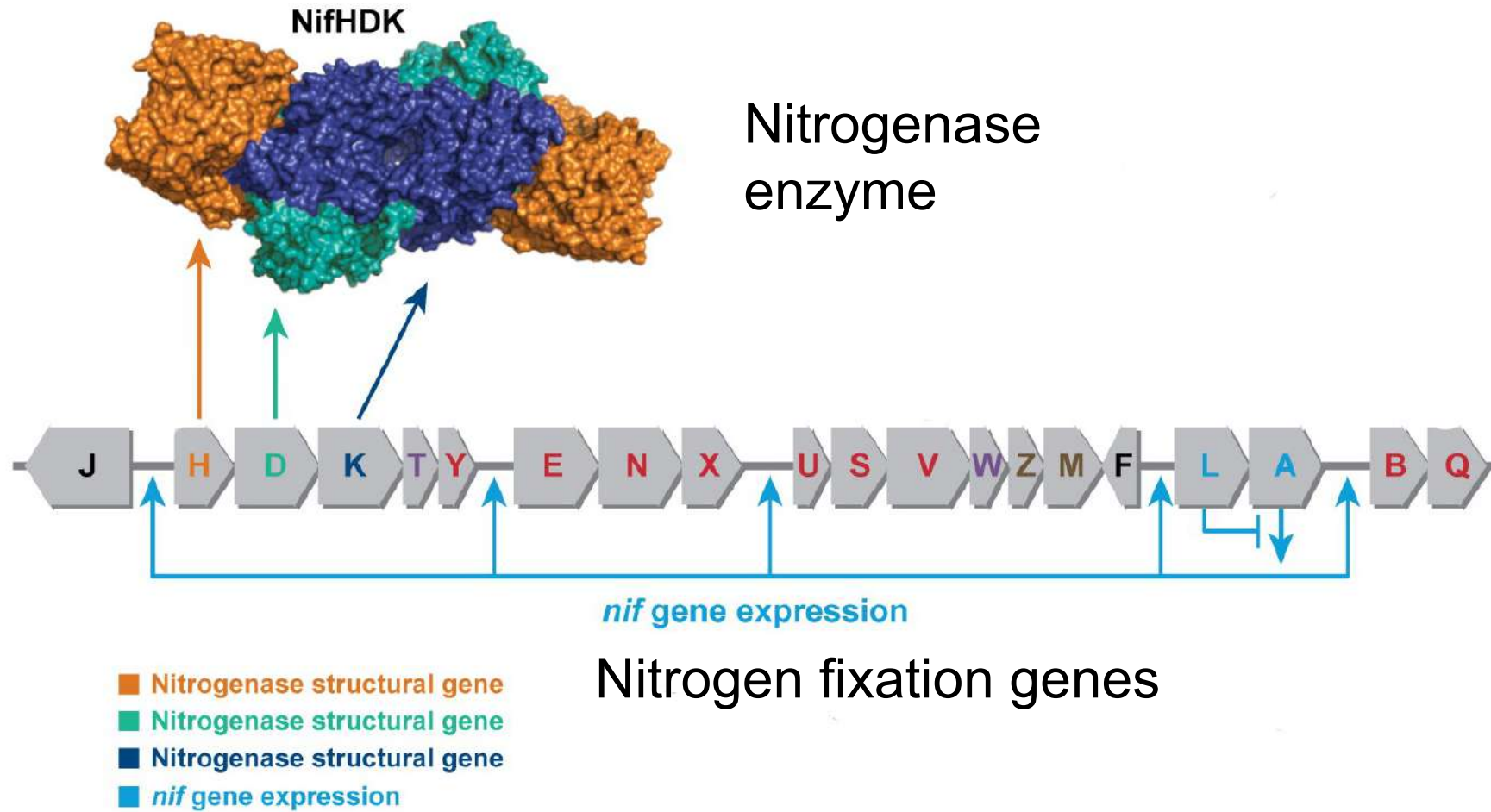
Engineering  
diazotrophs

Plant



Microbe

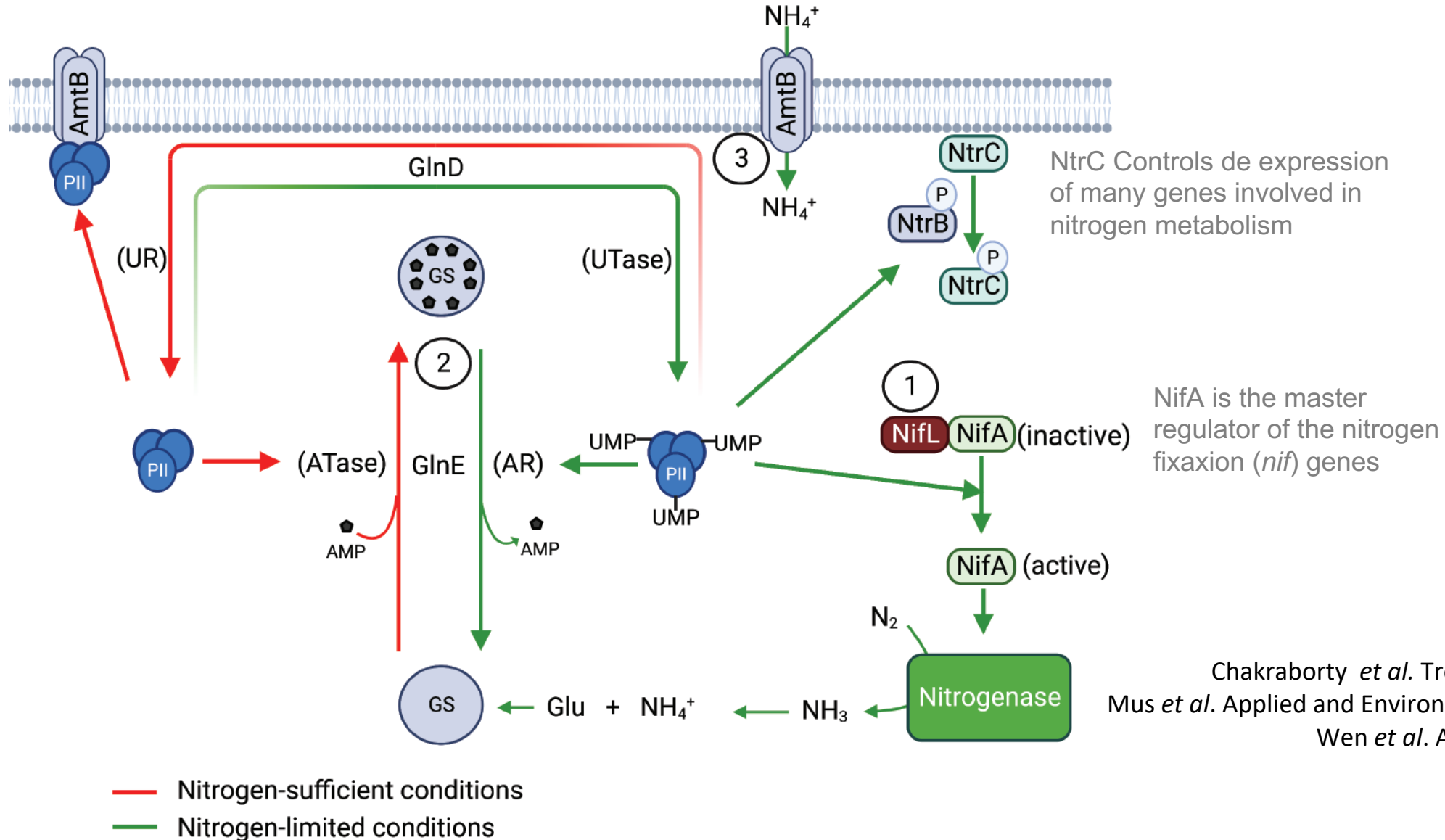
# Engineering diazotrophs



Rubio and Ludden, Annual Review of Microbiology 2008

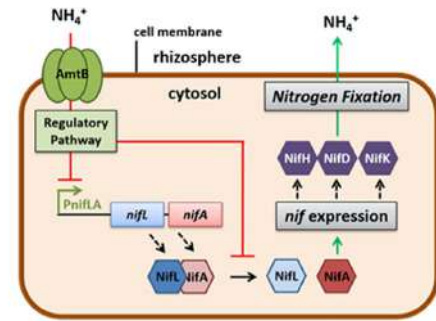


# Engineering diazotrophs

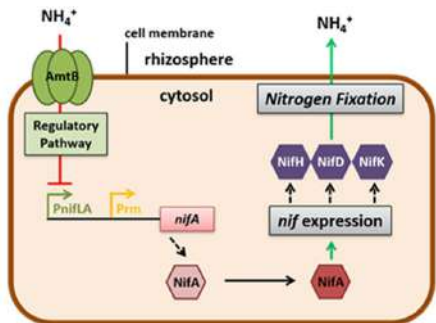


Chakraborty *et al.* Trends in Microbiology 2023  
 Mus *et al.* Applied and Environmental Microbiology 2022  
 Wen *et al.* ACS Synthetic Biology 2021

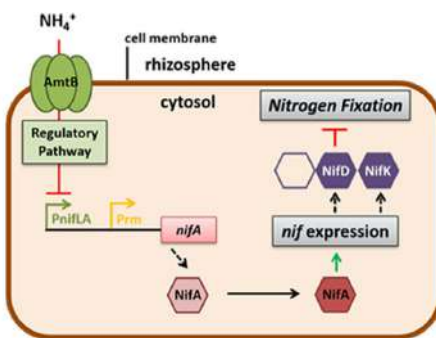
# Engineering diazotrophs



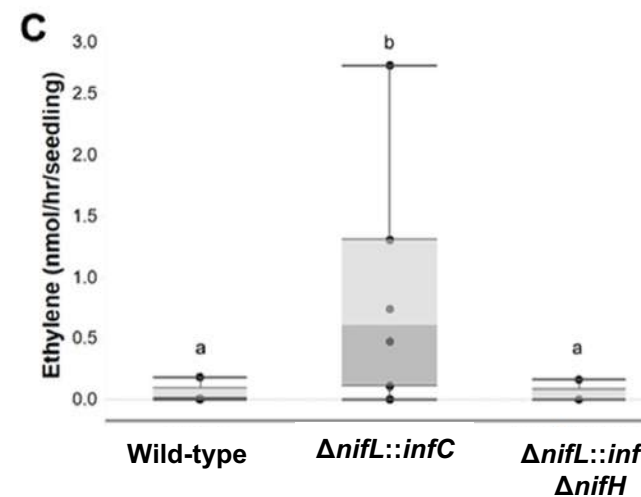
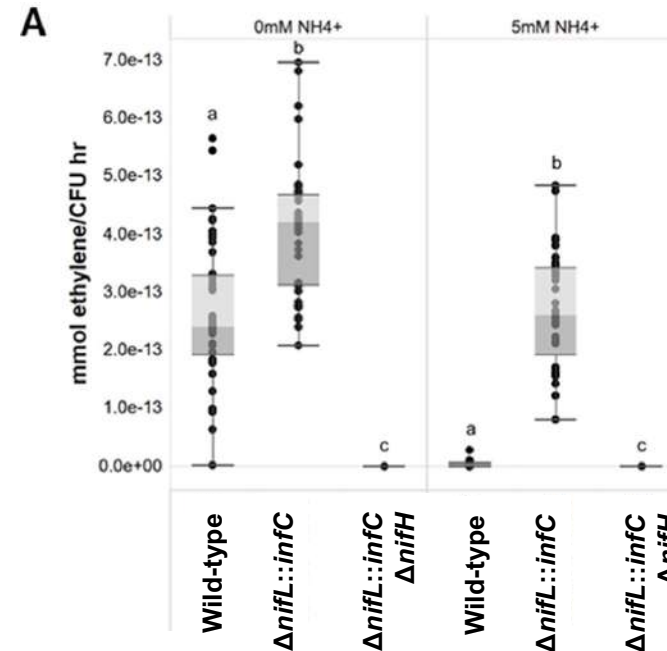
Wild-type



$\Delta nifL::infC$

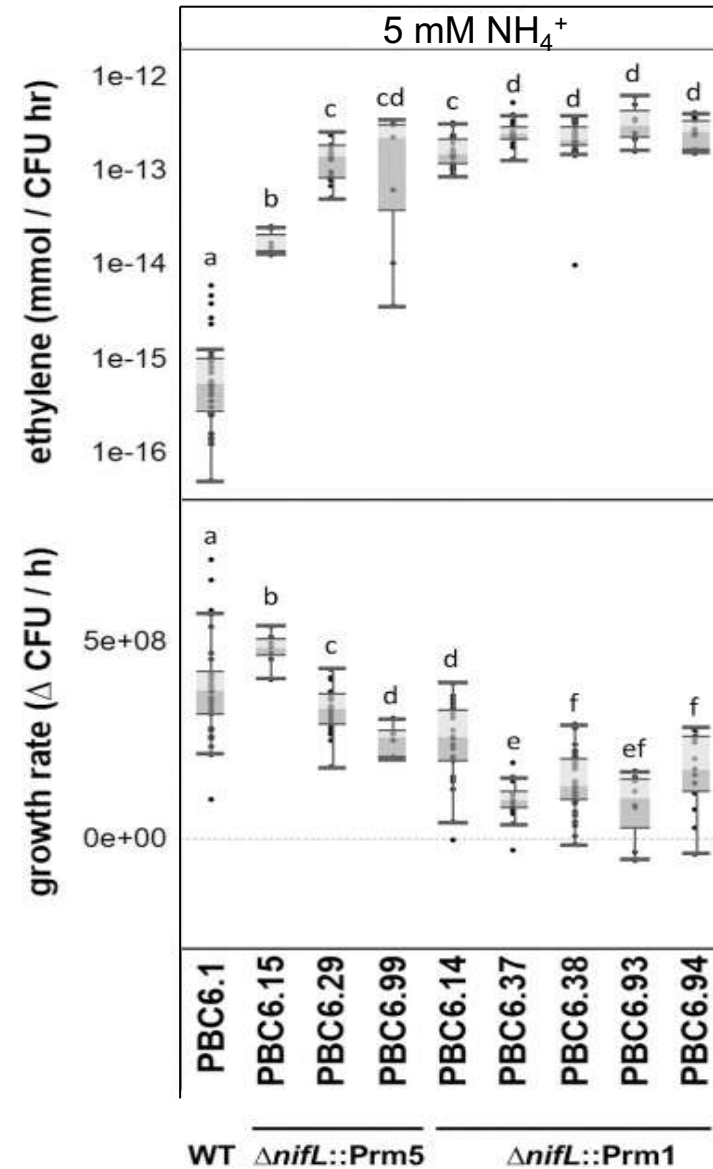


$\Delta nifL::infC$   
 $\Delta nifH$



# Fitness decrease in engineered diazotrophs

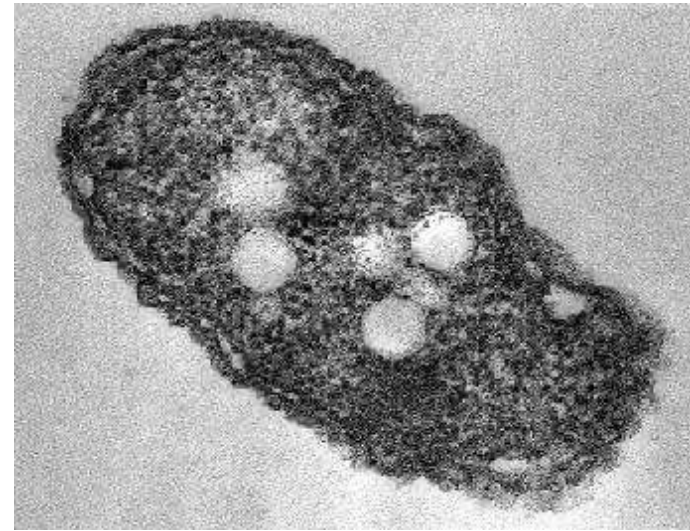
(Bloch *et al.*, 2020)



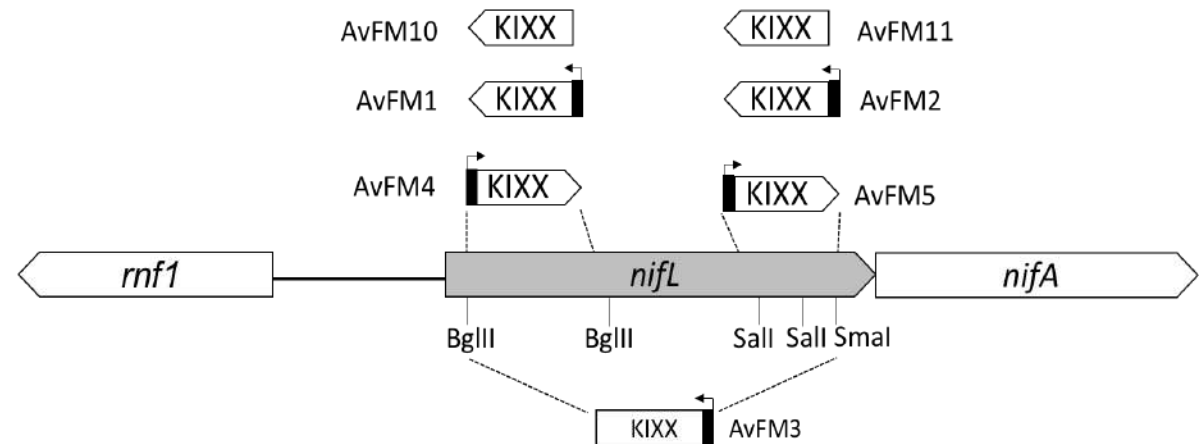


Map of the *nifLA* region of *Azotobacter vinelandii* showing restriction sites used for manipulations and the positions of KIXX and promoter inserts

(Mus *et al.*, Applied and Environmental Microbiology 2022)

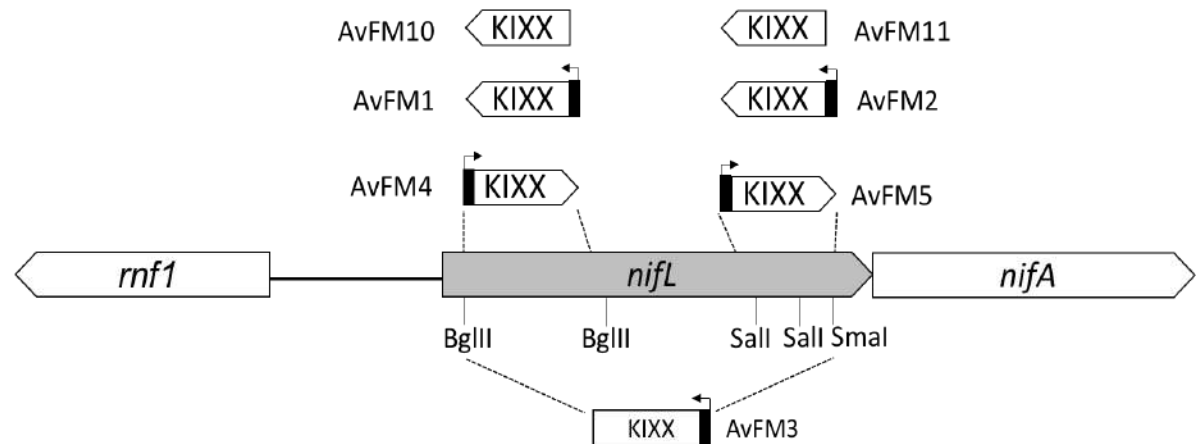
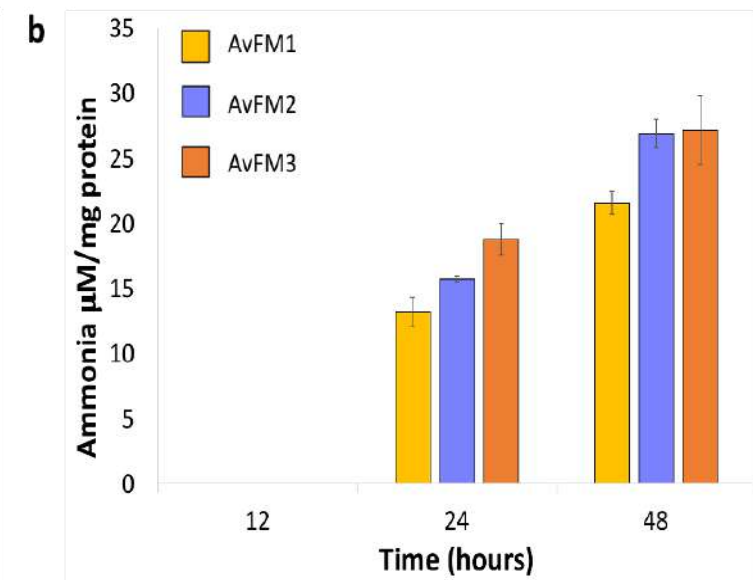
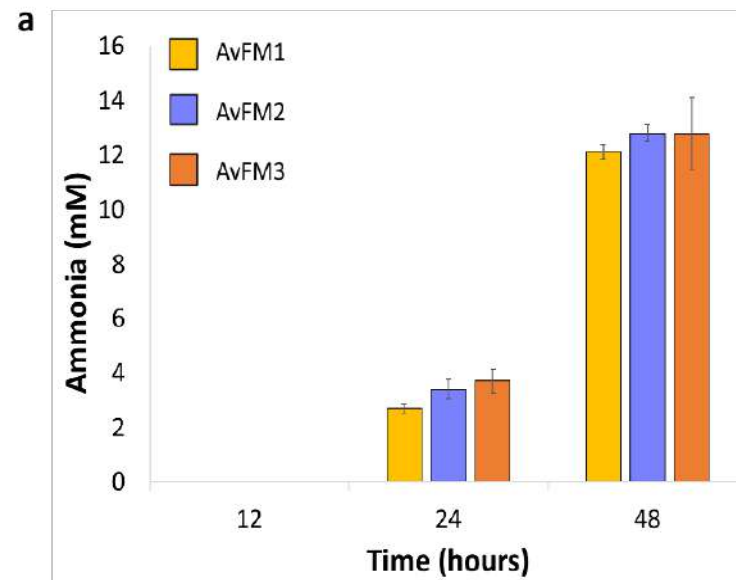


*Azotobacter vinelandii*



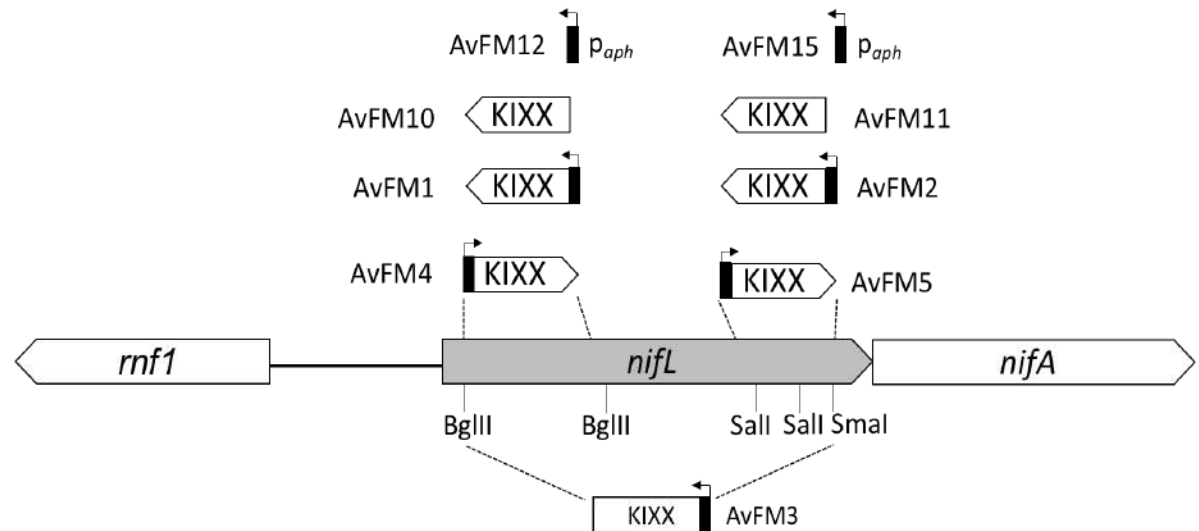
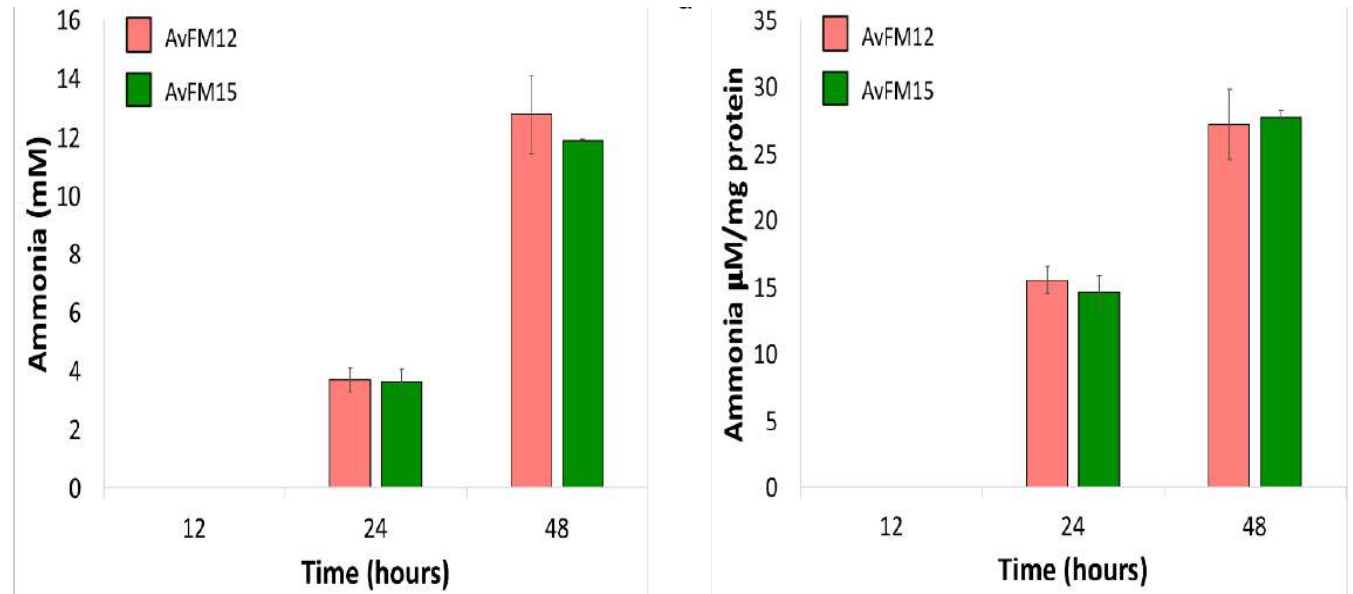
Extracellular ammonia concentrations in cultures the *nifL* mutant strains generated with the KIXX cassette containing the *aph* promoter (AvFM1, AvFM2, AvFM3), and the *nifL* mutant strains generated with the *aph* promoter (AvFM12, AvFM15) under diazotrophic conditions

(Mus *et al.*, Applied and Environmental Microbiology 2022)



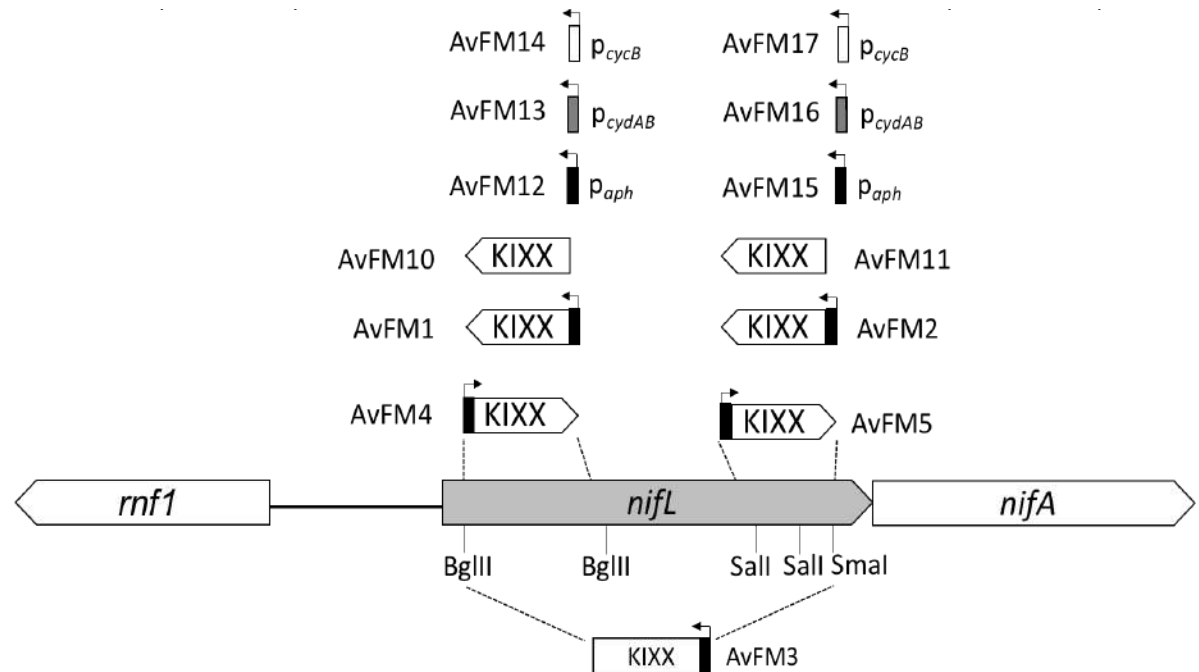
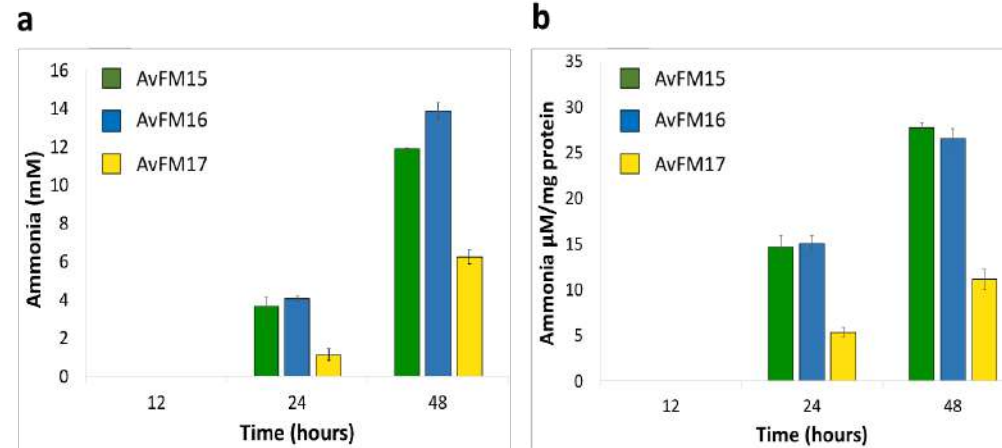
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(Mus *et al.*, Applied and Environmental Microbiology 2022)

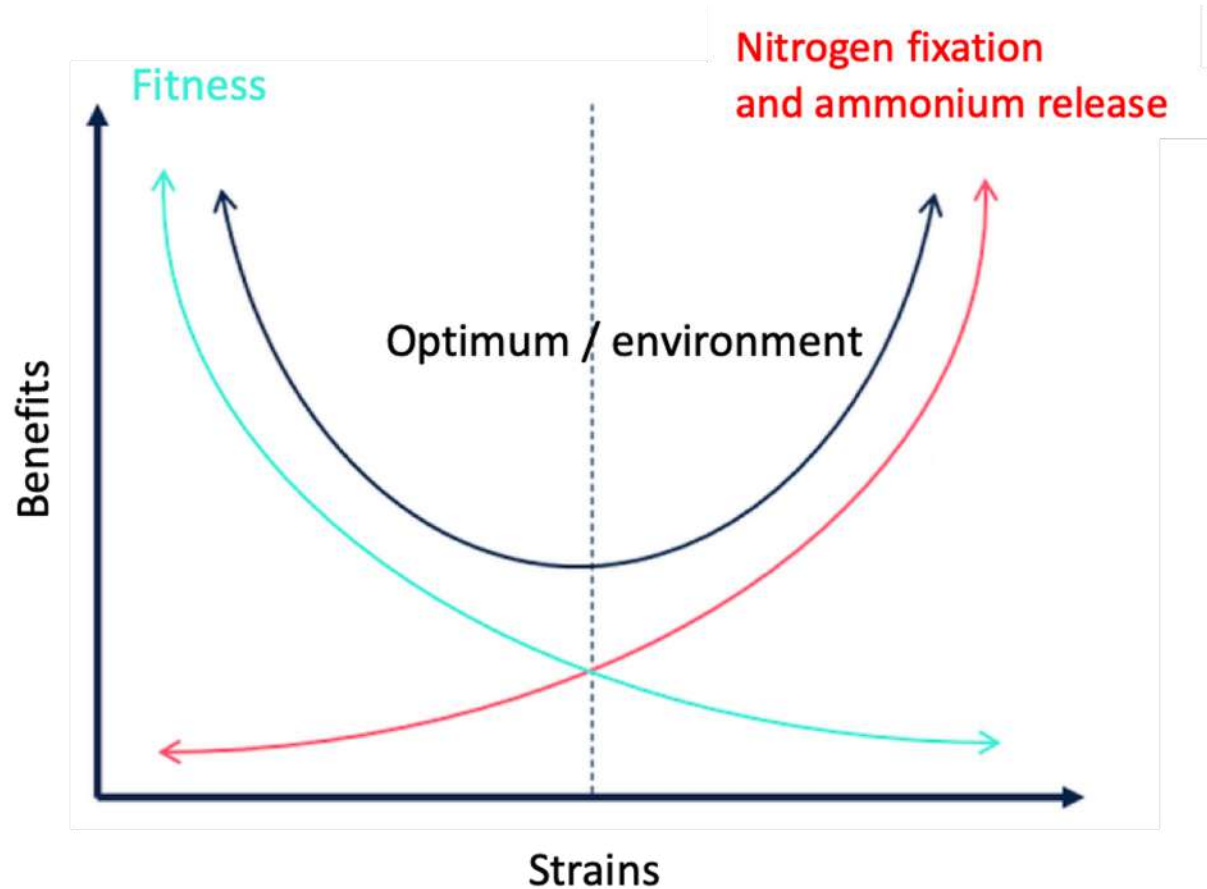


Extracellular ammonia concentrations in cultures of the  $\Delta nifL$  mutant strains generated with *aph*, *cydAB*, *cycB* promoters (AvFM15, AvFM16, AvFM17)

(Mus *et al.*, Applied and Environmental Microbiology 2022)



# Fitness decrease in engineered diazotrophs



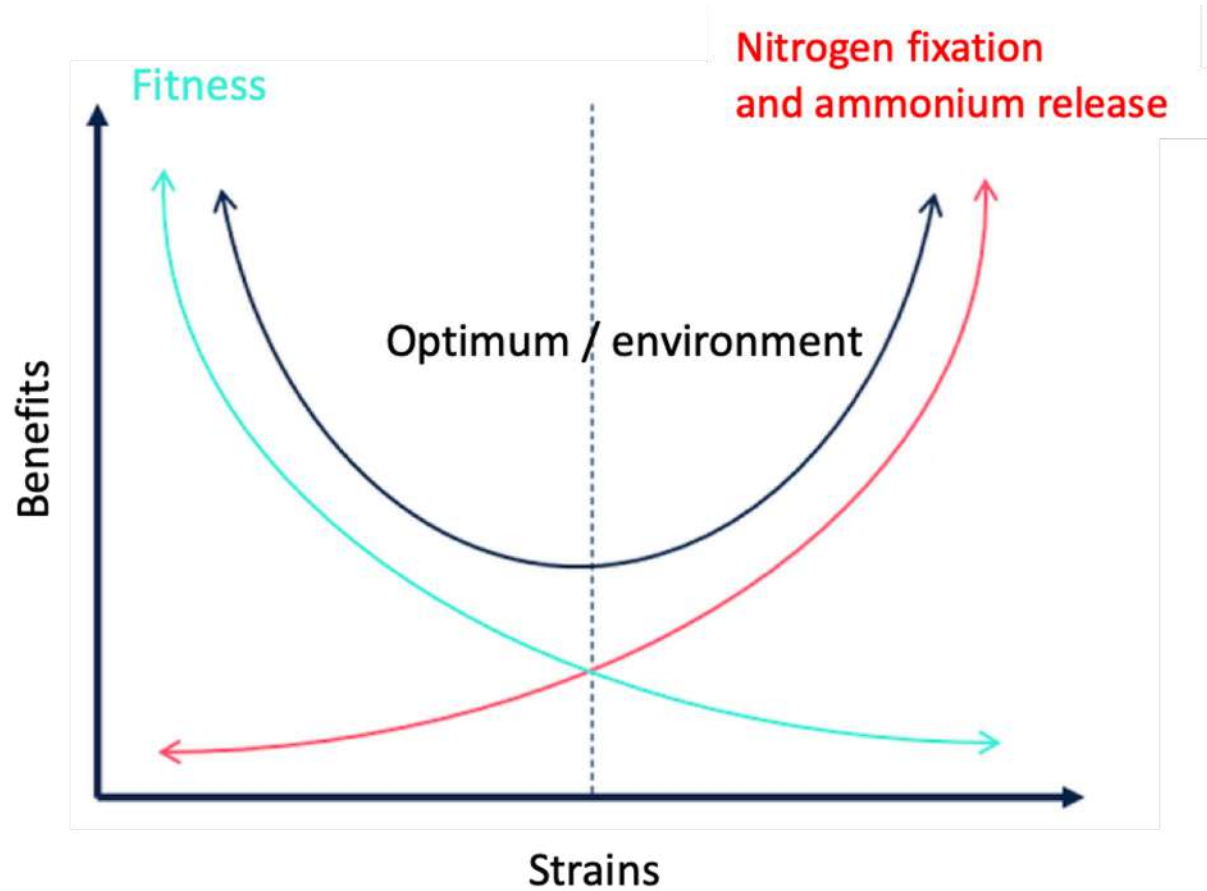
Synthetic biology

Bacterial synthetic communities (SynCom)

Bacterial co-isolation

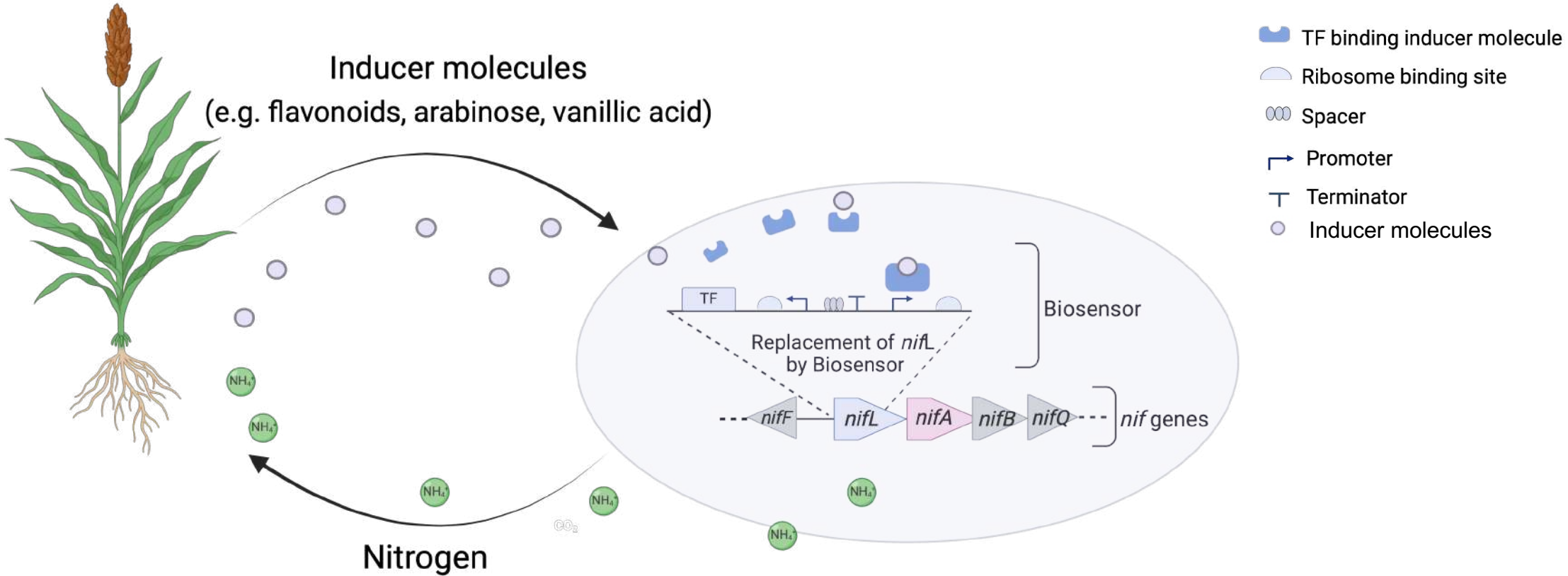
Arbuscular mycorrhizal fungi

# Fitness decrease in engineered diazotrophs



Synthetic biology

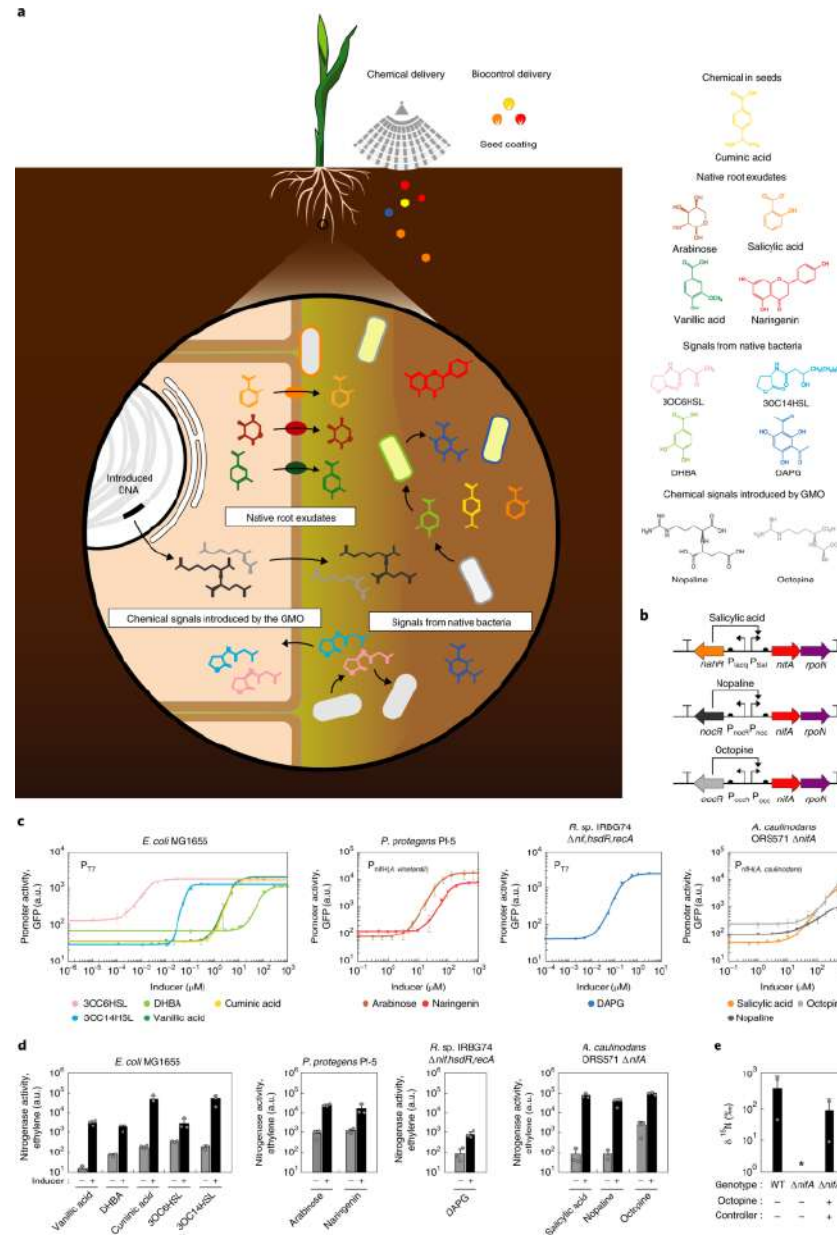
# Engineering inducible nitrogen fixation in response to plant signals



# Engineering inducible nitrogen fixation in response to plant signals



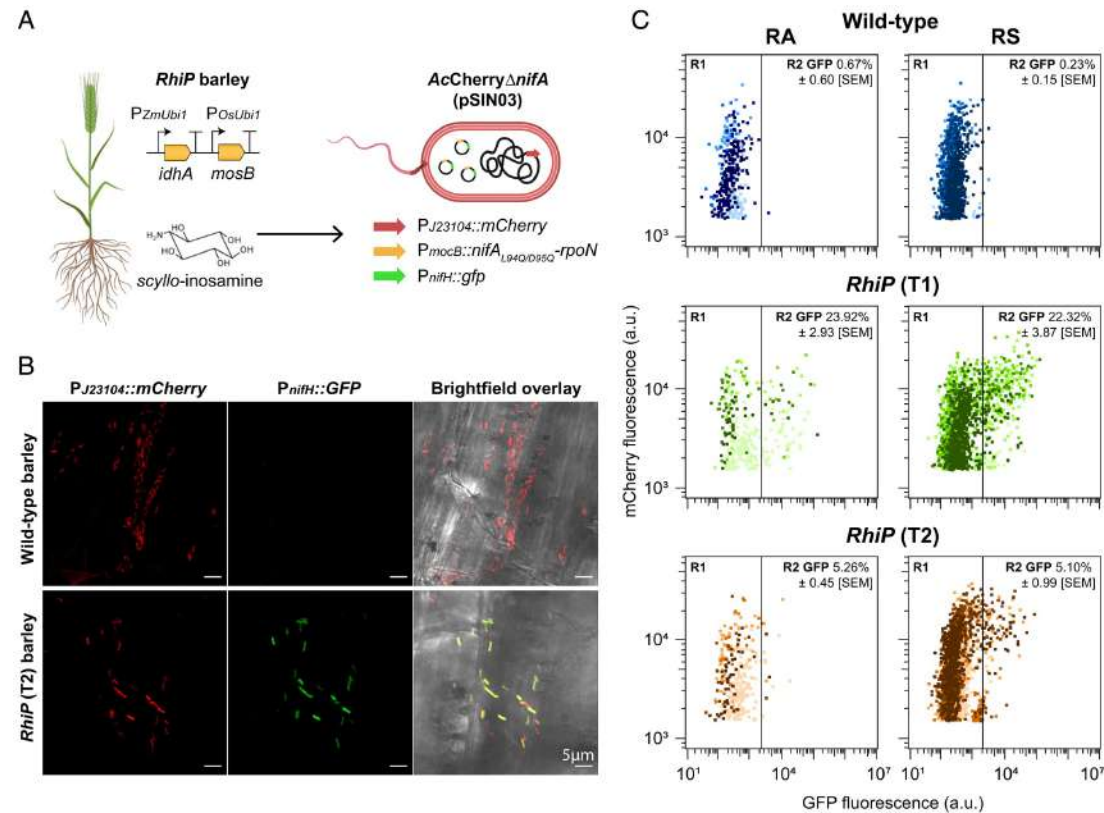
NSF-IOS #1753917



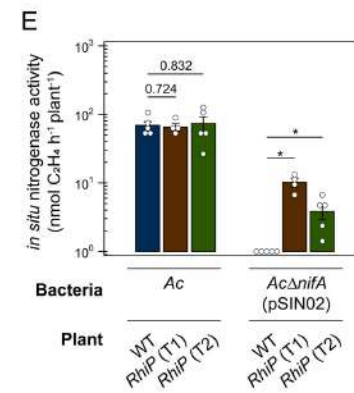
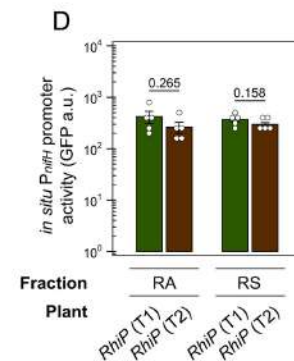
Ryu *et al.*, Nature Microbiology 2020



# Engineering inducible nitrogen fixation in response to plant signals



NSF-IOS #1753917



Haskett *et al.* PNAS 2022

# Looking for the best diazotroph chassis for synthetic biology

*Klebsiella variicola* strain A3

Gamma-proteobacterium

Isolated from sorghum

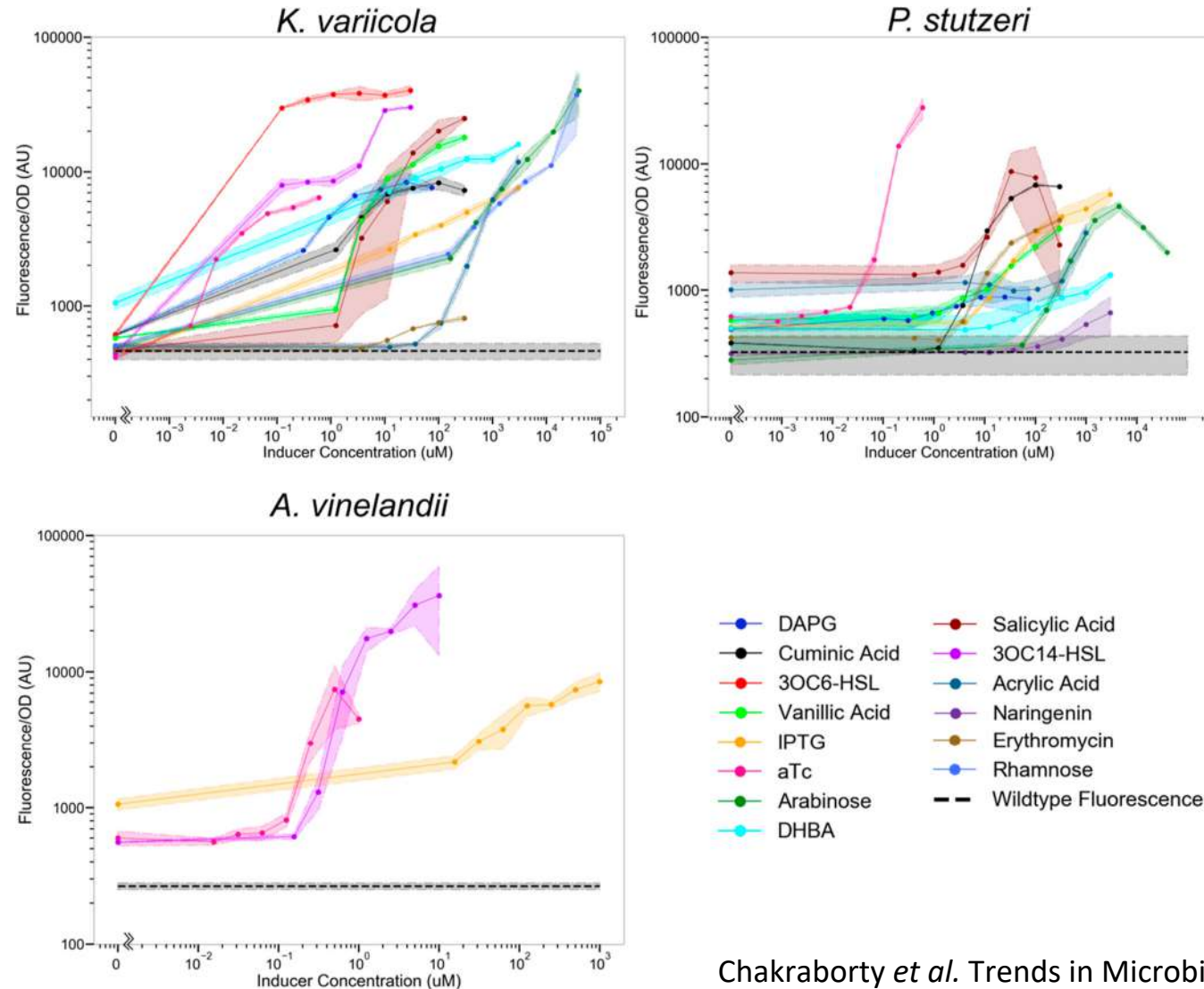
Genetically tractable

Non-pathogenic

Excellent nitrogen fixer



Dr. Maya Venkataraman  
Pfleger lab

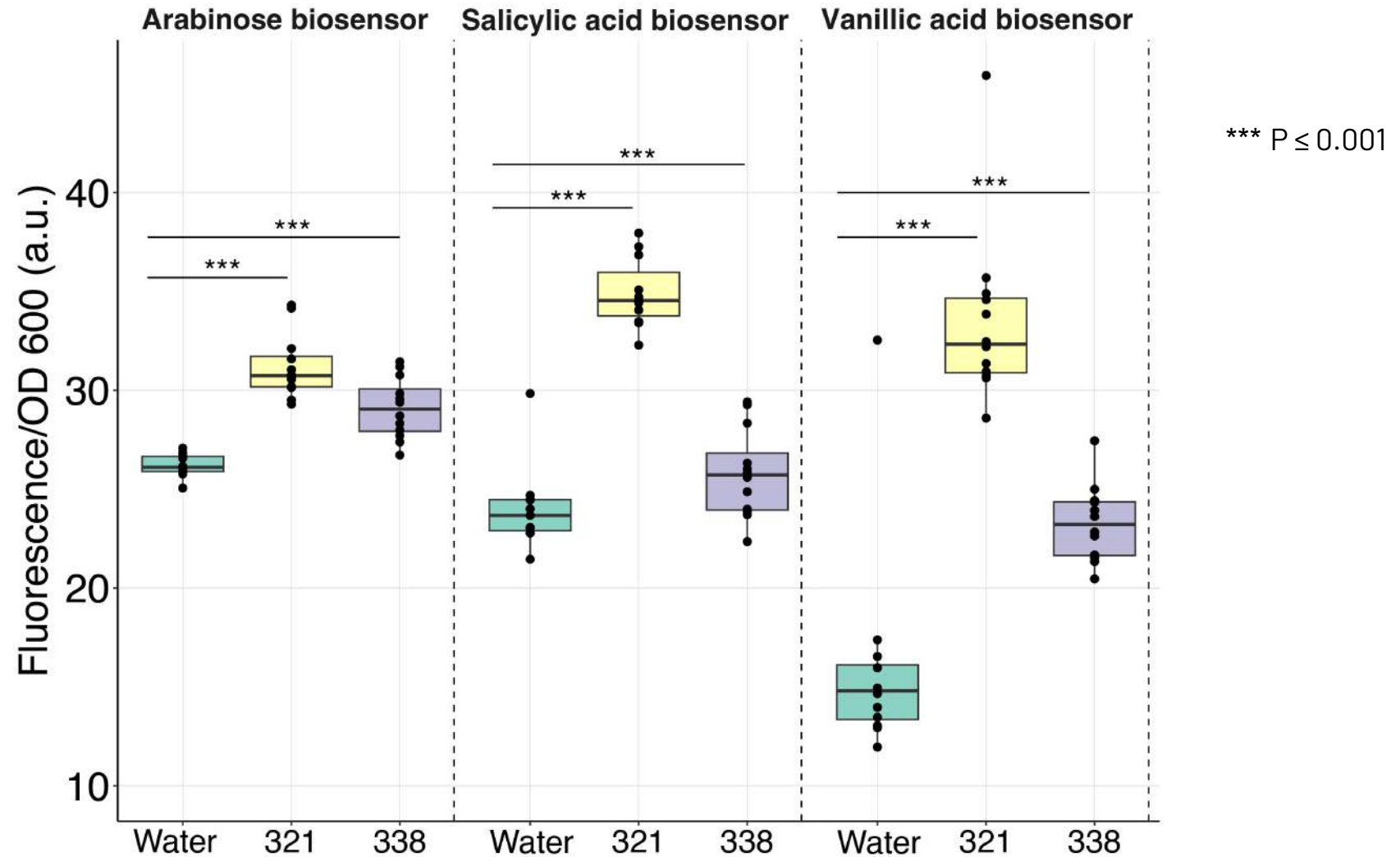


Chakraborty *et al.* Trends in Microbiology 2023  
Venkataraman *et al.* ACS Synthetic Biology 2023

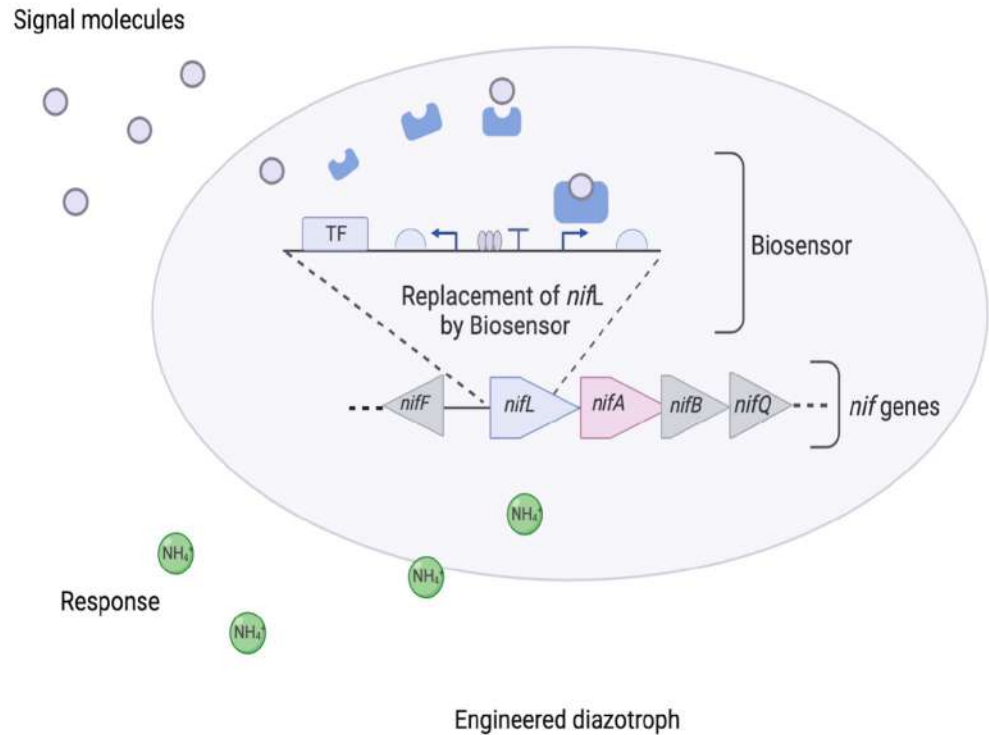
# Looking for biosensors induced by root exudates



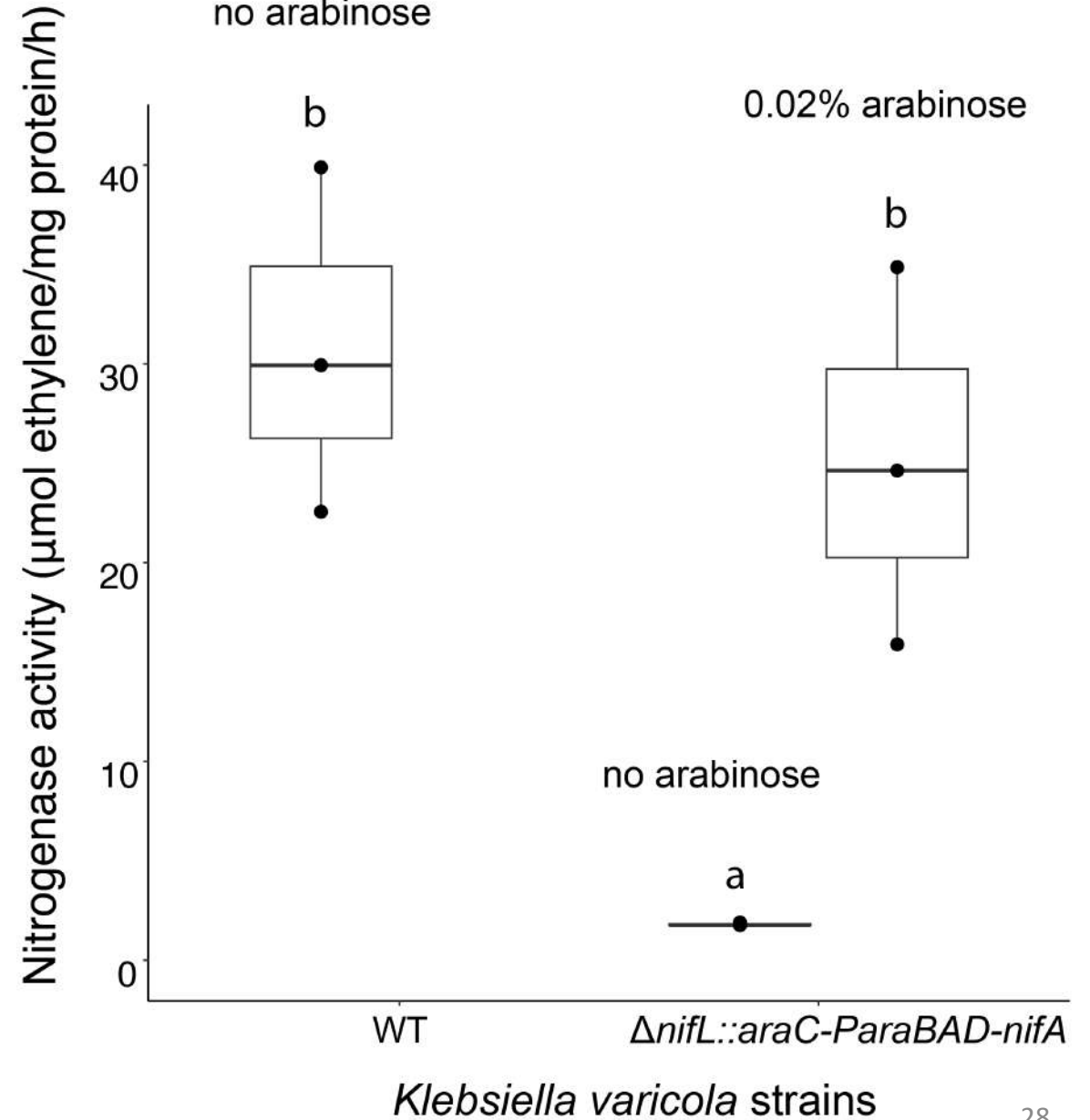
Valentina Infante



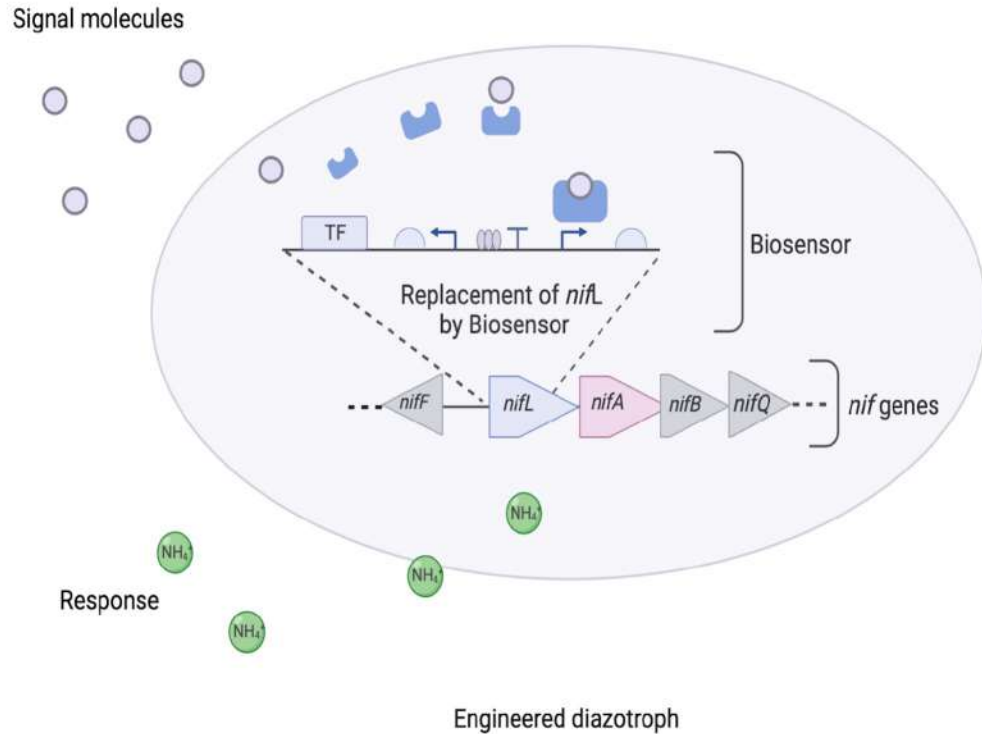
# Testing inducible fixation with an arabinose biosensor



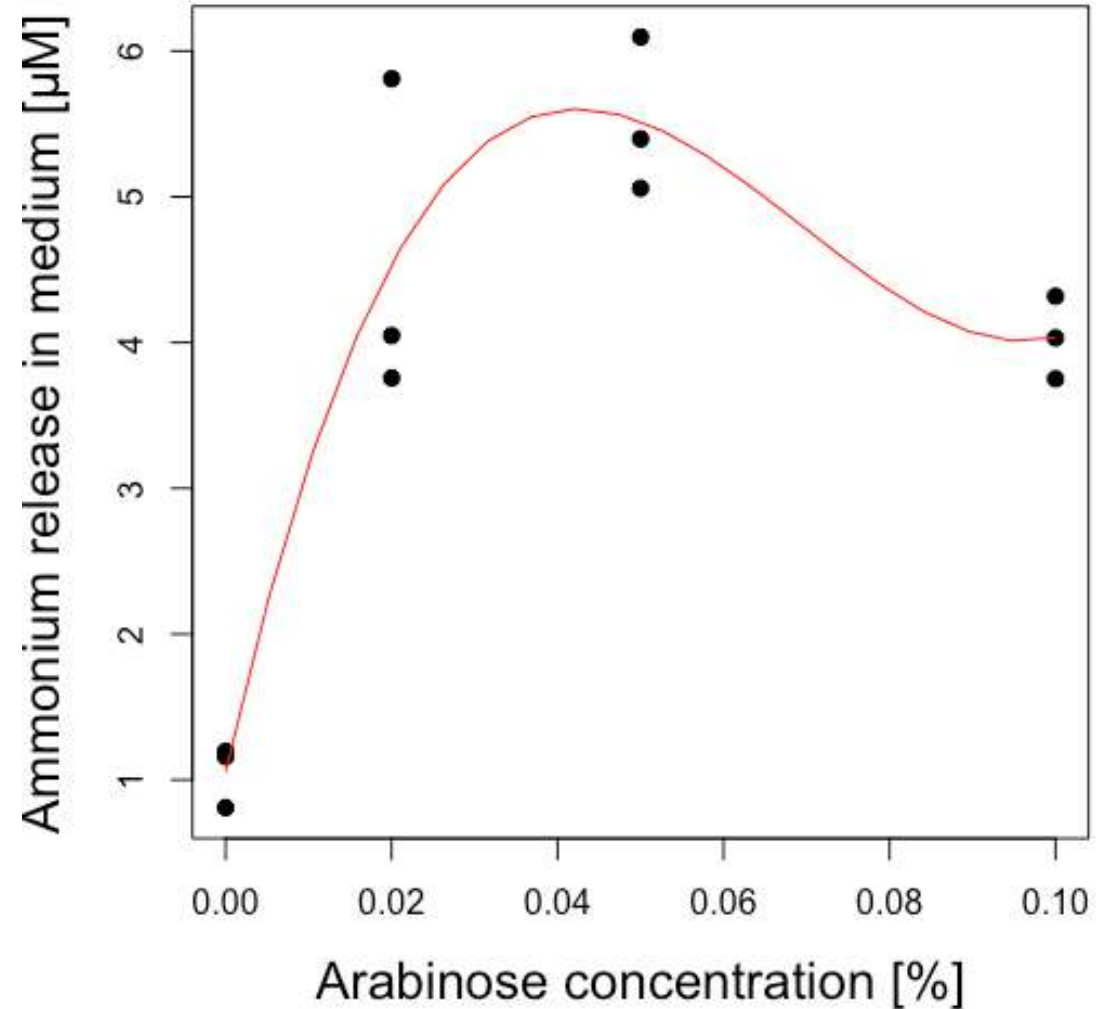
- Transcription factor
- Promoter
- Ribosome binding site
- Terminator
- Spacer



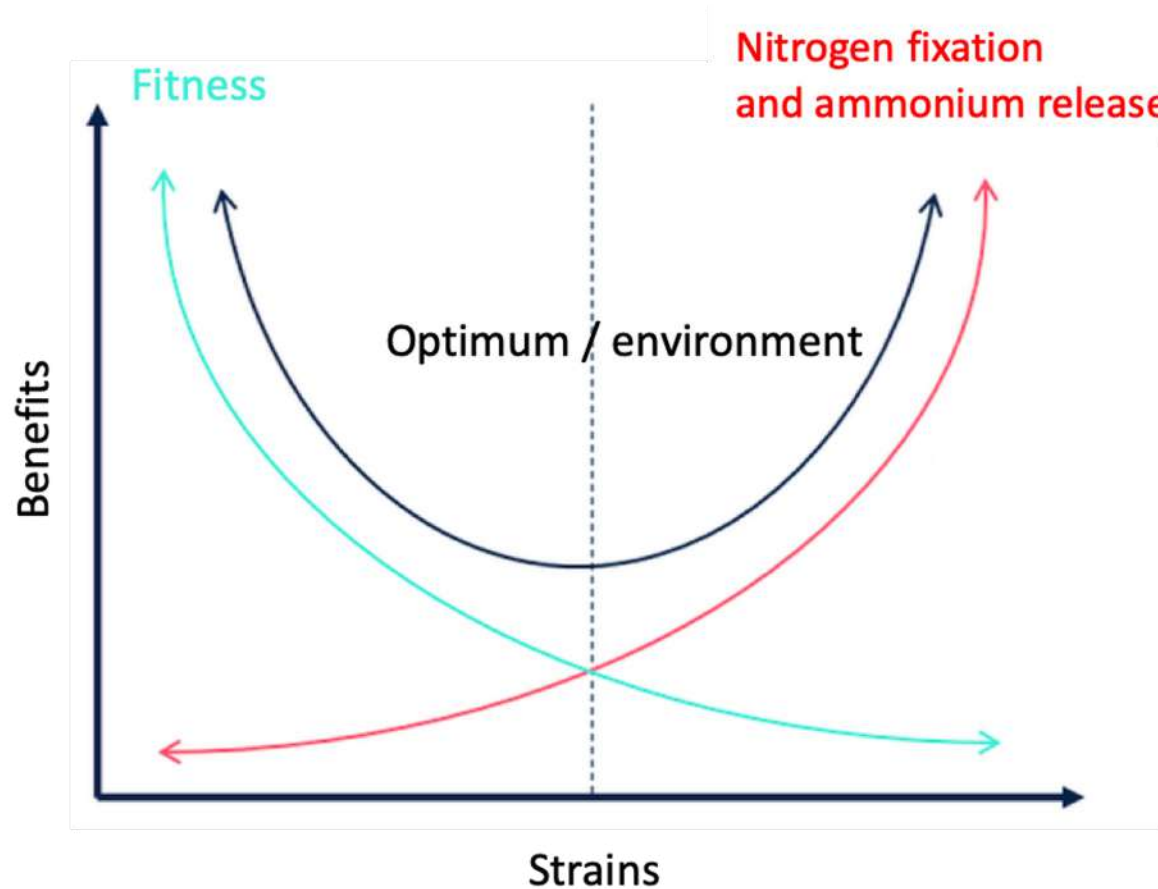
# Arabinose-inducible ammonium release



- Transcription factor
- Promoter
- Ribosome binding site
- Terminator
- Spacer



# Fitness decrease in engineered diazotrophs



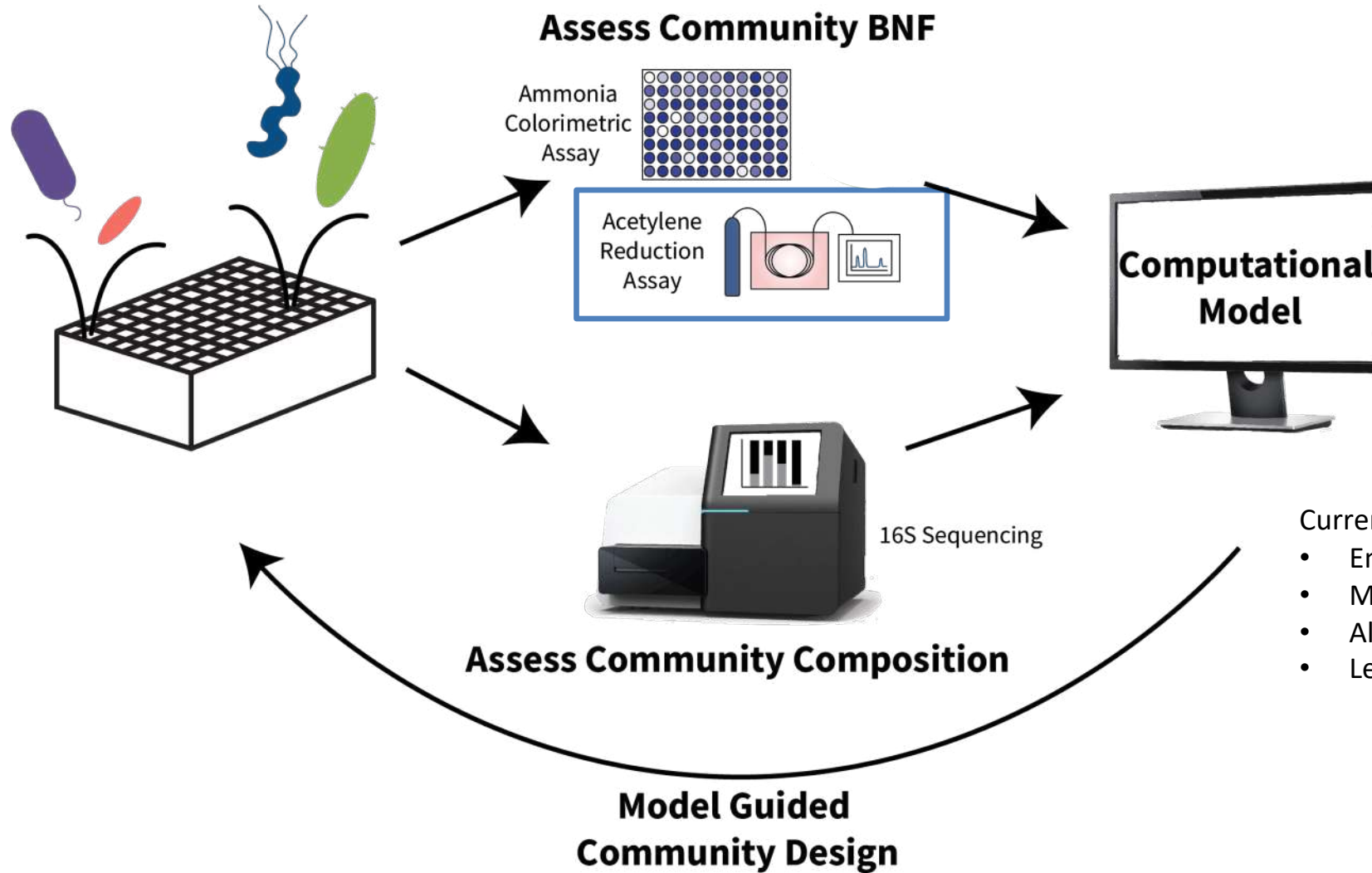
Synthetic biology

Bacterial synthetic communities  
(SynCom)



# Effect of community composition on nitrogen fixation

Growing sub-communities of a 12-member community *in vitro*



Dr. Claire M. Palmer  
Venturelli lab

## Current Dataset

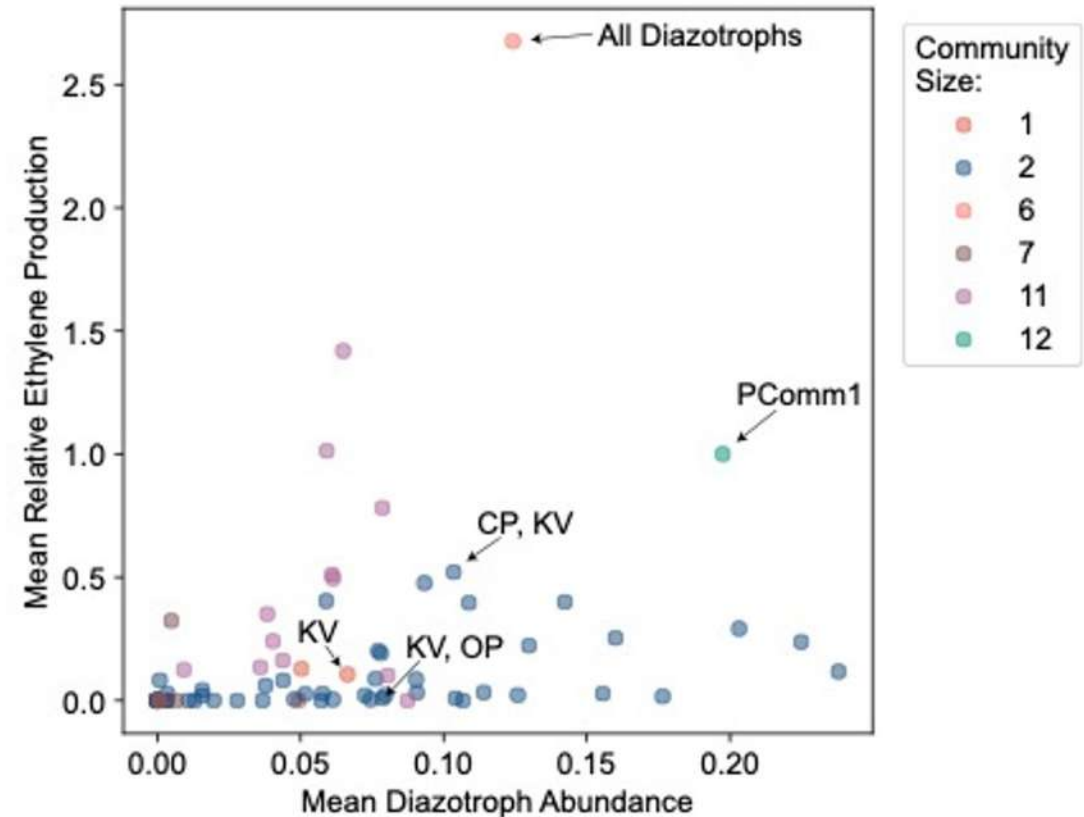
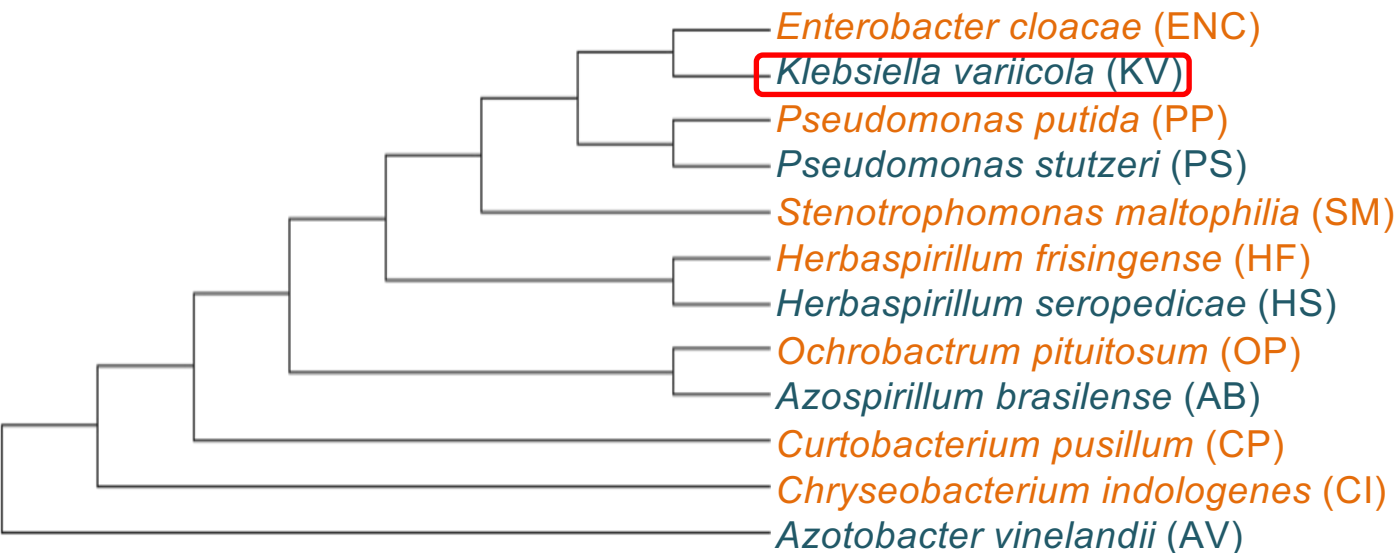
- Entire PComm1 community
- Monocultures
- All Possible Pairs
- Leave-one-out Communities

# Sub-communities provide insight into how non-fixers may both improve and hinder nitrogenase activity

Full 12-member Community: PComm1

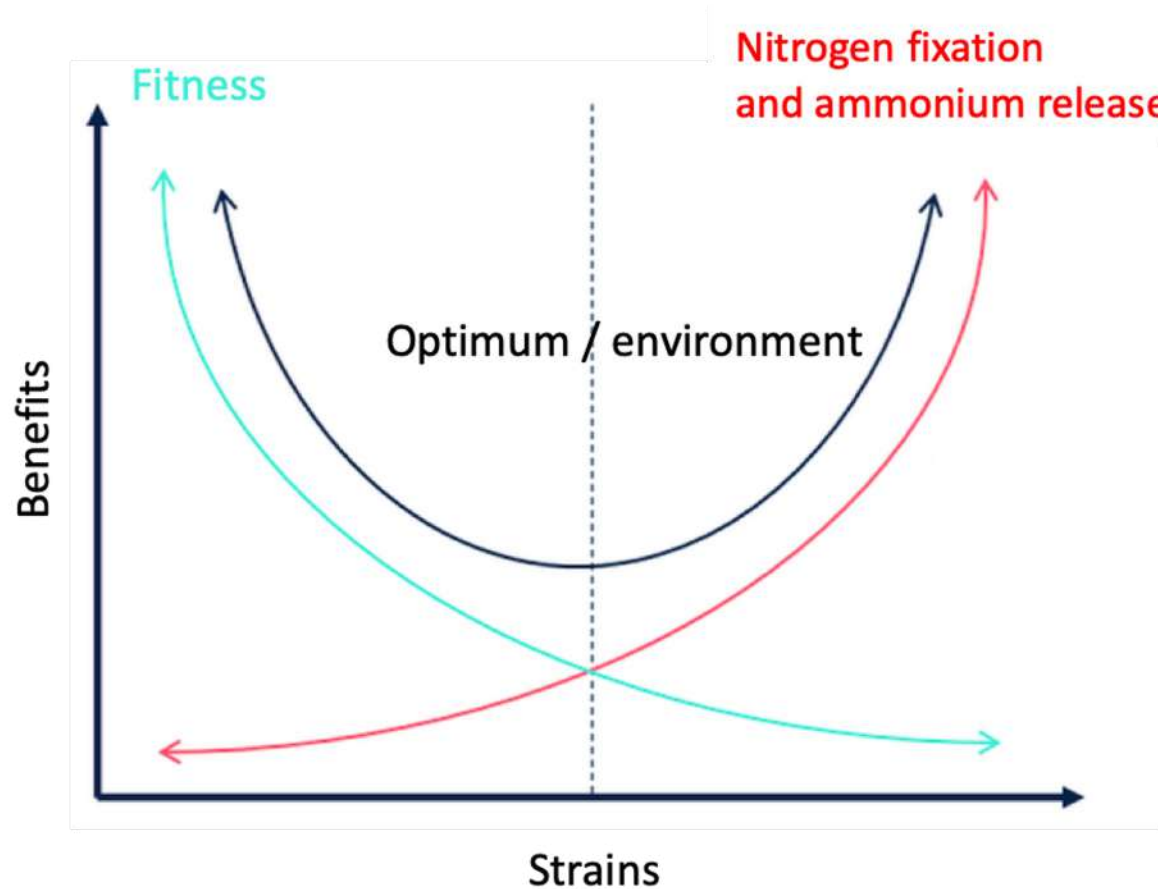
7-member community from Niu *et al.* (2017)

+ 5 tractable diazotrophs





# Fitness decrease in engineered diazotrophs

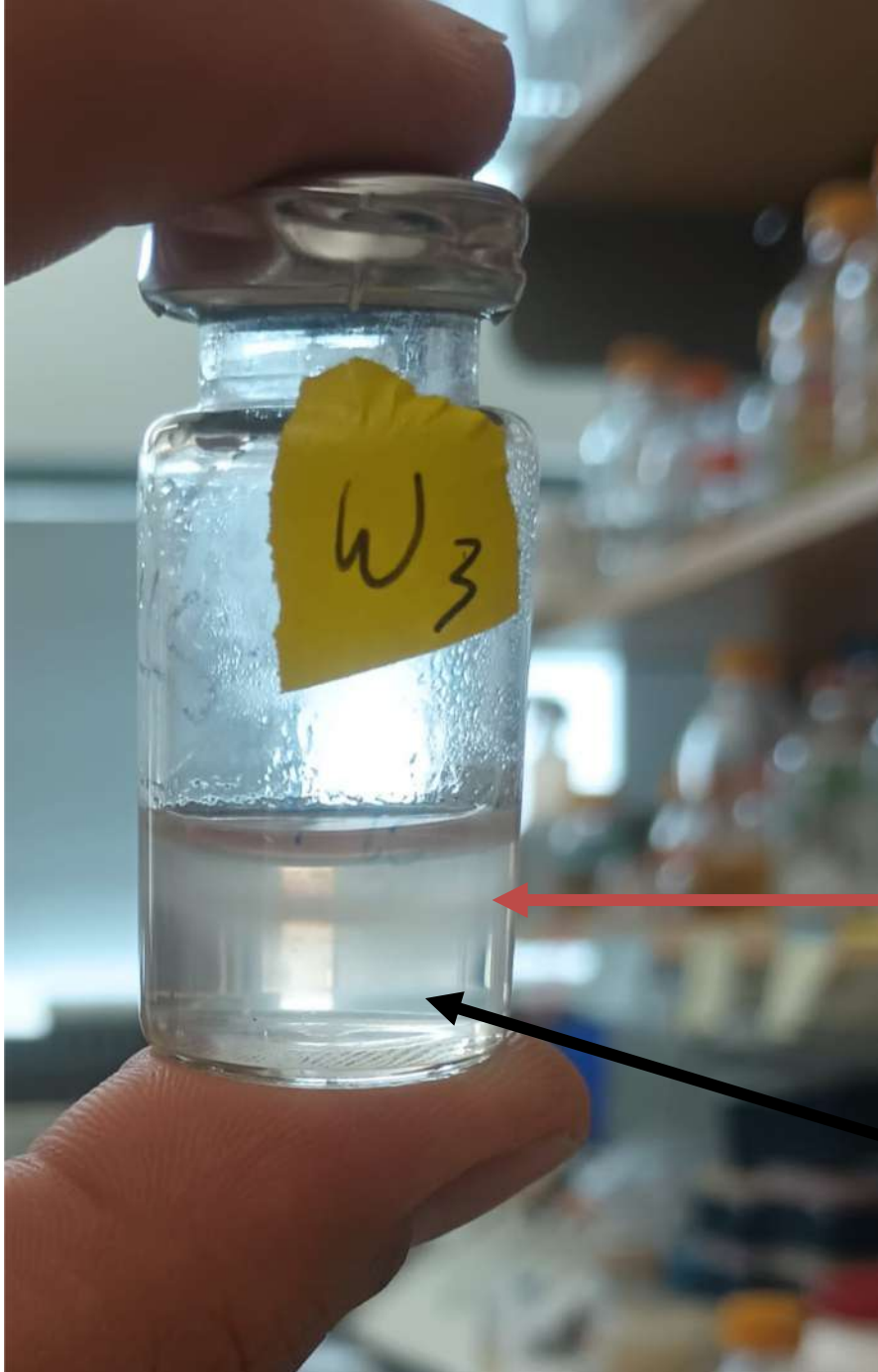


Synthetic biology

Bacterial synthetic communities (SynCom)

Bacterial co-isolation

# Can we isolate more diazotroph “helpers” ?



Diazotrophs

+

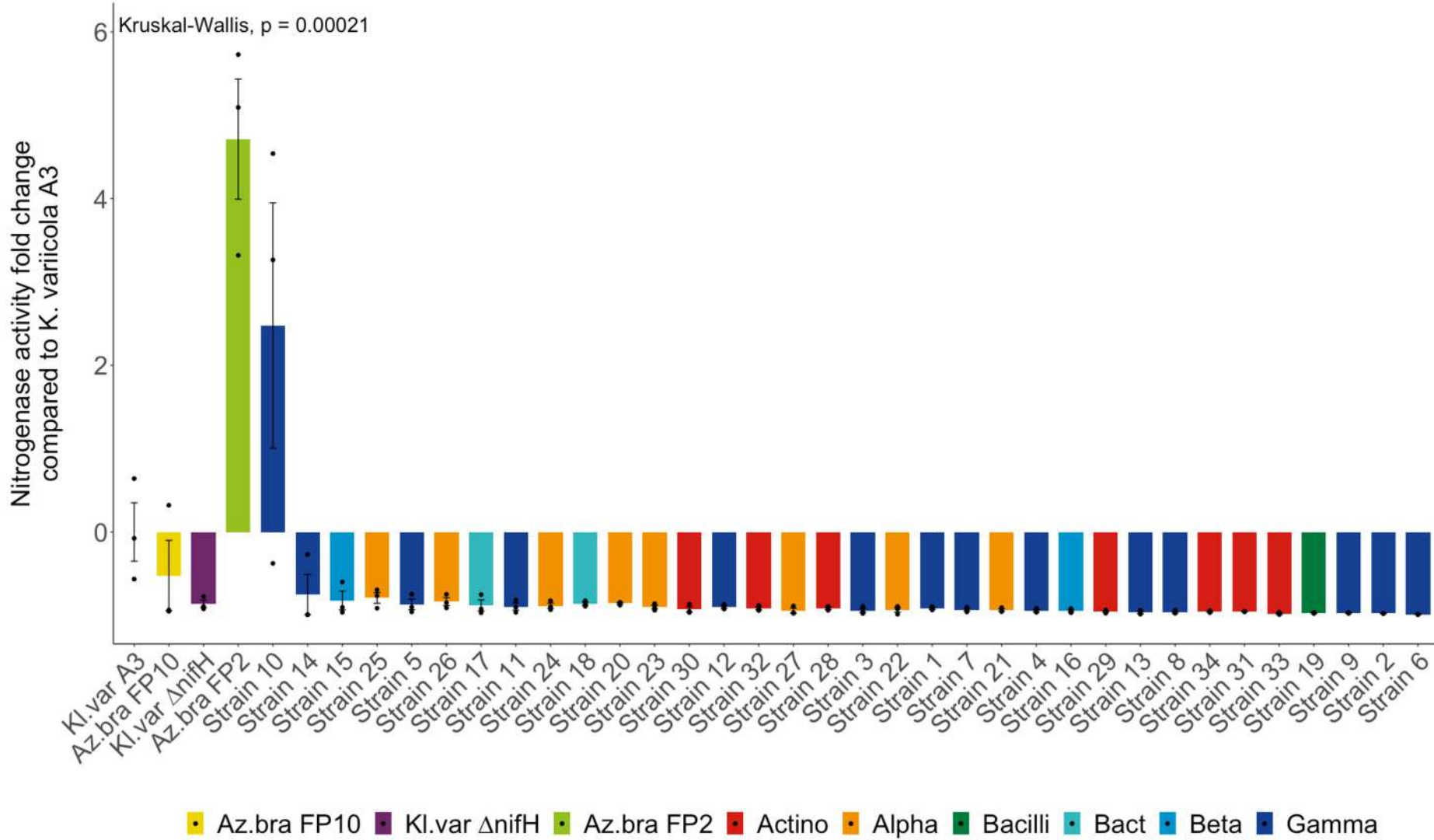
Non-diazotrophs  
(possible helpers)

Nitrogen-free semi-solid medium

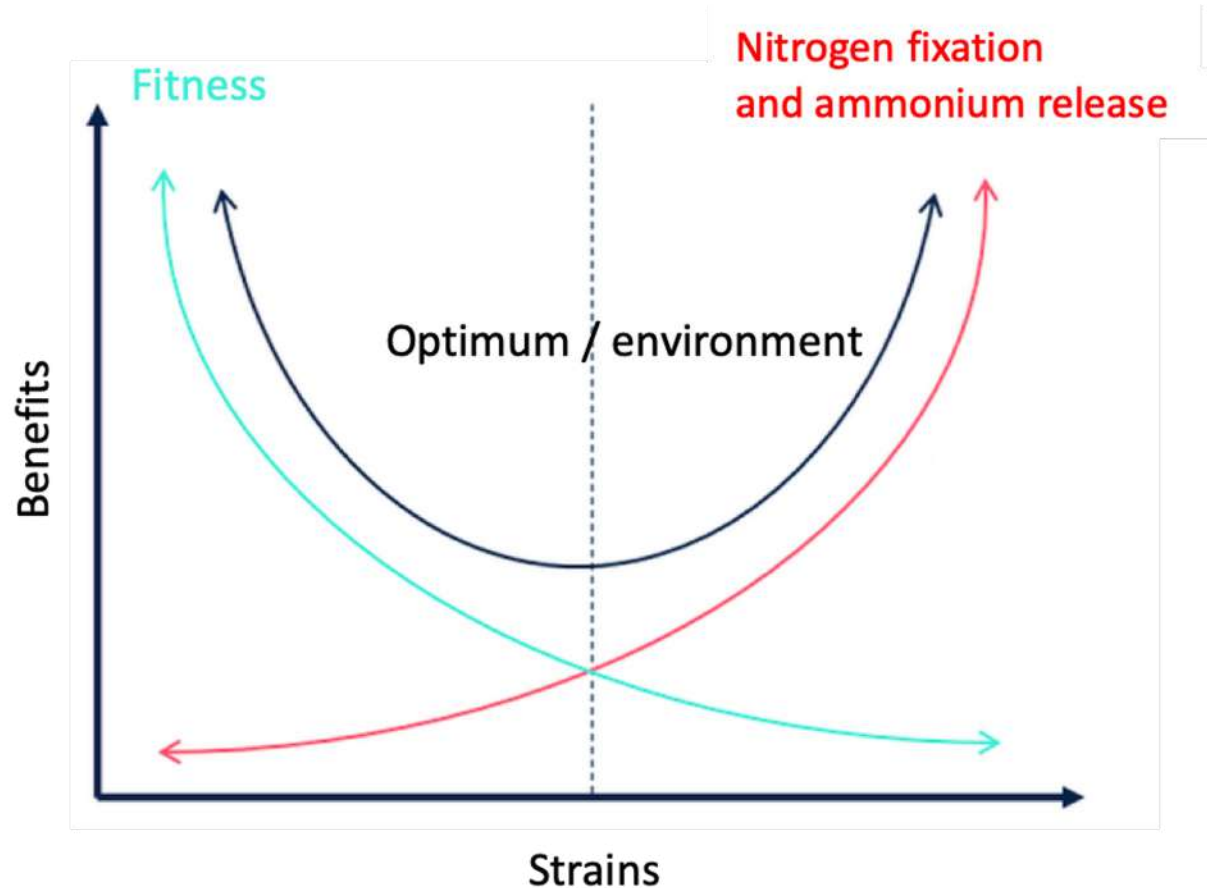


Dr. Paulo Ivan  
Fernandes Júnior  
(EMBRAPA)

# Identification of helper and competitor strains for *Klebsiella variicola* A3



# Fitness decrease in engineered diazotrophs



Synthetic biology

Bacterial synthetic communities (SynCom)

Bacterial co-isolation

Arbuscular mycorrhizal fungi

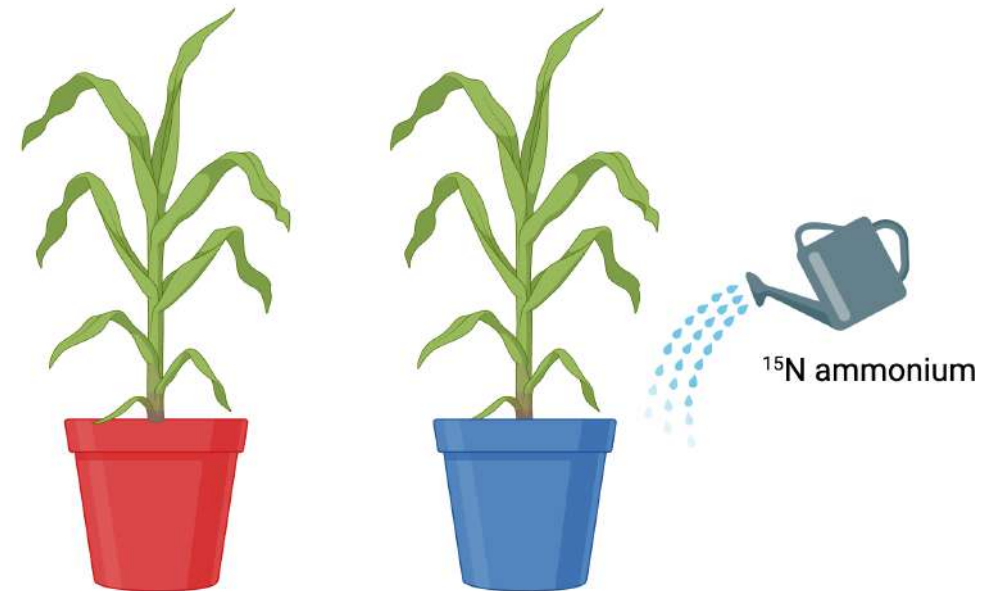
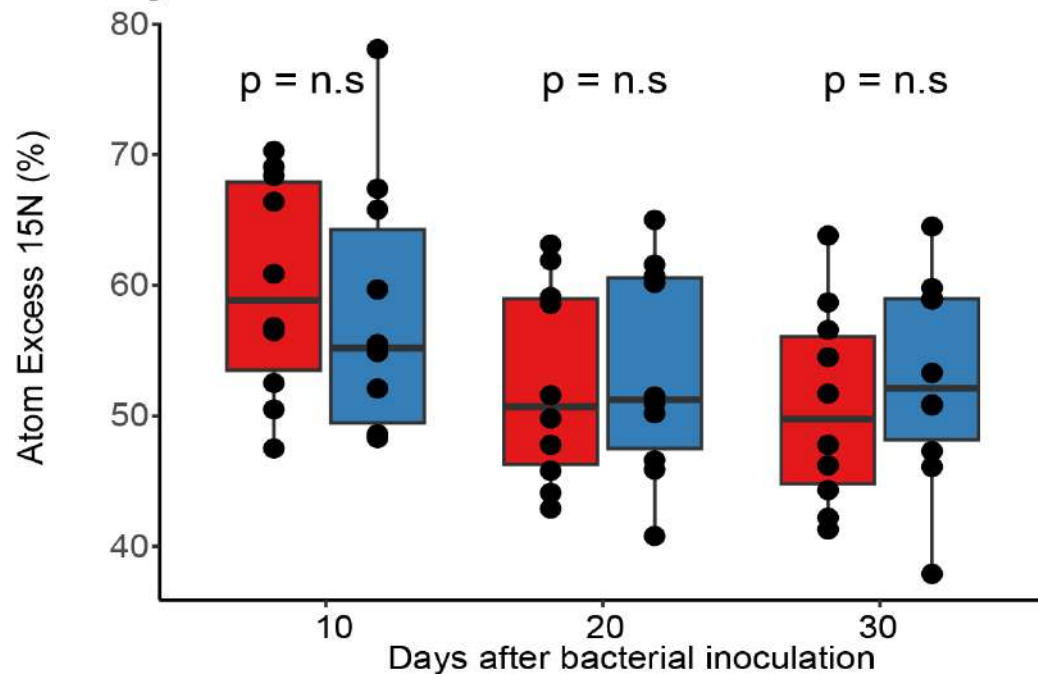
# Transfer of fixed nitrogen to corn mediated by arbuscular mycorrhizal fungi



Dr. Devanshi Khokhani  
University of Minnesota

$^{15}\text{N}$  dilution experiment (nitrogen-fixation evaluated by a decrease in  $^{15}\text{N}$  content)

## Non-mycorrhized treatment



*ΔnifD*: non-fixing mutant of *Azotobacter vinelandii*

*ΔnifL:km*: ammonium-excreting *Azotobacter vinelandii* published in Mus *et al.* (2022)



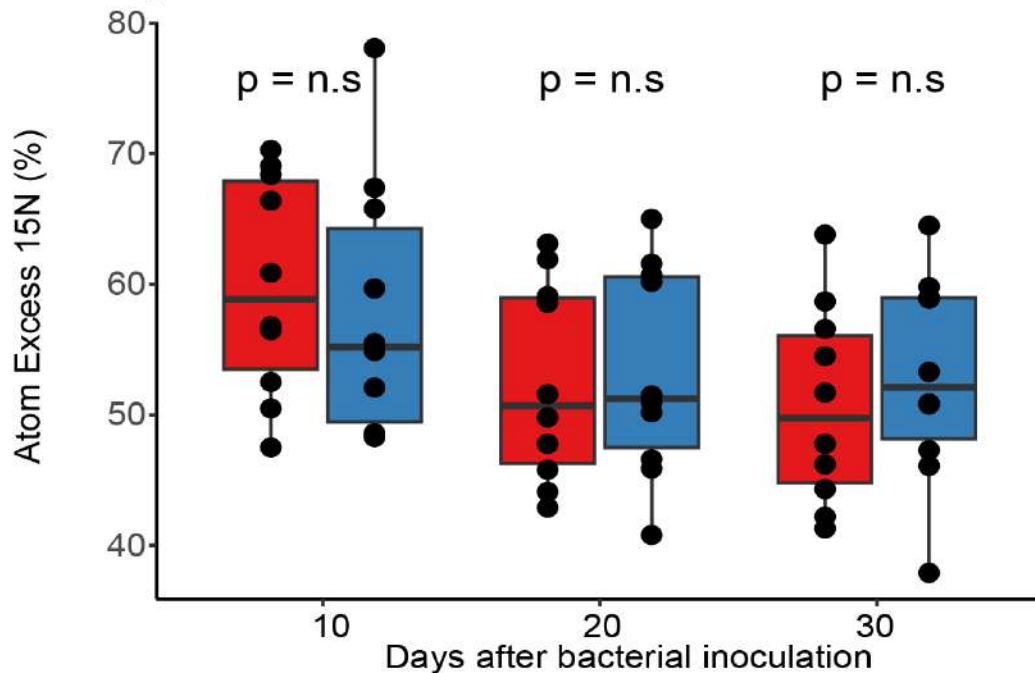
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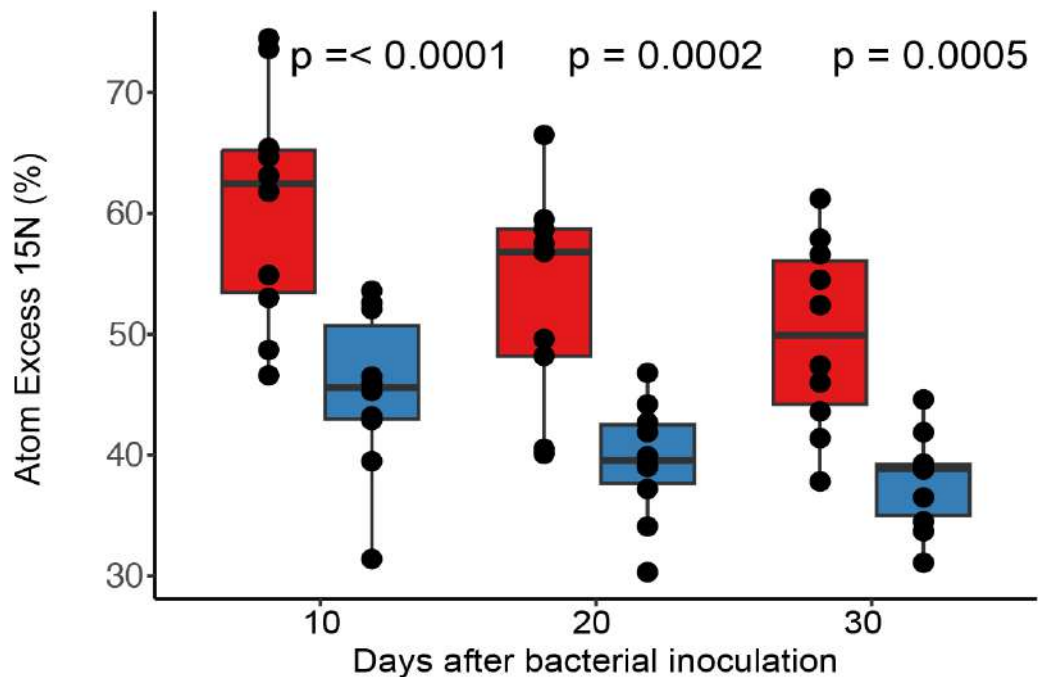
Dr. Devanshi Khokhani  
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### Non-mycorrhizal treatment

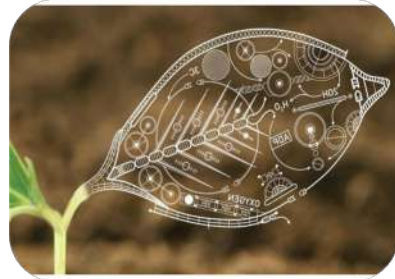


### Mycorrhizal treatment



# Current approaches to improve biological nitrogen-fixation in cereals

Engineering nitrogen-fixing plants



Engineering root nodules

Exploring plant natural diversity



Engineering diazotrophs

Plant



Microbe

# Are we there yet?

(Pankievicz *et al.*, BMC Biology 2019)



?

5-15 years



Now-10 years

Plant



Microbe





# Acknowledgements



## Ané lab members



Valentina Infante  
Biswajit Samal  
Junko Maeda  
April MacIntyre

## Key collaborators on projects presented



Brian Pflieger, University of Wisconsin - Madison  
Ophelia Venturelli, University of Wisconsin – Madison



Paulo Ivan Fernandes Júnior, EMBRAPA  
Devanshi Khokhani, University of Minnesota  
John Peters, University of Oklahoma