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

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

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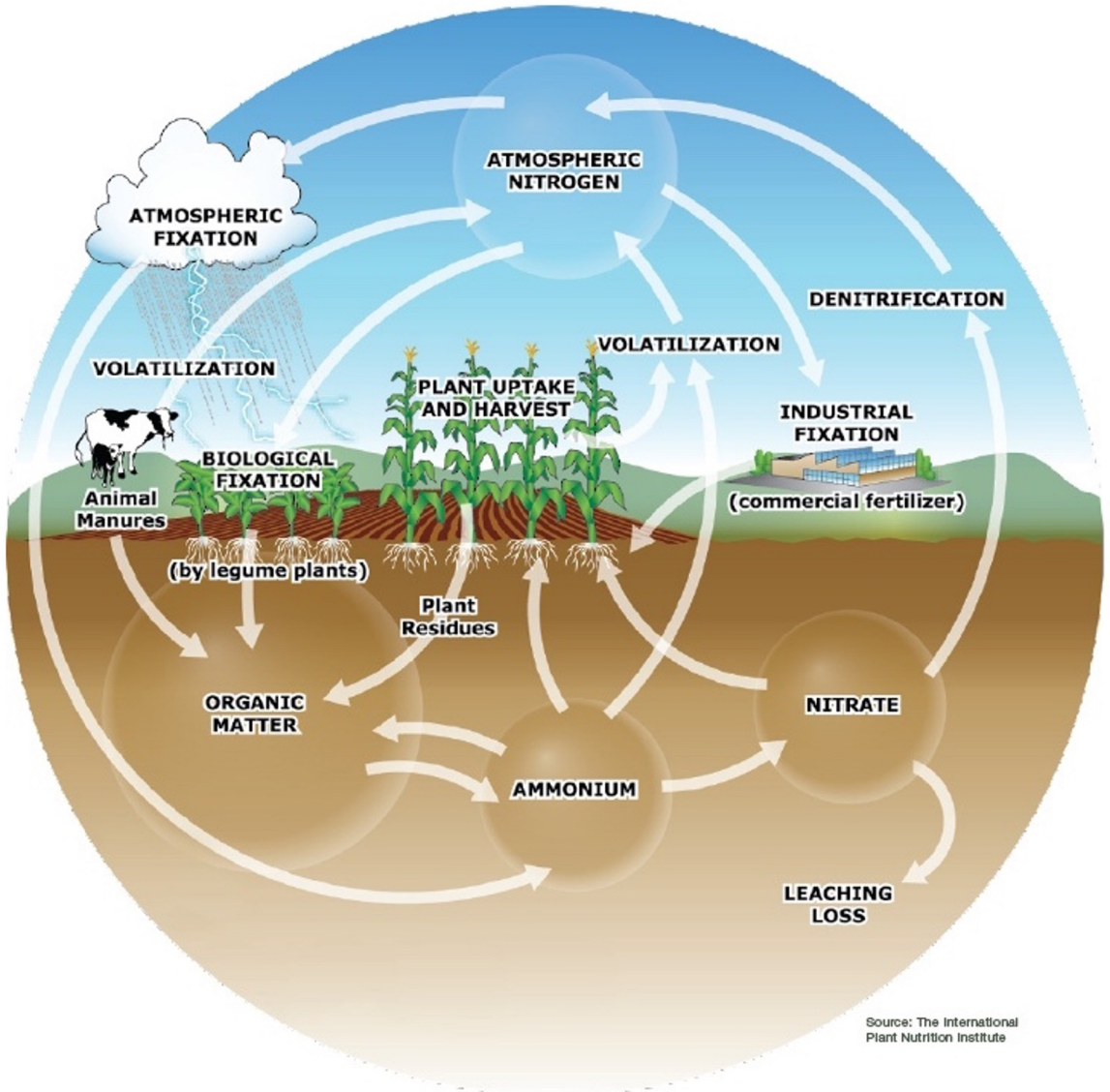
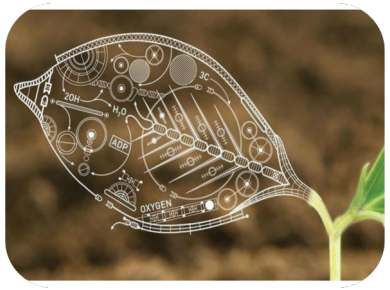
The nitrogen cycle and biological nitrogen fixation



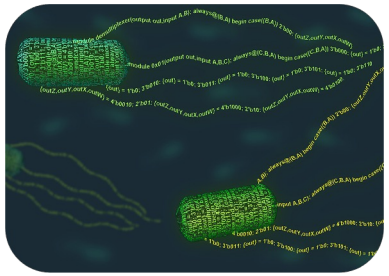
Current approaches to improve biological nitrogen-fixation in cereals

Engineering root nodules



Engineering nitrogen-fixing plants

Engineering diazotrophs

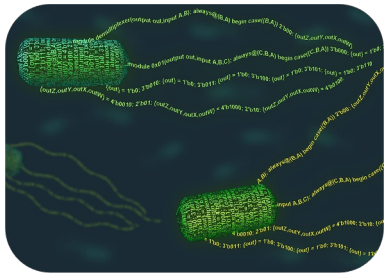


Exploring plant natural diversity

Microbe ⟷ Plant

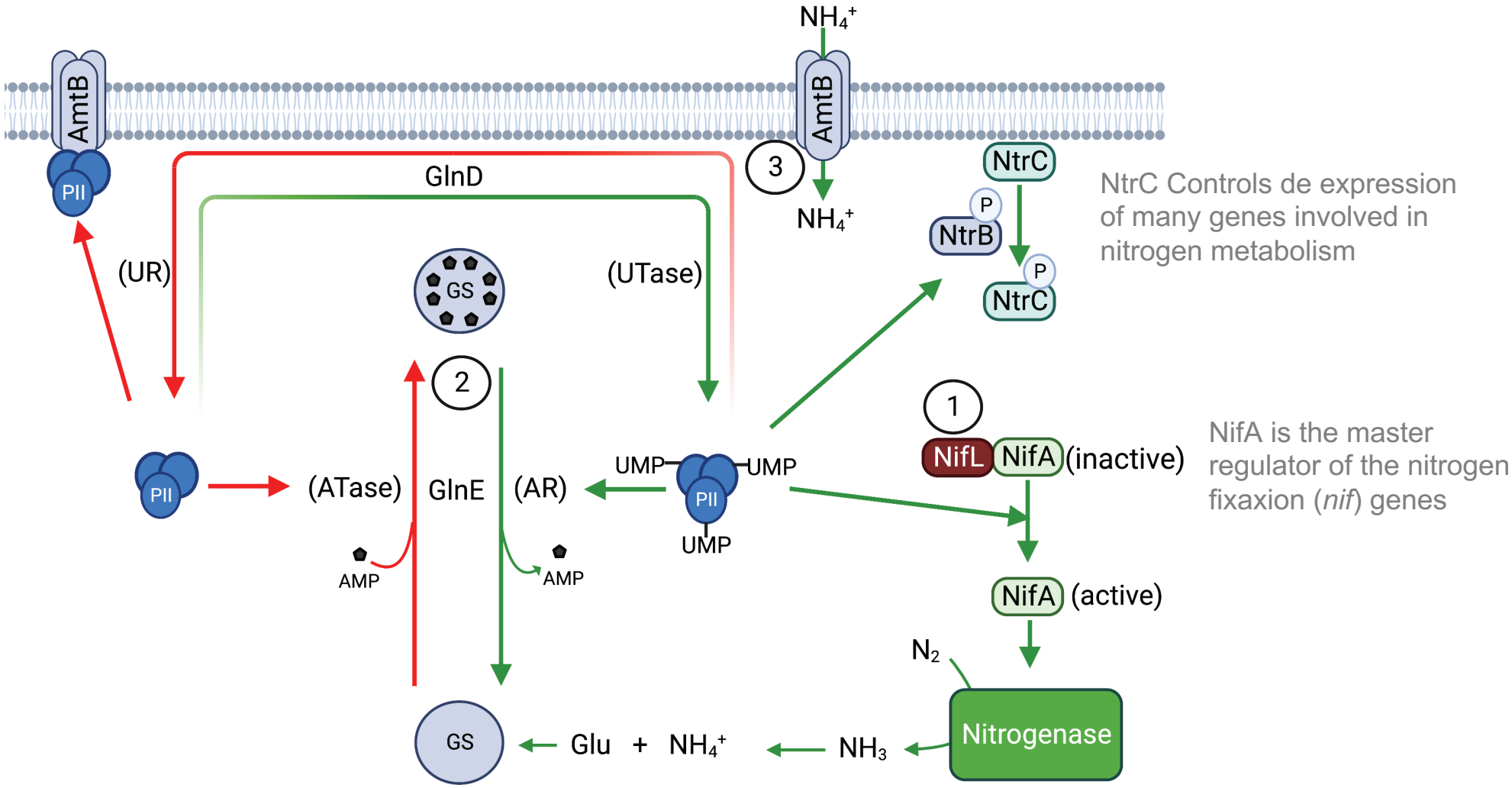
Current approaches to improve biological nitrogen-fixation in cereals

Engineering diazotrophs



Microbe ⟷ Plant

Bacterial engineering / remodeling



NtrC Controls de expression of many genes involved in nitrogen metabolism

NifA is the master regulator of the nitrogen fixaxion (*nif*) genes

NSF-IOS #1753917

Nitrogen-sufficient conditions

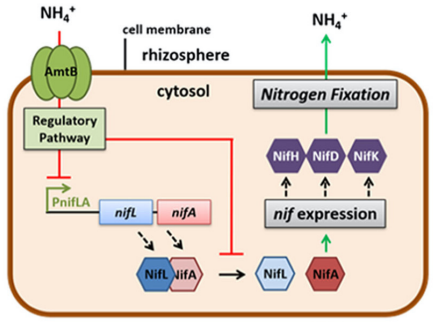
Nitrogen-limited conditions

Chakraborty *et al.* Trends in Microbiology 2023

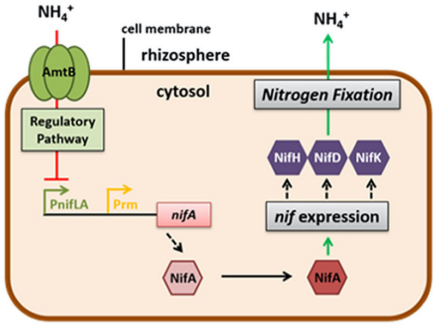
Mus *et al*. Applied and Environmental Microbiology 2022

Wen *et al*. ACS Synthetic Biology 2021

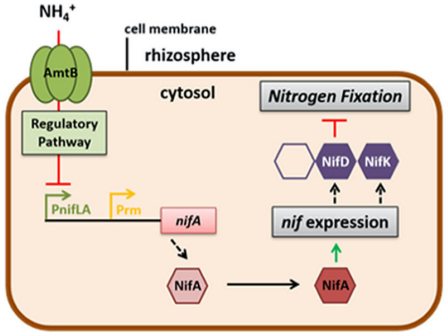
Bacterial engineering / remodeling



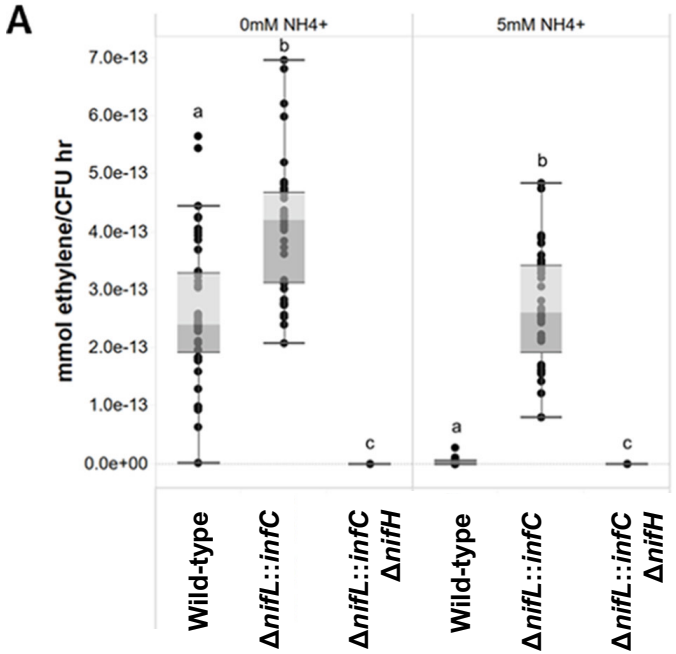
Wild-type

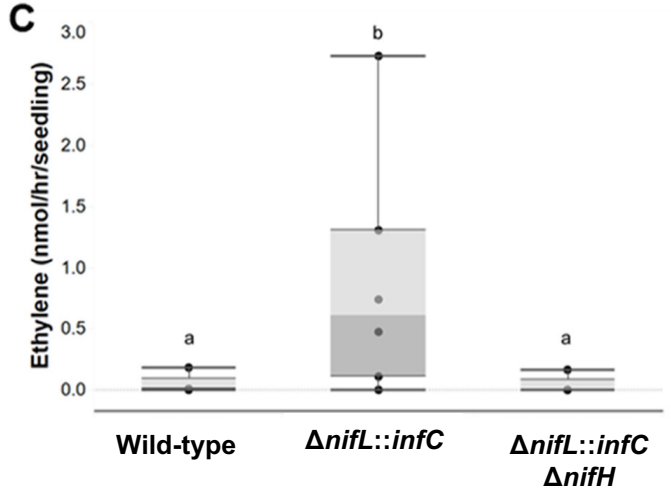


Δ*nifL*::*infC*



Δ*nifL*::*infC*
Δ*nifH*





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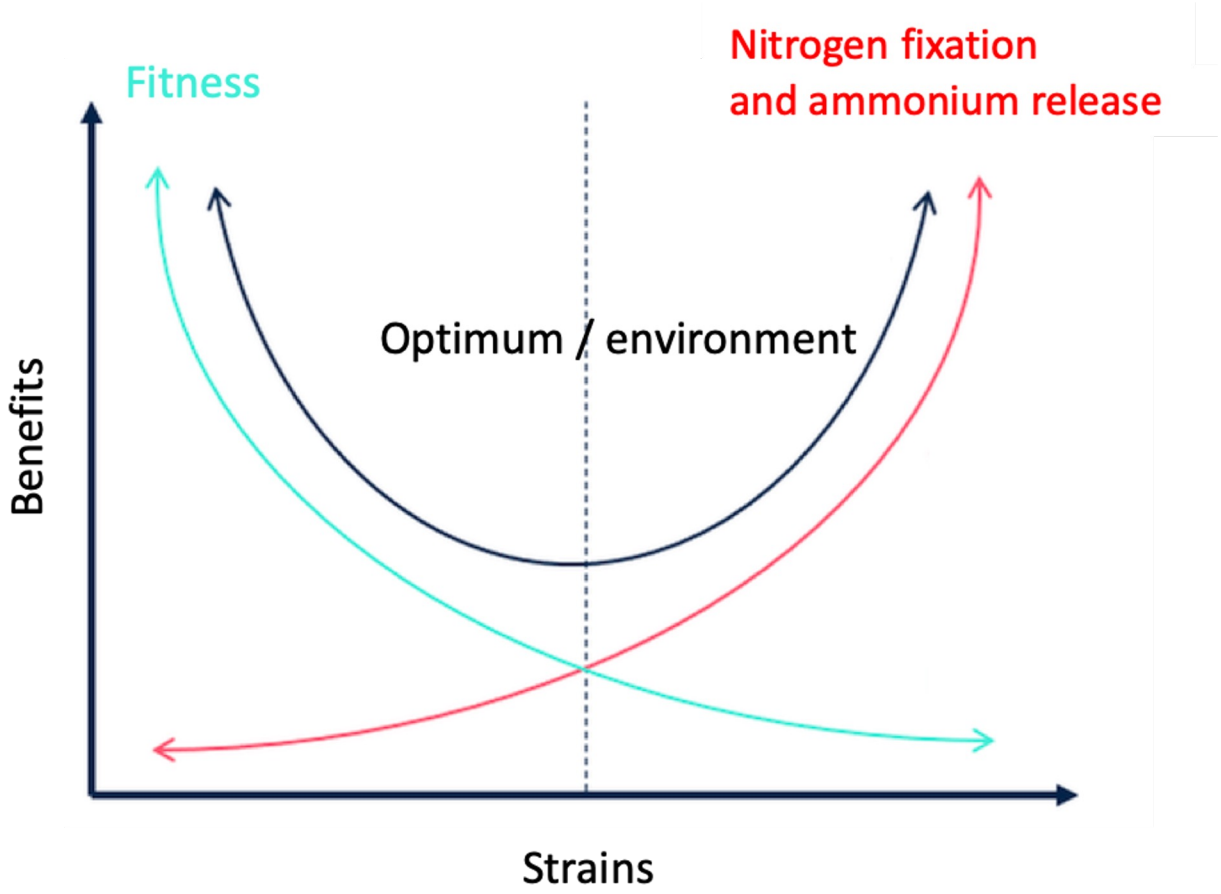
Wen *et al*. ACS Synthetic Biology 2021

Fitness decrease in engineered diazotrophs



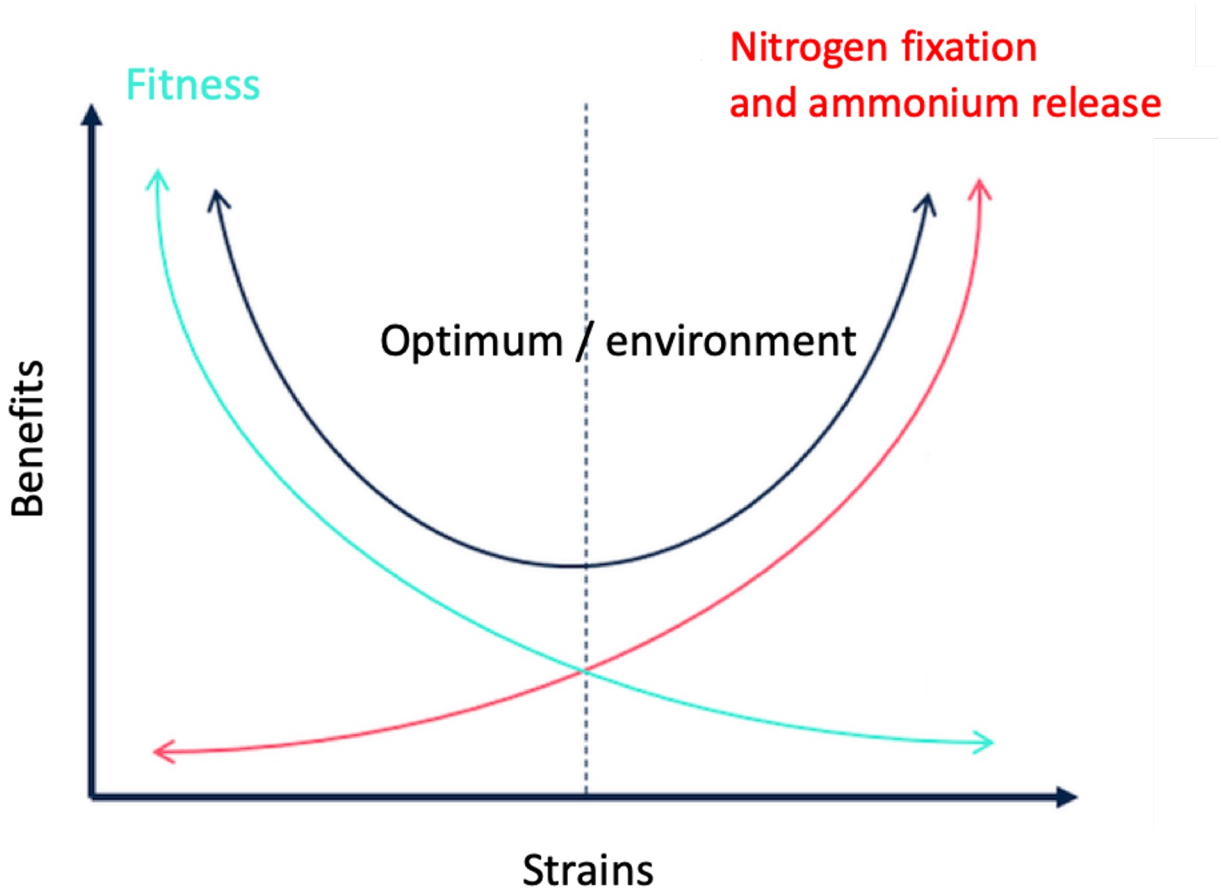
Synthetic biology

Bacterial synthetic communities (SynComs)

Bacterial co-isolation

Arbuscular mycorrhizal fungi

Fitness decrease in engineered diazotrophs



Synthetic biology

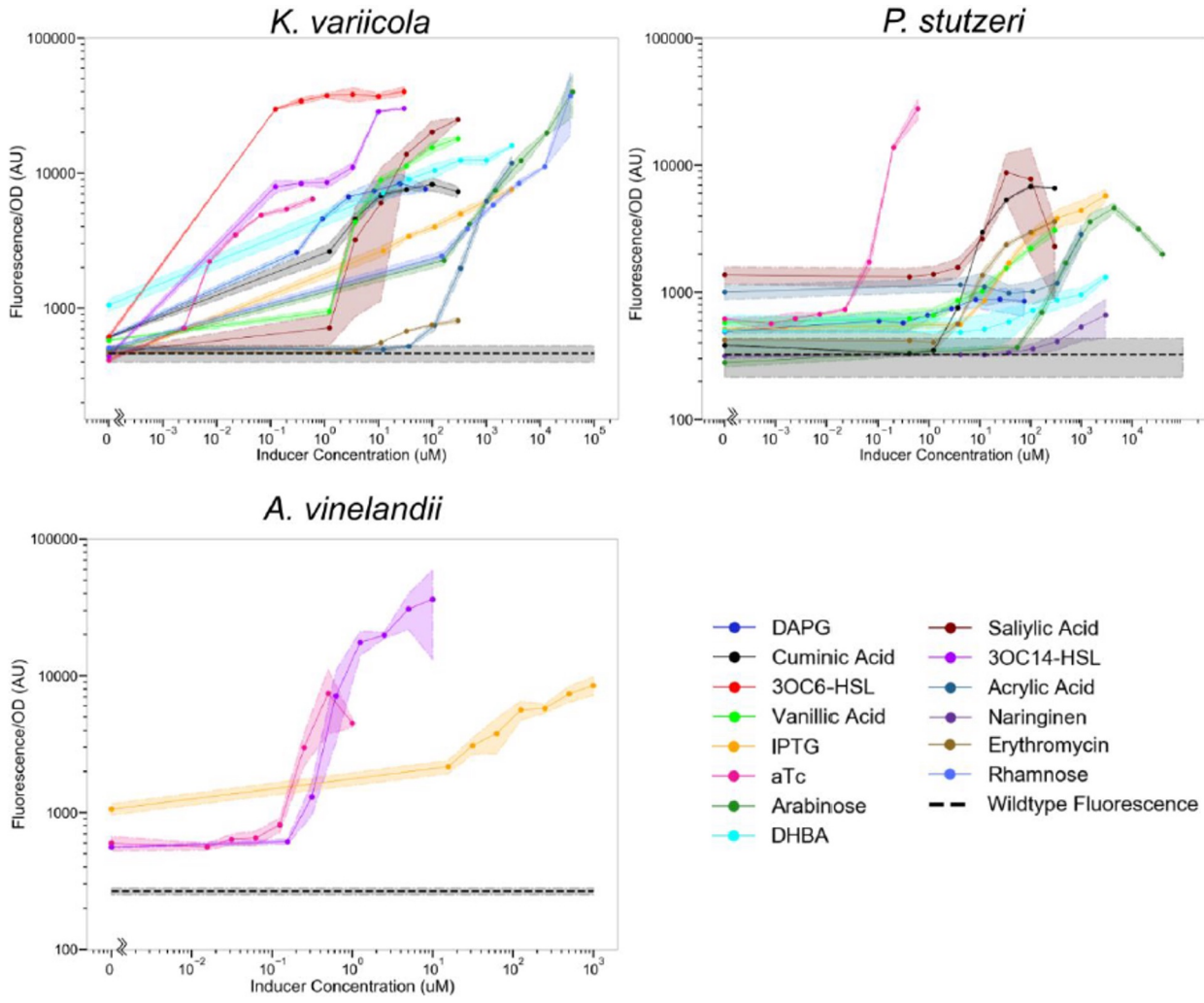
Looking for the best diazotroph chassis for synthetic biology

Best chassis so far:

- *Klebsiella* sp.
- *Pseudomonas* sp.

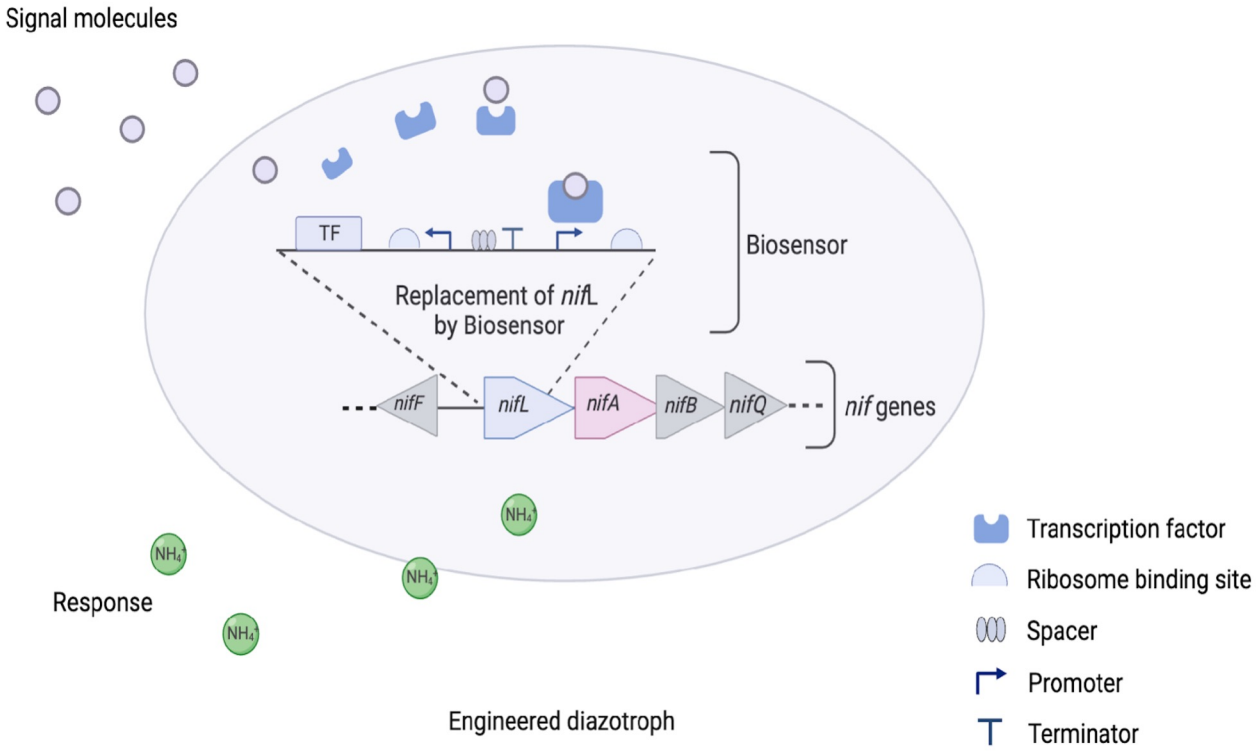
Biosensors currently optimized for:

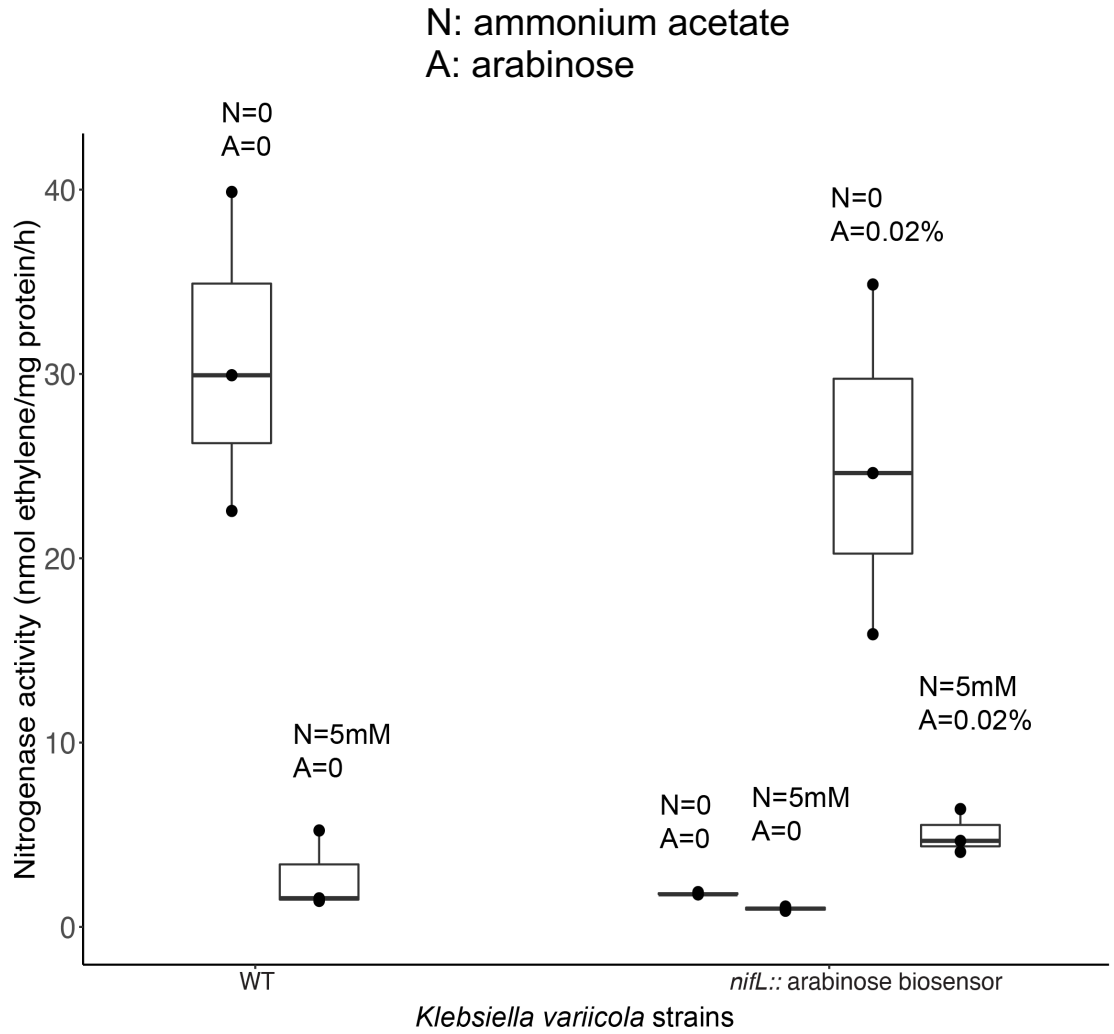
- Arabinose
- Cuminic acid
- Flavonoids: naringenin and luteolin
- p-Coumaric acid



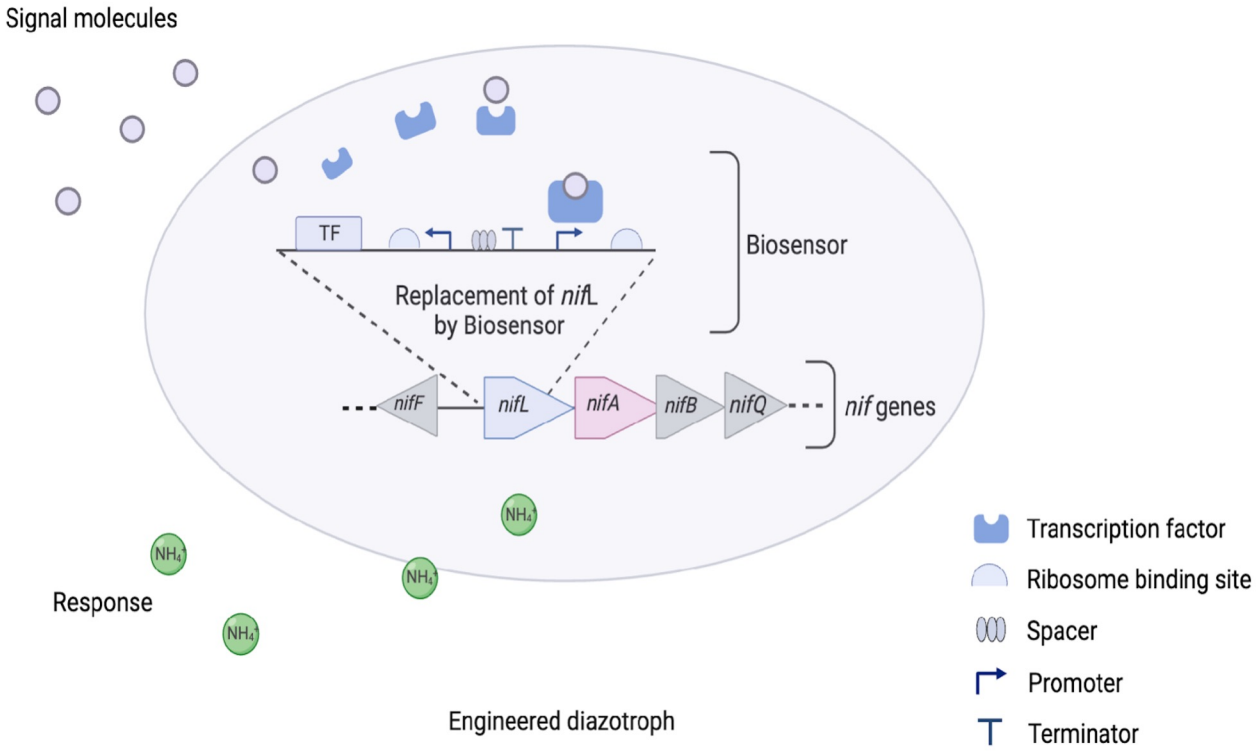
Chakraborty *et al.* Trends in Microbiology 2023
Venkataraman *et al.* ACS Synthetic Biology 2023

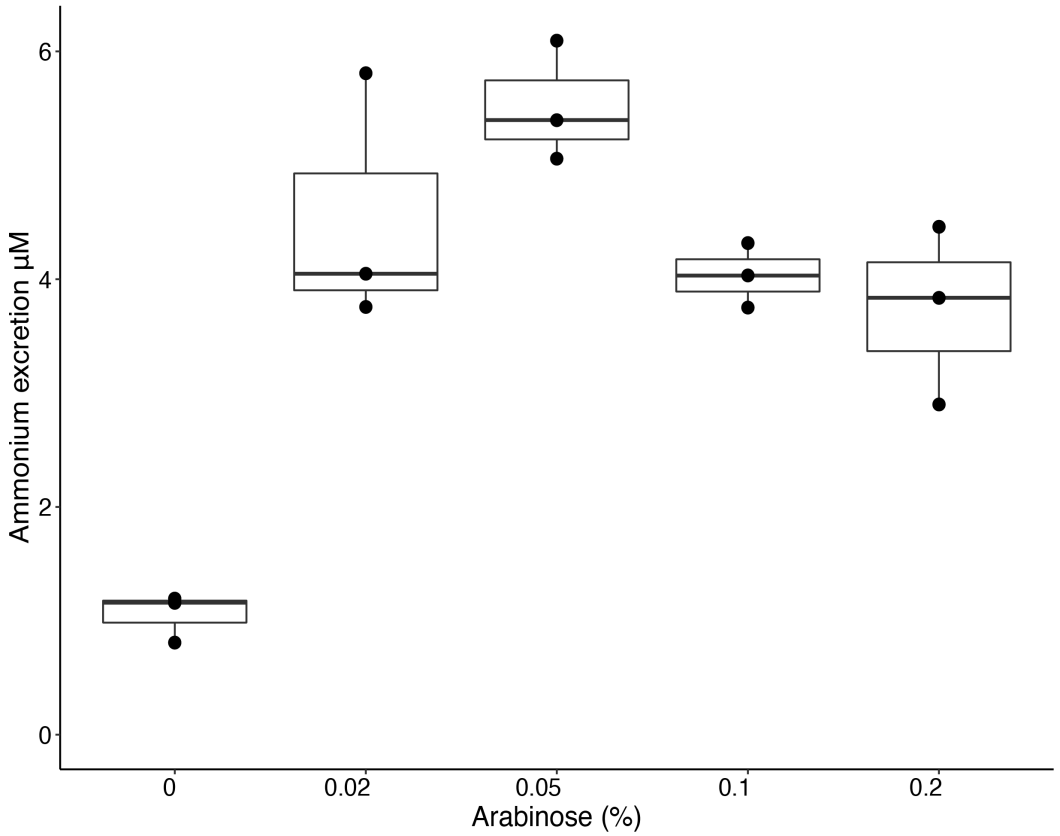
Inducible nitrogen fixation



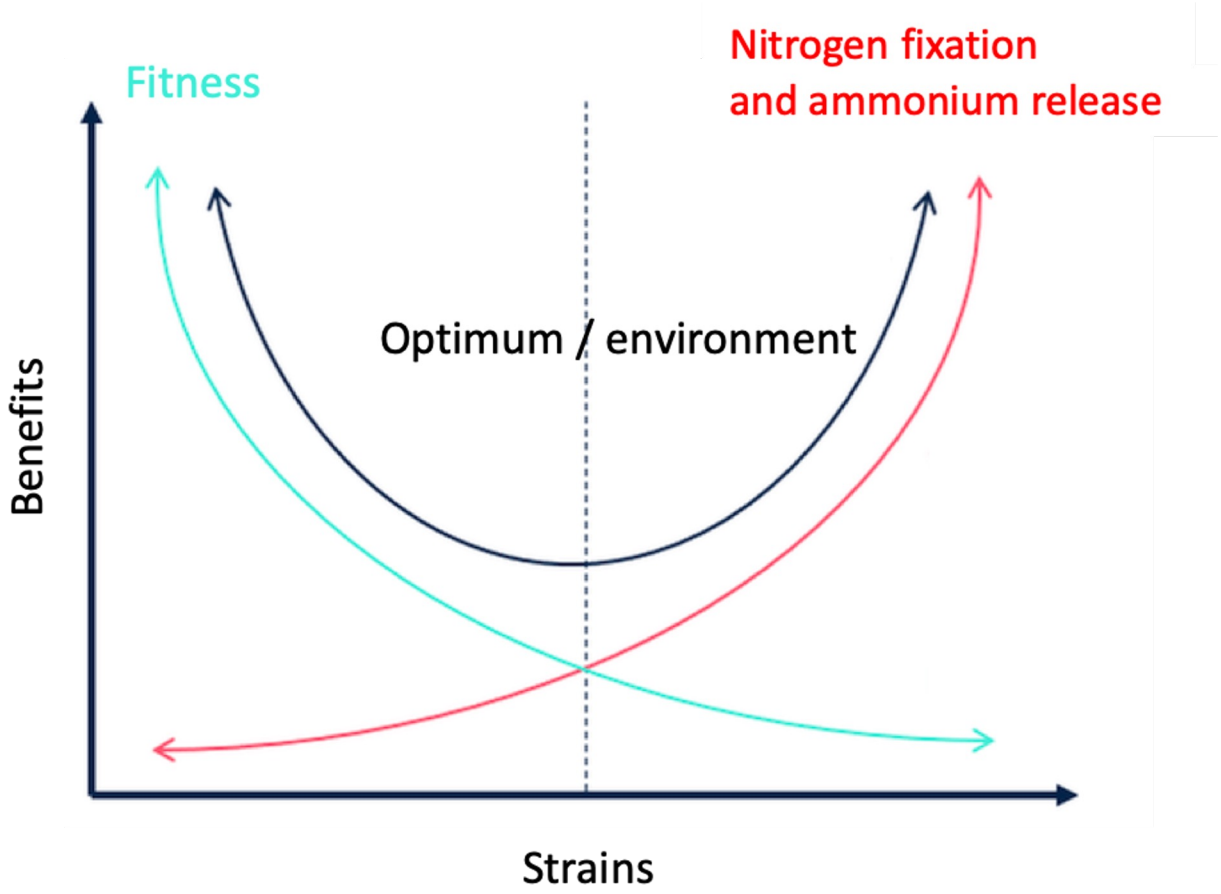


Inducible nitrogen fixation and ammonium excretion





Fitness decrease in engineered diazotrophs

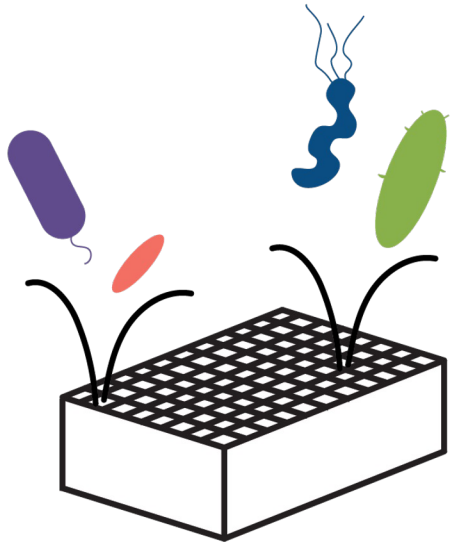


Synthetic biology

Bacterial synthetic communities (SynComs)

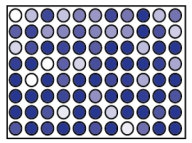
Effect of community composition on nitrogen fixation

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



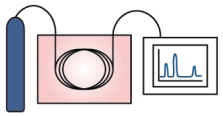
Assess Community BNF

Ammonia Colorimetric Assay



$^{15}N_2$

Acetylene Reduction Assay



Computational Model

16S Sequencing

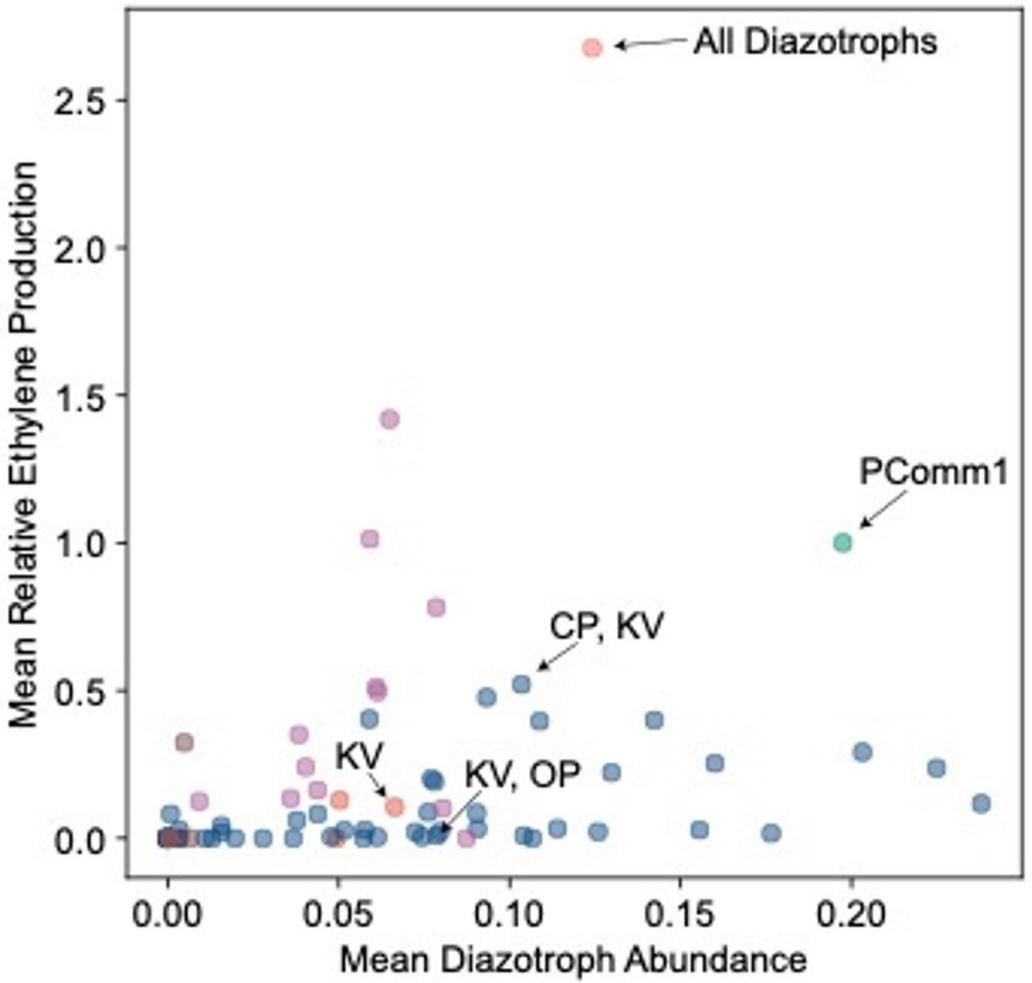
Assess Community Composition

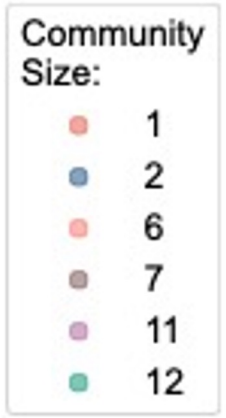
Model Guided Community Design

Current Dataset

- Monocultures
- All Possible Pairs
- Leave-one-out Communities

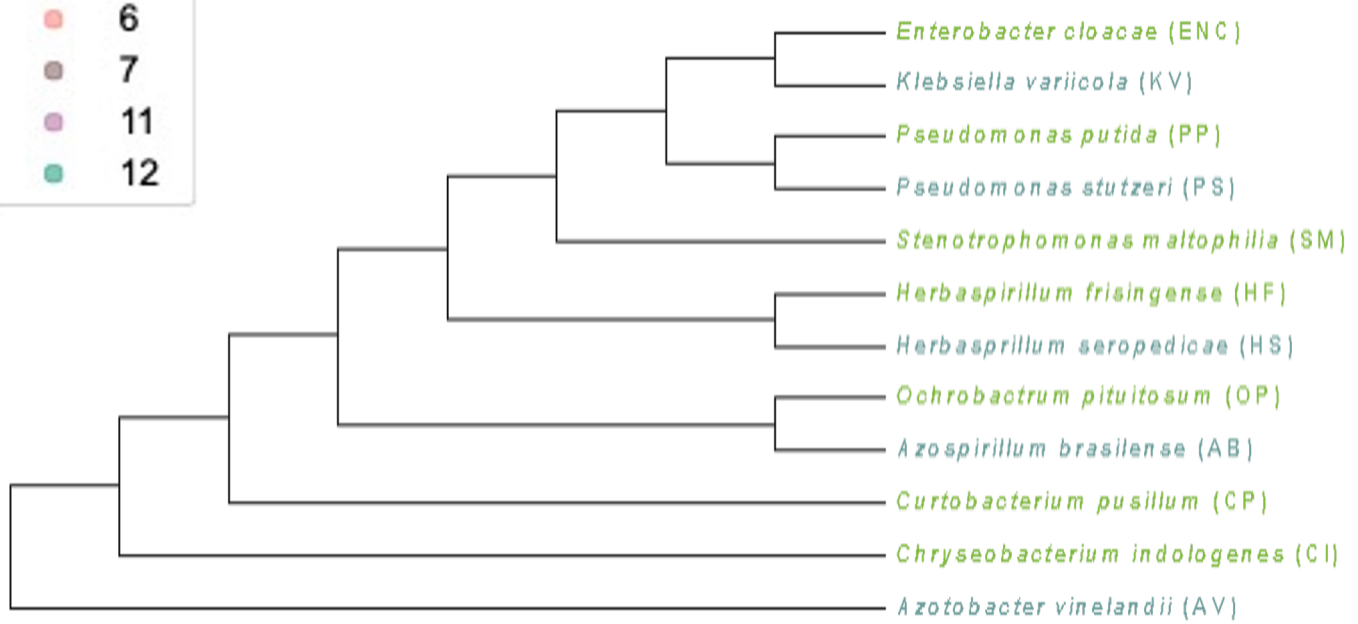
Sub-communities provide insight into how non-fixers may both improve and hinder nitrogenase activity



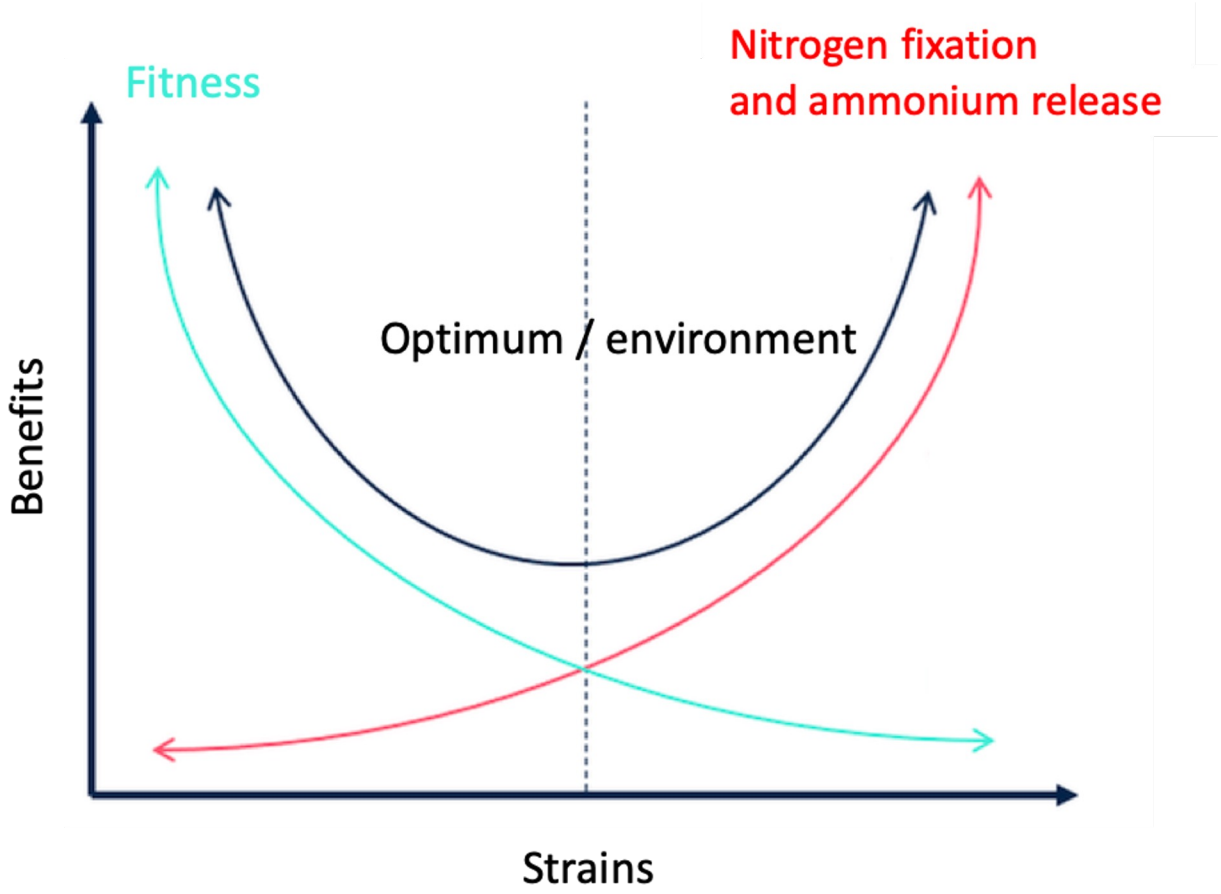


Full 12-member Community: PComm1

7-member community from Niu *et al.* (2017)
+ 5 tractable diazotrophs



Fitness decrease in engineered diazotrophs

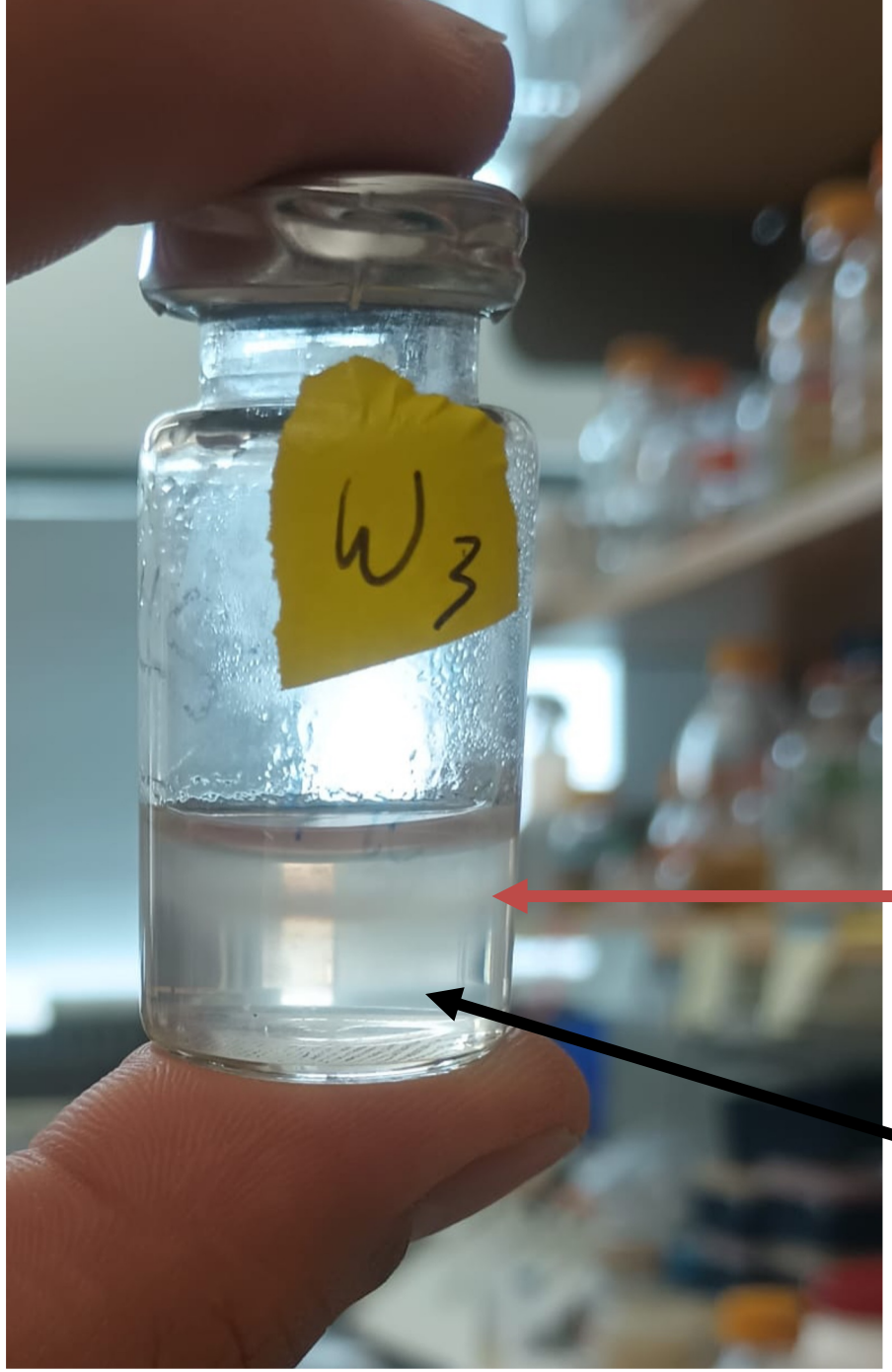


Synthetic biology

Bacterial synthetic communities (SynComs)

Bacterial co-isolation

Can we isolate more diazotroph “helpers” ?

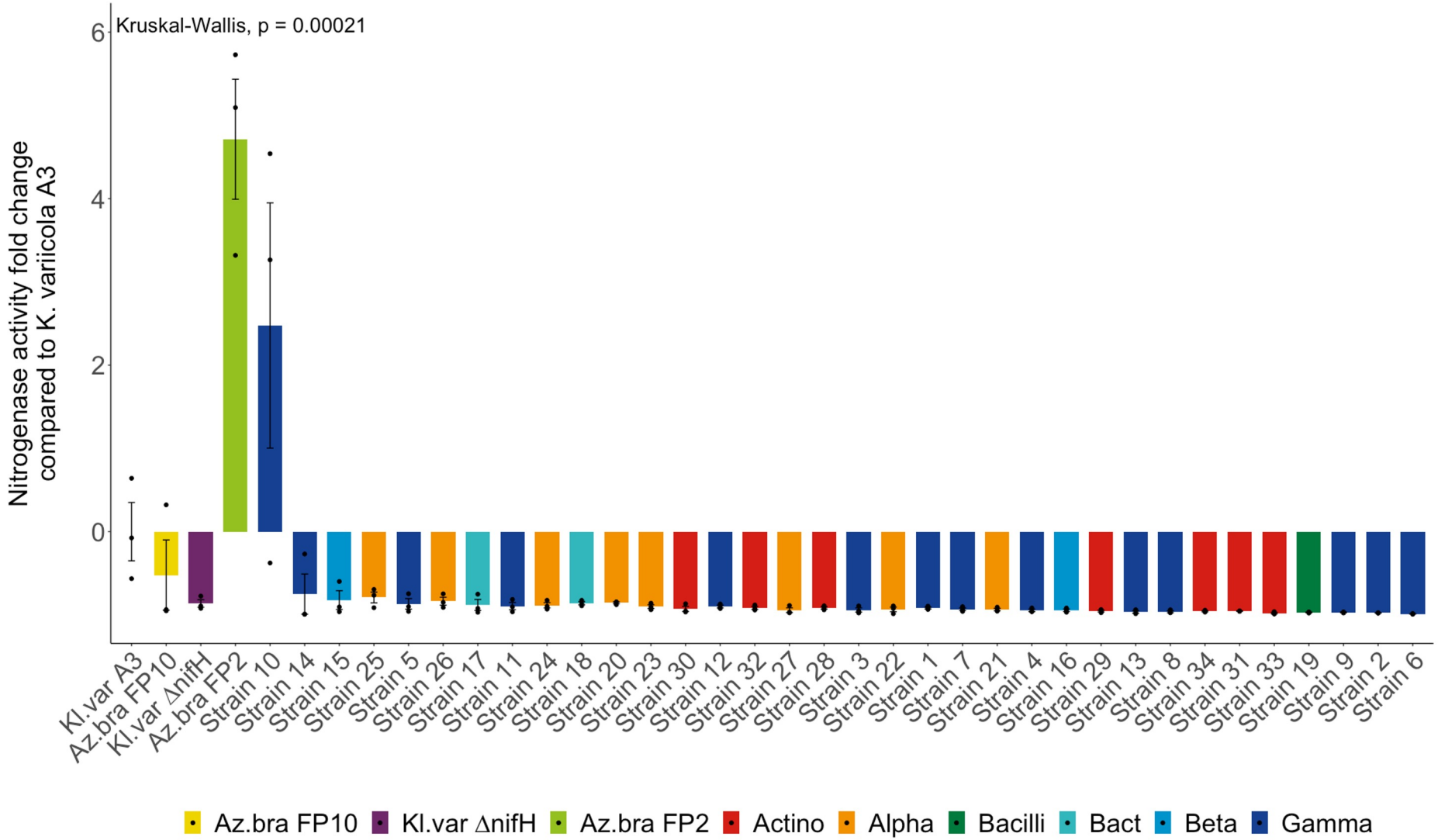


Diazotrophs
+
Non-diazotrophs
(possible helpers)

Nitrogen-free semi-solid medium

Dr. Paulo Ivan Fernandes Júnior
(EMBRAPA)

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



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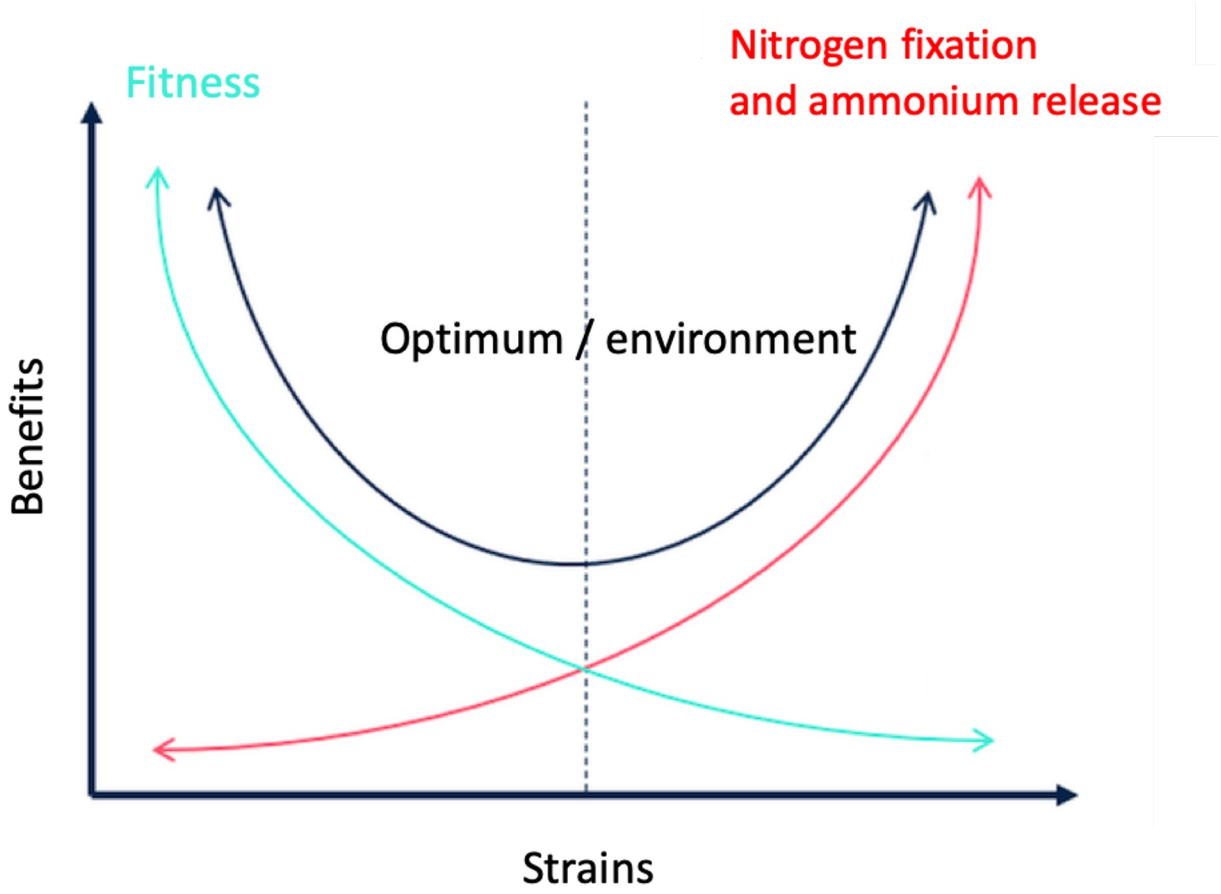

Synthetic biology

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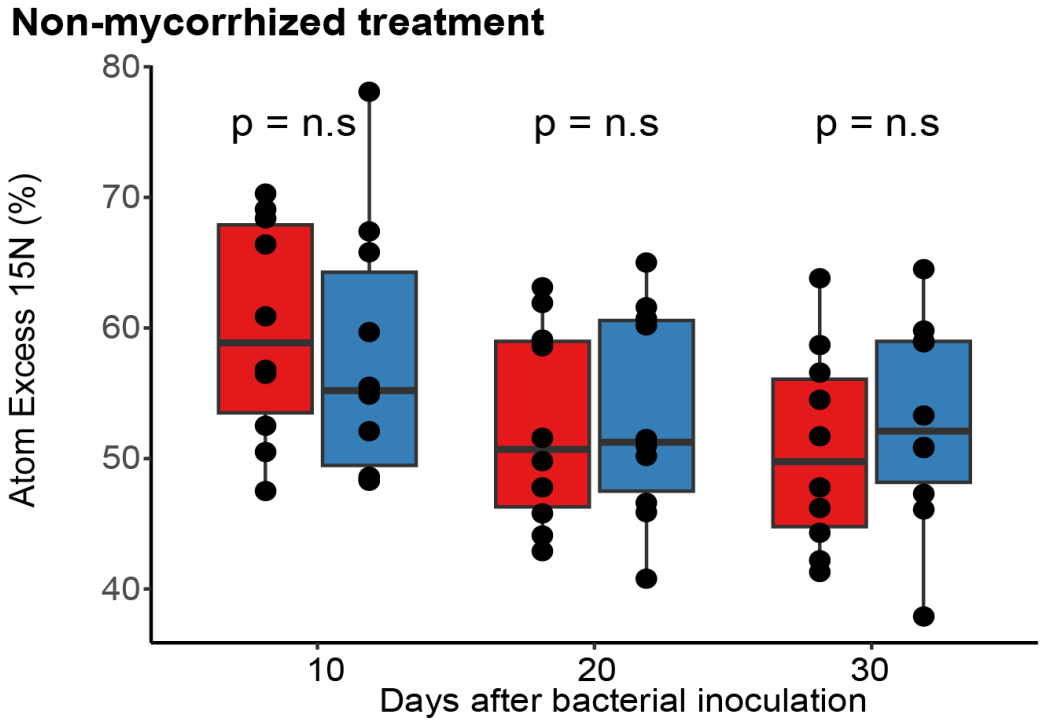
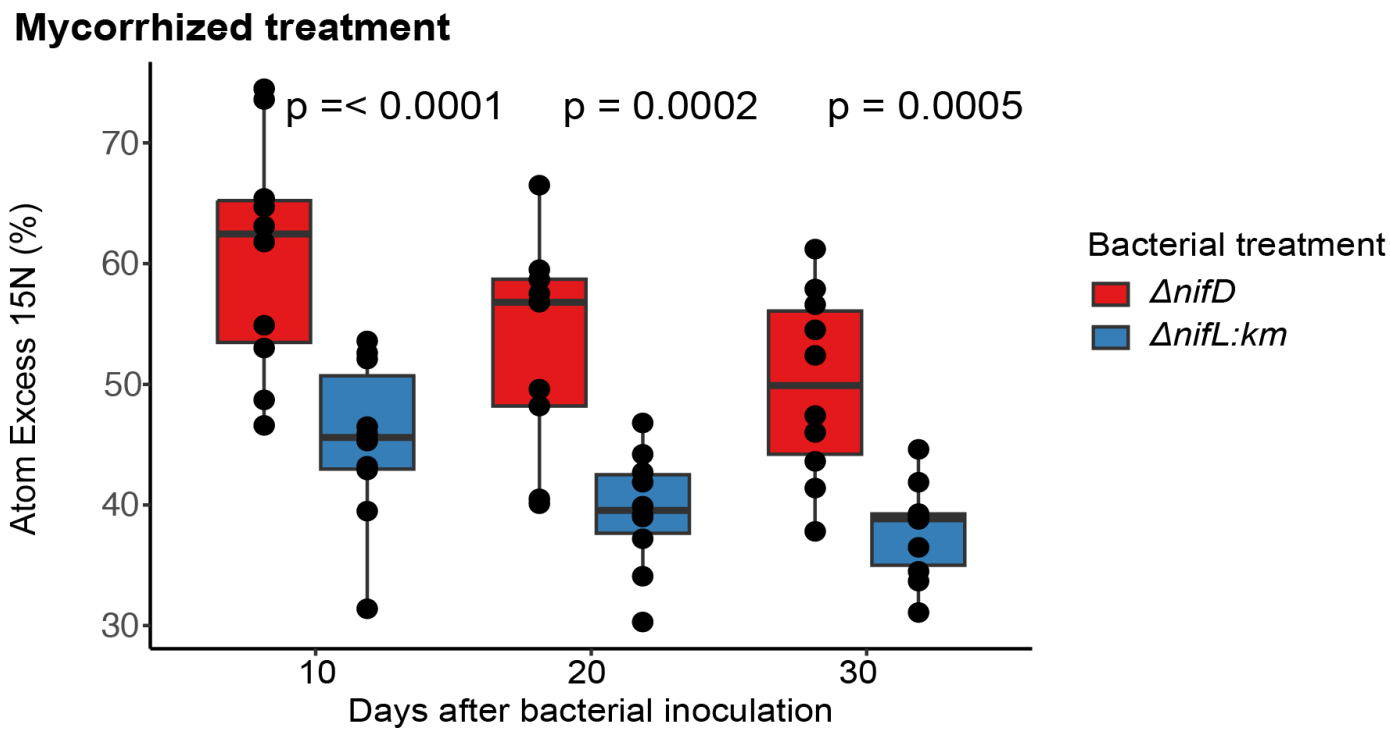
Bacterial co-isolation

Arbuscular mycorrhizal fungi

Transfer of fixed nitrogen to corn mediated by arbuscular mycorrhizal fungi

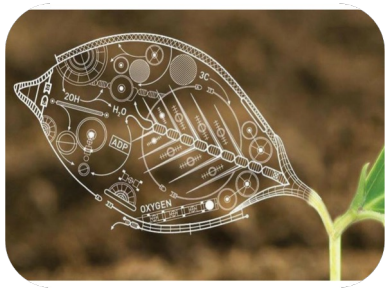
^{15}N dilution experiment (nitrogen-fixation evaluated by a decrease in ^{15}N content)
Δ*nifL:km :* Ammonium-excreting *Azotobacter vinelandii* published in Mus *et al.* (2022)
Δ*nifD:* non-fixing mutant of *Azotobacter vinelandii*





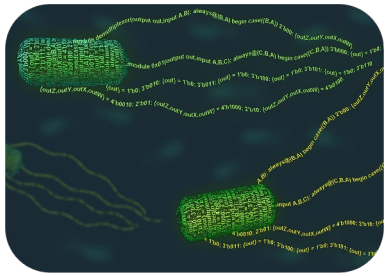
Current approaches to improve biological nitrogen-fixation in cereals

Engineering root nodules



Engineering nitrogen-fixing plants

Engineering diazotrophs



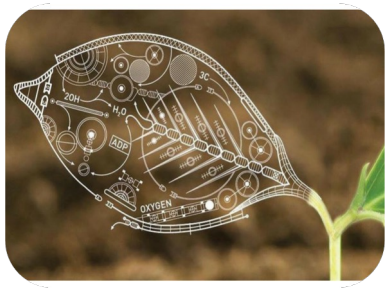
Exploring plant natural diversity

Microbe ⟷ Plant

Are we there yet?

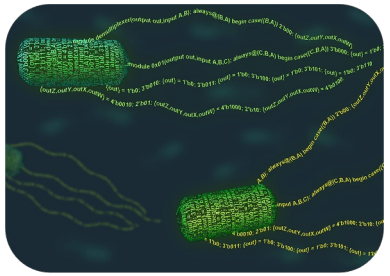
(Pankievicz *et al.*, **BMC Biology** 2019)

30 years?



50 years?

Now-10 years



5-15 years

Microbe ⟷ Plant

Acknowledgements

Ané lab members

Key collaborators on projects presented

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Claudia Calderón, University of Wisconsin - Madison
Sushmita Roy, University of Wisconsin – Madison

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John Peters, University of Oklahoma State
Christopher Voigt, Massachusetts Institute of Technology
Devanshi Khokhani, University of Minnesota
Wilfred Vermerris, University of Florida
Jason Wallace, University of Georgia