Why fix Nitrogen? Ecological, Evolutionary, and Economic Perspectives

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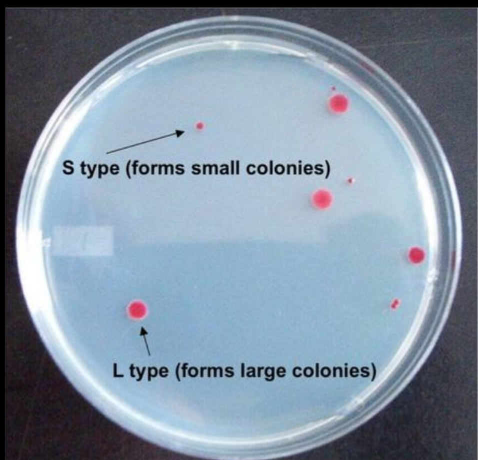
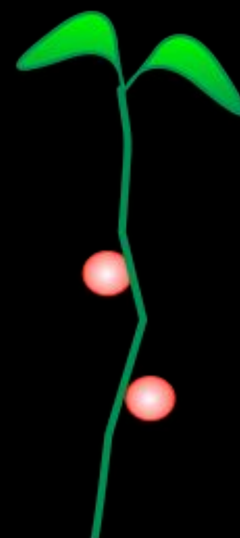
Model Organism
(*E. coli* +
Adaptive Dynamics)

Model Mutualism
(*Legume-rhizobia*)

Model Field Sites
(*LTAR, GLBRC,
Bodega Bay*)





BSc: Doebeli

PhD: Nuzhdin, Chesson, McElreath, Bronstein, Strauss

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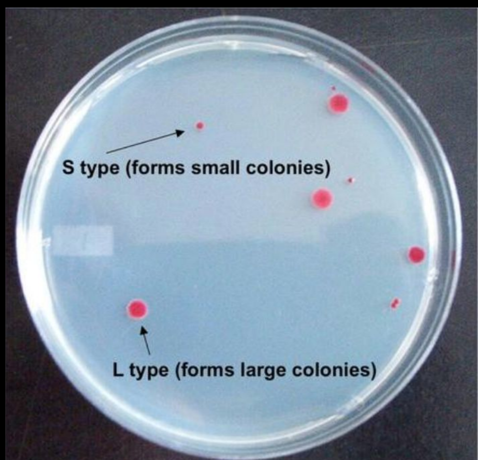
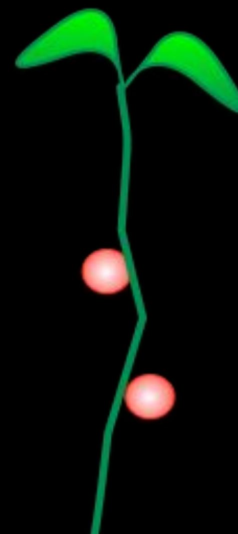




Questions:

What promotes beneficial interactions?

How does diversity arise and persist?

The Paradox of Diversity in Mutualisms

Antagonistic coevolution

- readily generates negative frequency-dependent selection
- can maintain numerous alleles / phenotypes

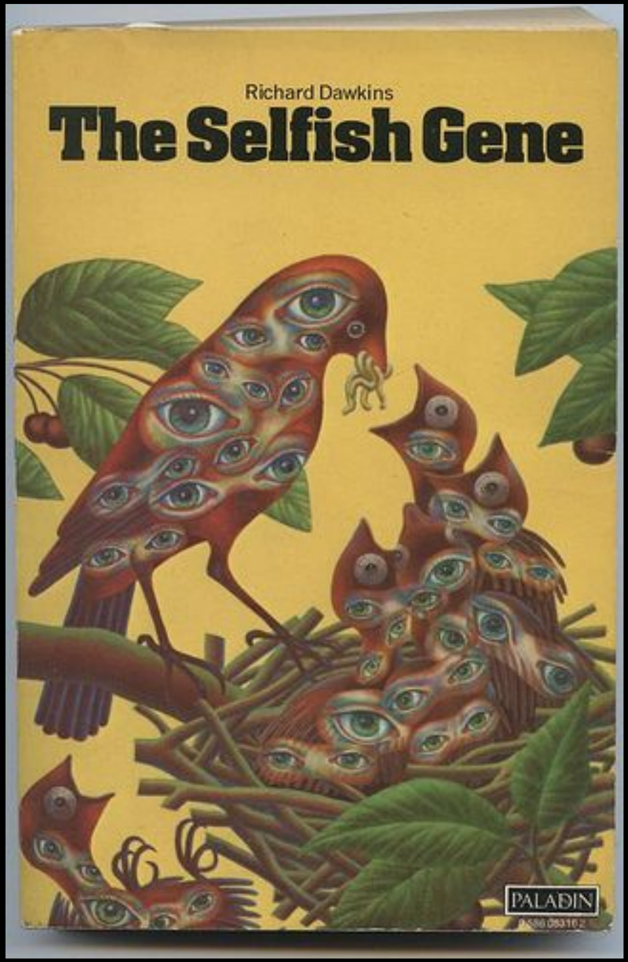
Mutualistic coevolution

- predicted to generate positive frequency dependent selection
- many models show coexistence of only 2 alleles / phenotypes

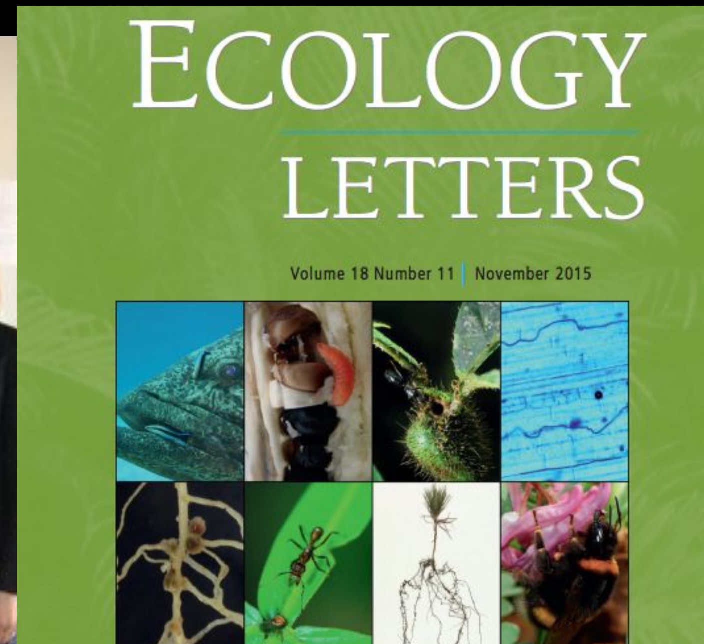
Yet, mutualisms are diverse… are they undergoing antagonistic coevolution?

Cheating in Mutualisms:

Why perform a costly act that benefits another species?

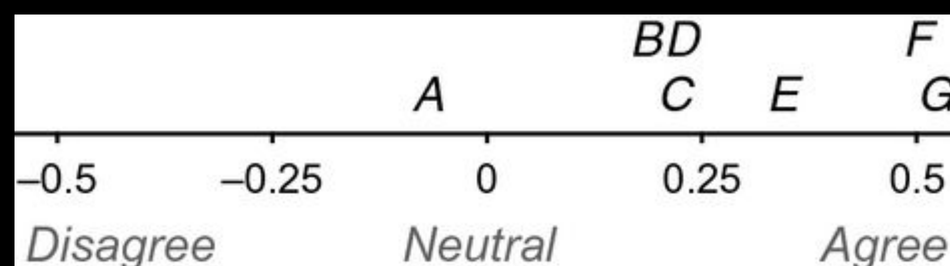


"When is a mutualist a cheater?" **NCEAS 2012**



Jones et al. 2015

7 common definitions of cheating



Cheaters must prosper!

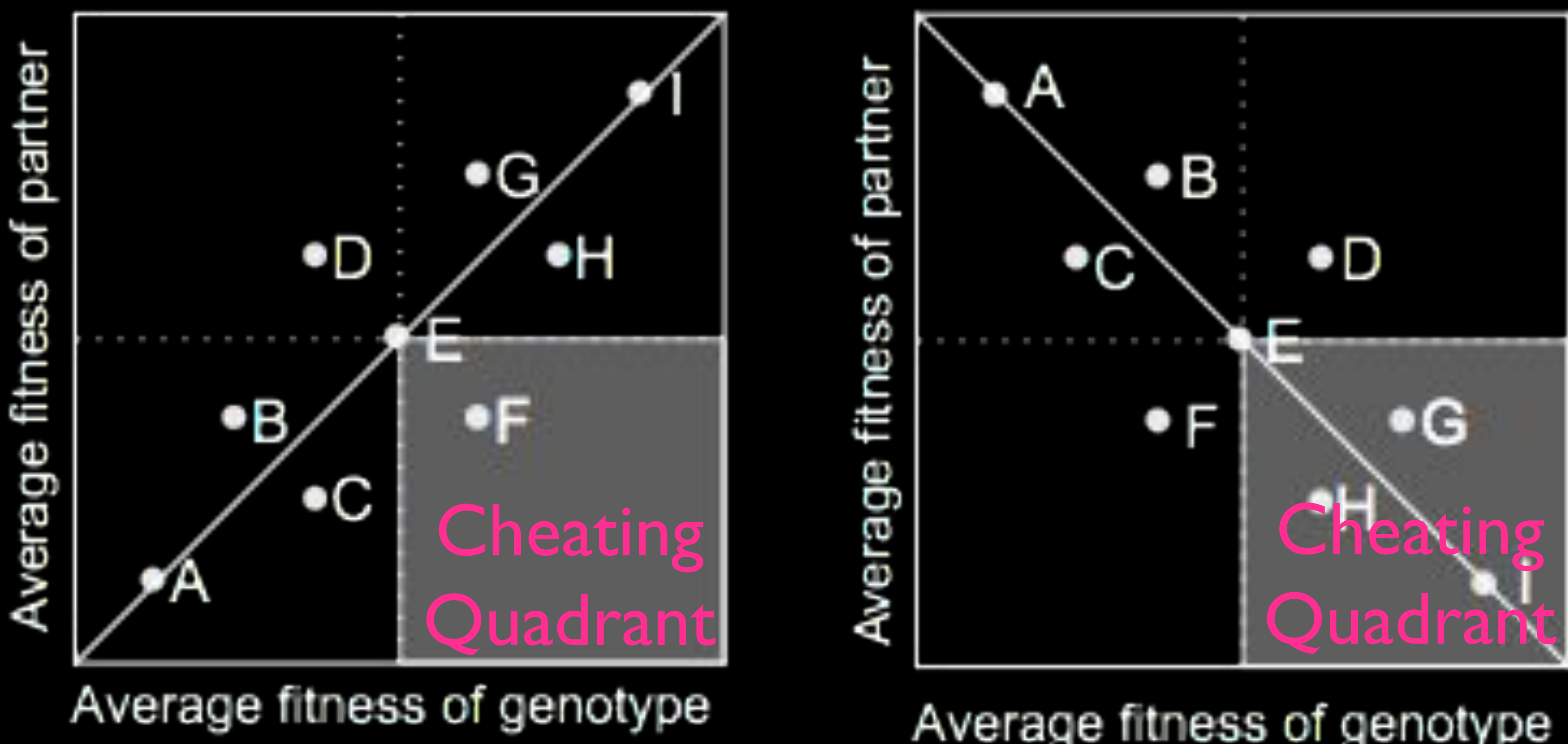
In terms of FITNESS, a cheater must have increased relative fitness while decreasing its partner's relative fitness

Otherwise, they can't threaten the mutualism!

Very few examples: hard to collect these data? Or perhaps cheating is rare?

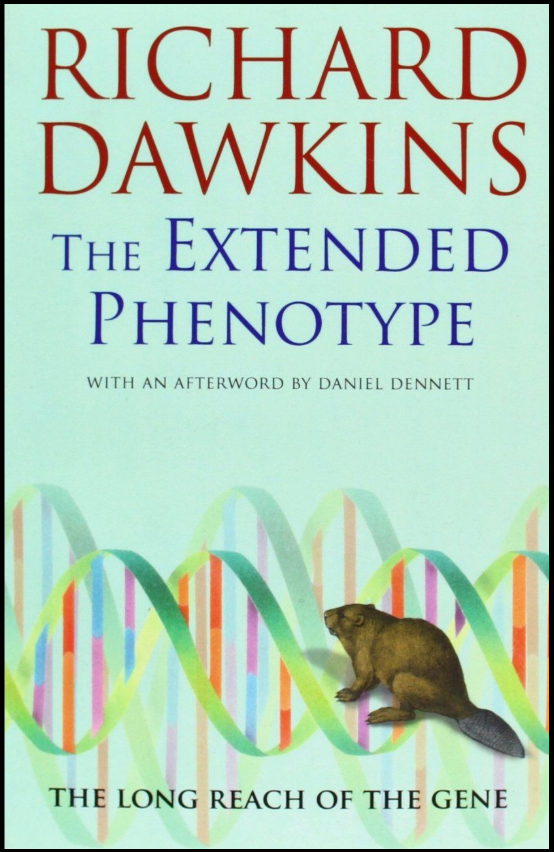
Jones et al. 2015

Cheaters can occur under fitness alignment or conflict



Jones et al. 2015

What determines conflict? Whose traits are they anyway?

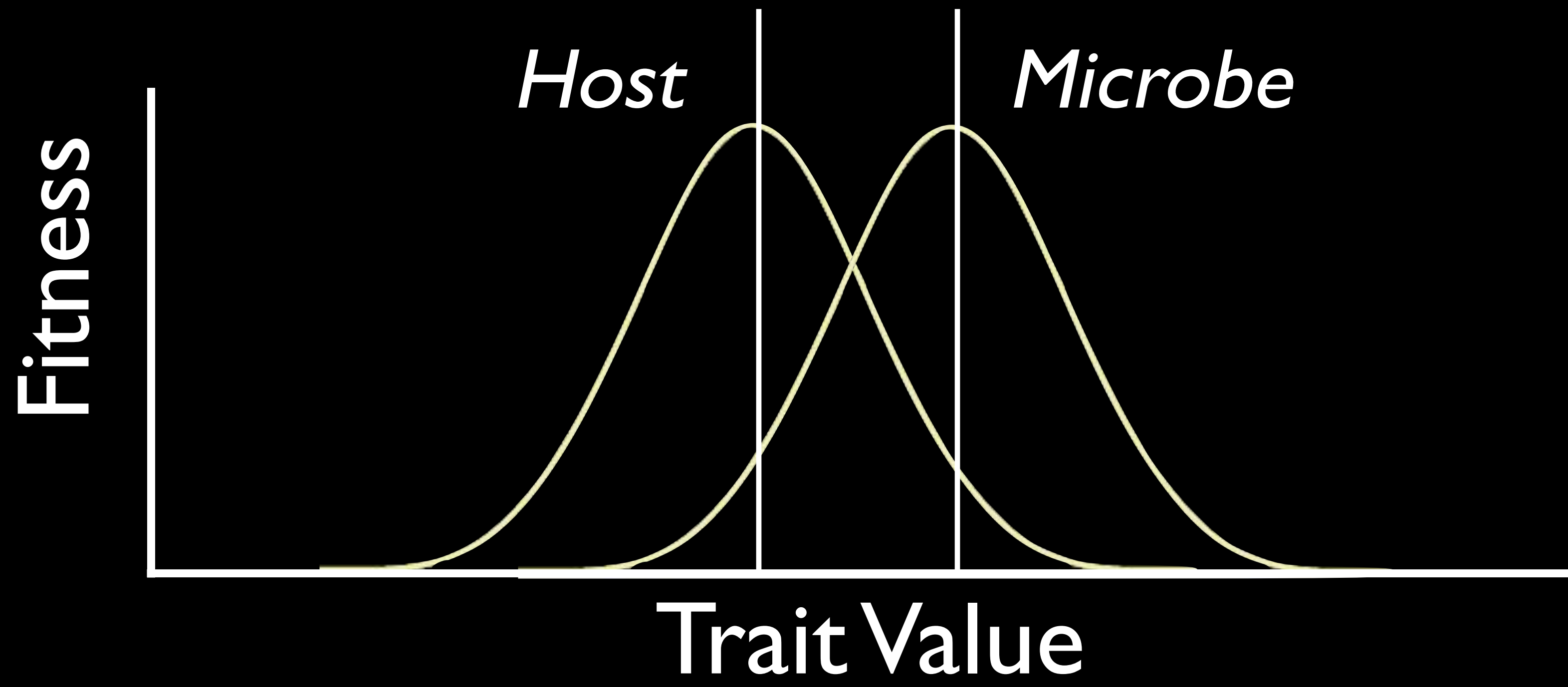


Two *fundamentally different* types of microbially-mediated host traits:

1) One-dimensional traits
2) Two-way exchange

One-dimensional Traits With Multi-genomic Basis

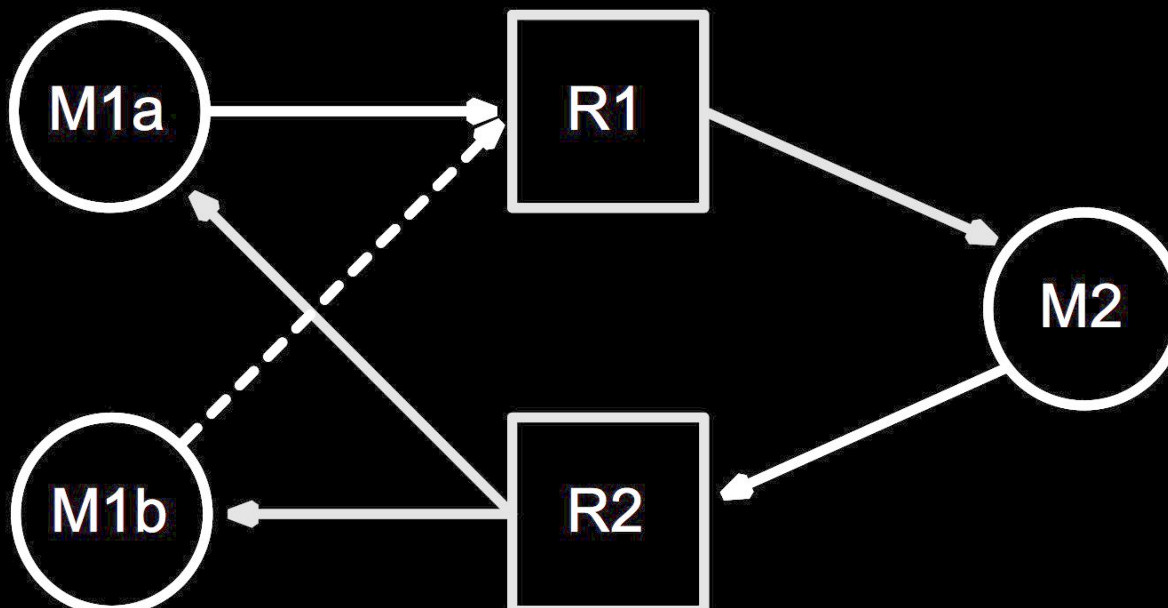
alignment *conflict* *alignment*



e.g., flowering time, root length, …

Pop gen coevol: Simonsen et al. 2020

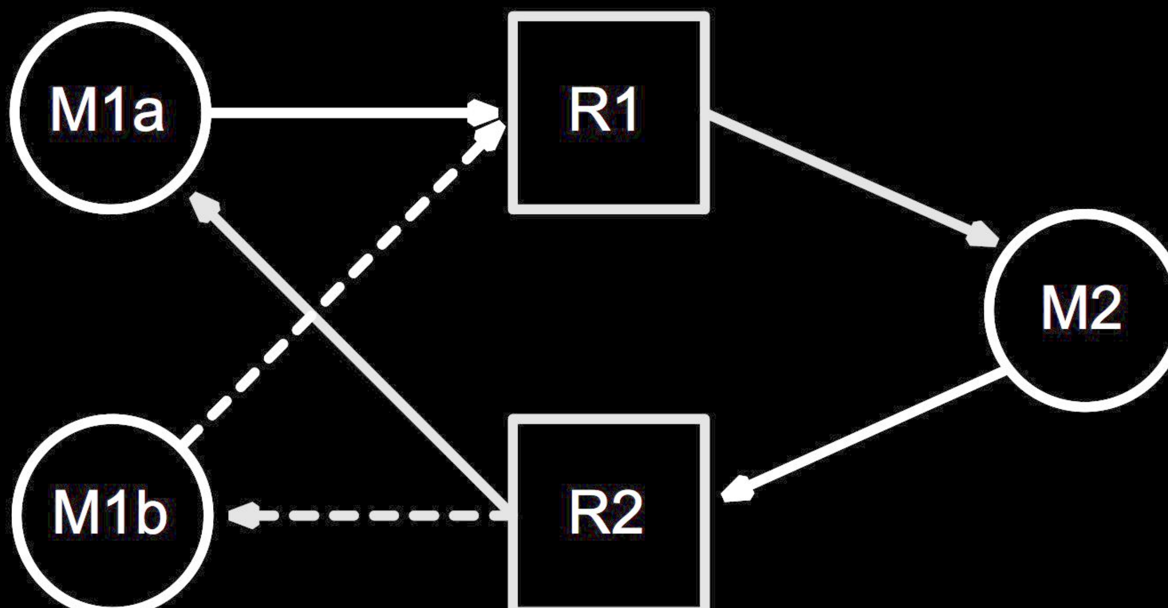
Two-way Exchange: Conflict



M1b benefits by producing less R1, which decreases fitness of partner M2

Two-way Exchange: Alignment

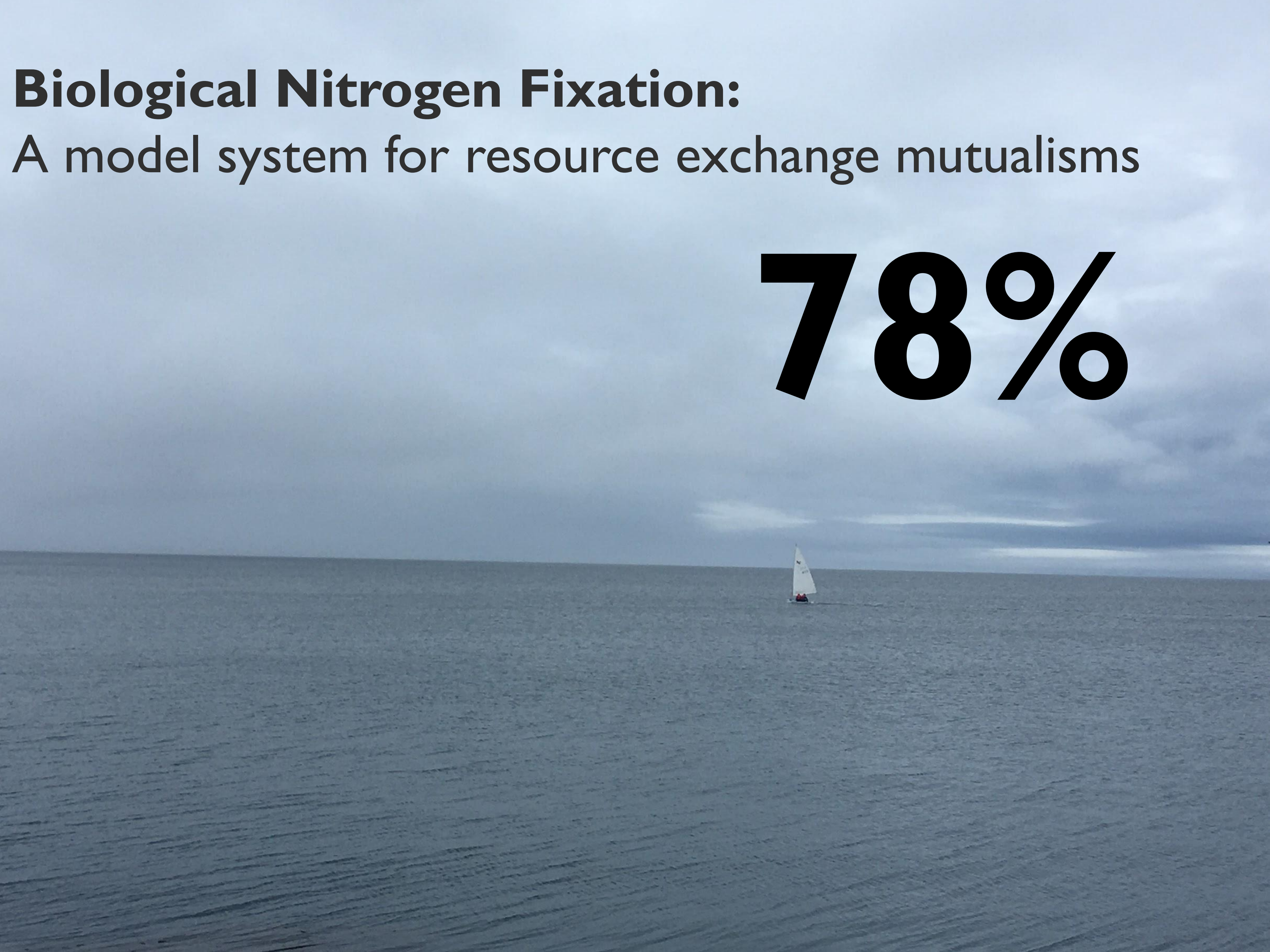
Giving less ≠ Cheating



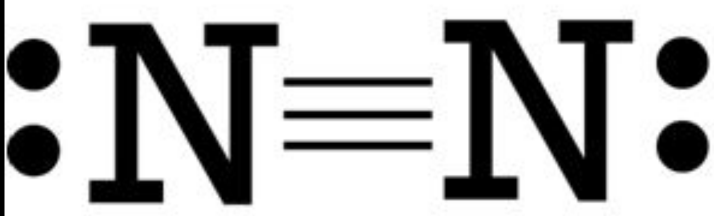
Potential mechanisms coupling R2 provision to R1 production: reciprocity, sanctions, partner fidelity feedback

Biological Nitrogen Fixation:

A model system for resource exchange mutualisms

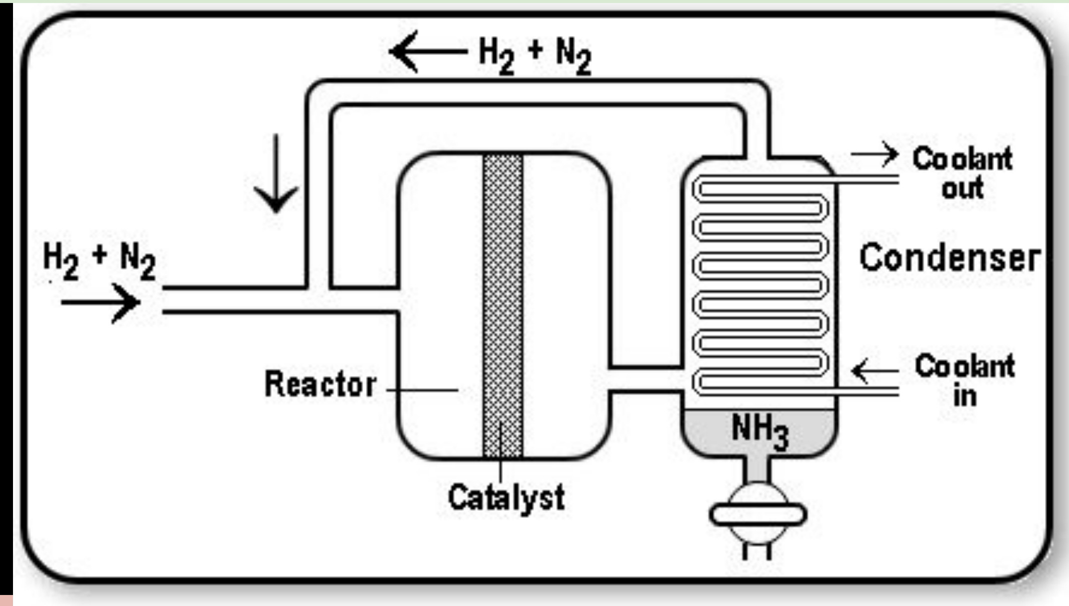


78%

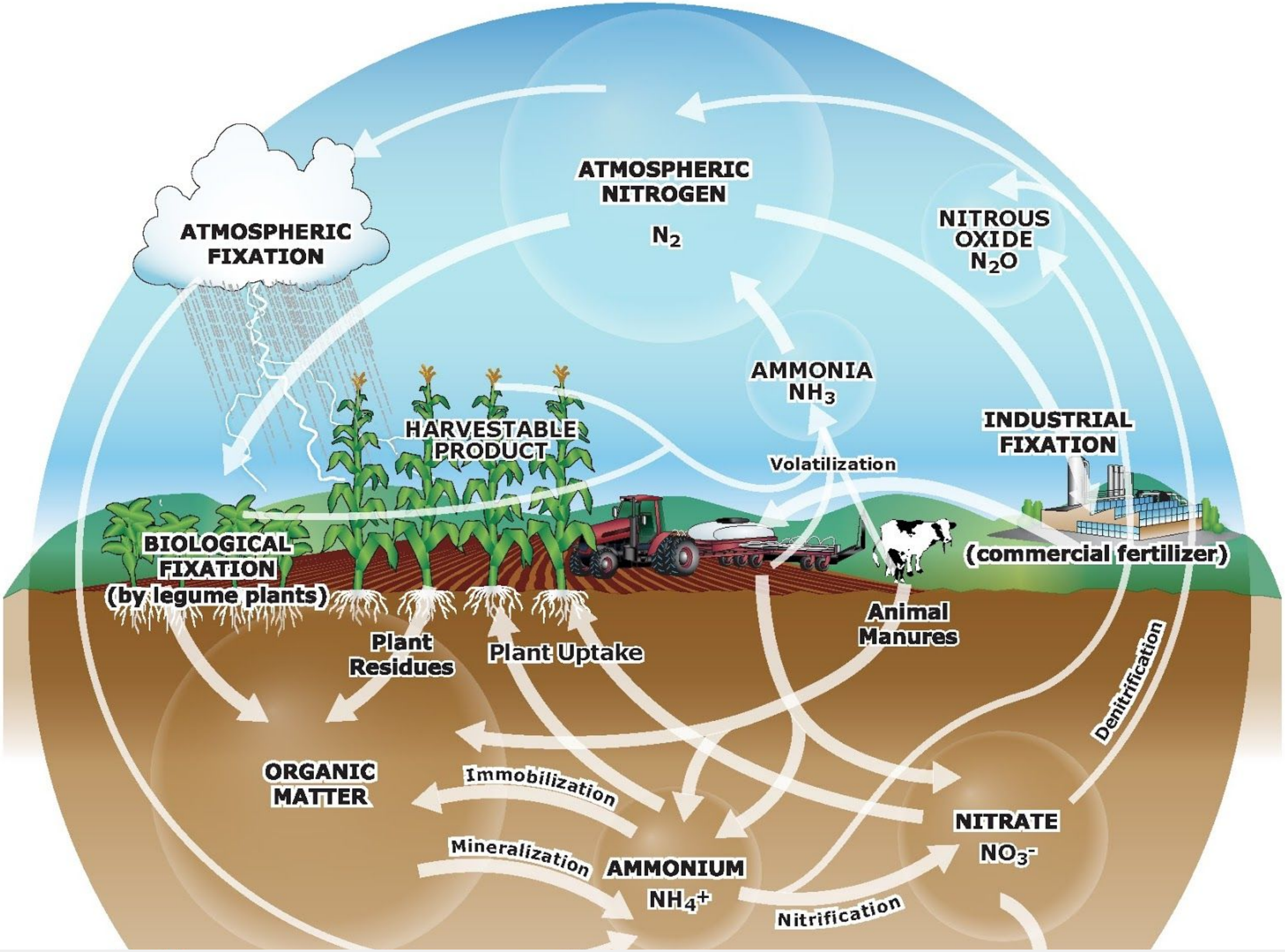


Lightning

Haber-Bosch Process

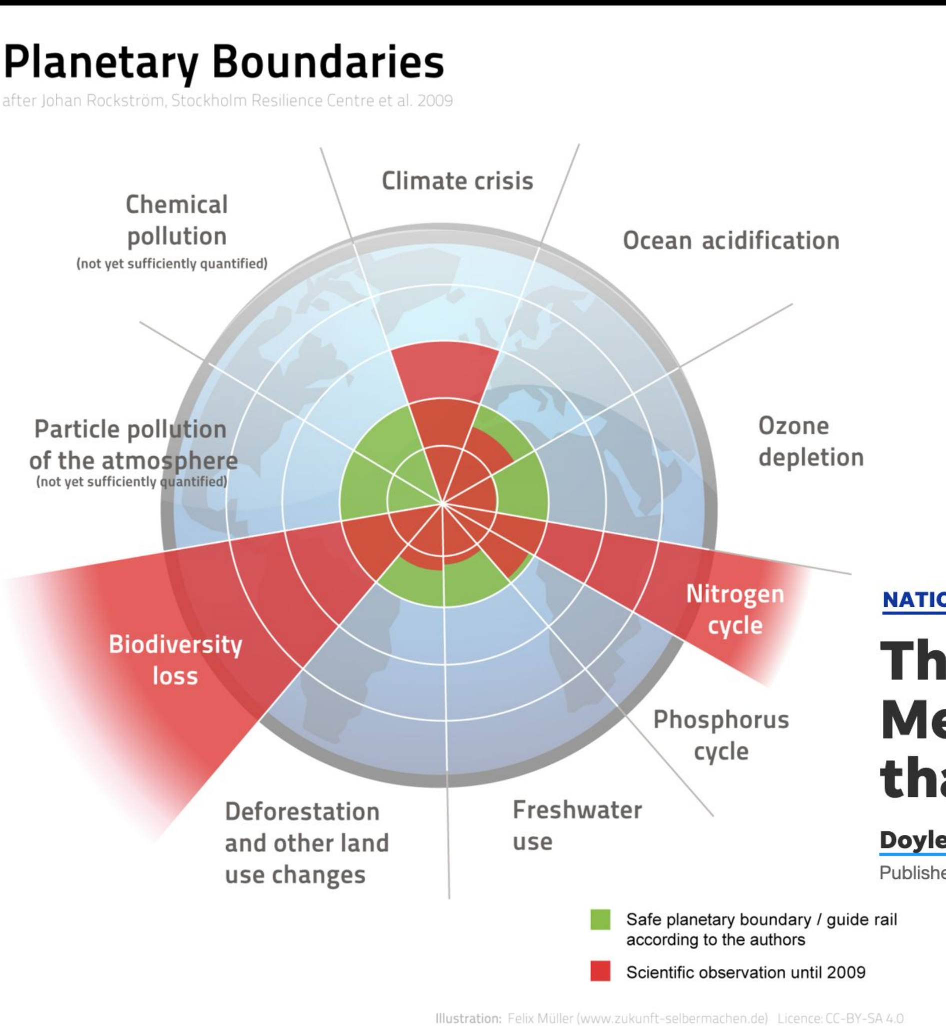


Biological N-Fixation



~195 megatonnes/year N converted

We are beyond the N planetary boundary



NATION

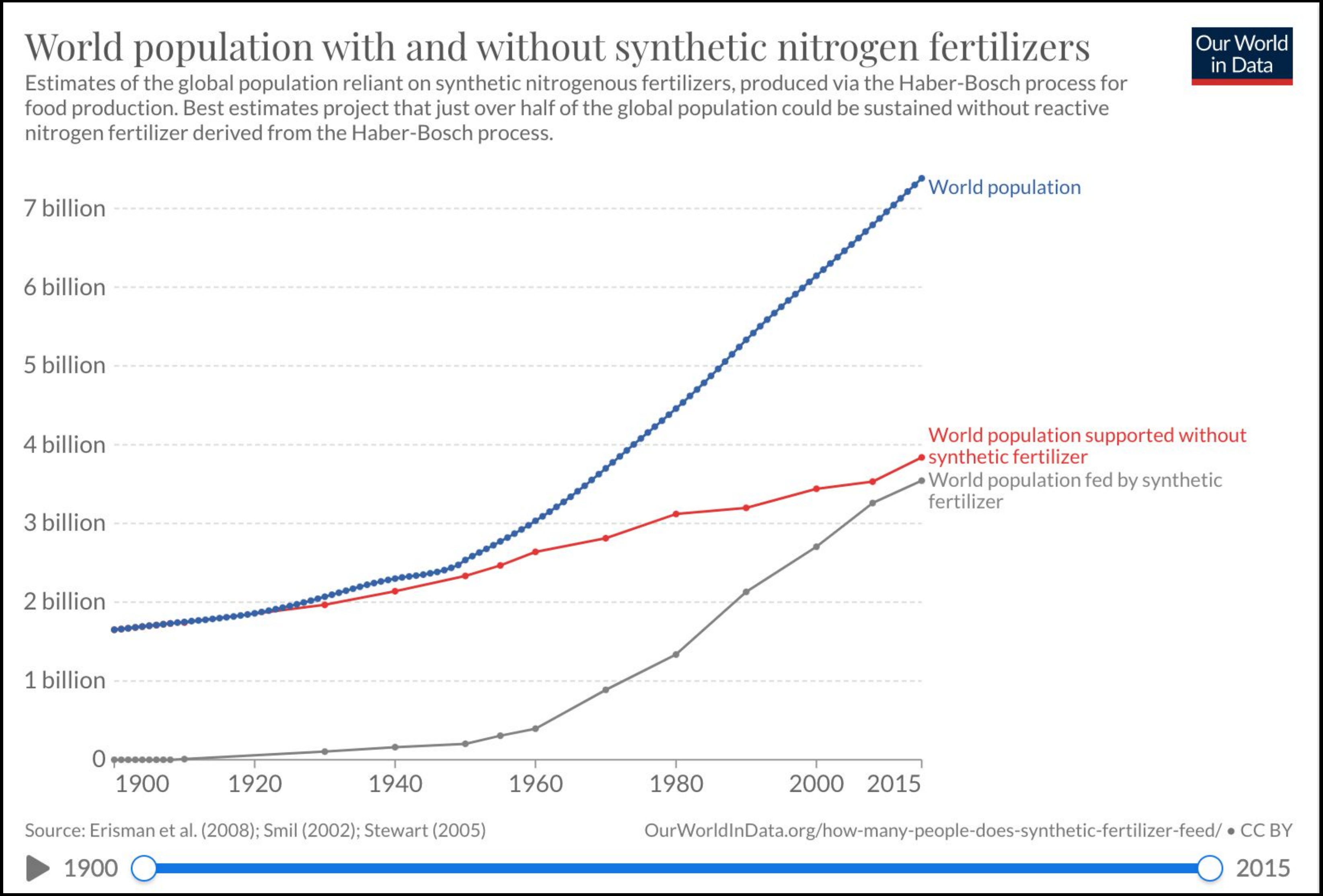
There's a 'dead zone' in the Gulf of Mexico this summer that's bigger than Connecticut

Doyle Rice USA TODAY

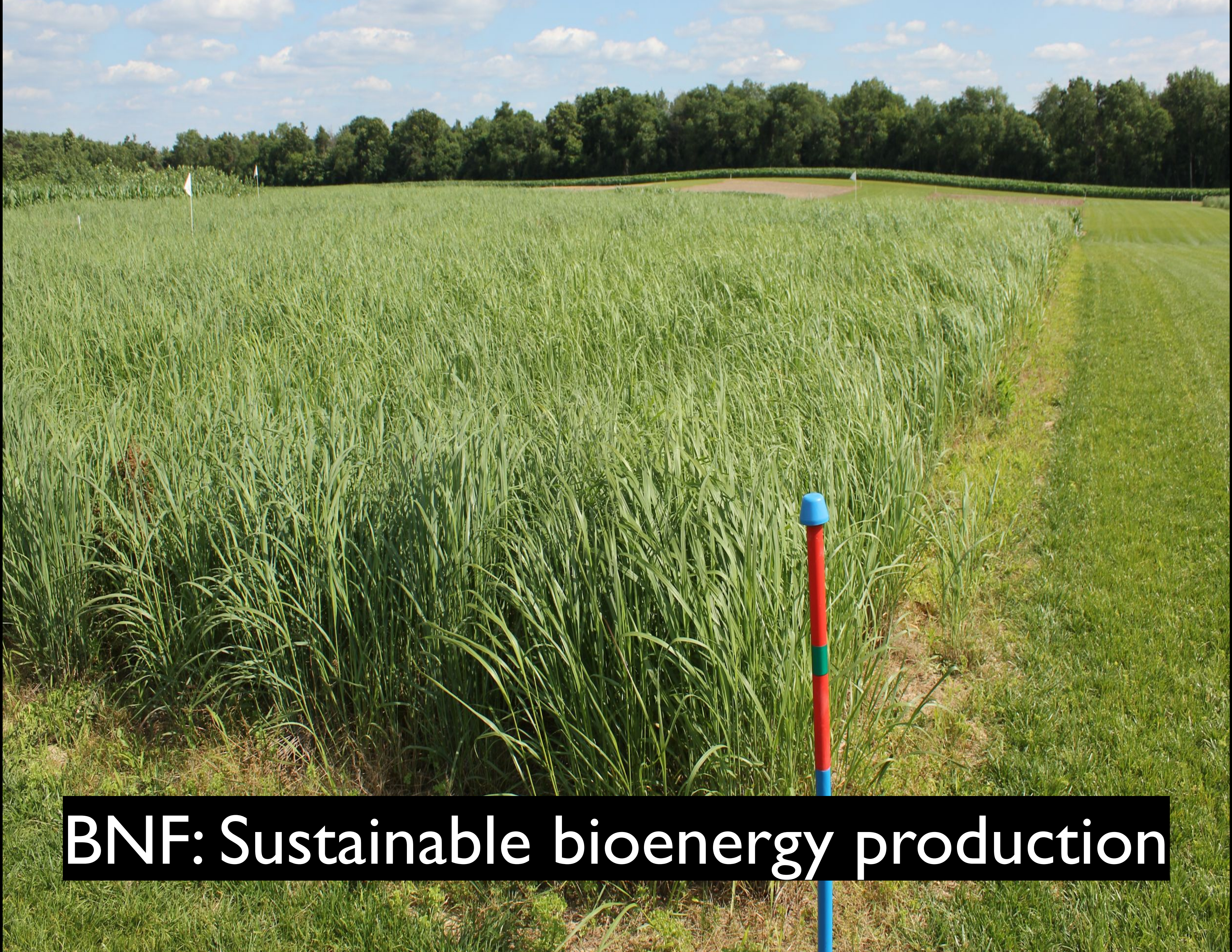
Published 7:34 p.m. ET Aug. 3, 2021

Steffen et al. 2015.

40-50% of Humans due to Haber-Bosch

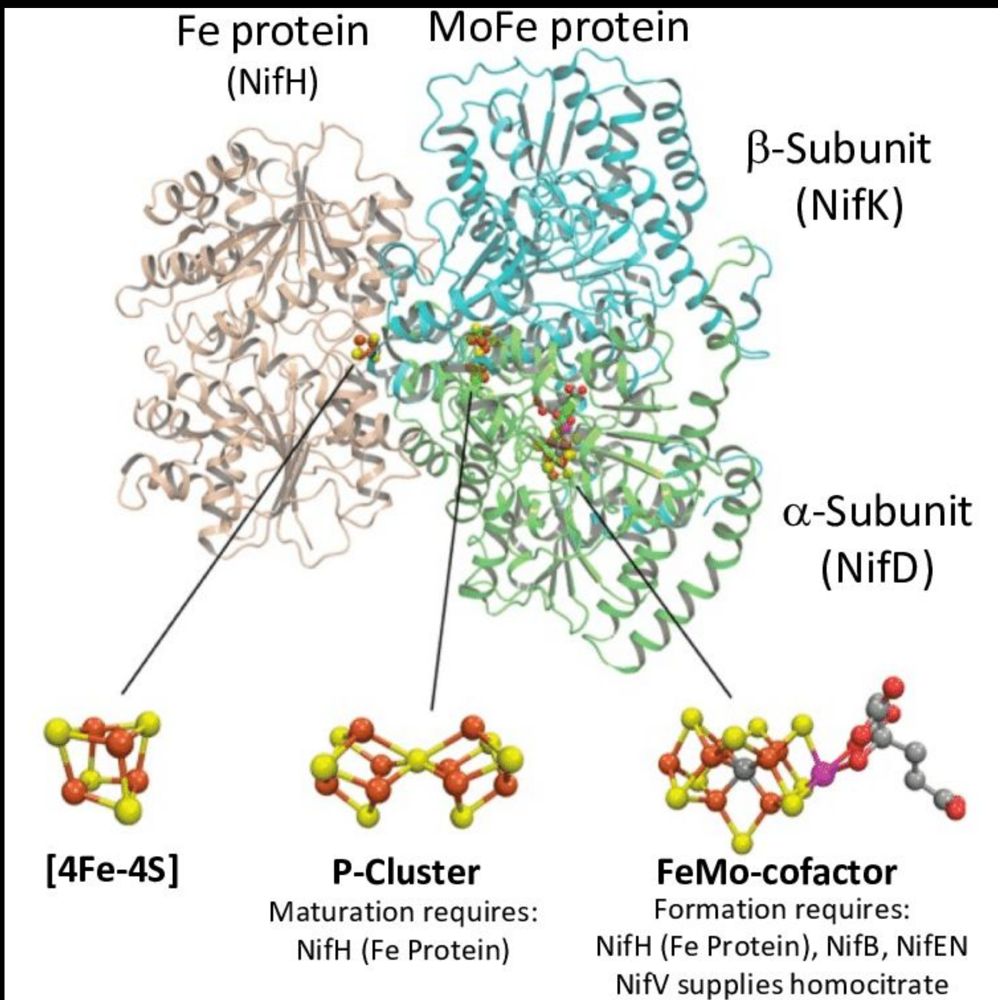


BNF: ~30% of protein in human diet


BNF: Sustainable bioenergy production

Nitrogenase: One and Done

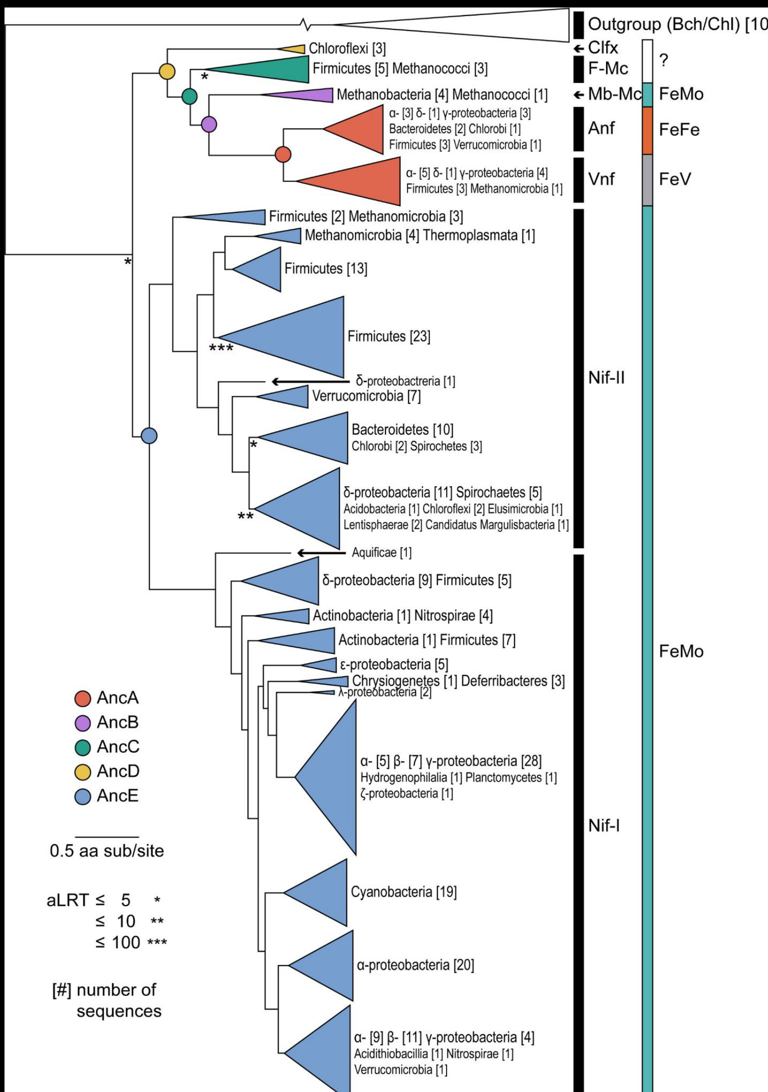
$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,Pi$$

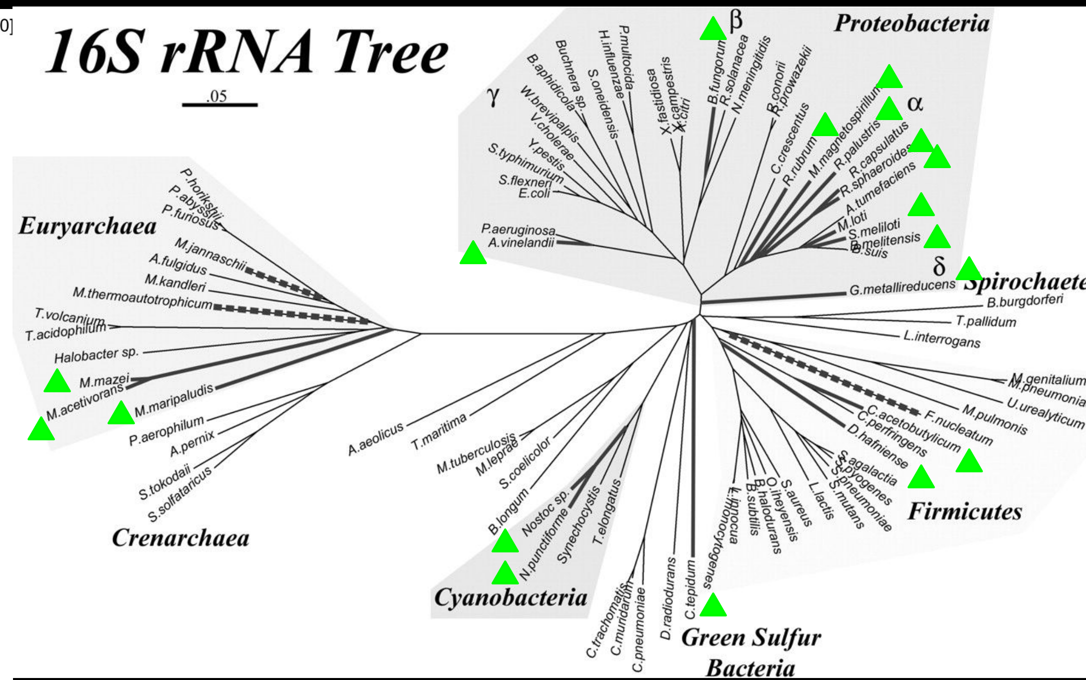


- 3 forms (Fe-Fe, Fe-Vn, Fe-Mo)
- Highly conserved proteins: *nifHDKEN*
- No other enzymes known that can reduce dinitrogen (the "superoxide dependent nitrogenase" SDN system was concluded to not exist: MacKellar et al. 2016)
- Irreversibly inhibited by O_2
- Lots of effort trying to engineer into plants...

Jimenez-Vincente et al. 2018

Phylogenetic Distribution of N-fixation

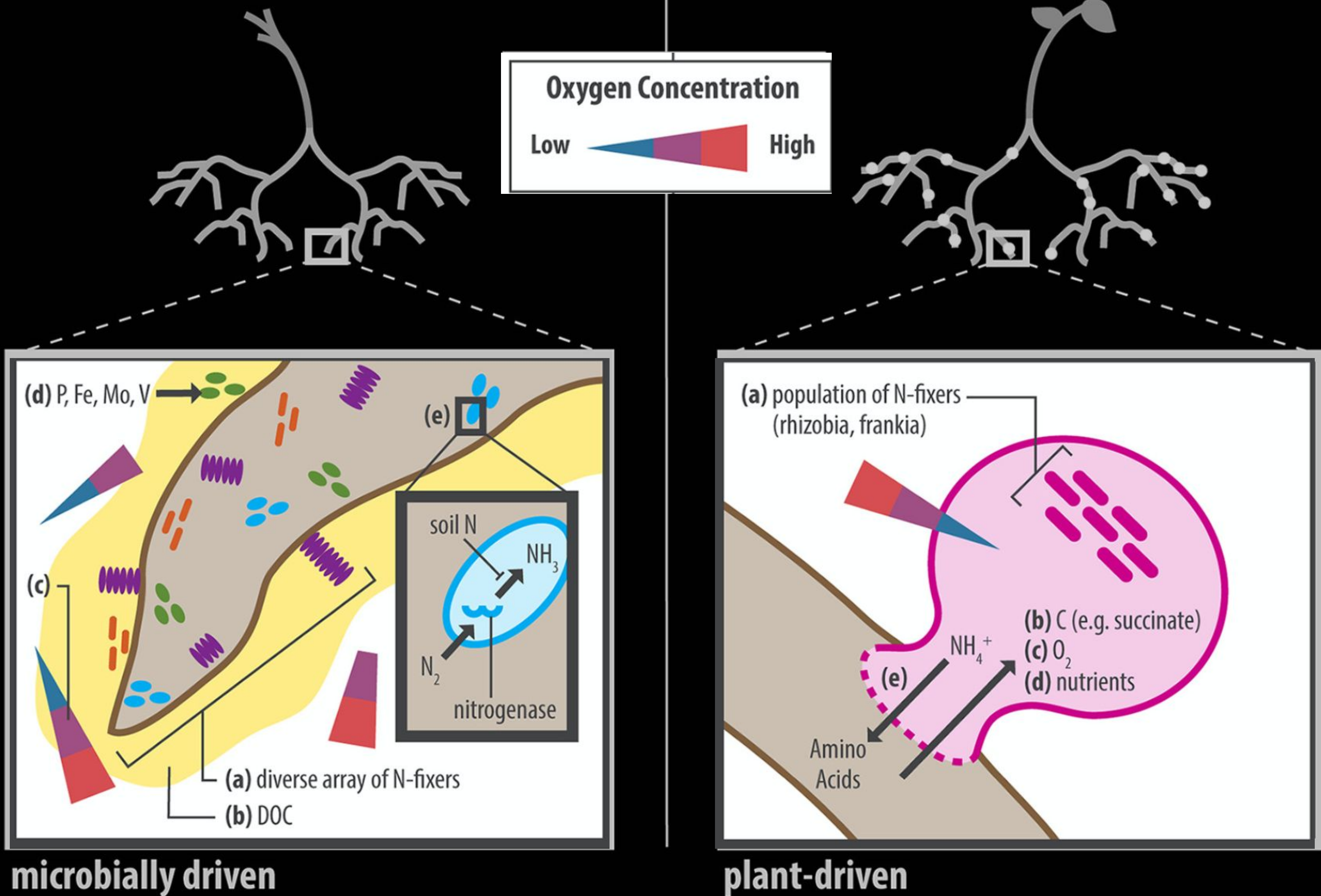




- Fe-Mo may have evolved first
- Paraphyletic with ancient HGT

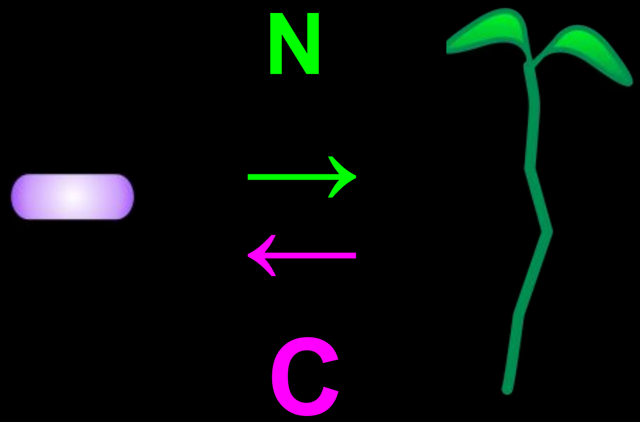
Raymond et al. 2004, Garcia et al. 2020

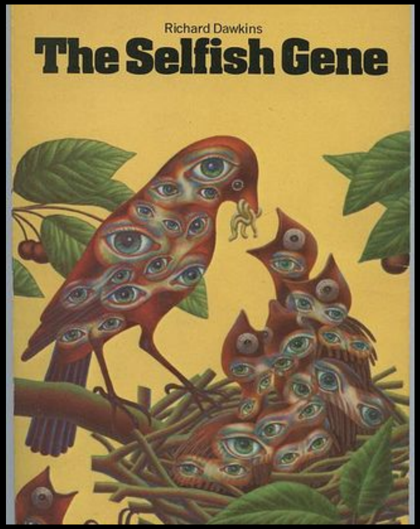
Associative vs Symbiotic



Smercina et al. 2019 "To Fix or Not to Fix"

N-fixation *seems* like costly cooperation



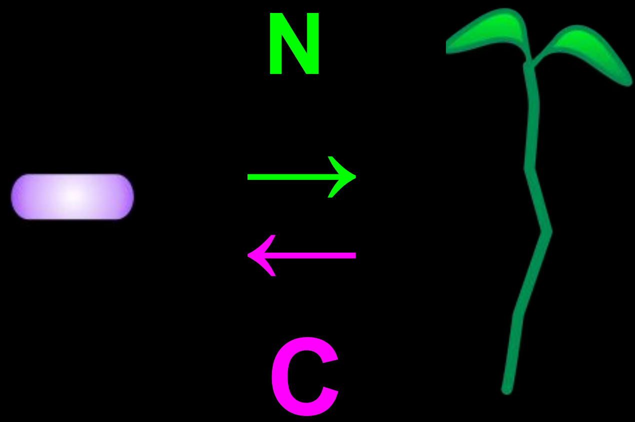


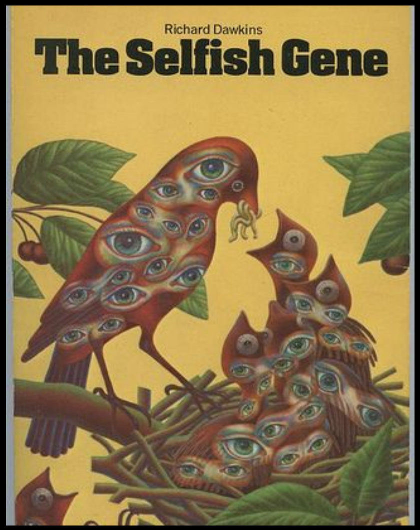
Partner choice: (Robinson 1969, Heath & Tiffin 2009, Sachs et al 2010)

Sanctions / reciprocity: (Kiers et al. 2003, Simms et al. 2006, Oono et al. 2009, 2010)

→ *Selection on the symbiont to fix nitrogen at the expense of its own potential fitness*

Possible ***cost-free*** reasons for N-fixation





1 -- Microbes fix nitrogen because they need nitrogen and plants sometimes benefit

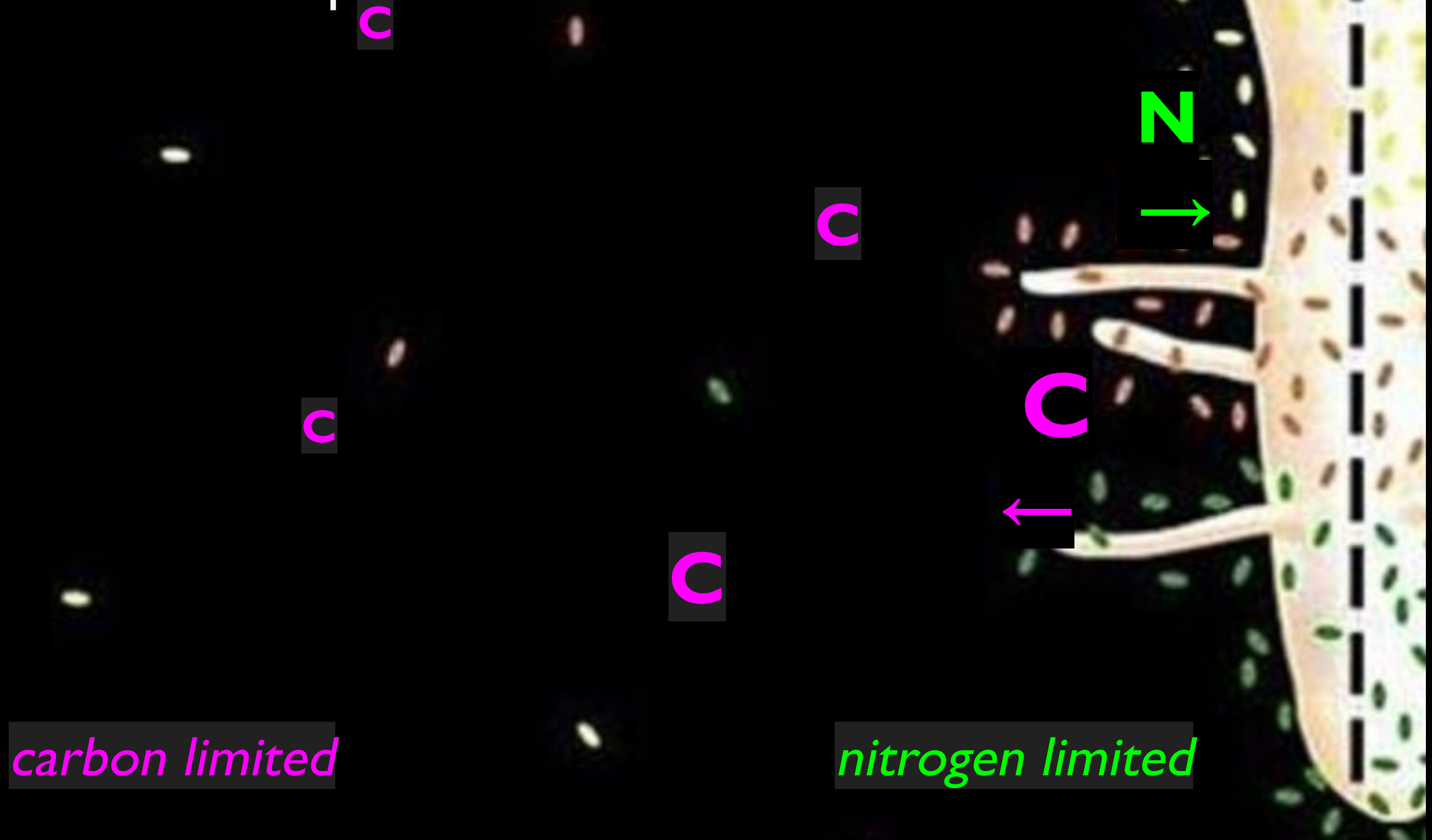
2 -- Microbes fix nitrogen because plants give them luxury carbon

3 -- Nitrogen fixation doesn't happen as part of an exchange of carbon for nitrogen, but rather the plant is using the microbe as an organelle in metabolic dependency

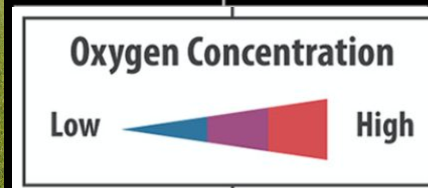
Free-living N-fixers

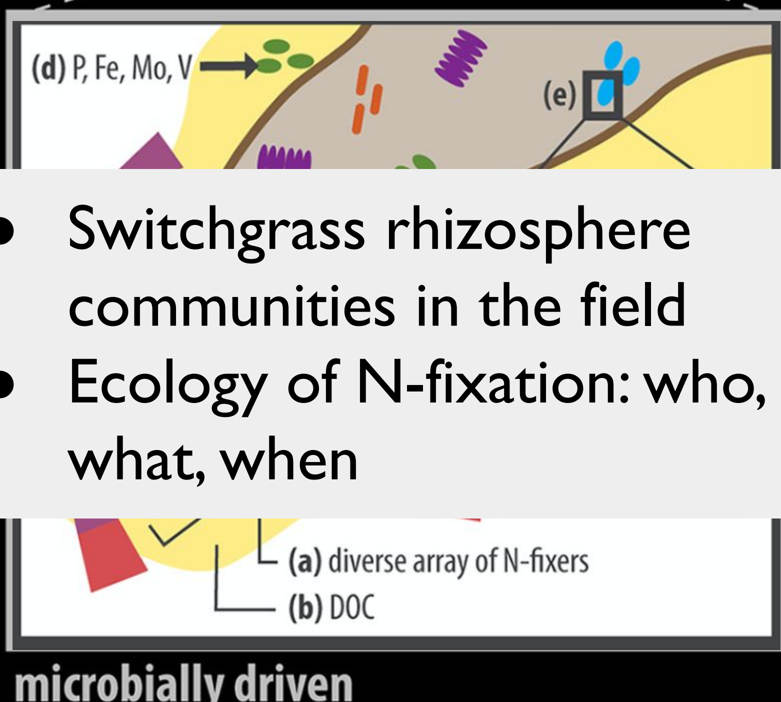
~ Screening *sensu* Archetti et al. 2011

<u>Passive trade</u>: results in byproduct benefit for plant

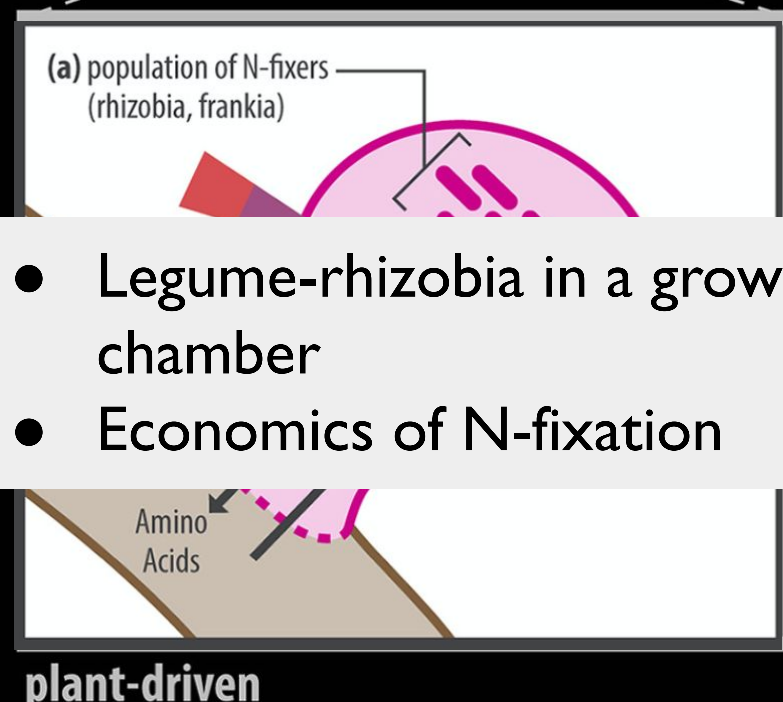


Associative vs Symbiotic





- Switchgrass rhizosphere communities in the field
- Ecology of N-fixation: who, what, when

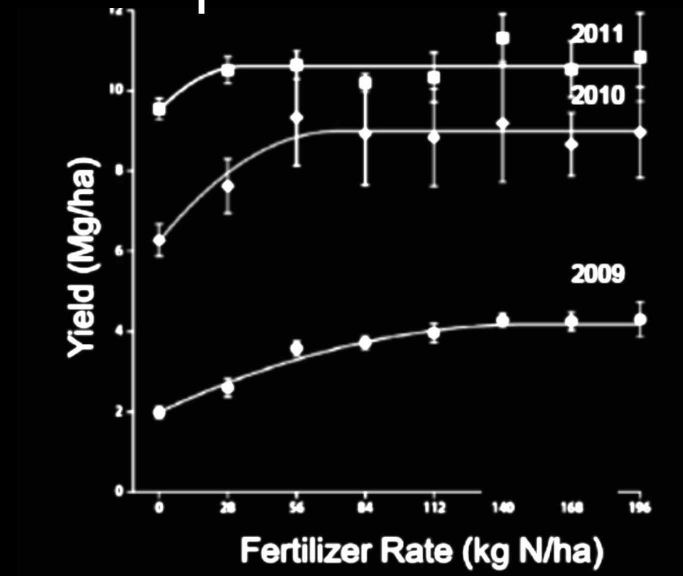


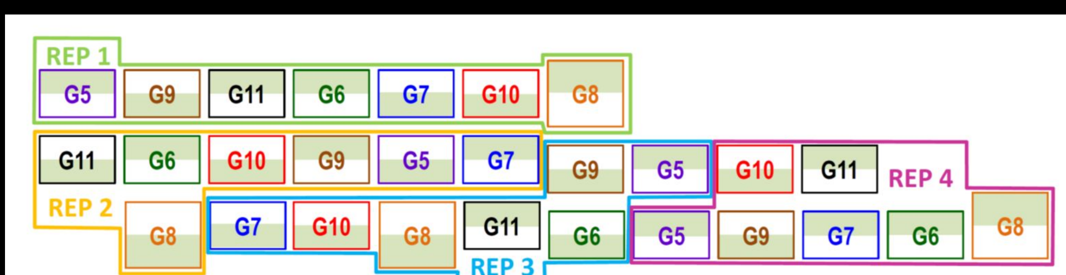
- Legume-rhizobia in a growth chamber
- Economics of N-fixation

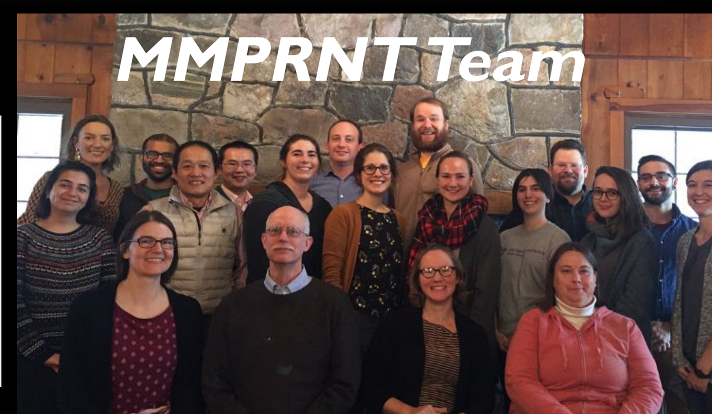
Smercina et al. 2019 "To Fix or Not to Fix"

Great Lakes Bioenergy Research Center
Switchgrass on marginal lands

Response to Fertilizer

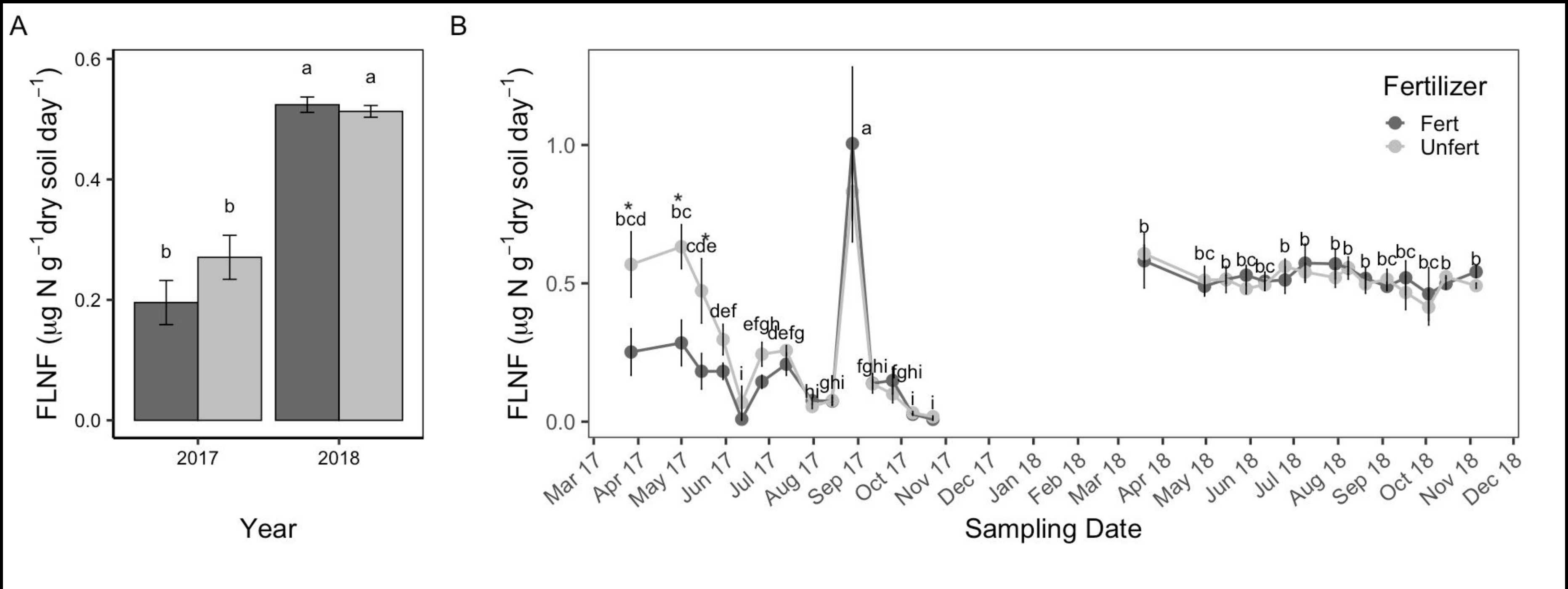






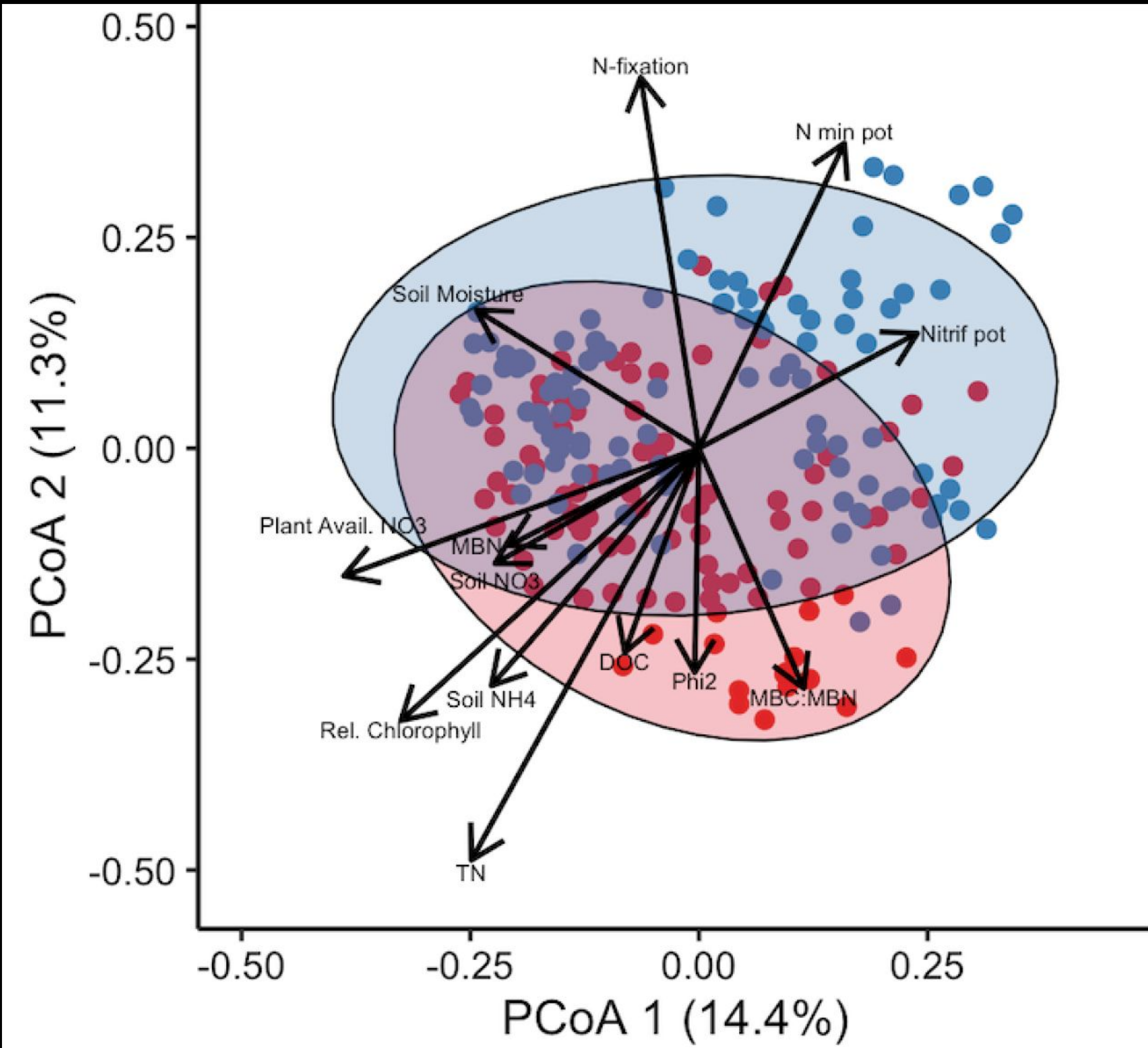
Rhizosphere N-fixation potential:

Varies by year, within year, not by fertilization



Smercina et al. in submission

nifH Communities Diverse & Variable

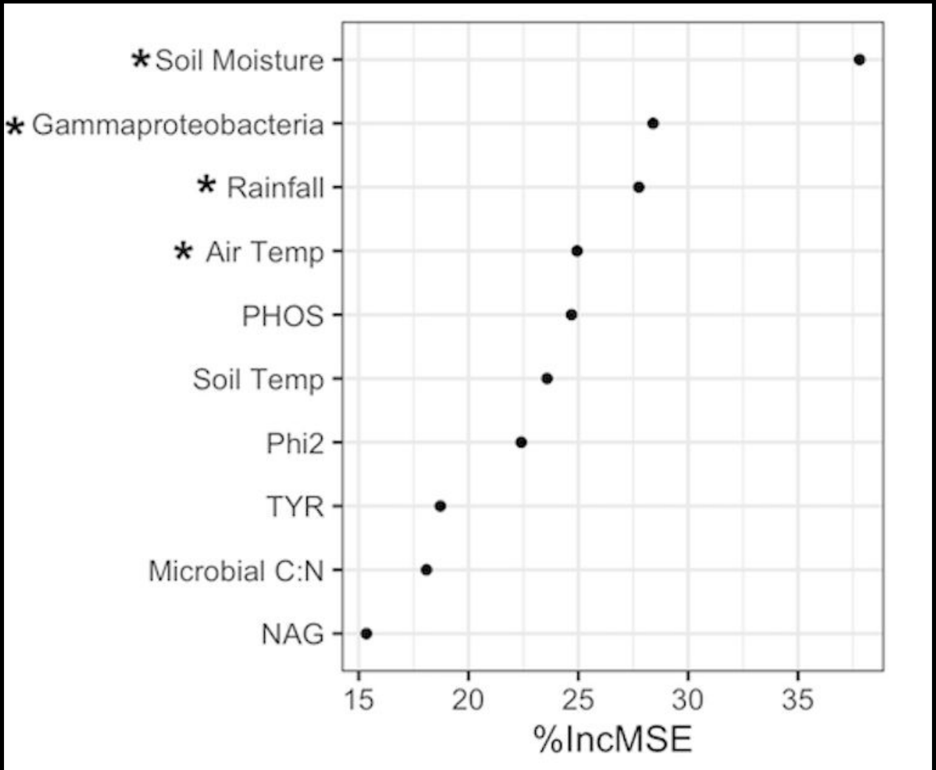


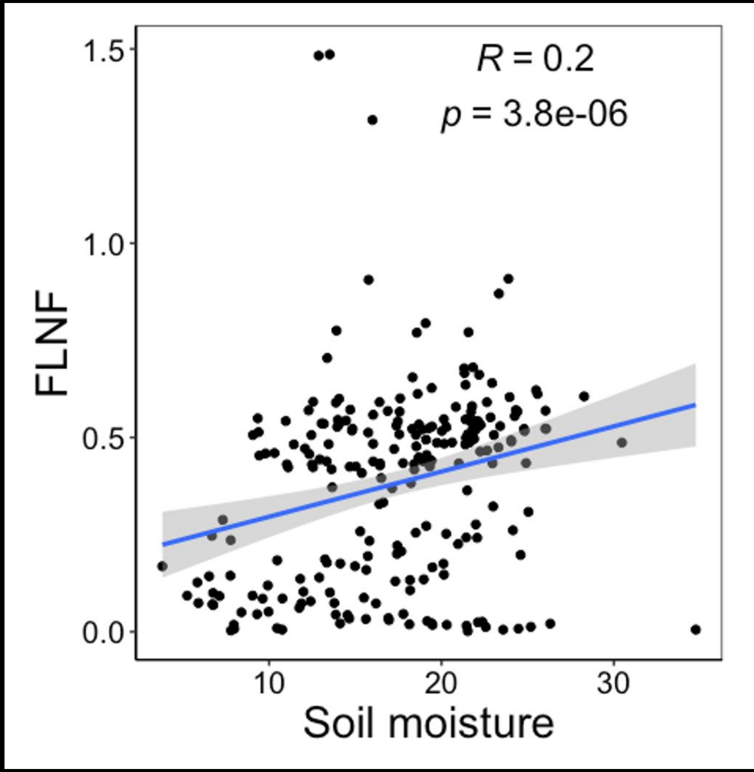
Top Indicator Taxa:
Bradyrhizobium (10)
Desulfovibrionales
Methylococcaceae
Paenibacillaceae (2)
Burkholderiaceae (2)
Rhodobacteraceae
Ectothiorhodospiracea
e

Sample Date: $R^2 = 0.0095$, $p = 0.0009$	**Year:** $R^2 = 0.0541$, $p = 0.0001$
Field Rep: $R^2 = 0.0638$, $p = 0.0001$	**Fertilizer** $R^2 = 0.0151$, $p = 0.0001$

Smercina et al. in submission

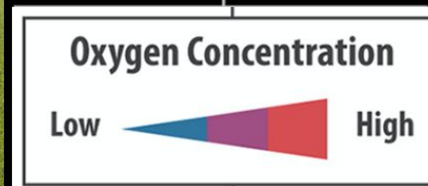
FLNF Variation ~ soil moisture!





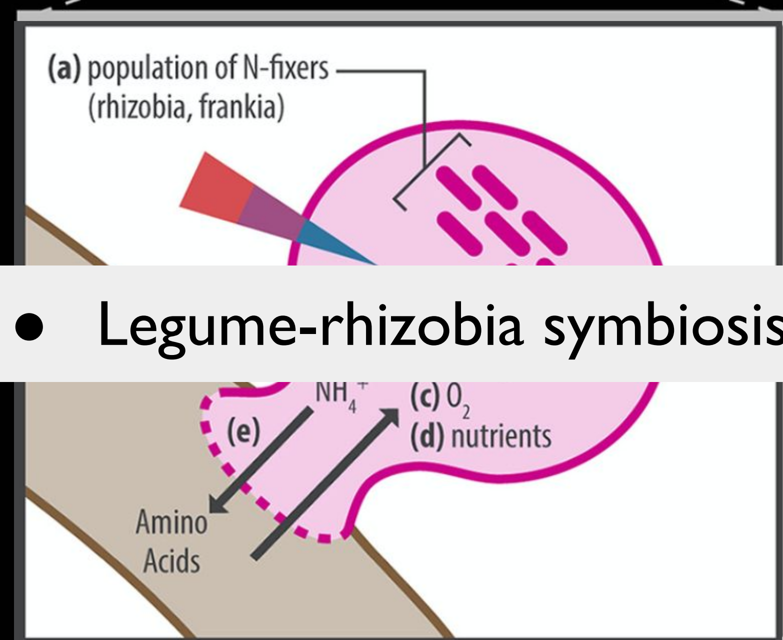
Smercina et al. in submission

Associative vs Symbiotic



- Switchgrass rhizosphere communities: diverse, many taxa correlate with N-fixation rates. *We have some in culture…*
- Soil moisture big driver, see also Roley et al. 2018

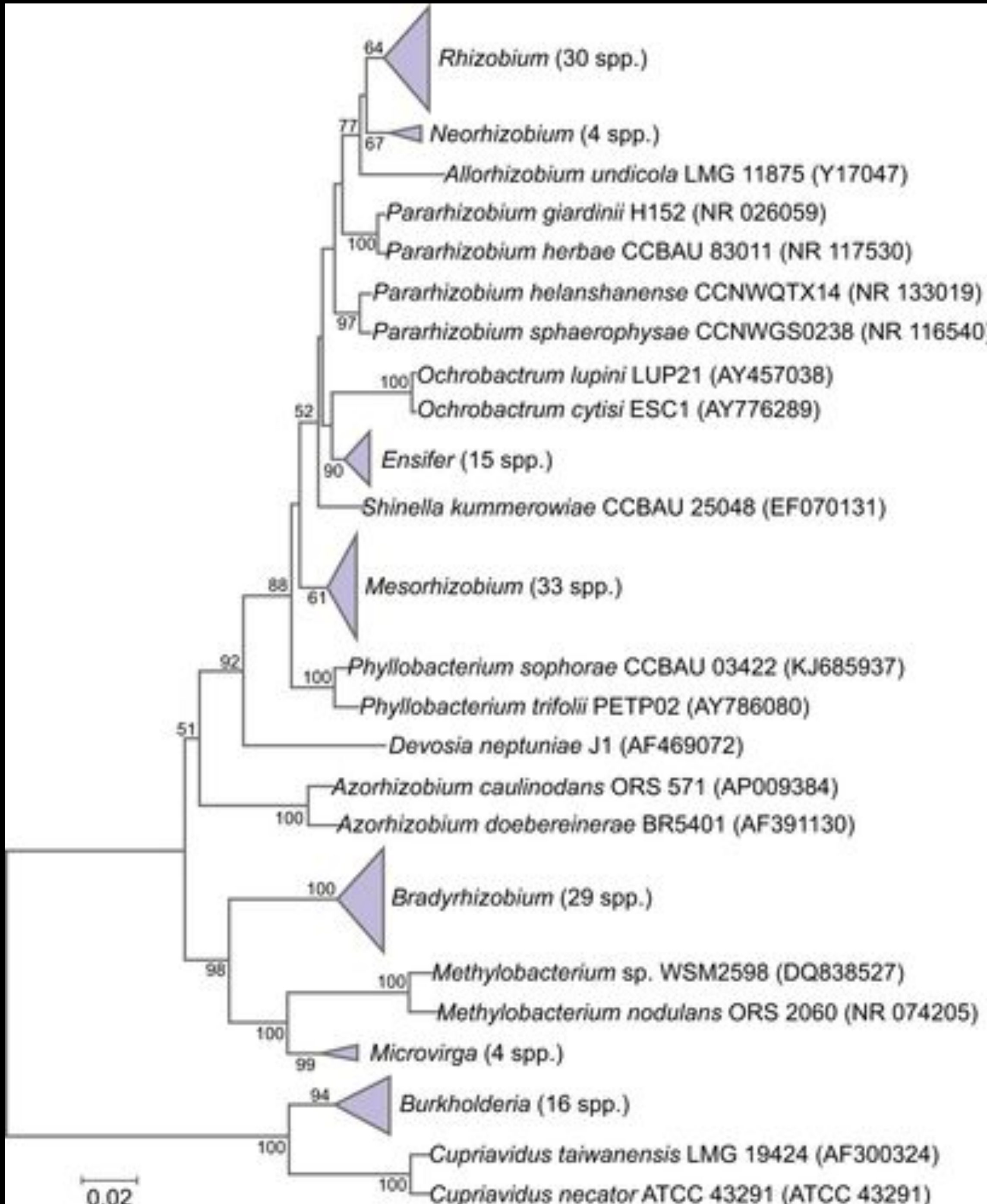
microbially driven



- Legume-rhizobia symbiosis

plant-driven

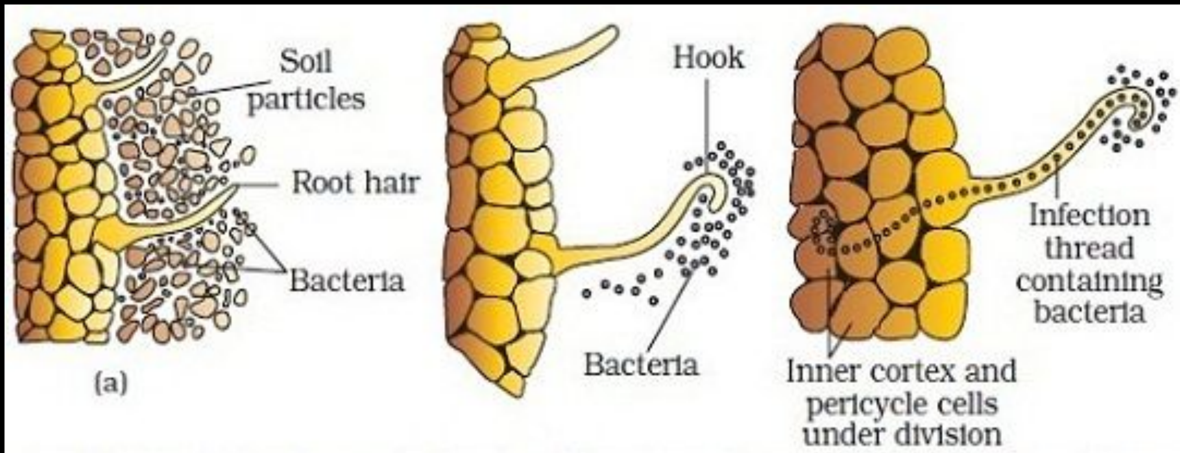
Smercina et al. 2019 "To Fix or Not to Fix"

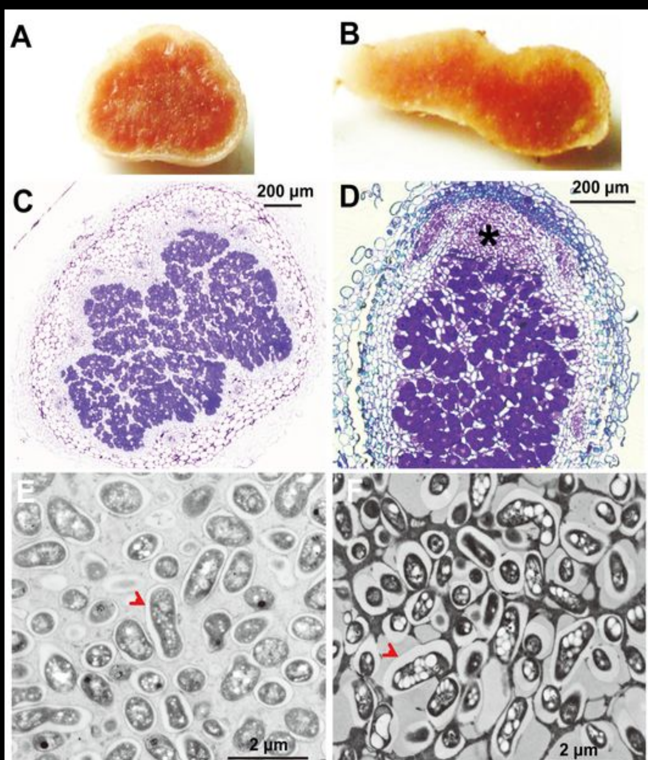


Rhizobia

Phylogenetically diverse (paraphyletic)

Beta-proteobacteria arose through HGT of nod factor genes from Alpha-proteo



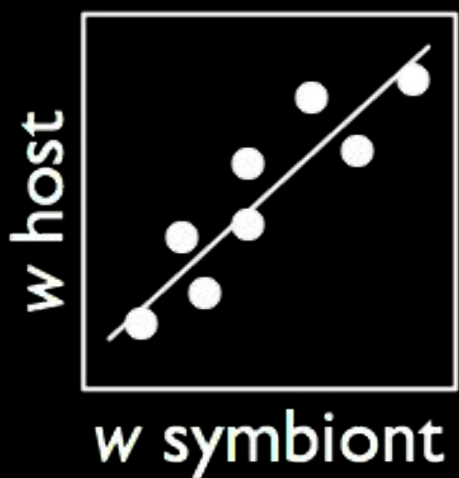


Horizontal transmission

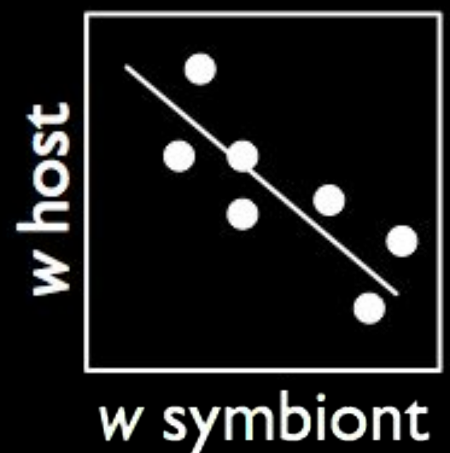
Nodule is essentially clonal

Bacteria are endosymbiotic

Is there fitness conflict?

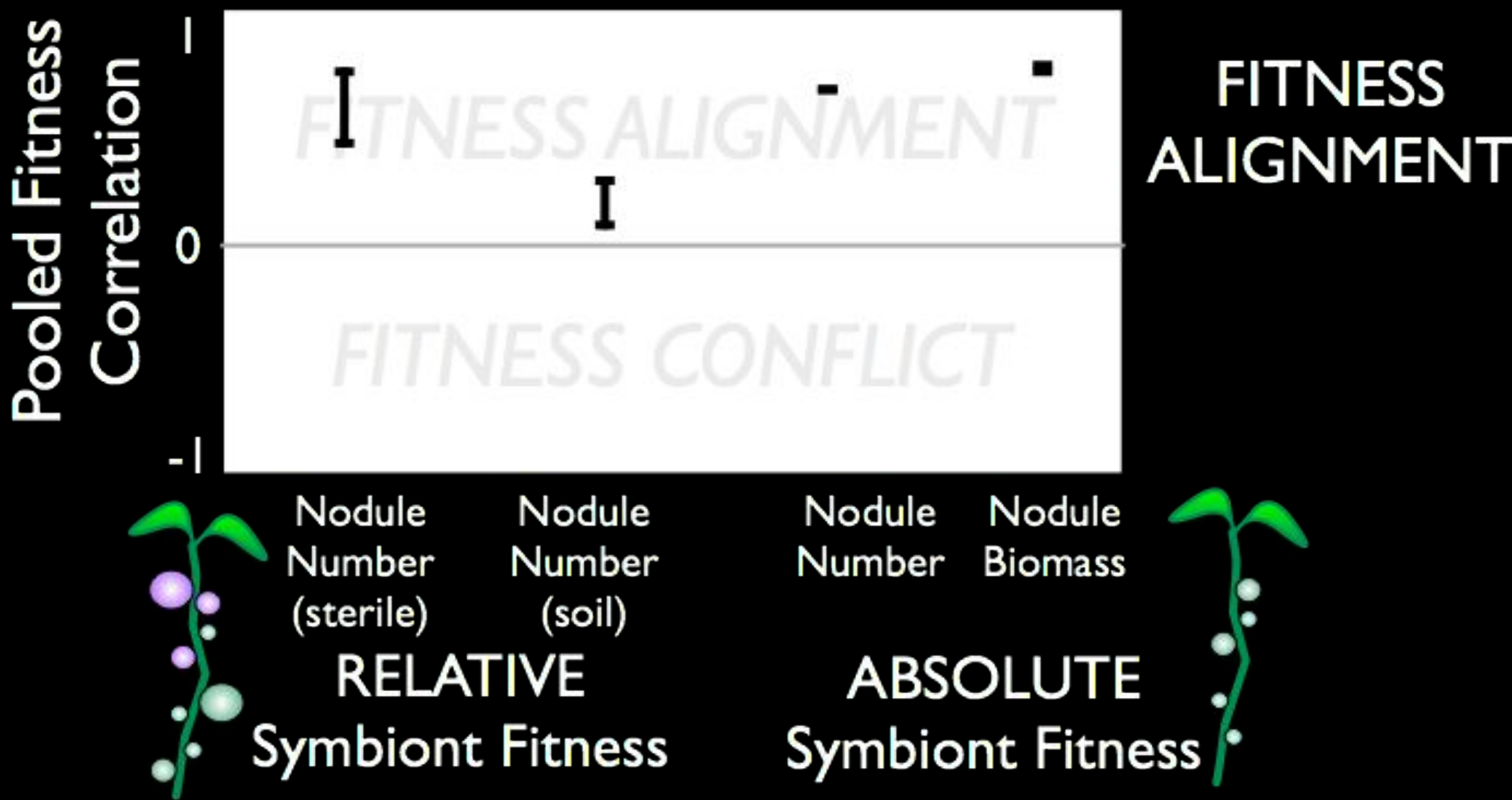


Fitness Alignment



Fitness Conflict

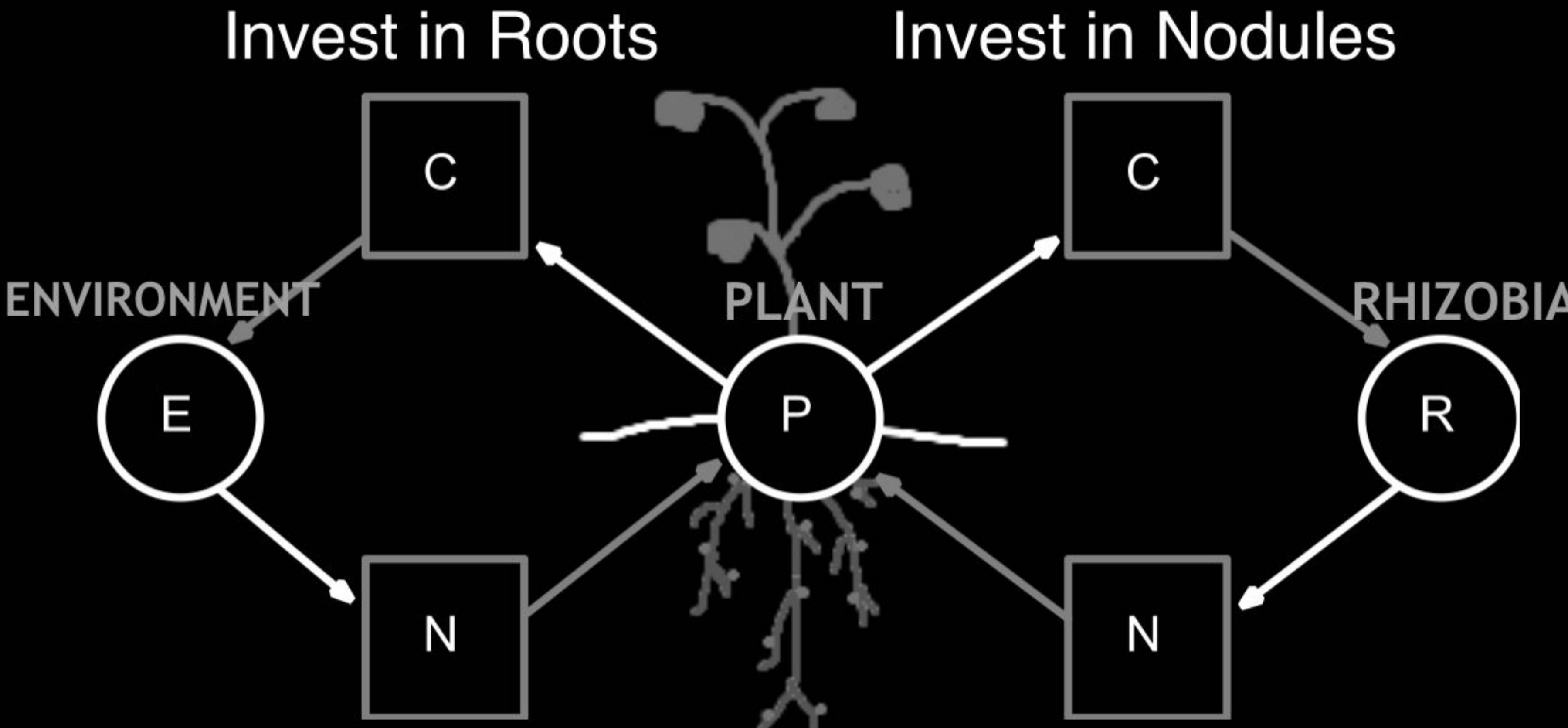
Rhizobia-legume fitness alignment



Ineffective rhizobia are “defective”, not “defectors”

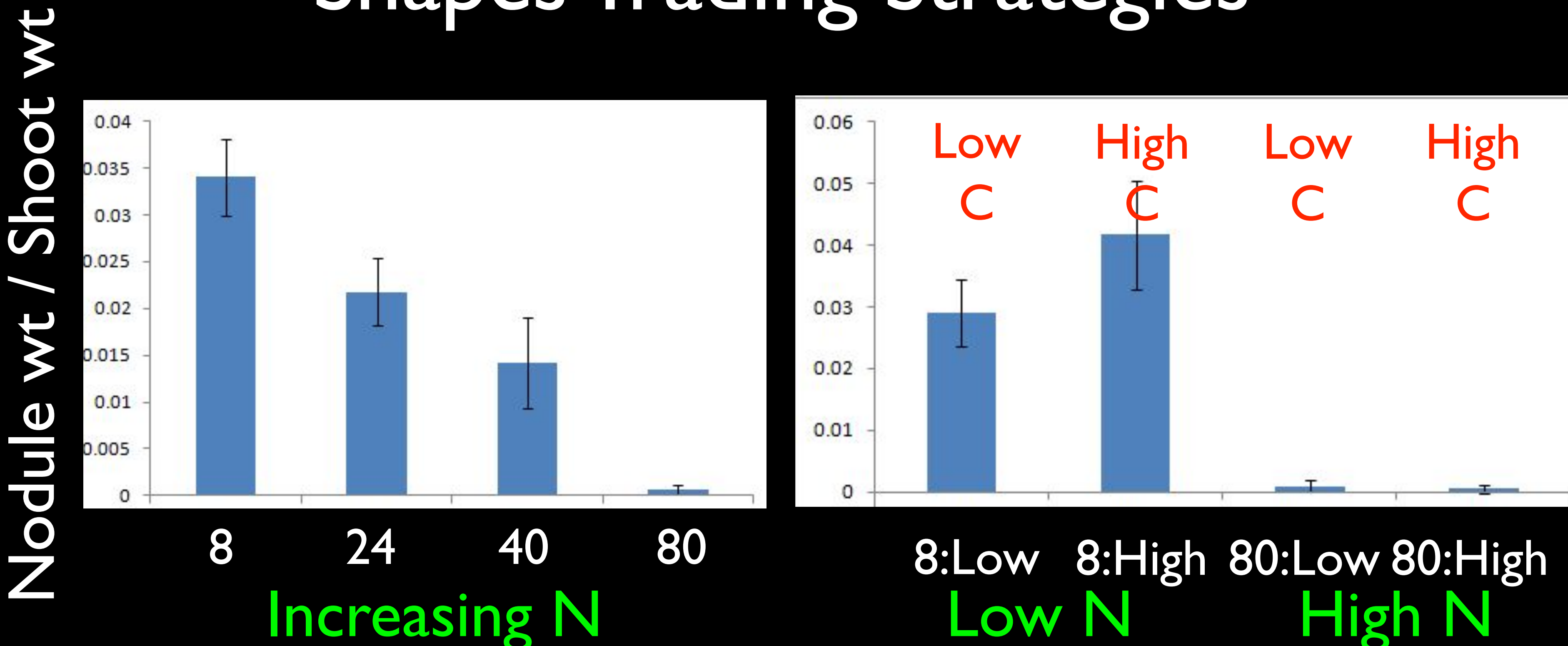
Friesen (2012) New Phytologist

Quantifying Mutualism via Nutrient Manipulation



Colleen Friel, Teresa Clark, Shachar-Hill, Grman

Resource Environment Shapes Trading Strategies



- Plants give less to nodules when N is available
- Plants pay more for N when C is cheap

Friel et al. (2019)

Economic model predicts when trade benefits both partners

$g_P = \min [Y_{NP}(f_{NP} + X/(P \times T)), Y_{CP}(f_{CP} - X/P)]$

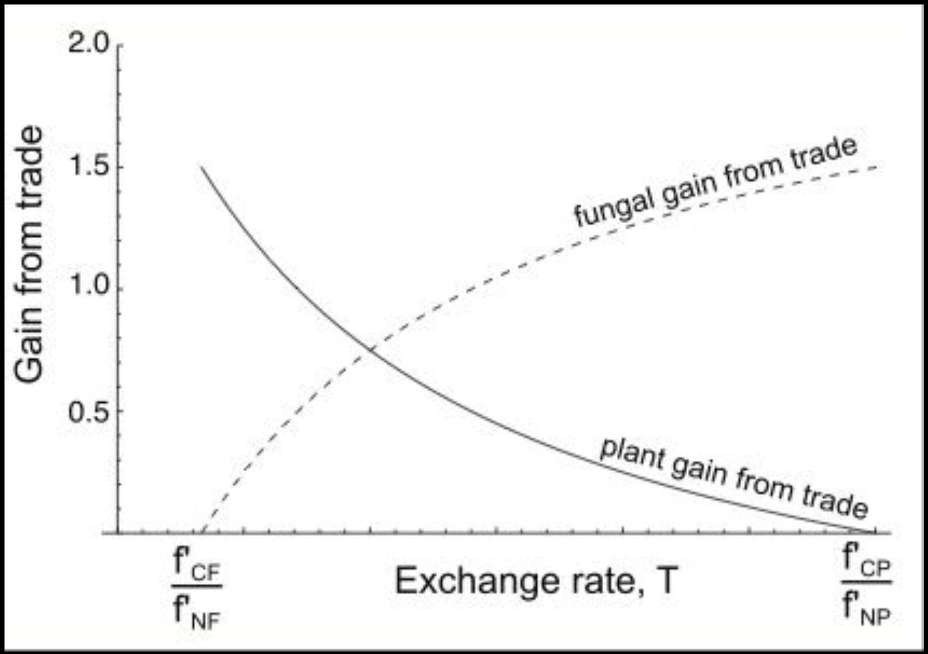
$g_R = \min [Y_{NR}(f_{NR} - X/(R \times T)), Y_{CR}(f_{CR} + X/R)]$

P: plant biomass; R: nodule biomass; X: amount exchanged; T: trade ratio; Y: yield; f: production

Leibeg's Law of the Minimum: growth determined by limiting resource

Grman et al. 2012; Clark et al. 2019

Leibeg's Law of the Minimum with 2 resources
Comparative Advantage: both gain from trade



__Not__ trading is costly, since your excess resource can't be used for growth!

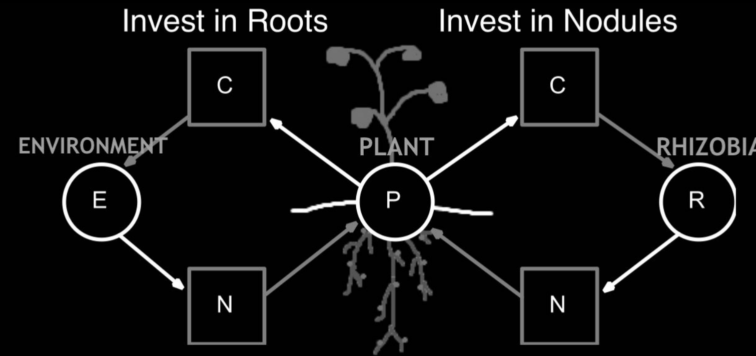
(Schwartz & Hoeksema 1998, De Mazancourt & Schwartz 2010, Grman et al. 2012)

Empirical Measurements

Parameter	Interpretation (units)
A_{NP}	Plant allocation to nutrient uptake ([g C root] [g C plant]$^{-1}$)
Y_{NP}	Plant carbon yield per unit nutrient ([g plant C m^{-2}] [g plant N^{-1} m^{-2}])
f'_{NP}	Plant nutrient uptake rate ([g N m^{-2}][s^{-1}] [g root C m^{-2}]$^{-1}$)
V_{CP}	Plant max carbon uptake (photosynthesis) rate ([g C fixed m^{-2}][s^{-1}][g shoot C m^{-2}]$^{-1}$)
K_{CP}	Plant carbon half-saturation constant ([g C] [μmol m^{-2} s^{-1}])
A_{CF}	Fungal allocation to carbon uptake ([g C intra-radical][g C fungus]$^{-1}$)
Y_{NF}	Fungus carbon yield per unit nutrient ([g fungal C m^{-2}][g fungal N^{-1} m^{-2}])
f'_{NF}	Fungus nutrient uptake rate ([g N m^{-2}][s^{-1}][g extraradical C m^{-2}]$^{-1}$)
V_{CF}	Fungus max carbon uptake rate ([g C m^{-2}][s^{-1}][g intraradical C m^{-2}]$^{-1}$)
K_{CF}	Fungus carbon half-saturation constant ([g C][μmol m^{-2} s^{-1}])
P	Plant biomass ([g plant C] m^{-2})
F	Fungal biomass ([g fungus C] m^{-2})
N	Nutrient available in the environment ([g N][g soil]$^{-1}$)
C	Carbon (light) available in the environment (μmol m^{-2} s^{-1})
X	Amount of carbon exchanged ([g C] m^{-2} s^{-1})
T	Exchange (trade) ratio of carbon for nutrient ([g C][g N]$^{-1}$)

019

Benefit from Rhizobia Depends on External Nitrogen Environment



– rhizobia

+ rhizobia

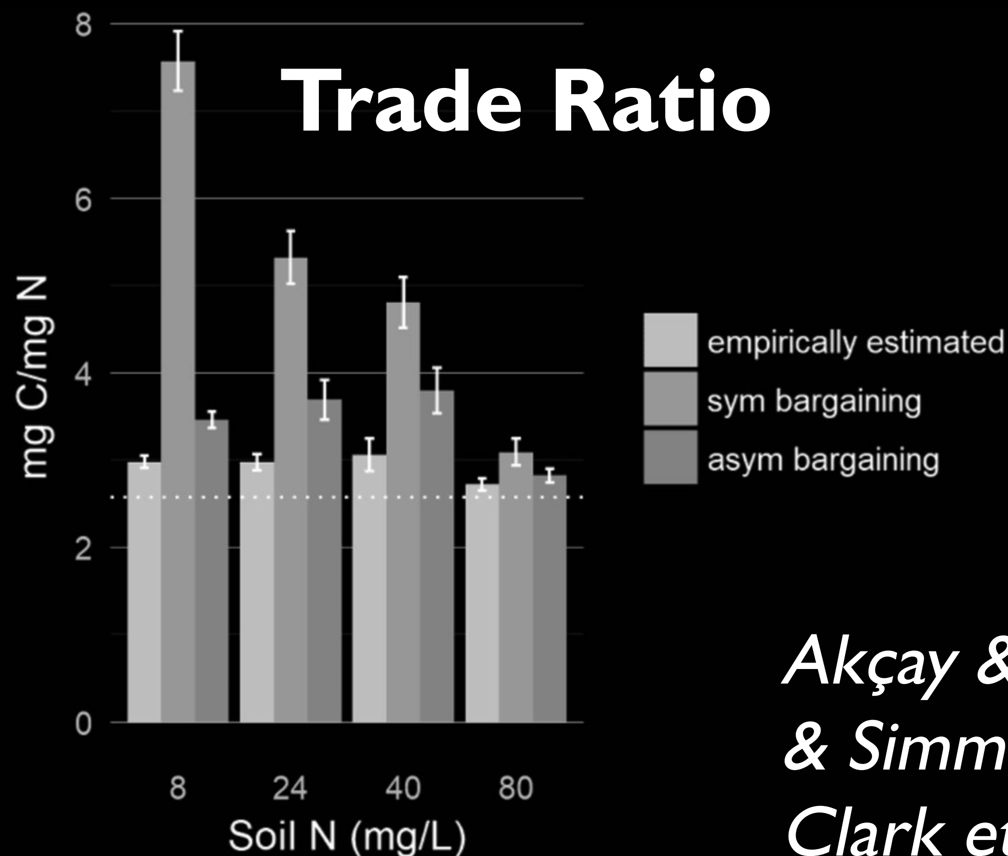
8 24 40 80

Clark et al. 2019

Exchange rate: determined by Nash bargaining

Maximize joint gain from trade: $(g_{PX} - g_{P-})(g_{RX} - g_{R-})$

Asymmetric bargaining: $(g_{PX} - g_{P-})^{\beta}(g_{RX} - g_{R-})^{1-\beta}$



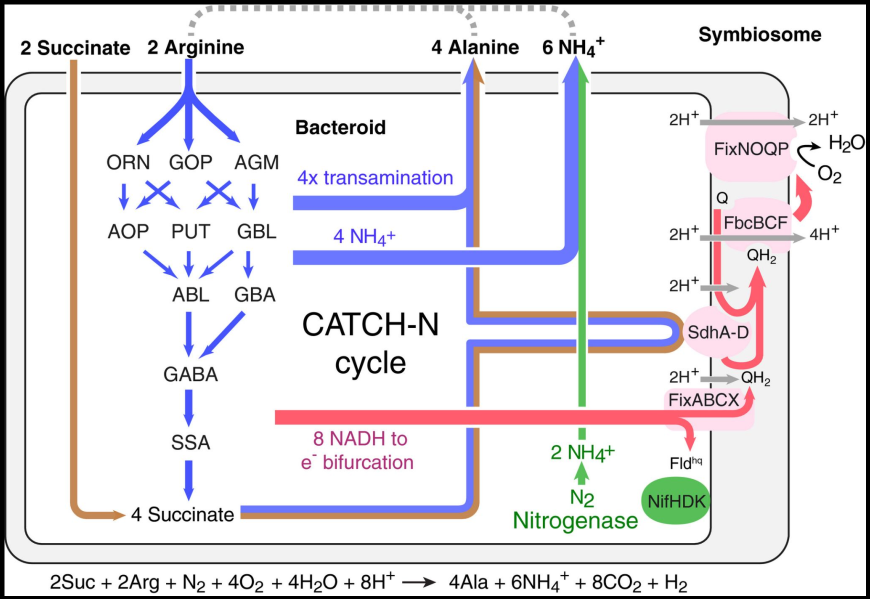
- Asymmetry exponent: ~0.9 to ~0.6 as soil N increases
- Plant has more bargaining power than rhizobia

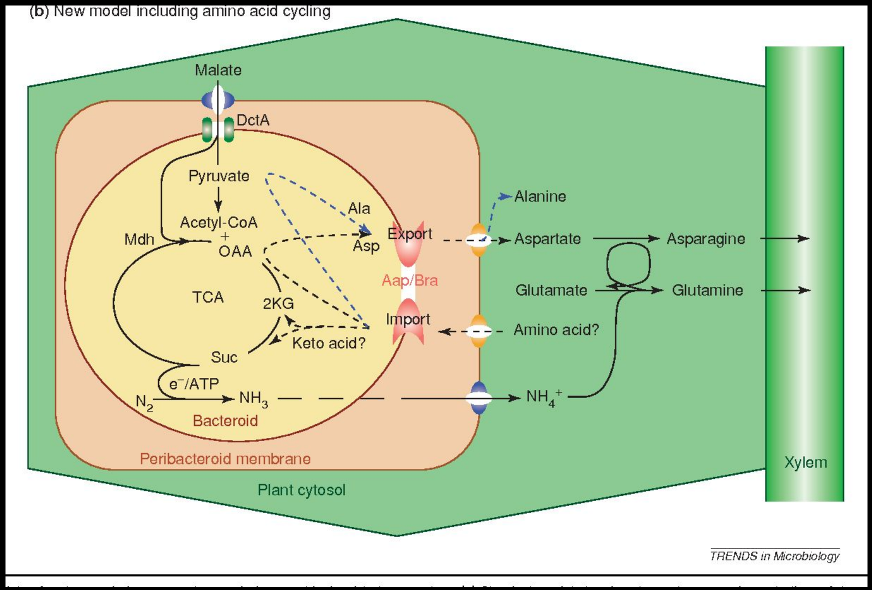
Akçay & Roughgarden 2007; Akçay & Simms 2011; German et al. 2012; Clark et al. 2019

Grand Ambition

Can we determine 'negotiation' rules using metabolic modeling parameterized with genomic data?

- Much more complex than C ⟵⟶ N...





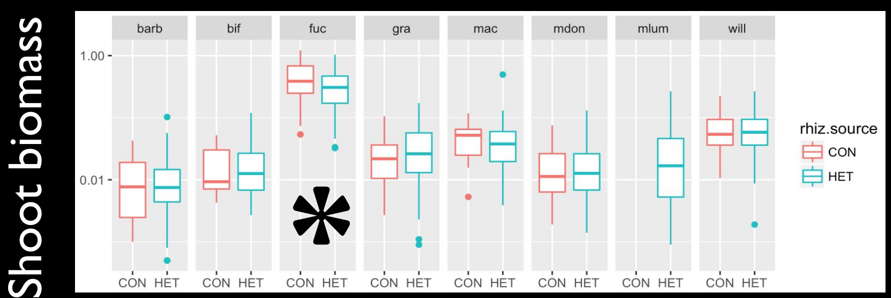
Outstanding questions:

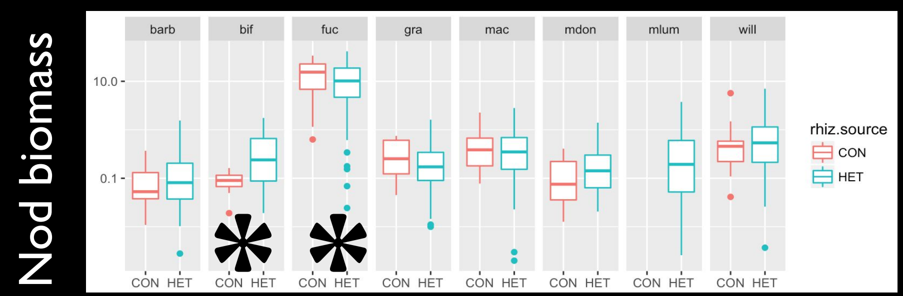
-Are cheaters actually rare (or are we just bad at finding them)?

-Why is there diversity in mutualism?

- *Frequency-dependent nodulation…?*

-Why is there diversity in specialization?





-How can we predict N fixation rates by microbes?

-What is the role of the hundreds of "third parties"?

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