

# Factors governing a synthetic microbial mutualism

DOE Early Career Program Award  
DE-SC0008131  
2012 - 2018

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Indiana University



@mckinlab  
@JakeMcKinlay

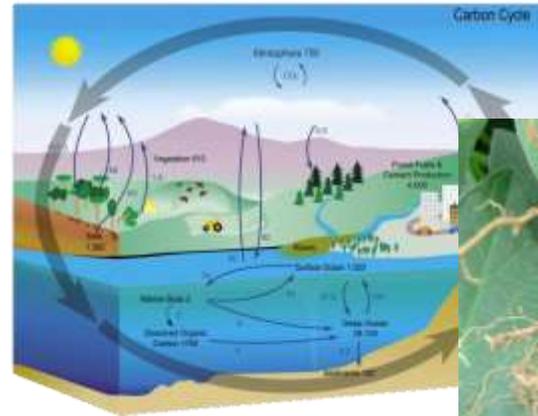


# Overview

1. Development of a synthetic H<sub>2</sub>-producing bacterial coculture
2. Discovery highlights from the coculture system
3. Unanticipated projects stemming from the coculture system
4. Other impacts of the award on my research program and the people involved in it

# Microbial cross-feeding is important

- Global elemental cycles
- Agriculture
- Biotechnology

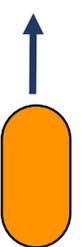


Lignocellulose



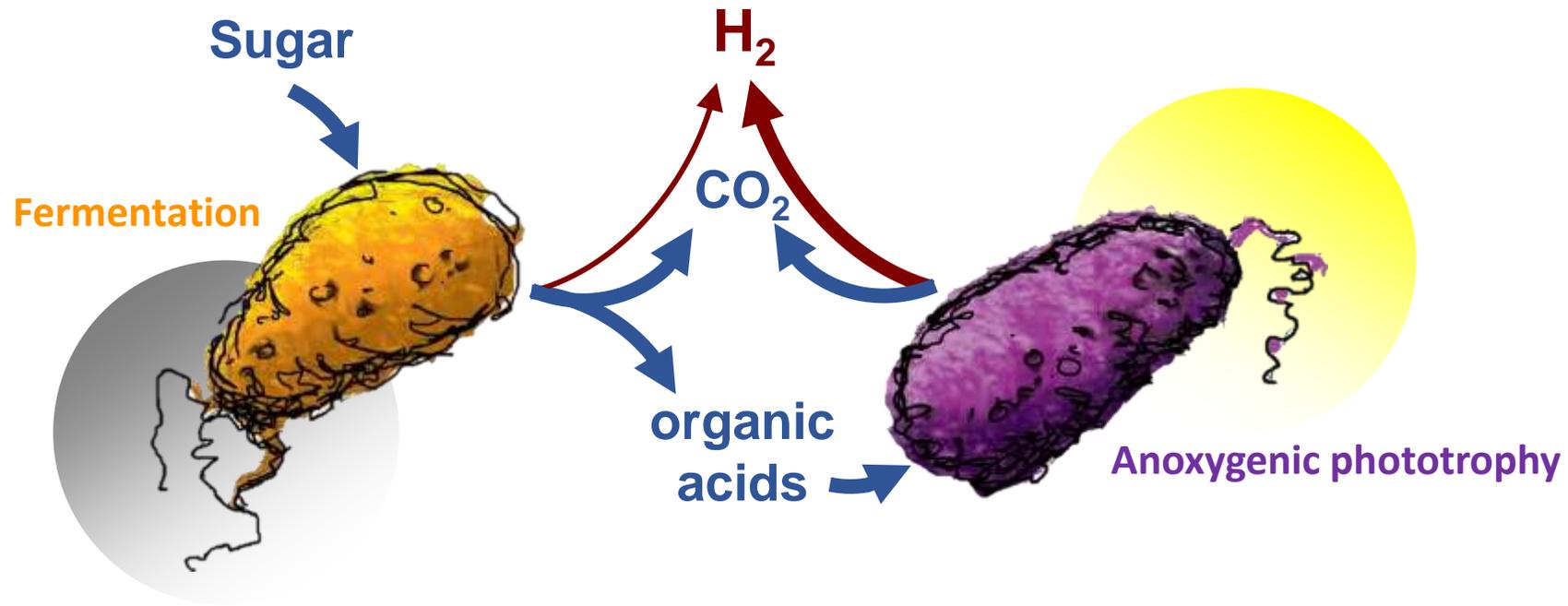
Glucose

Fuel



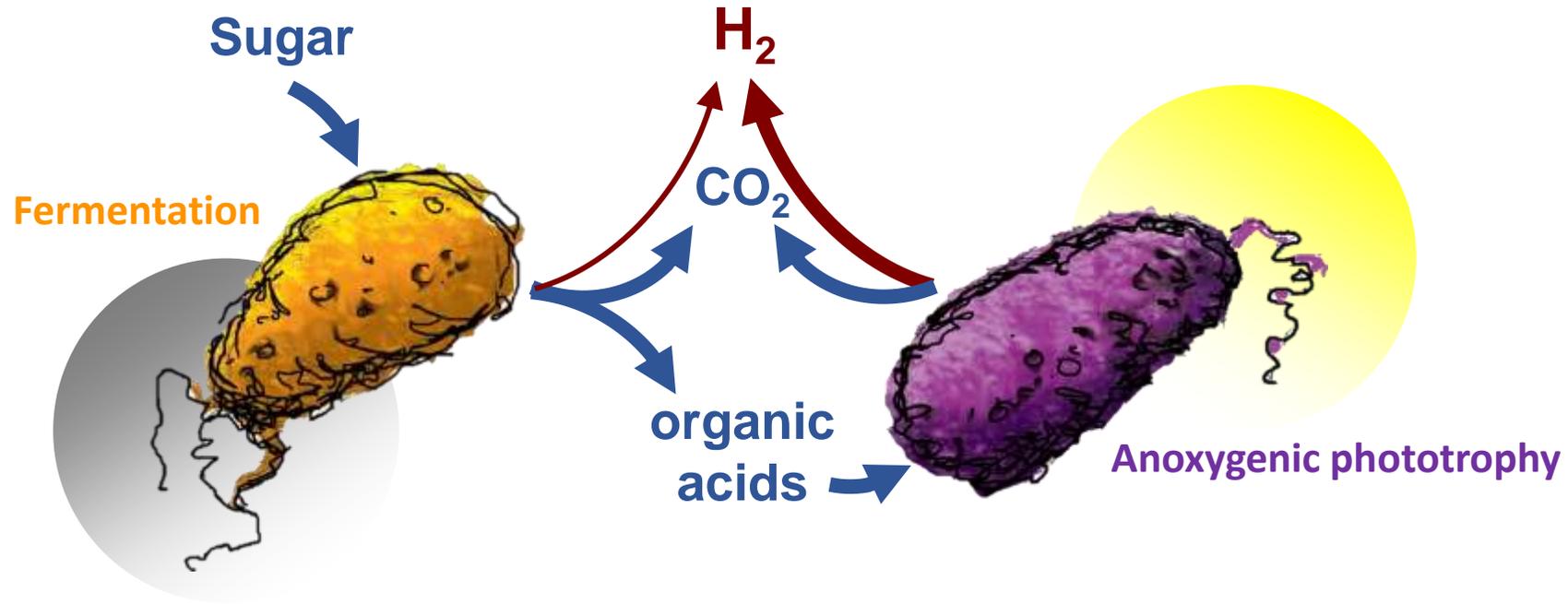
- **Understanding the factors that govern cross-feeding interactions has implications for our environment, health, and industries**

# Cross-feeding of fermentation products



- The unidirectional excretion of organic acids can lead to cross-feeding
- Resembles an anaerobic food-web

# Traditional cocultures lacked stable coexistence and reproducibility



- Fast-growing fermentative bacteria paired with slow-growing phototrophs
- Establishing stable synthetic communities is rarely trivial

# Building a practical coculture to study cross-feeding



**Ryan Fritts**

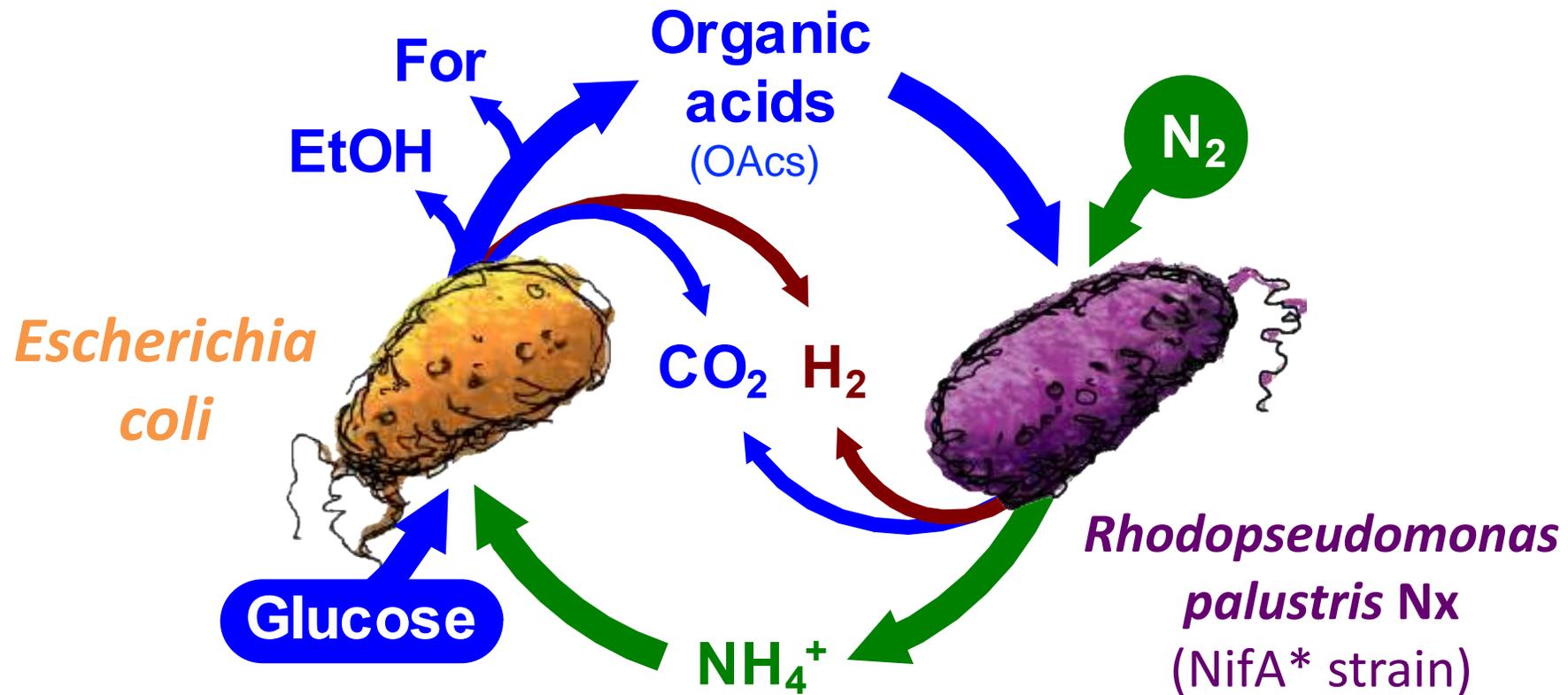


**Dr. Breah LaSarre**



**Dr. Ali McCully**

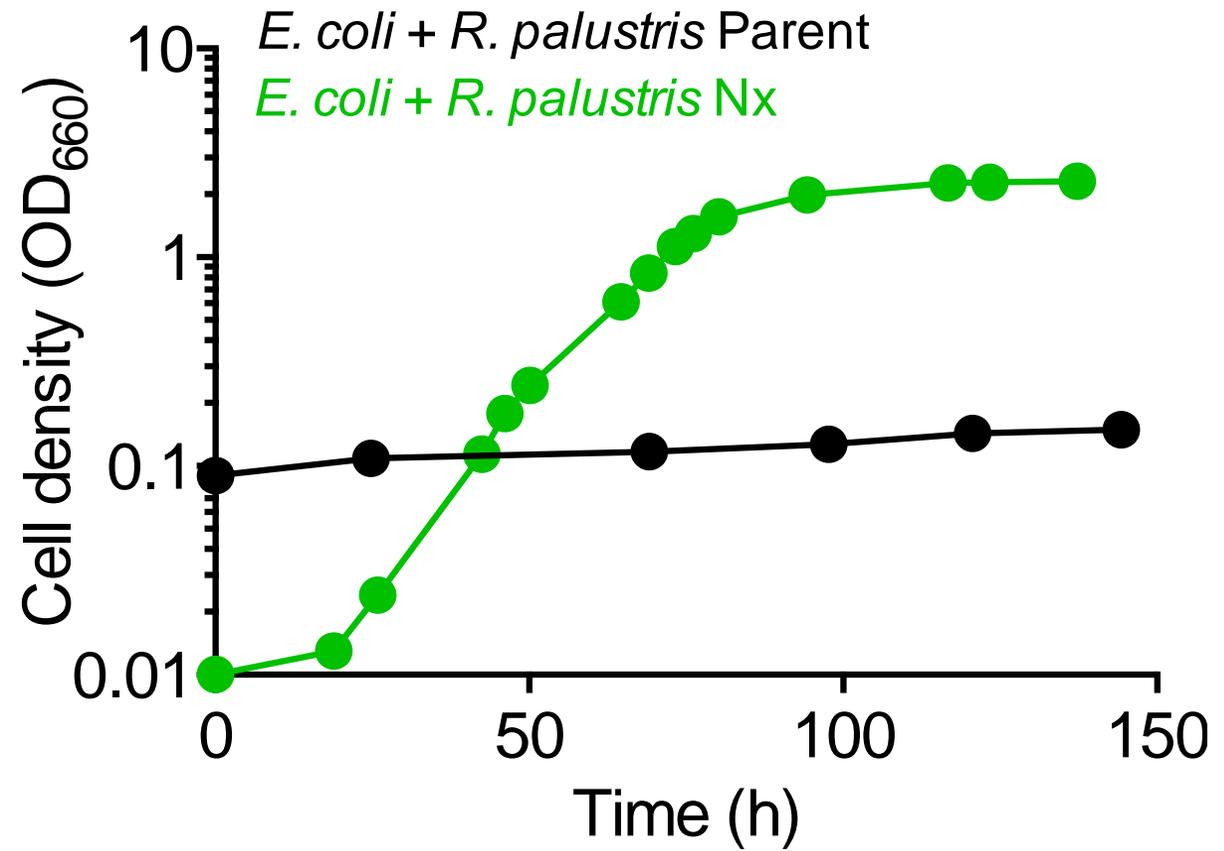
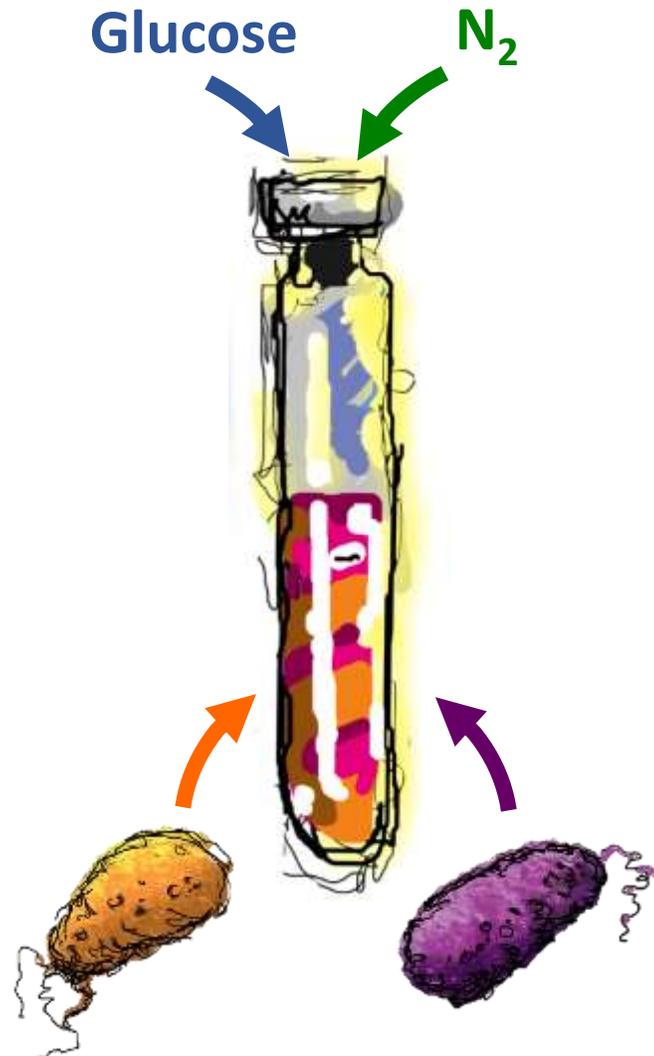
# Reciprocal cross-feeding of ammonium could lead to a stable mutualism



LaSarre et al. 2017. ISME J  
McCully et al. 2017. Env. Microbiol

McCully et al. 2017. mBio  
McCully et al. 2018. Appl. Environ. Microbiol.

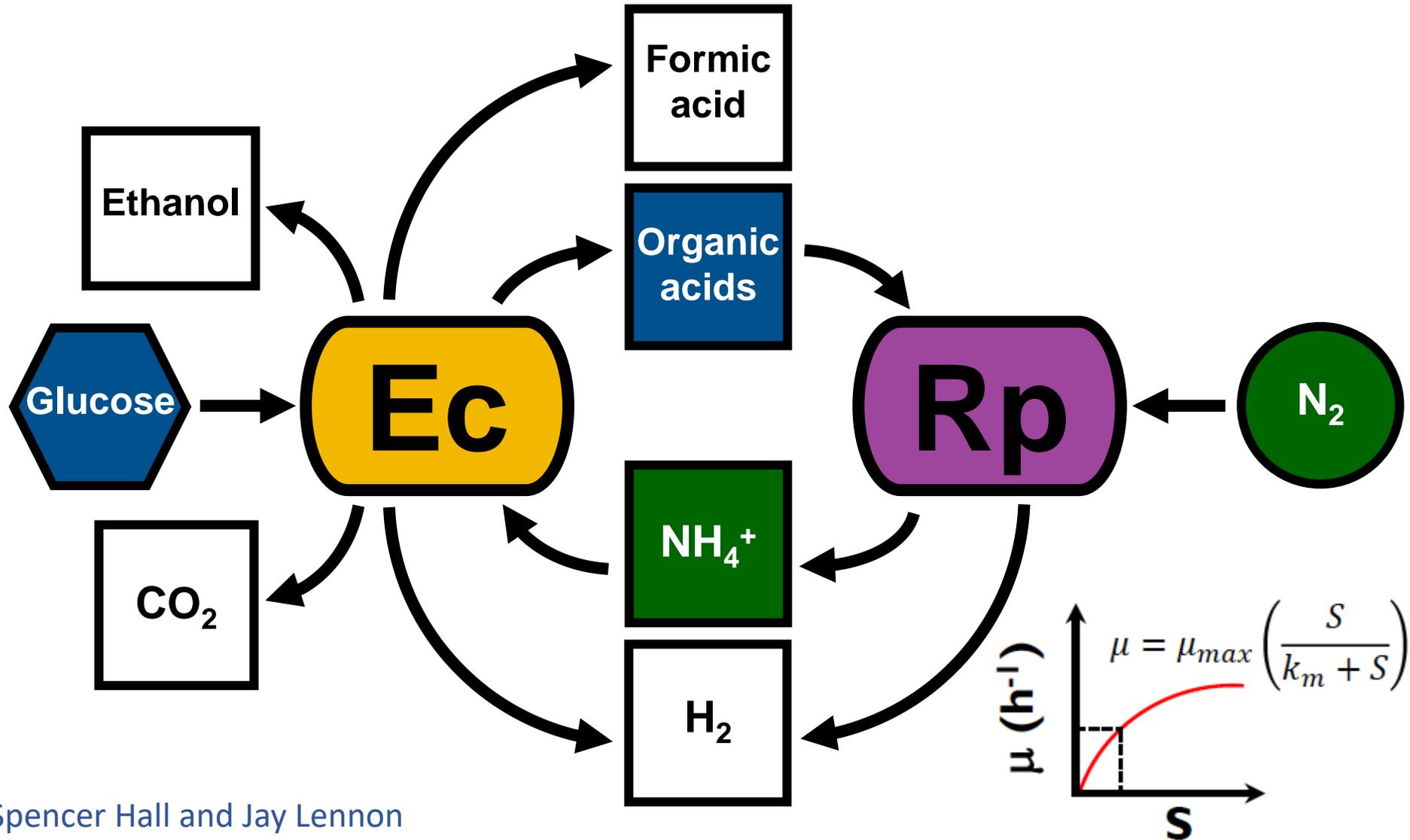
# *R. palustris* Nx mutation results in cross-feeding of ammonium to *E. coli*



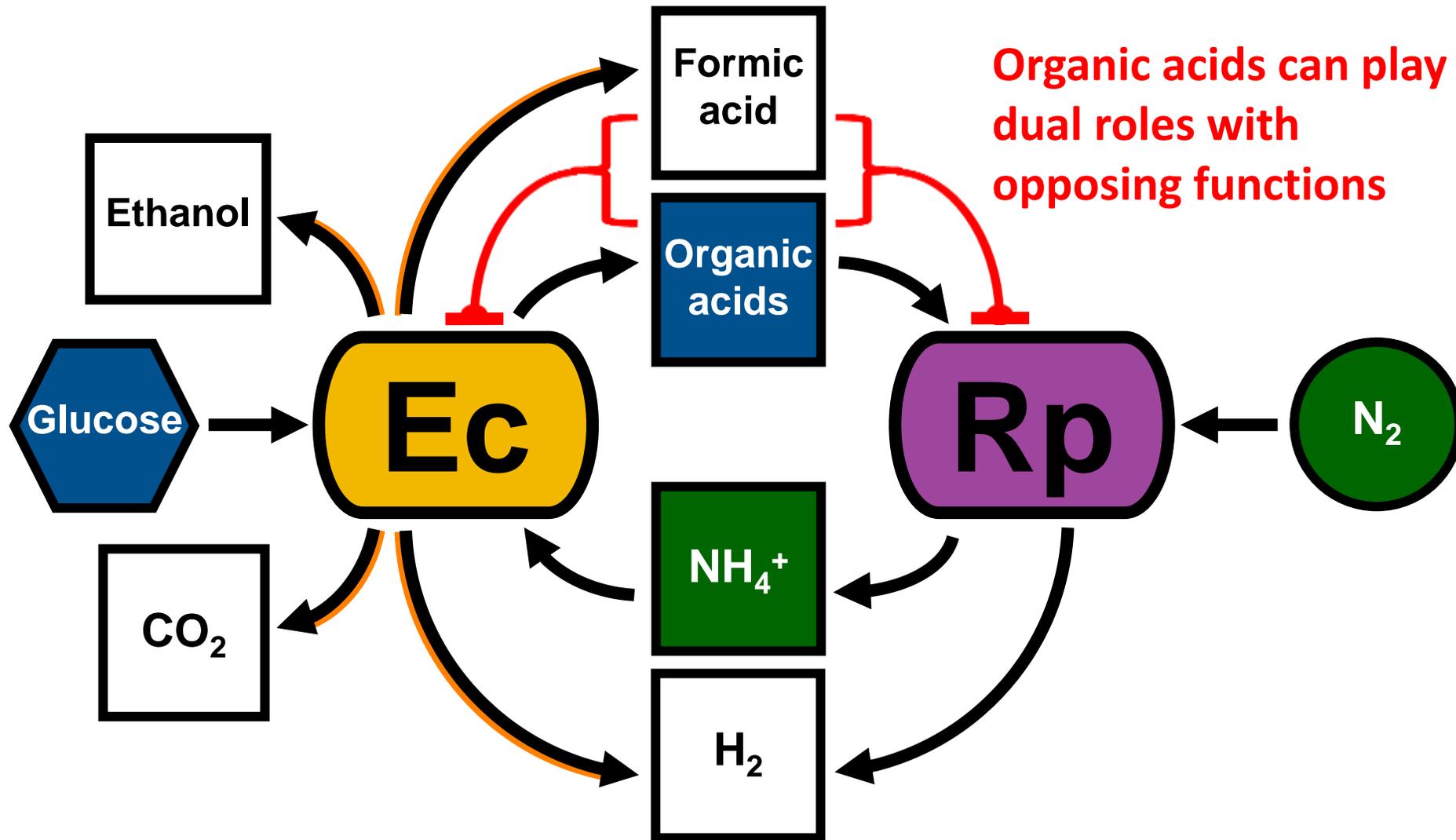
Coculture growth in phosphate buffered minimal medium

- Cocultures move to equilibrium from starting ratios spanning >6-orders of magnitude

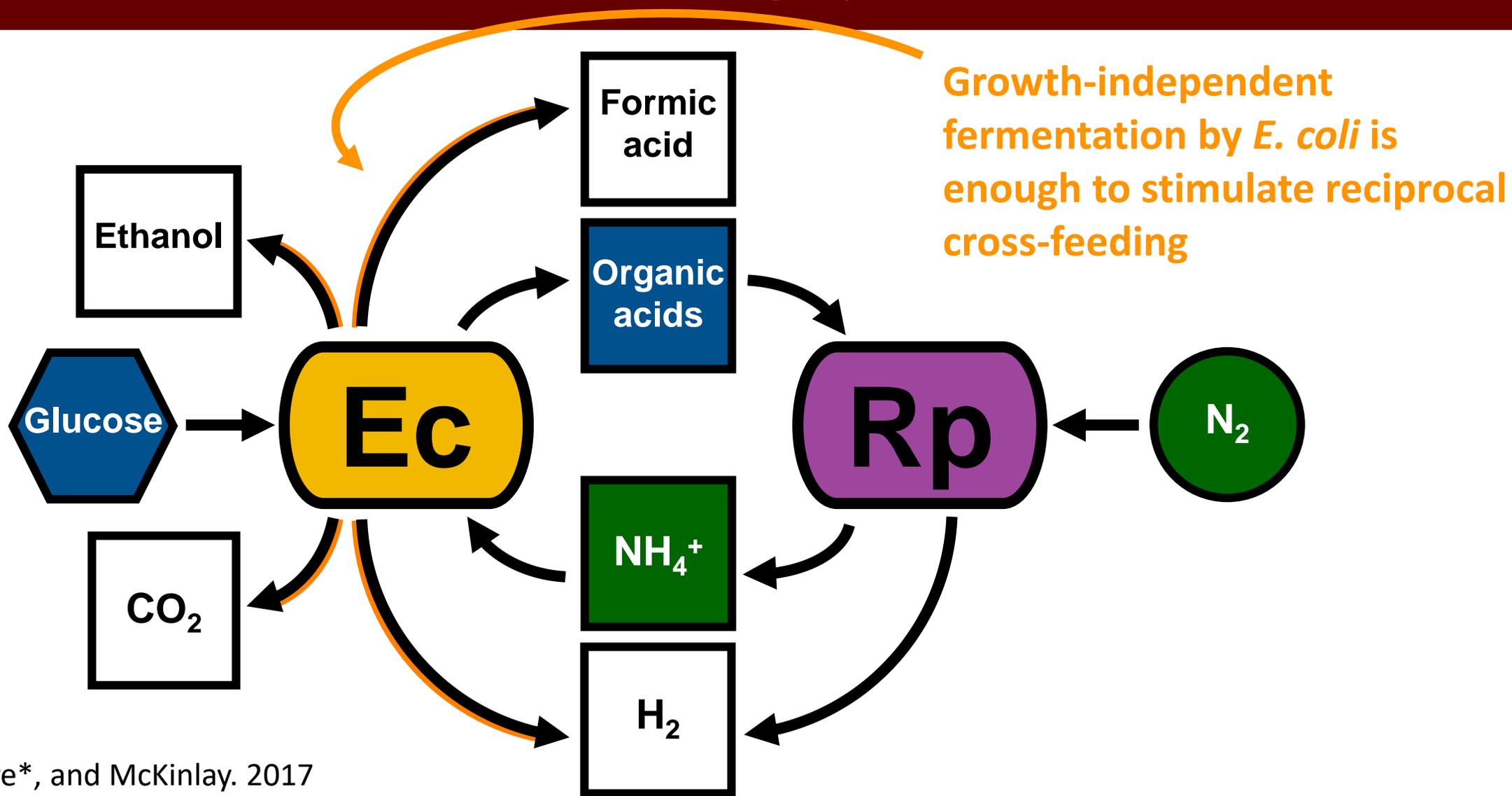
# A kinetic model for simulating coculture trends



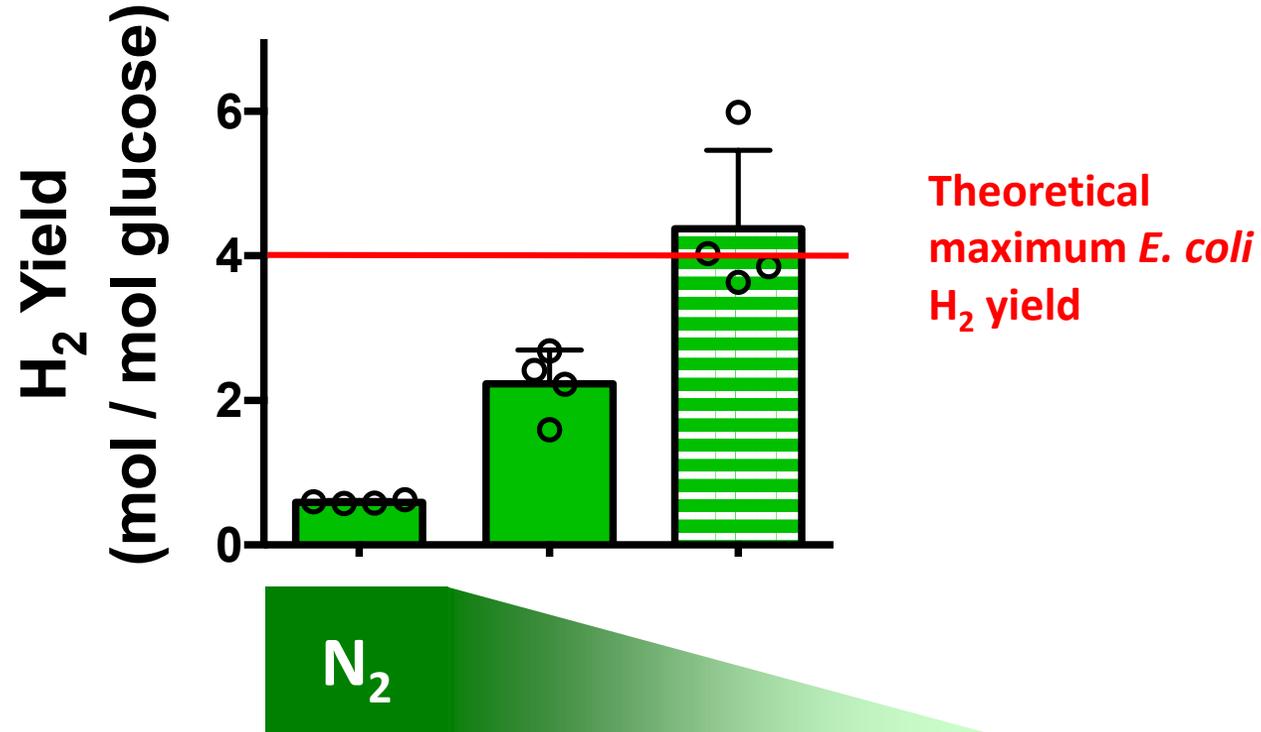
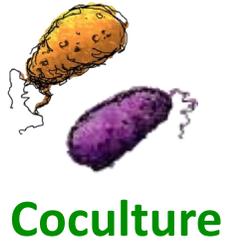
# Accumulation of organic acids can inhibit growth



# Growth-independent cross-feeding can maintain a mutualism through periods of starvation



# Nitrogen-deprived cocultures also have high a H<sub>2</sub> yield



- H<sub>2</sub> yields can exceed the theoretical maximum for a fermentation

# *Competition during cross-feeding*

Competition for communally valuable cross-fed metabolites

## McKinlay Lab



Dr. Ali  
McCully



Dr. Breah  
LaSarre



Jennifer  
Gliessman



Jeffrey  
Mazny

## Drummond Lab



Dr. Evgeny  
Pilipenko



Prof. Allan  
Drummond

## Lynch Lab



Dr. Megan  
Behringer

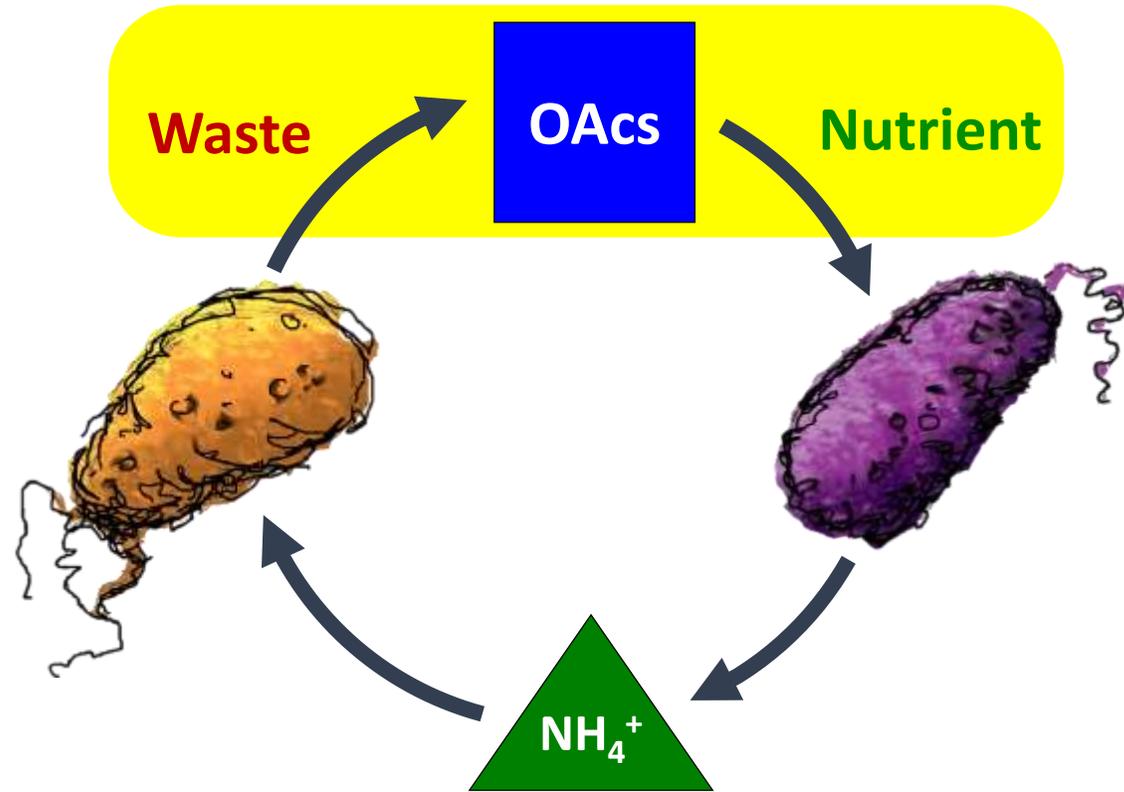


Prof. Mike  
Lynch

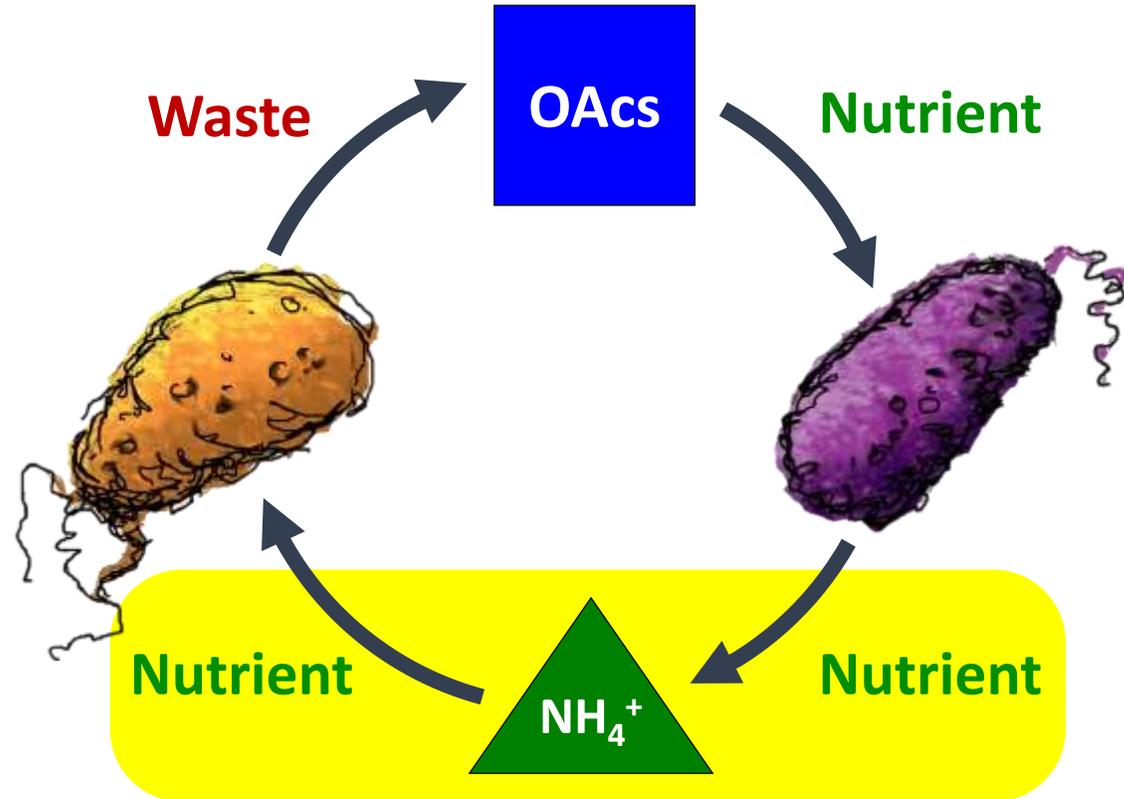
McCully, LaSarre, and McKinlay. 2017. mBio

McCully, Behringer, Gliessman, Pilipenko, Mazny, Lynch, Drummond, and McKinlay. 2018. Appl. Environ. Microbiol.

# Some cross-fed metabolites are of value to only one partner

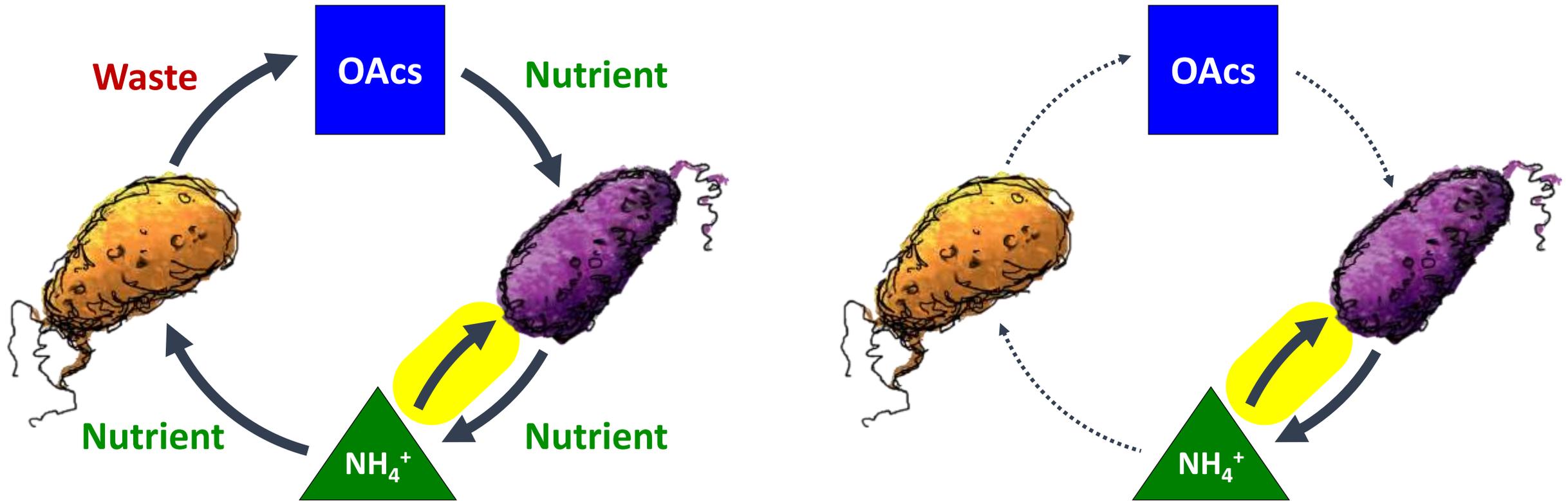


# Other cross-fed metabolites are of value to both partners



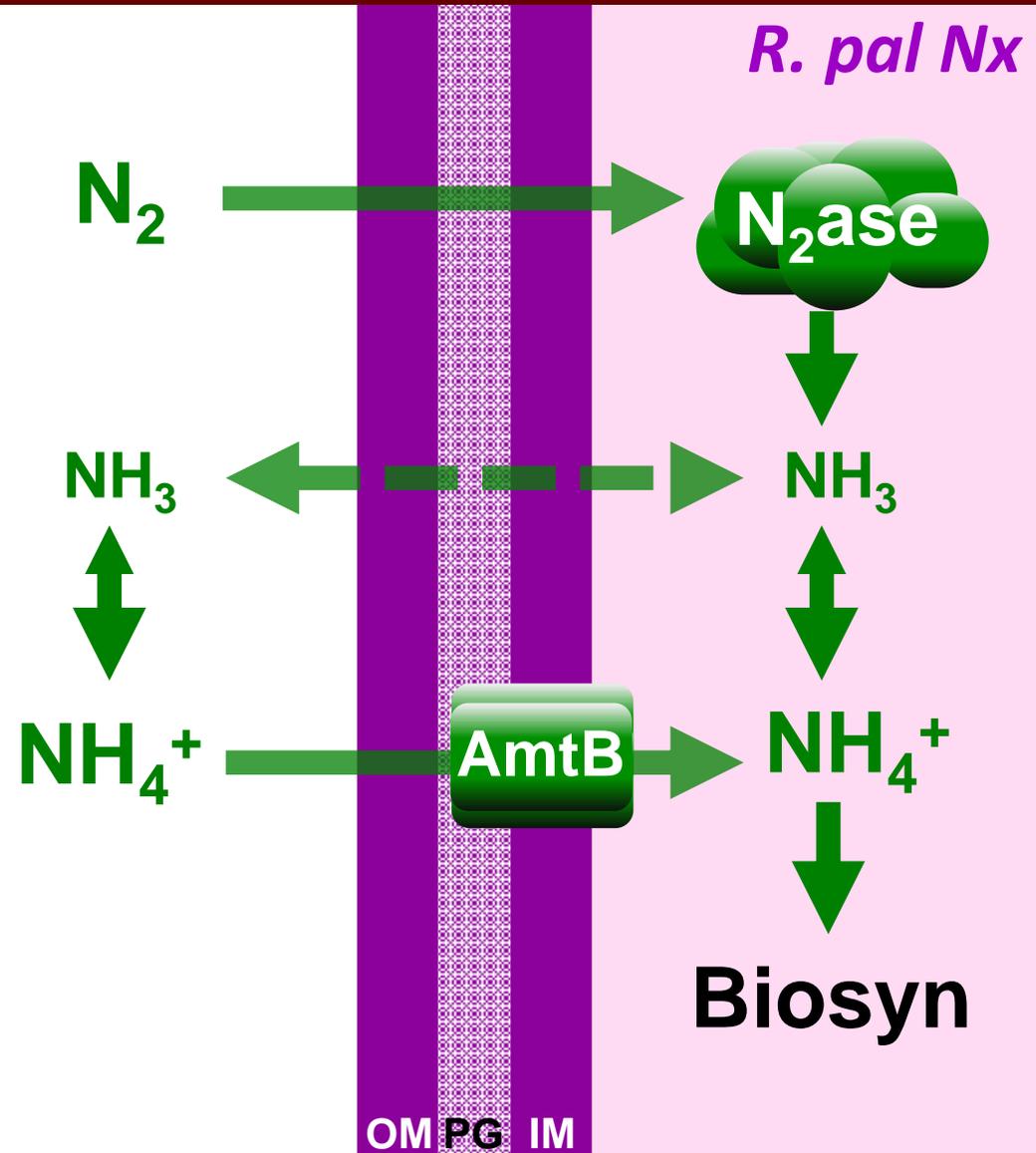
- Other microbial examples
  - Nitrogen transfer from N<sub>2</sub>-fixing bacteria to other bacteria, fungi, and plants
  - Transfer of vitamin B<sub>12</sub> between bacteria and abundant marine algae

# Do mutualistic partners compete for communally-valuable cross-fed nutrients?

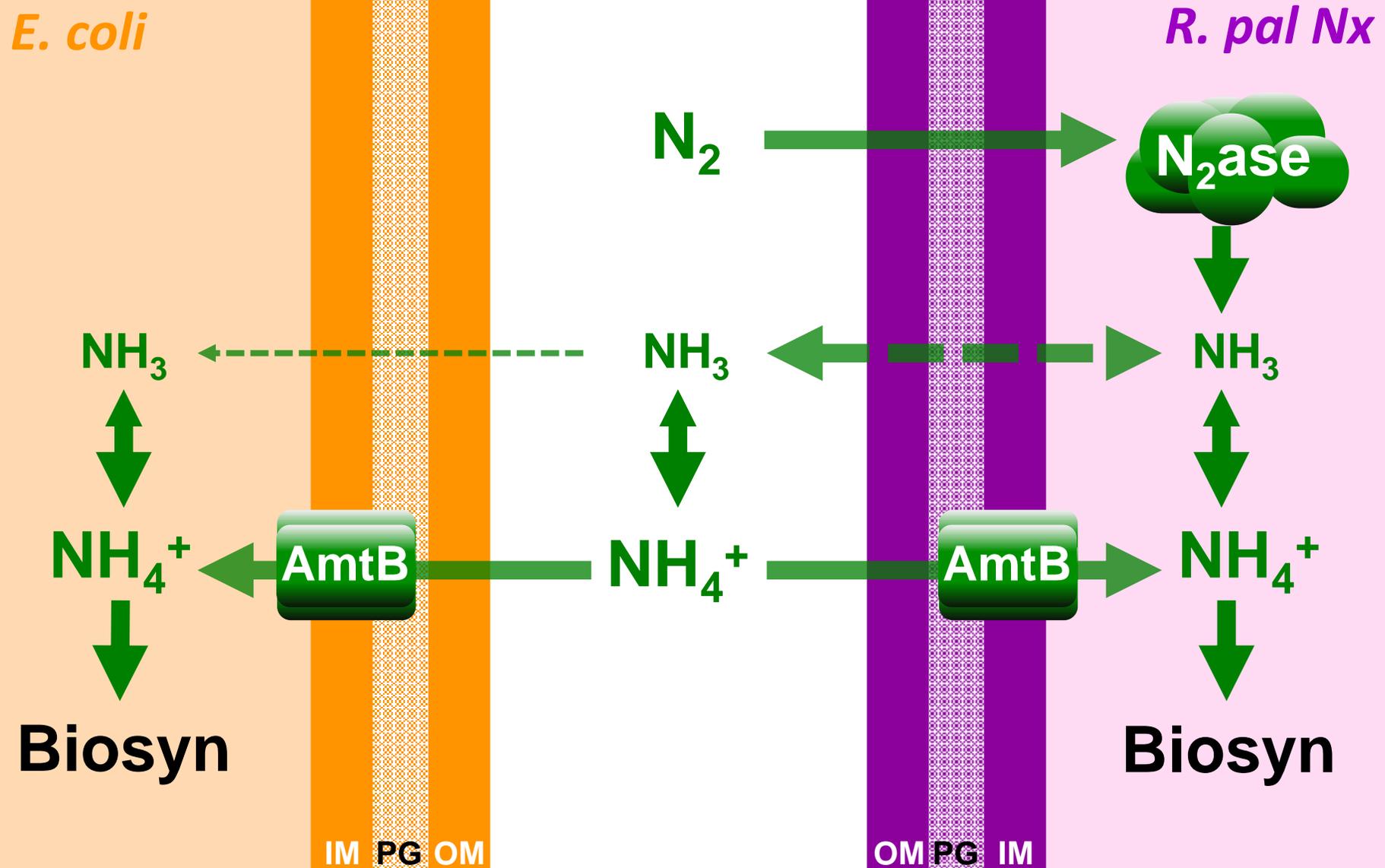


- Model predicts that competition for  $\text{NH}_4^+$  must be biased in favor of *E. coli* or the mutualism will collapse

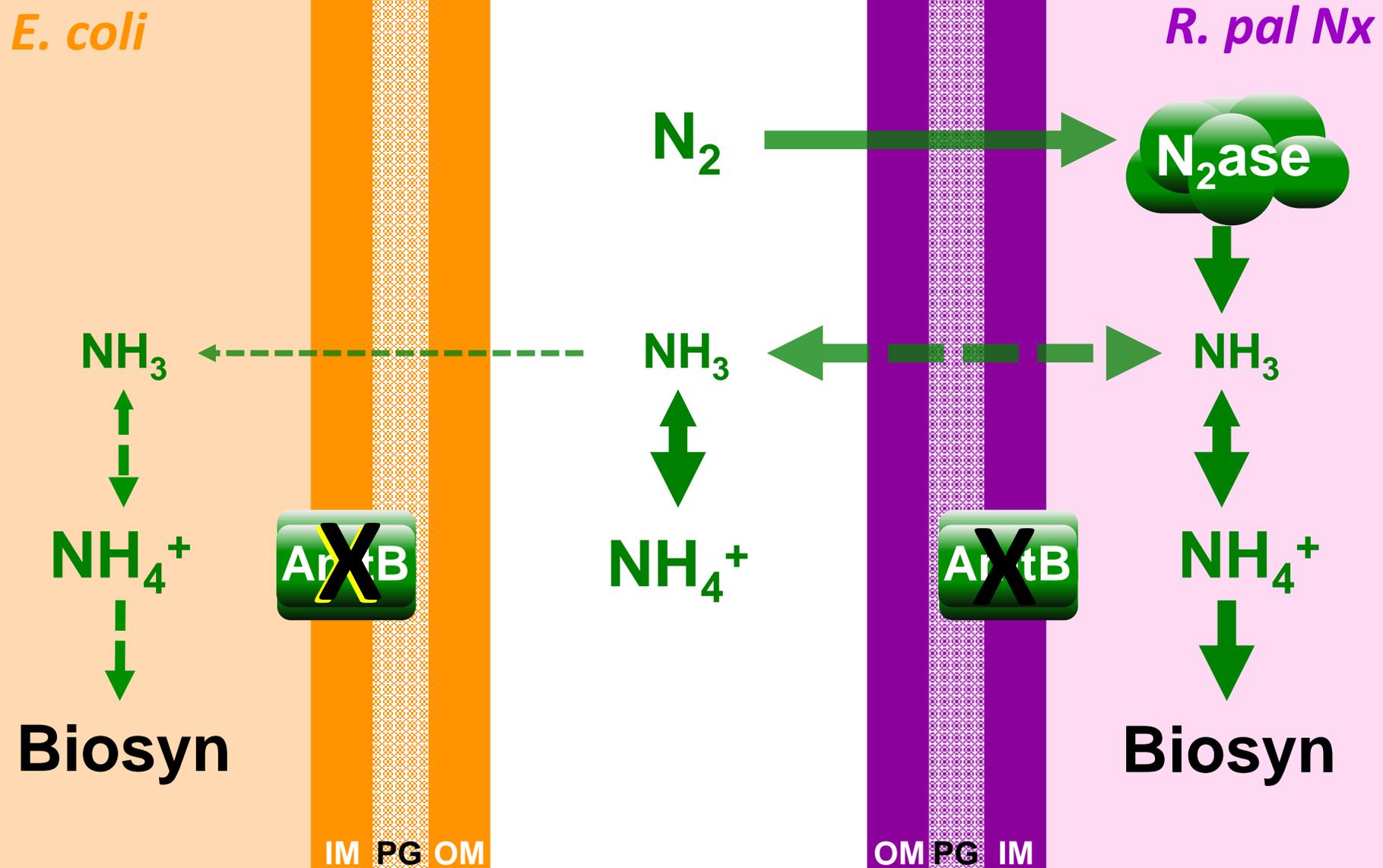
# How is competition for ammonium occurring?



# Competition is likely occurring at the level of AmtB $\text{NH}_4^+$ transporters



# Coculture collapses whenever *E. coli* is lacking AmtB



# *E. coli* AmtB is upregulated in coculture

Fold-changes for WT *E. coli* transcripts and proteins in coculture vs monoculture

RNA-seq			Proteomics		
Gene	Description	Fold change	Gene	Description	Fold change
rutACDEFG	Nitrogen scavenging from pyrimidines	157	argT	Lys/Arg/Orn binding protein	11
nac	Nitrogen assimilation control	97	ddpA	D-ala dipeptide permease	6
ddpX	D-ala dipeptidase	76	bfr	Bacterioferritin	5
csgB	Curli	64	gss	Glutathionylspermidine synthetase/amidase	4
argT	Lys/Arg/Orn binding protein	61	potF	Putrescine-binding periplasmic protein	4
patA	Putrescine aminotransferase	59	modA	Molybdate-binding periplasmic protein	4
glnK	Nitrogen regulation	37	gabD	Succinate-semialdehyde dehydrogenase	4
<b>amtB</b>	<b>NH<sub>4</sub><sup>+</sup> Transporter</b>	<b>24</b>	<b>amtB</b>	<b>NH<sub>4</sub><sup>+</sup> Transporter</b>	<b>4</b>

# Most of the upregulated genes are controlled by NtrC, the master transcriptional activator of the nitrogen starvation response

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csgB	Curli	64	gss	Glutathionylspermidine synthetase/amidase	4
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- Deleting *ntrC* can also lead to a collapse of the mutualism

# *Mutualism favors the scavenger*

Emergence of a nascent mutualism

## McKinlay Lab



Ryan  
Fritts



Dr. Jordan  
Bird

## Joint Genome Institute



Community Science Program

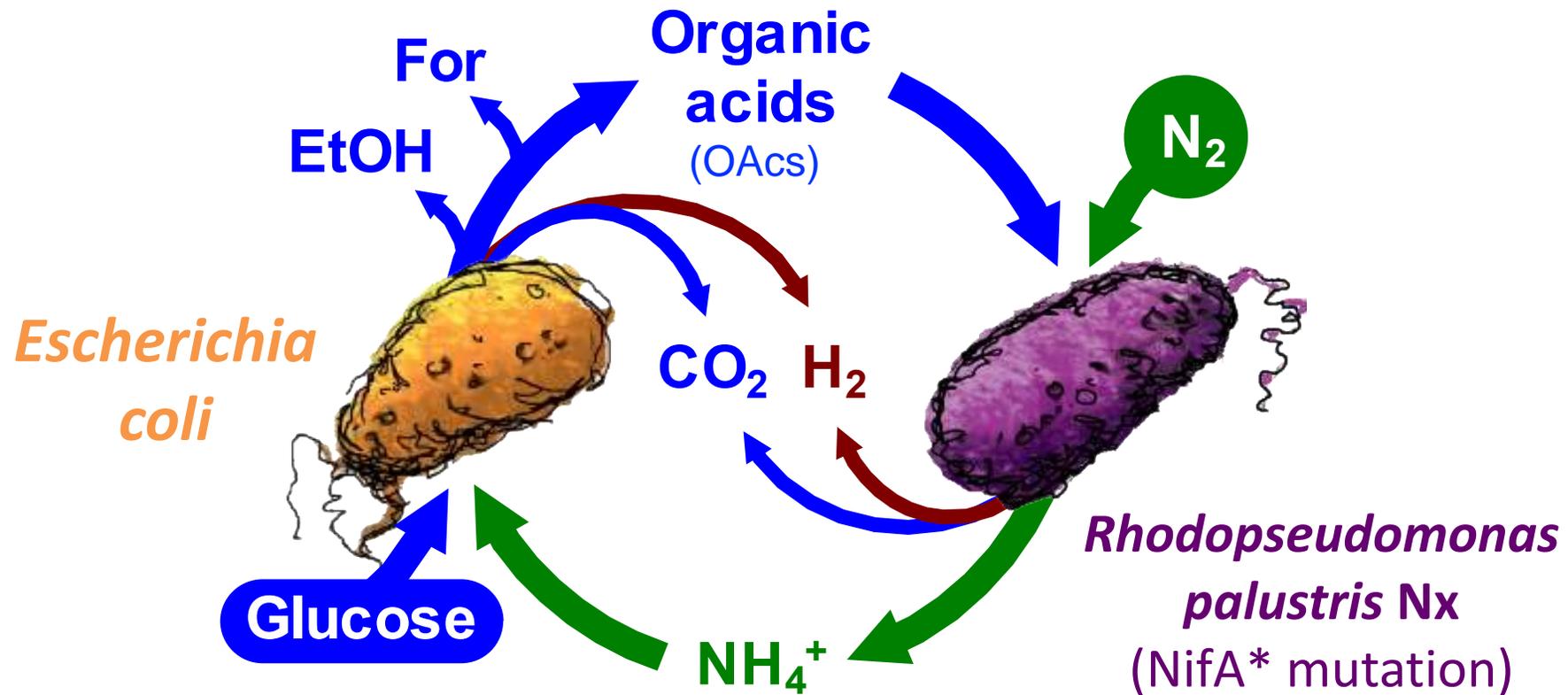


Dr. Megan  
Behringer



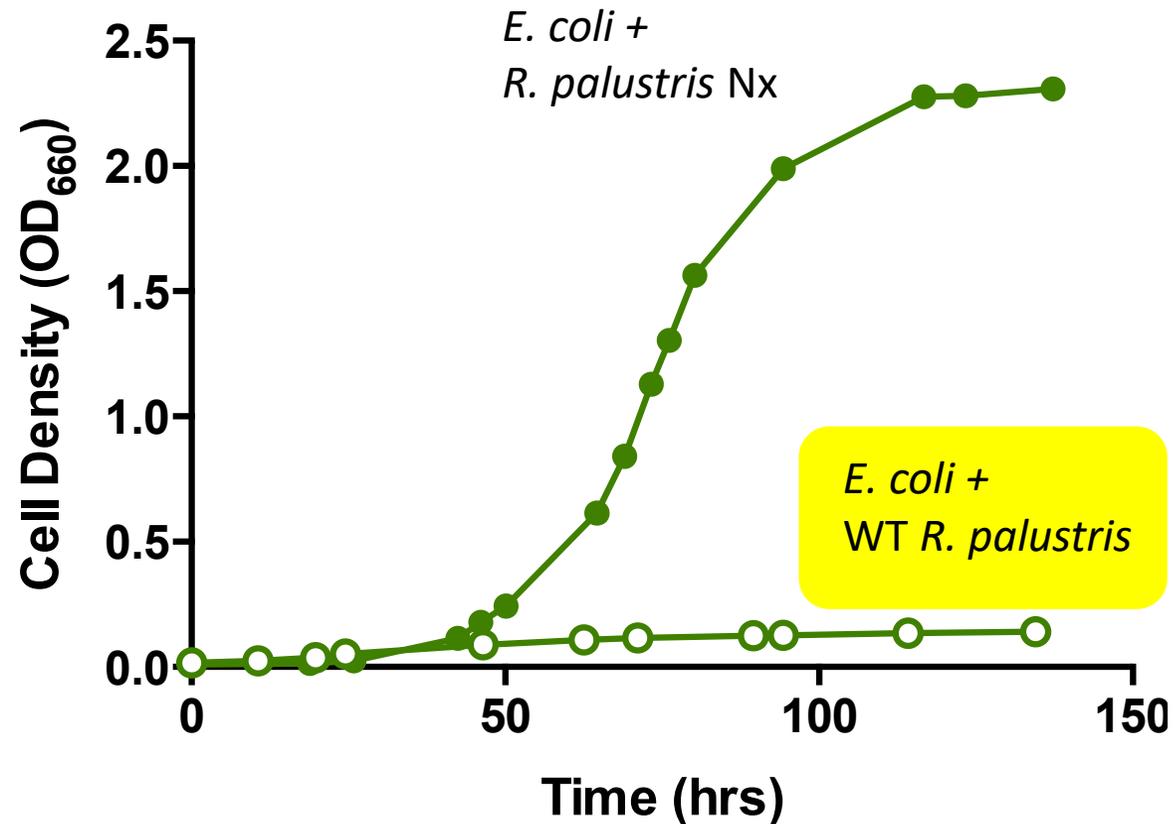
Prof. Mike  
Lynch

# A carefully engineered mutualism



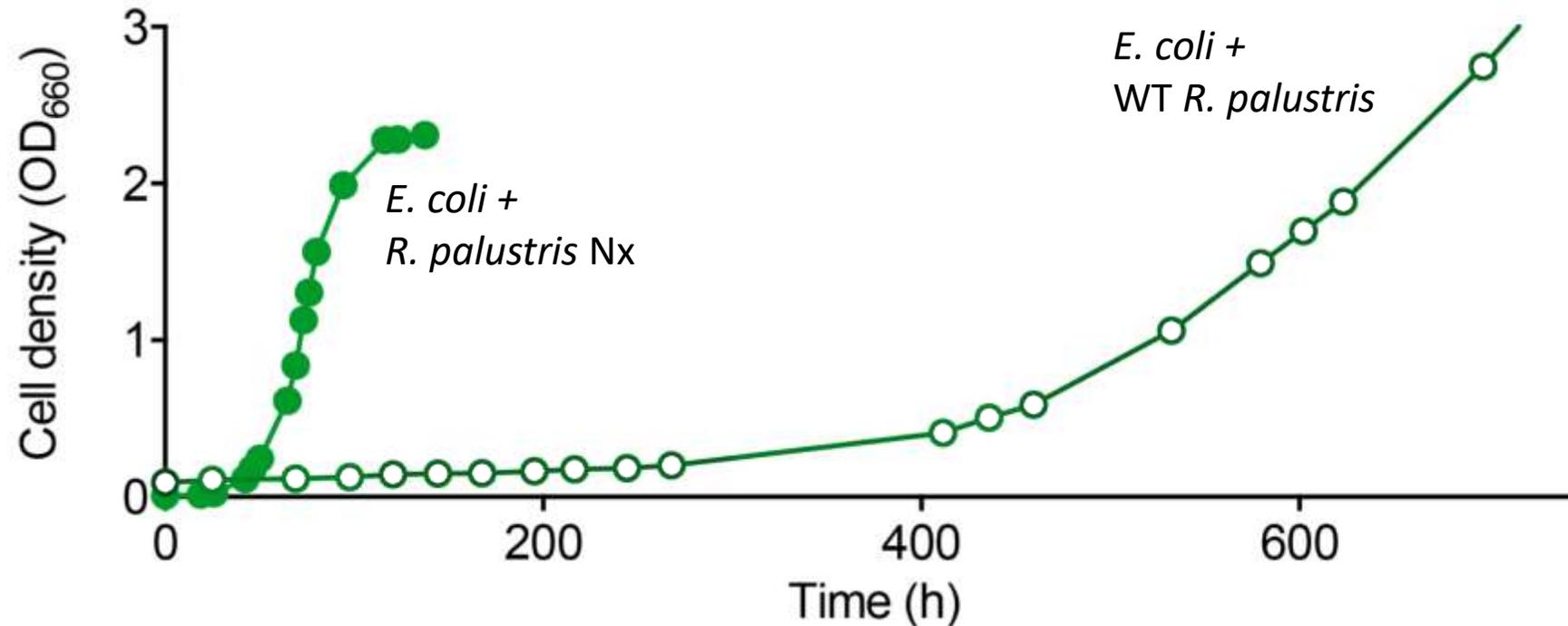
Could such a mutualism arise naturally?

# Wildtype *R. palustris* does not support coculture growth in the short term



- Cocultures with wildtype *R. palustris* can be used to enrich for spontaneous mutants that support coculture growth

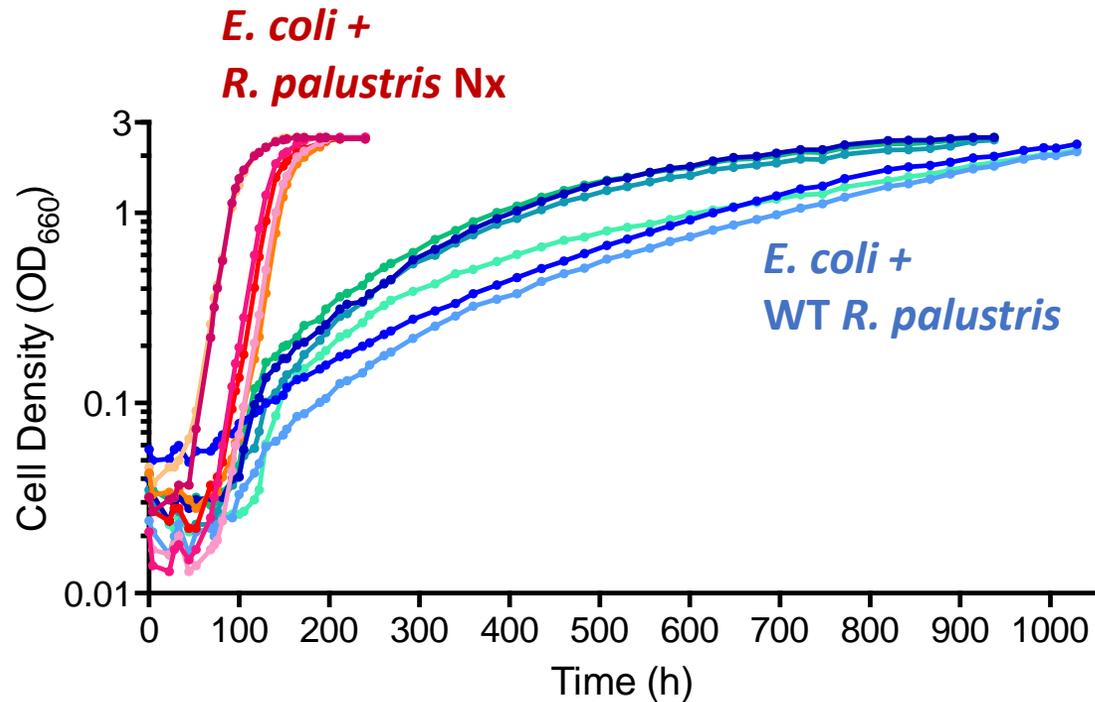
# Cocultures with wildtype *R. palustris* eventually grow



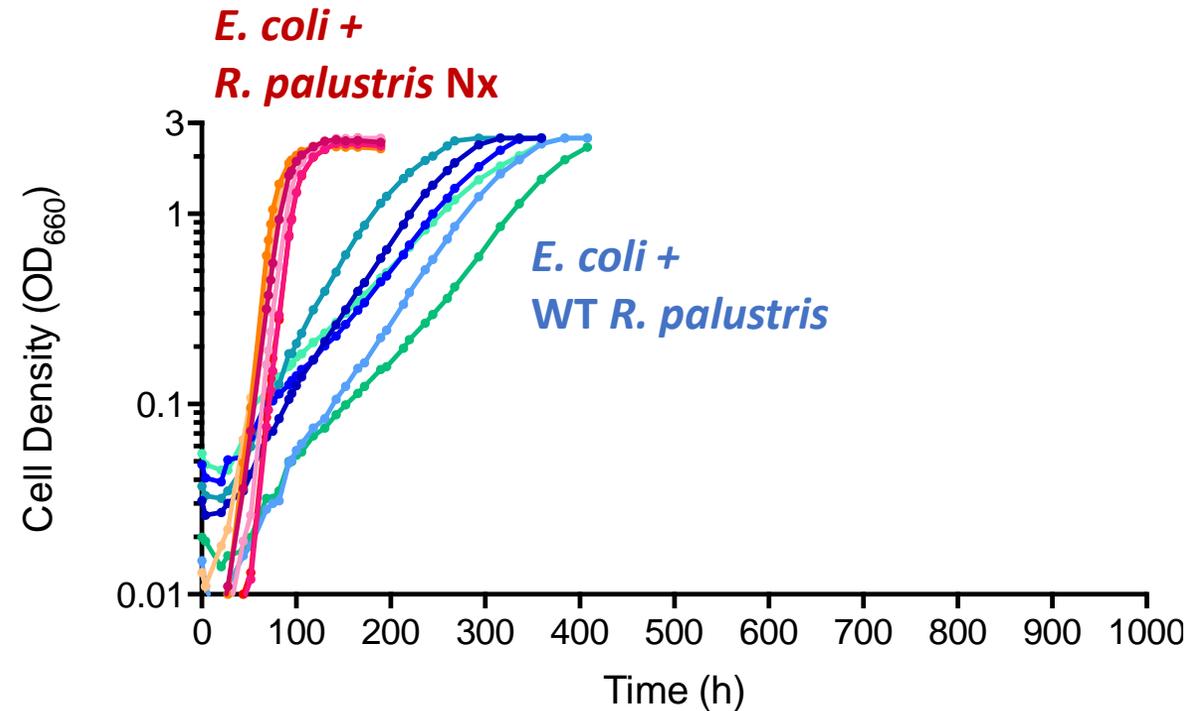
- Nascent mutualistic cross-feeding can emerge relatively quickly

# Coculture growth improves through serial transfers

## Transfer 2

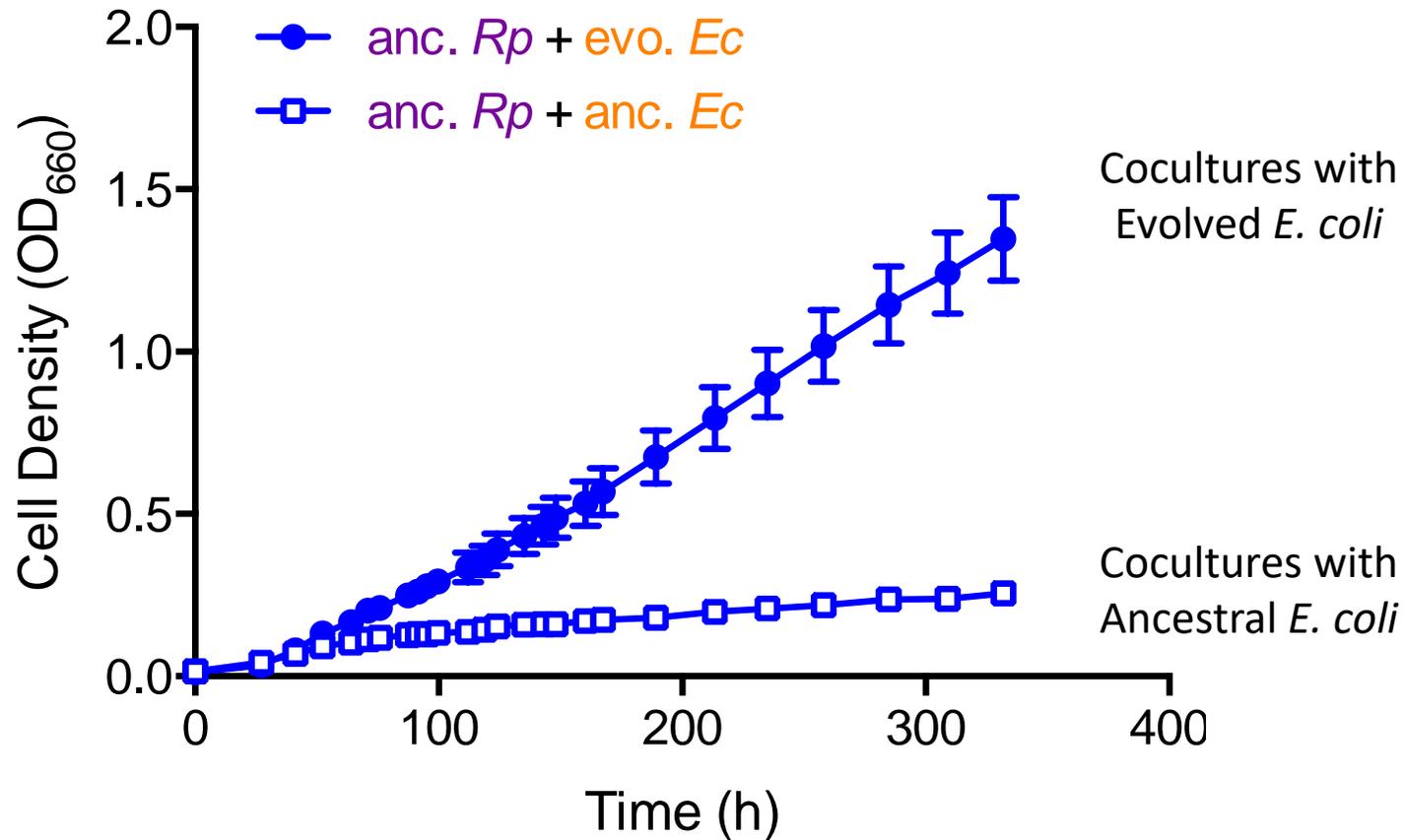


## Transfer 25



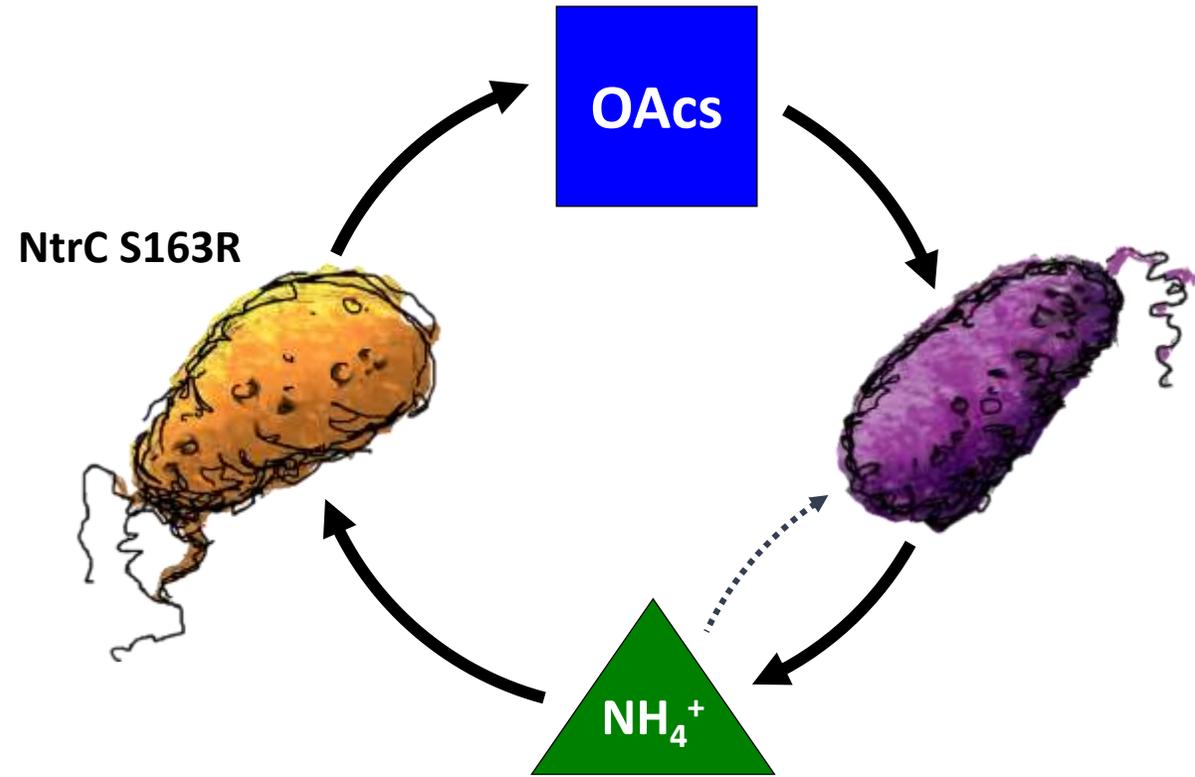
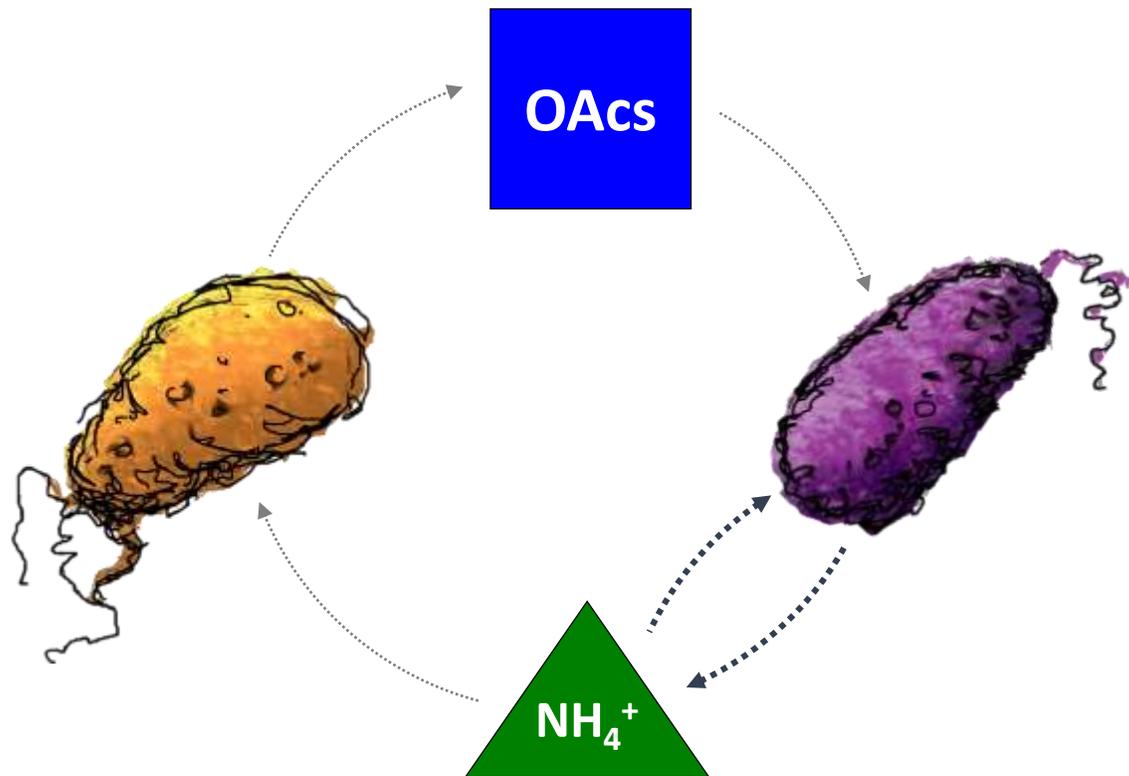
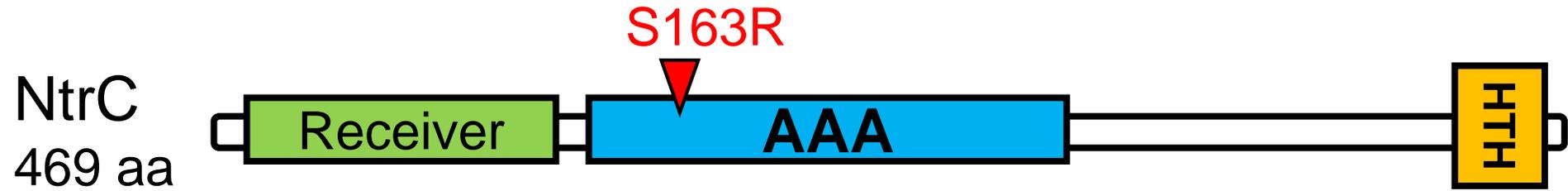
- What mutations are responsible for establishing nascent cross-feeding?

# Evolved *E. coli* alone is sufficient to support cross-feeding!



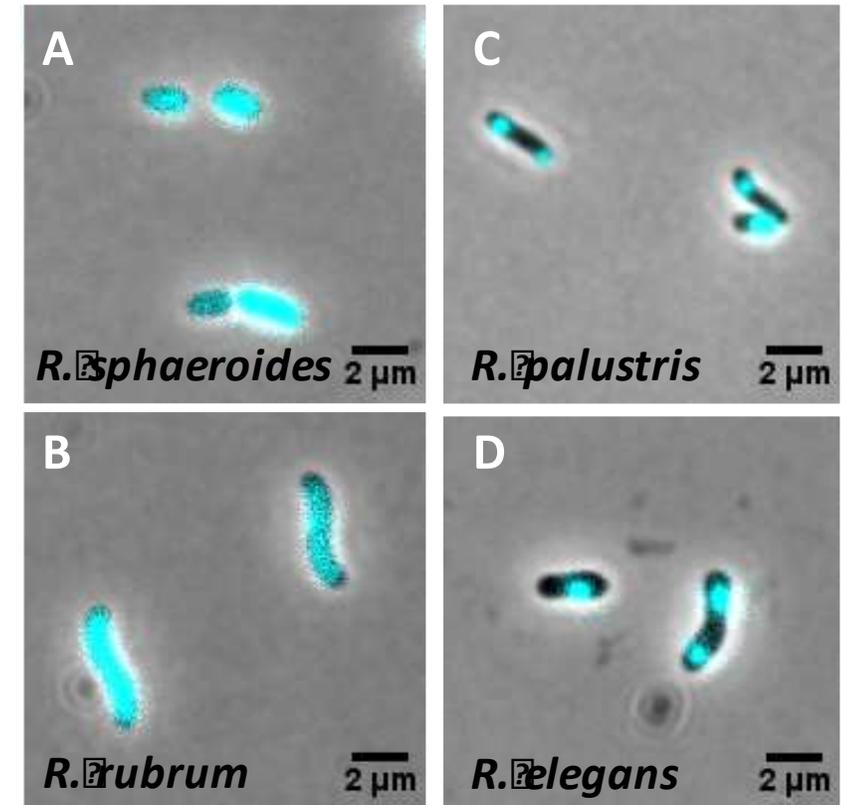
- Genetically-enhanced *R. palustris* ammonium excretion is not required

# Evolved *E. coli* lines have a common mutation in NtrC

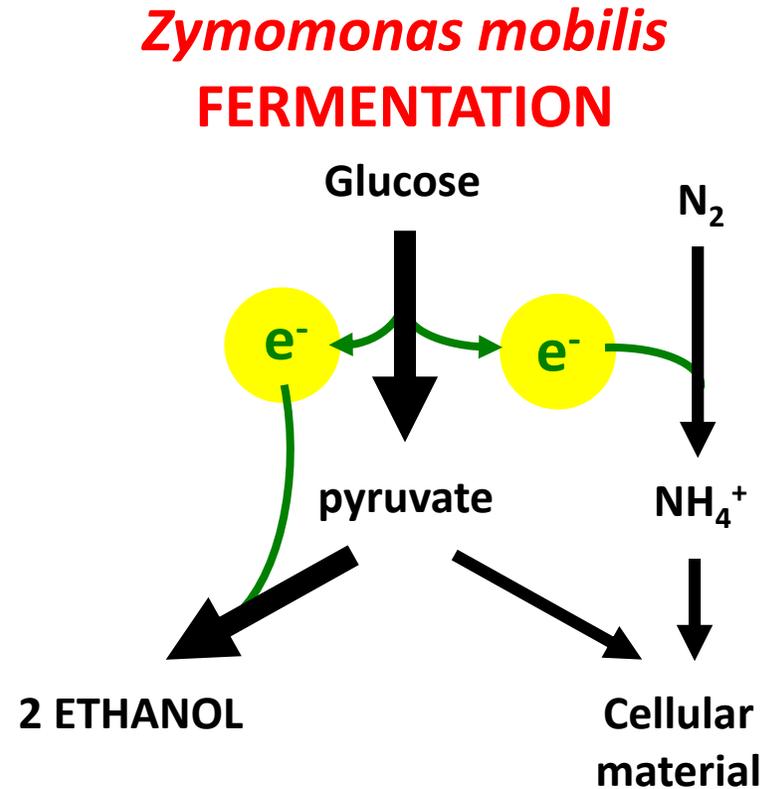


# Unanticipated projects stemming from the coculture system

1. Polar localization of *R. palustris* photosystems
2. Using N<sub>2</sub> as an inexpensive nitrogen source for ethanol production

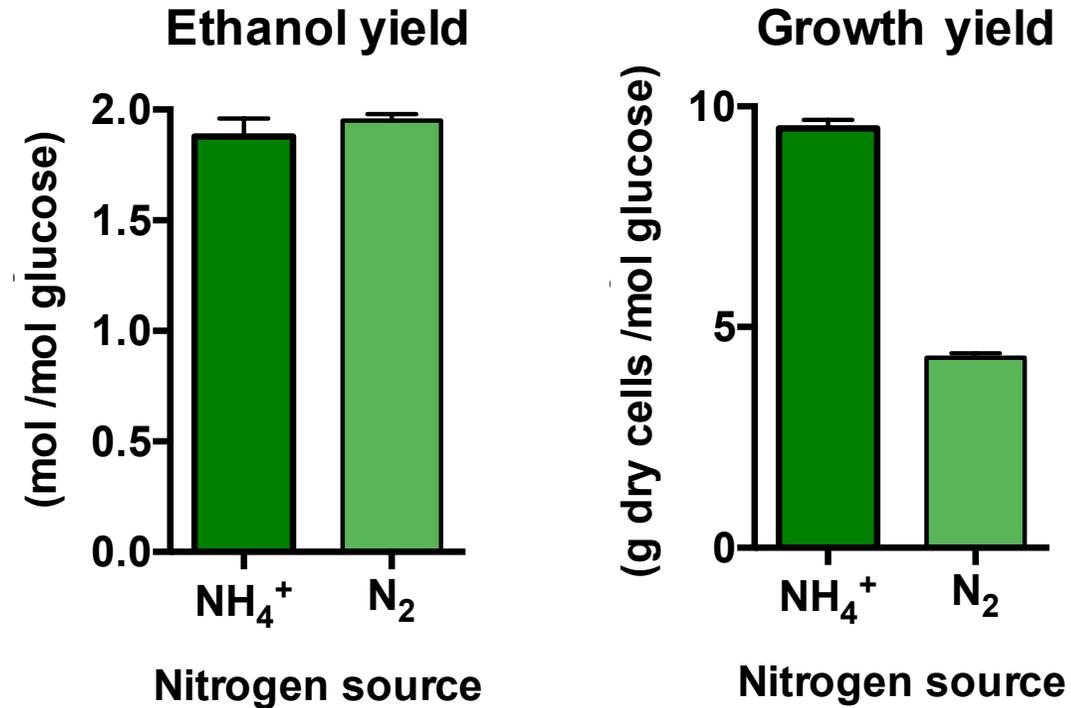


# N<sub>2</sub> fixation in ethanol-producing *Z. mobilis*



- How does *Z. mobilis* partition electrons between ethanol production and N<sub>2</sub> fixation/growth?

# N<sub>2</sub> as a fertilizer for ethanol production



- Using N<sub>2</sub>-fixing *Z. mobilis* could save an ethanol facility >\$1 million per year

\*Kremer, \*LaSarre, Posto, and McKinlay. 2015. PNAS \*equal contribution

Also supported by an Oak Ridge Associated Universities Ralph E. Powe Junior Faculty Enhancement Award

# Impact of Early Career award on my research program

## Grants

- JGI Community Science Program (PI)
  - Sequencing of naturally-evolved cross-feeding relationships
- DoD Multidisciplinary University Research Initiative (co-PI)
  - Evolution of cooperative microbial communities
- Defense University Research Instrumentation Program (PI)
  - High-throughput capabilities in anaerobic microbiology
- NSF CAREER (PI)
  - Impact of bacterial motility and adhesion on cross-feeding interactions

# Impact of Early Career award on my research program

## People

- Dr. Breah LaSarre
  - Joined lab as a postdoc in 2013
  - Applied to lab after reading DOE award press release
  - NIH NRSA fellowship from 2014-2017
- Dr. Alexandra 'Ali' McCully
  - Joined lab as a graduate student in 2014
  - Graduated in 2018 with numerous awards
  - Now a postdoc with Prof. Alfred Spormann at Stanford
  - Recently received a Simon's Foundation Postdoctoral Fellowship
- Ryan Fritts





# The McKinlay Lab



Tony  
Zmuda

Alekhya  
Govindaraju

Amee  
Sangani

Dr. Breah  
LaSarre

Jennifer  
Gliessman

Ryan  
Fritts

Jeffrey  
Mazny

Dr. Ali  
McCully

Elizabeth  
Parent

Dr. Jordan  
Bird

Ella  
Chuang

## Coculture collaborators:

**Lennon Lab** – Jay Lennon

**Lynch Lab** – Mike Lynch, Megan Behringer

**Drummond Lab** – Allan Drummond, Evgeny Pilipenko

## Coculture funding:

US Department of Energy

Army Research Office

National Science Foundation

Joint Genome Institute

IU College of Arts and Sciences



@mckinlab

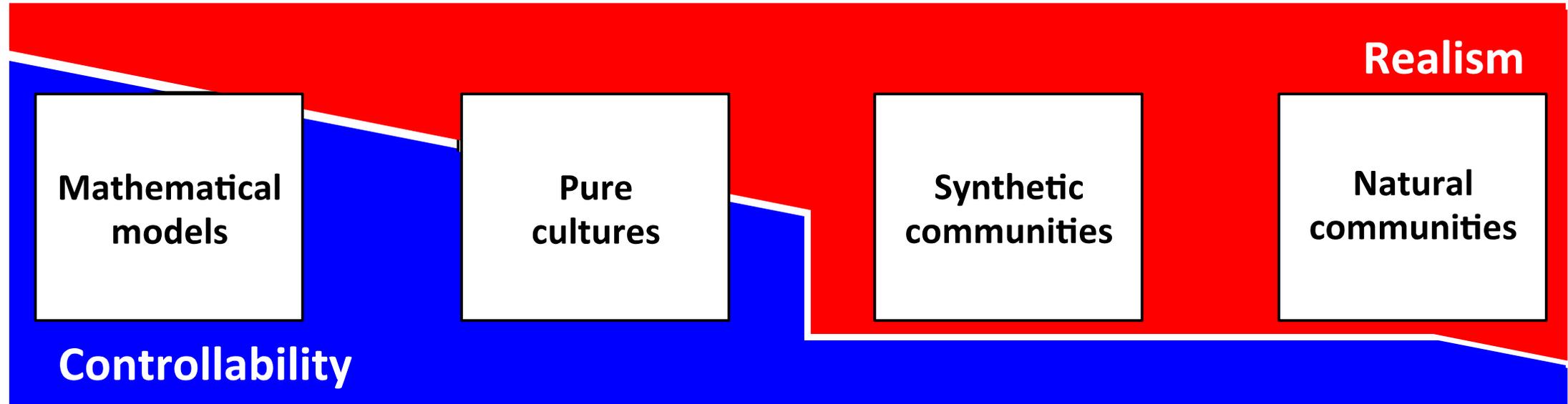
@JakeMcKinlay

# Impact of Early Career award on my research program

## Publications and Patents

1. **McKinlay**, Oda, Rühl, Posto, Sauer, Harwood. 2014. Non-growing *Rhodopseudomonas palustris* increases the hydrogen gas yield from acetate by shifting from the glyoxylate shunt to the tricarboxylic acid cycle. J Biol Chem. 289: 1960-1970.
  2. Gordon and **McKinlay**. 2014. Calvin cycle mutants of photoheterotrophic purple non-sulfur bacteria fail to grow due to an electron imbalance rather than toxic metabolite accumulation. J Bacteriol. 196: 1231-1237.
  3. Kremer, LaSarre, Posto, **McKinlay**. 2015. N<sub>2</sub> gas is an effective fertilizer for bioethanol production by *Z. mobilis*. PNAS. 112: 2222-2226.
  4. McCully and **McKinlay**. 2016. Disrupting Calvin cycle phosphoribulokinase activity results in proportional increases to both H<sub>2</sub> yield and specific H<sub>2</sub> production rate. Int J H2 Energy. 41: 4143-4149.
  5. LaSarre, McCully, Lennon, **McKinlay**. 2017. Microbial mutualism dynamics governed by dose-dependent toxicity of cross-fed nutrients. ISME J. 11:337–348.
  6. Fritts, LaSarre, Stoner, Posto, **McKinlay**. 2017. A *Rhizobiales*-specific unipolar polysaccharide adhesin contributes to *Rhodopseudomonas palustris* biofilm formation across diverse photoheterotrophic conditions. Appl Environ Microbiol. 83: doi:10.1128/AEM.03035-16
  7. McCully, LaSarre, **McKinlay**. 2017. Growth-independent cross-feeding modifies boundaries for coexistence in a bacterial mutualism. Environ Microbiol. 19: 3538-3550.
  8. McCully, LaSarre **McKinlay**. 2017. Recipient-biased competition for an intracellularly generated cross-fed resources is required for coexistence in a bacterial mutualism. mBio. 8: e01620-17
  9. McCully, Behringer, Gliessman, Pilipenko, Mazny, Lynch, Drummond, **McKinlay**. 2018. A nitrogen starvation response is important for *E. coli* to coexist in a mutualistic cross-feeding relationship with *Rhodopseudomonas palustris*. Appl Environ Microbiol. 84:e00404-18
2015. McKinlay, JB, TA Kremer, B LaSarre, AL Posto. Culture conditions that allow *Zymomonas mobilis* to assimilate N<sub>2</sub> gas as a nitrogen source during bio-ethanol production. (submitted). <https://patents.google.com/patent/WO2016109286A1/en>

# Synthetic microbial communities are useful experimental systems...



... provided that a reasonable level of control can  
be achieved