# Guided microbial remodeling: flipping the switch for better nutrient availability and uptake by crops

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PIVOT BIO

# Pivot Bio's Commitment

Pivot Bio is on a mission to replace all nitrogen fertilizer with microbes that adhere to the crop's root system and feed the crop each day

# Cereal Crops

## Feed the World

CORN WHEAT RICE SORGHUM

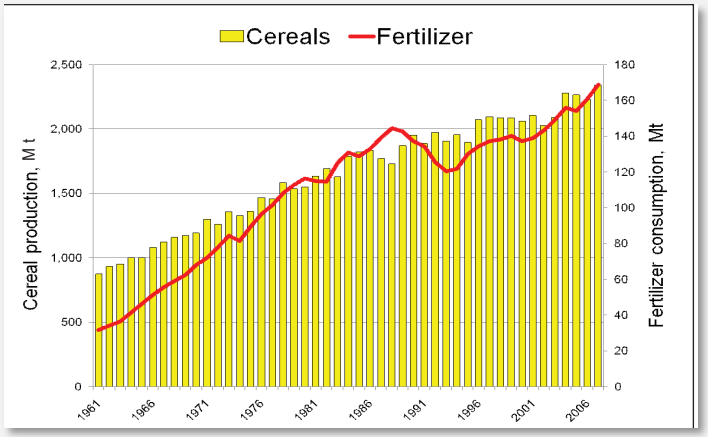
50 - 60%

of Worldwide Dietary Energy

Source: Food and Agricultural Organizations of the United Nations, USDA

# Fertilizers

## Grow the World's Food




40 - 60%

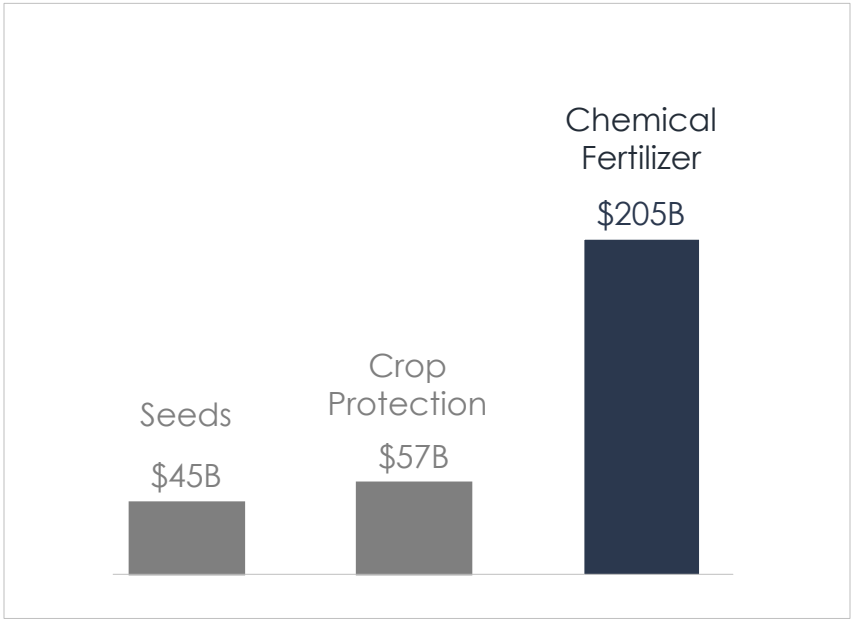
of World Food Production

Source: World cereal production and fertilizer production, 1961-2007 (IFA Statistics, 2007; FAOSTAT, 2008)

# Chemical fertilizer industry didn't exist 100 years ago

We have become dependent on fertilizer


The fertilizer market is huge



# Nitrogen fixation in agriculture

## SYNTHETIC

Haber-Bosch Process

Convert $N_2$ to ammonia via high temp and pressure

## BIOLOGICAL

Legume-Rhizobia Symbiosis

Bacteria convert $N_2$ to ammonia in exchange for sugars

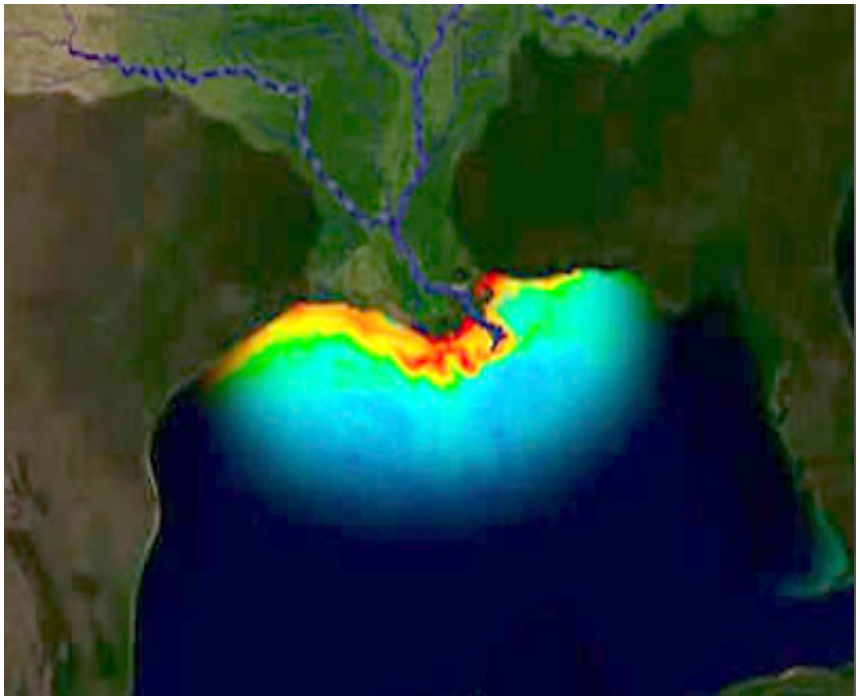
## BIOLOGICAL

Cereals

Transgenic plants
Mutualistic microbes

Source: Stokstad, Science Vol 353, Issue 6305 (2016)

# Intensification of fertilizer use has had unintended environmental consequences



Global nitrogen production of ~118 million metric tons annually

- Burns 3% of the world's natural gas
- Contributes ~13% of anthropogenic
- Results in estimated ecosystem and health damages of $157 billion annually
- Contributes to hypoxic 'dead zones'
- Disrupts terrestrial and aquatic ecosystems

*Nature Plants* **3**, Article number: 17012, March 1, 2017
National Oceanic and Atmospheric Administration (NOAA)

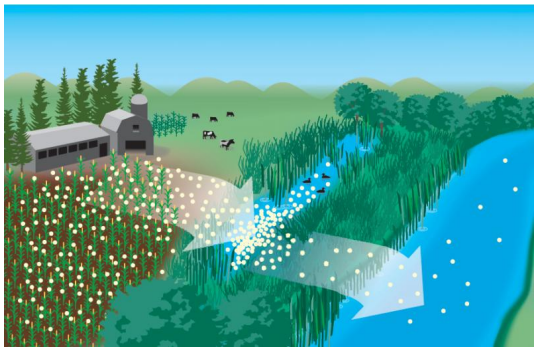
# Significant opportunity for improvements

Many fields are under fertilized

unrealized yield potential

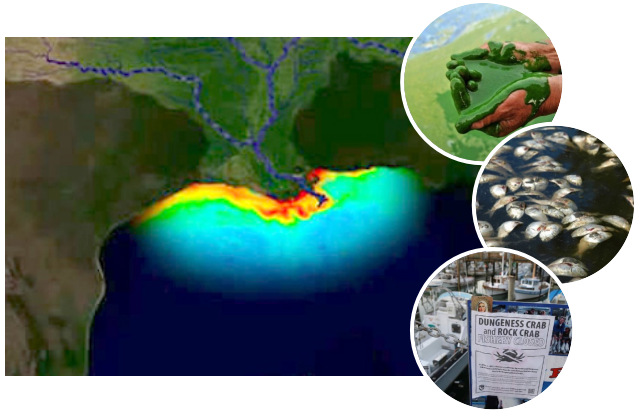
Up to 40% of acres are over fertilized

unnecessary fertilizer expense



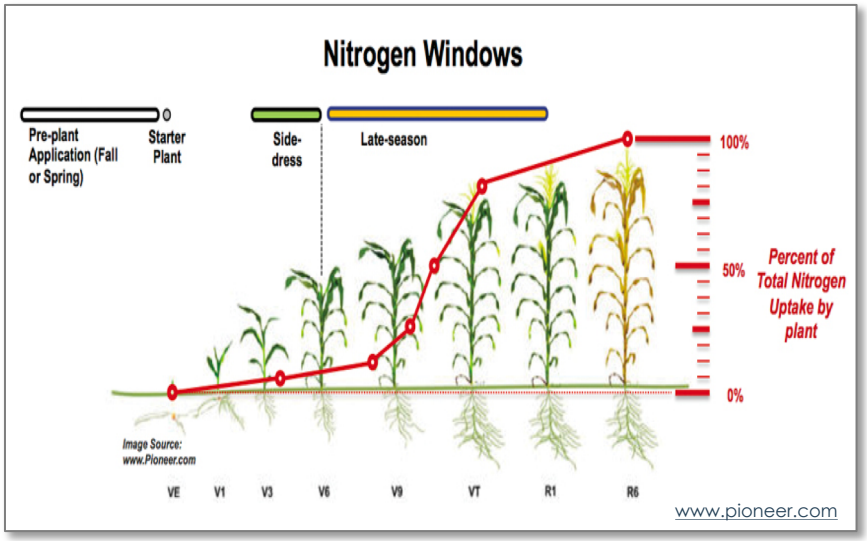
Nitrogen runoff and GHG emissions

massive environmental impact




Sources:
The social costs of nitrogen. Keeler et al. Science Advances, 2016, 10.1126/sciadv.1600219
Fertilizer Nitrogen Recovery Efficiency of Furrow-Irrigated Corn. Roberts et al. Agronomy Journal, 2016. 10.2134/agronj2016.02.0092

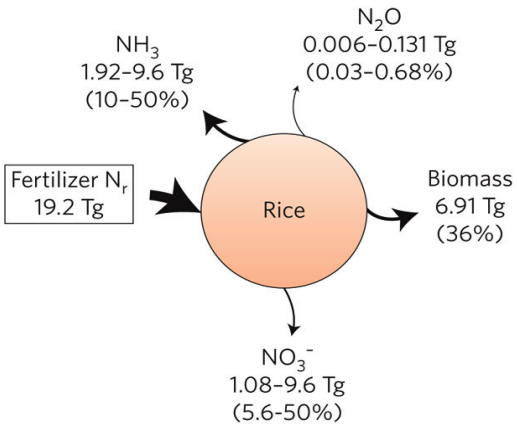
# Matching nutrient supply with plant needs

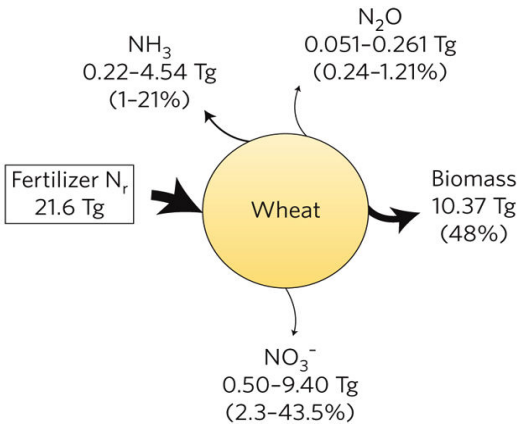


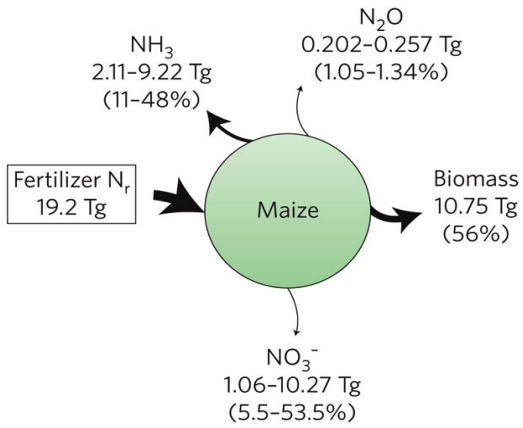

- 10% of total N needed is taken up from VE to V6
- 70% of total N needed between V6 and tassel

- Rapid period of growth from V6 to VT
- Yield is impacted by low N at V6 to V10

# Nitrogen budgets of three major cereal crops




- **Half of the N produced by Haber-Bosch is applied to rice, wheat and corn**
- **546 million ha of global cropland**
- **30-60% N retained as biomass, and the remaining is lost to the environment**

NATURE PLANTS 3, 17074 (2017) | DOI: 10.1038/nplants.2017.74 | www.nature.com/natureplants

# Long-term nitrogen use causes evolution of less-cooperative mutualists

- 22 year N-addition ecological study
- Compared growth effects of natural soil communities or single microbes from N-fertilized and non-fertilized plots
- Data showed Rhizobium strains from N-fertilized treatments produced 17-30% less crop biomass and had reduced chlorophyll content
- N inputs cause the evolution of rhizobia that provide fewer growth benefits to their hosts

Weese et al. EVOLUTION 69-3: 631-642 doi:10.1111/evo.12594

# In 10 years the fertilizer industry could be very different

TODAY

**3%**

Global energy used in fertilizer production

**>$200B**

Fertilizer market size; chemical commodity

10 YEARS

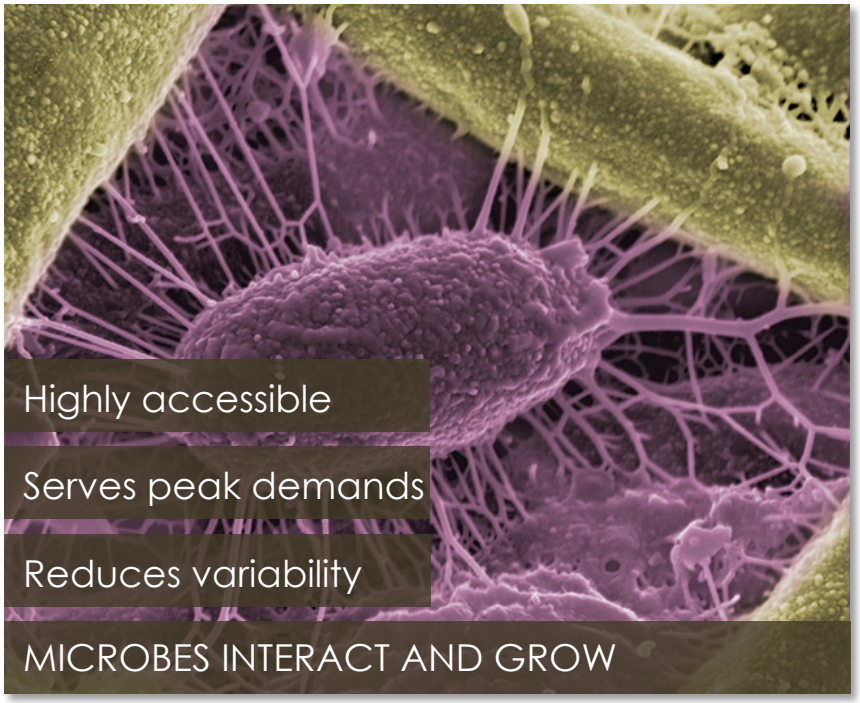
**Biological**

Seed is coated with fertilizer
Pricing is value-based

*Nature Plants* **3**, Article number: 17012, March 1, 2017

# Pivot develops microbes that fertilize crops

CHEMICALS ARE STATIC AND DEGRADE



Highly accessible

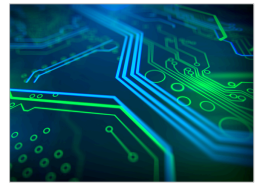
Serves peak demands

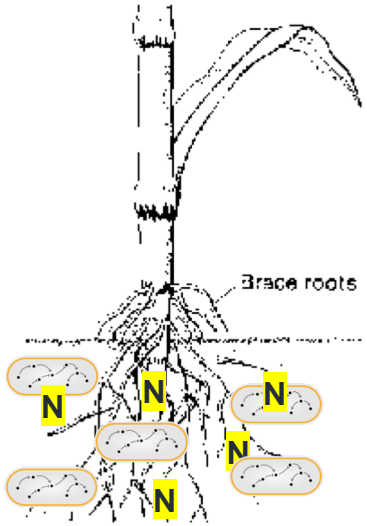
Reduces variability

MICROBES INTERACT AND GROW

# Metrics of a successful nitrogen biofertilizer

- Express nitrogenase genes in the presence of fertilizer
- Robust rhizosphere colonization in field conditions
- Release of fixed N from microbe
- Transfer of fixed N to host plant

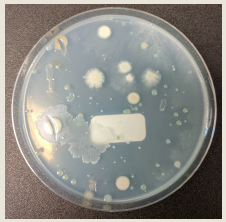


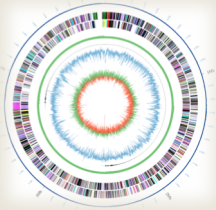



# Pivot's approach to strain optimization

1 Identification of microbes that interact with crops and carry the innate genetics for a trait of interest

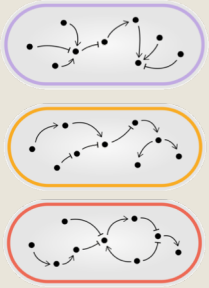
prospecting

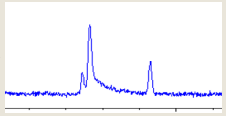




2 Directed improvement of microbial strains through intragenomic rearrangement of regulatory nodes

design/build





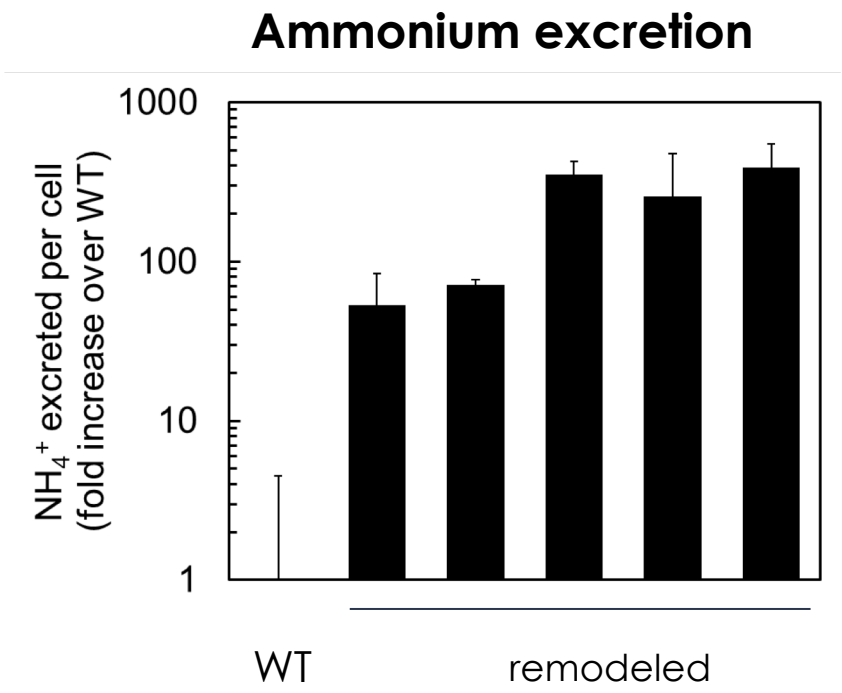
3 Inoculation of crops with the remodeled microbes to screen for improved microbial phenotypes and crop traits

test

# Optimized nitrogen release in remodeled bacteria

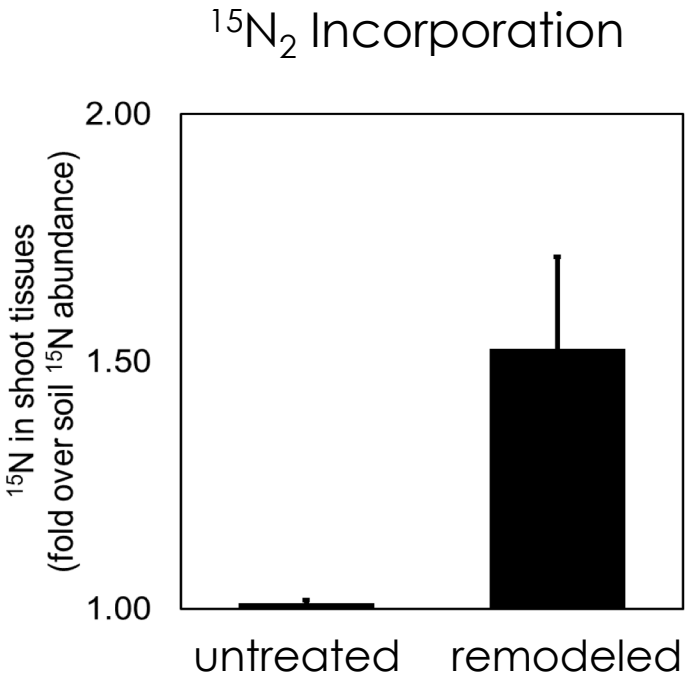
## Ammonium excretion assay

- culture microbes anaerobically in N-free media
- measure ammonium in media
- a direct measure of N released by microbial cells



# Optimized nitrogen transfer in remodeled bacteria

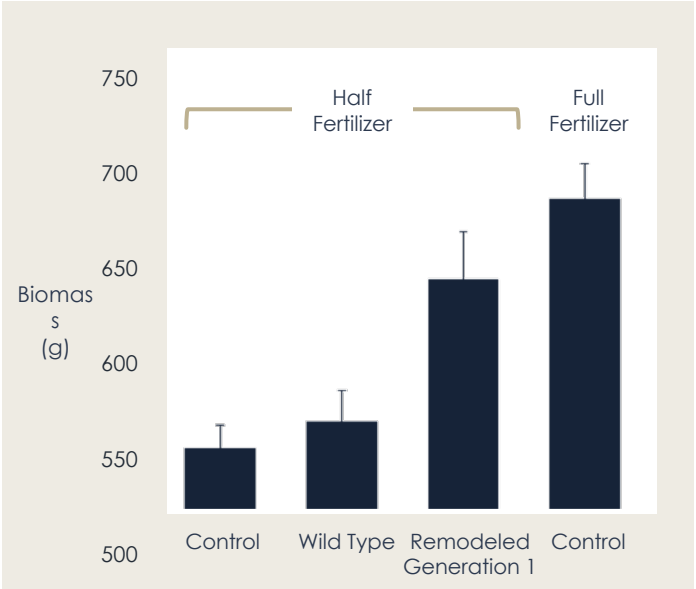
*Setaria italica*



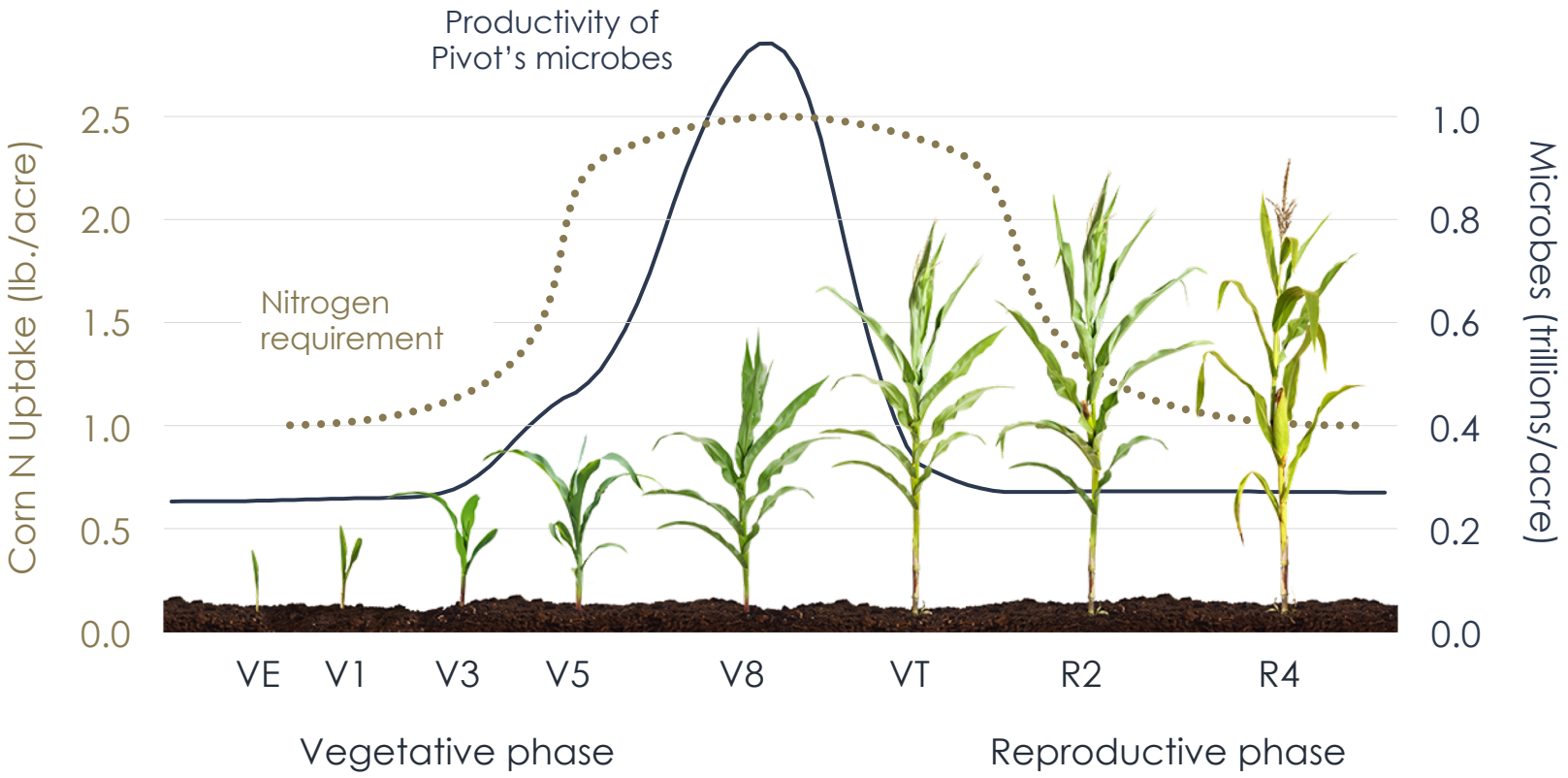

## *Setaria* $^{15}N_2$ incorporation assay

- small grass as model organism
- 27 days of growth
- 3 days exposure to $^{15}N_2$
- immediate sampling of shoot tissues for Isotope Ratio Mass Spec (IRMS) analysis

# Remodeled bacteria provide fixed nitrogen in corn

# Pivot designs microbes to produce nitrogen when most needed

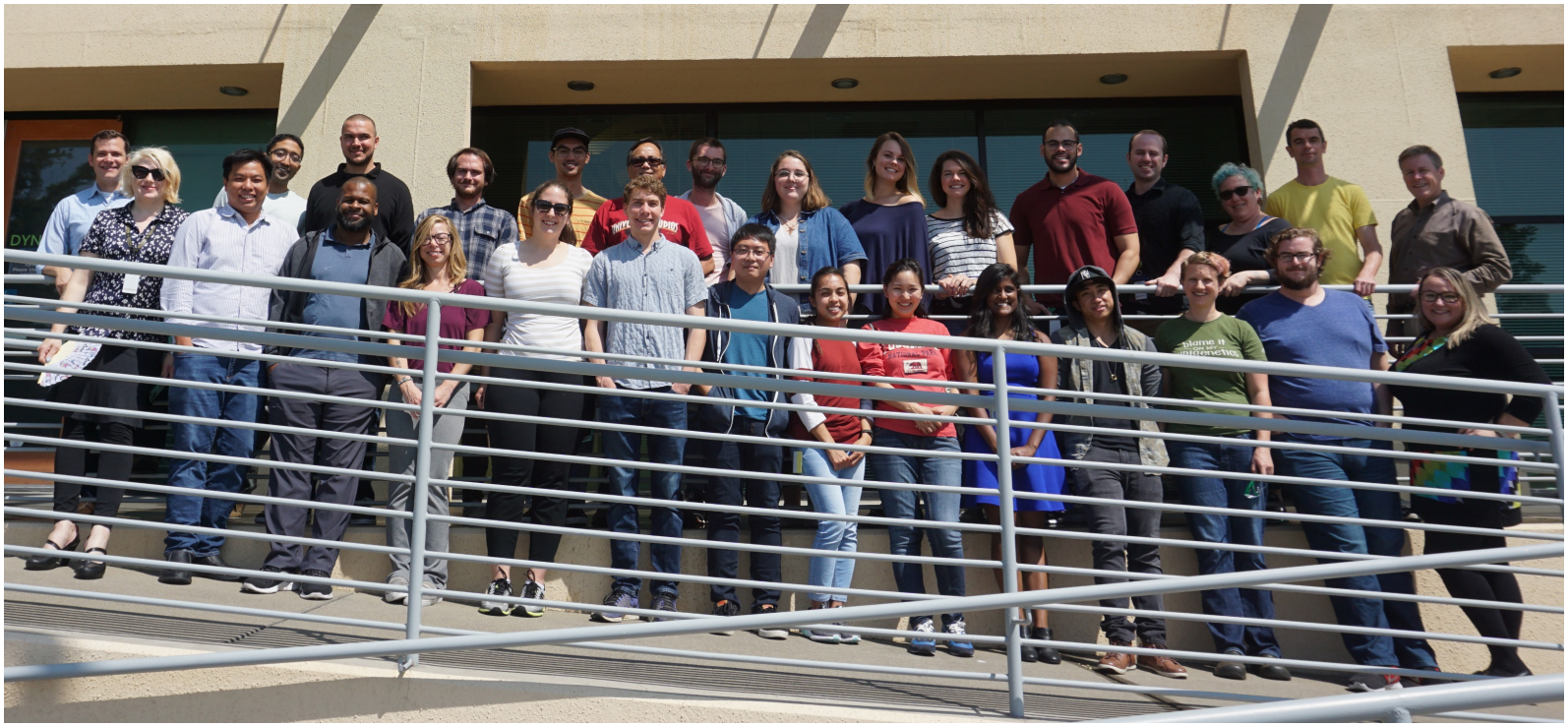


# Our commitment

Pivot Bio is on a mission to replace nitrogen fertilizer with microbes that thrive in the crop's root system and feed the crop each day.

**"We're on a journey to improve agriculture, for farmers, our kids and future generations."**

# The Pivot Bio Team



FUNDING

Bill & Melinda Gates foundation · NSF · DARPA · Data Collective · MGV · Prelude Ventures · Spruce Capital Partners LLC