

A NEW NITROGEN CYCLE IN AGRICULTURE FOR BIOENERGY CROPS

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Acknowledgements



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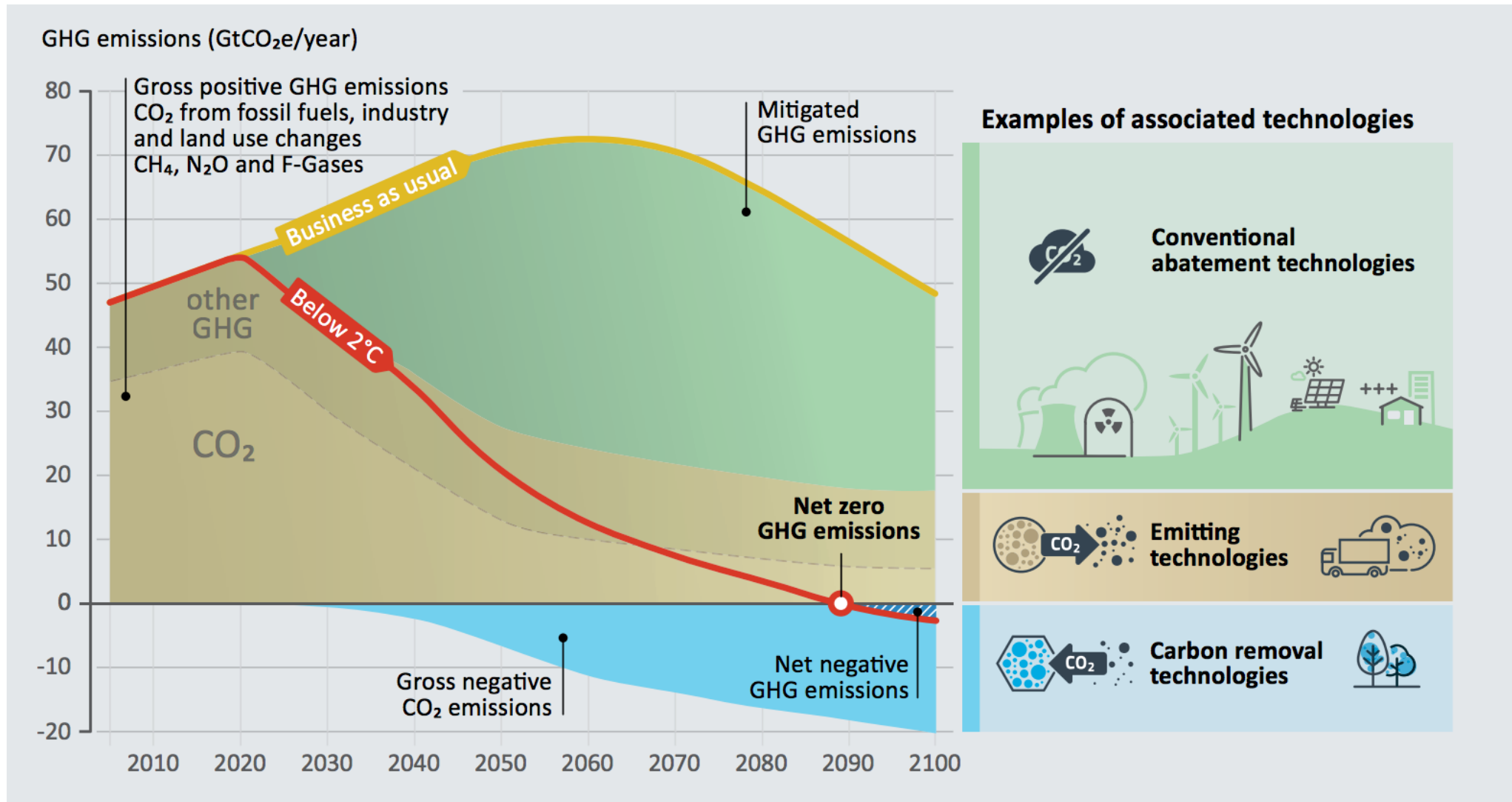


Miriam
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Lee

All paths to <math><2^{\circ}\text{C}</math> warming require GHG mitigation and negative emissions



GHG emissions from agriculture are difficult to decarbonize

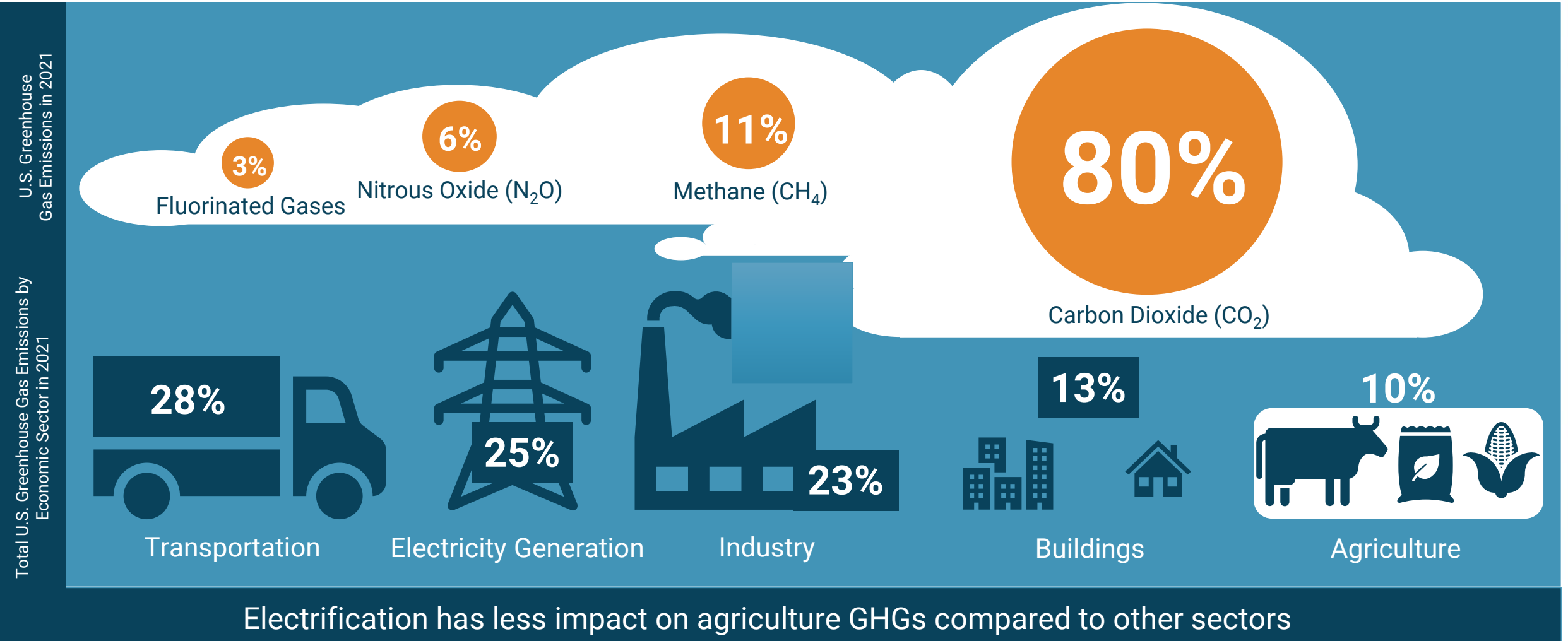
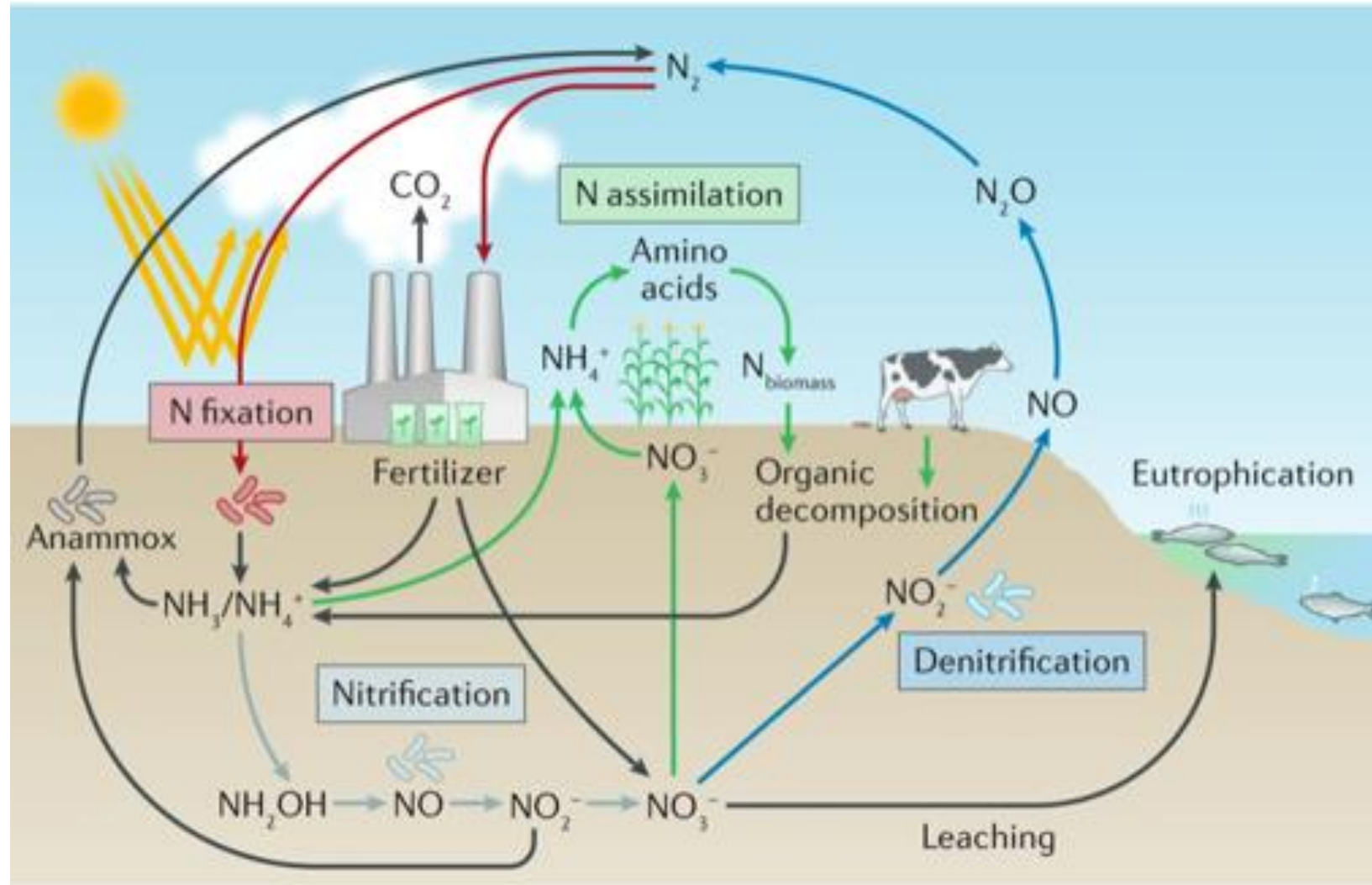


Figure 1: Greenhouse Gas Contribution in the US.

Graphic adapted from University of Nebraska-Lincoln Institute of Agriculture and Natural Resources
Data from Environmental Protection Agency, Inventory of U.S. Greenhouse Gas Emissions and Sinks (2021)

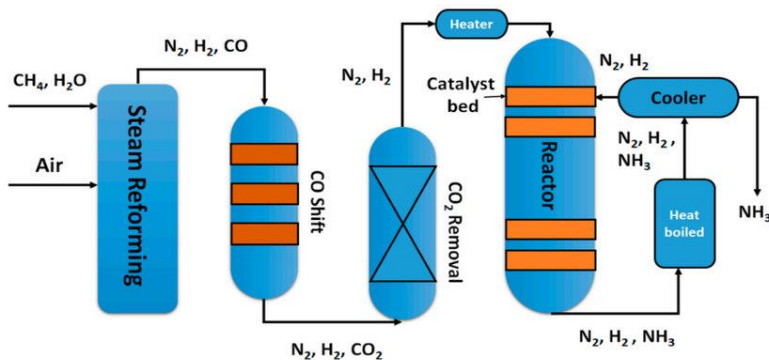
The agricultural nitrogen cycle emits CO₂ and N₂O (6% total GHGs)



The challenges of the current agricultural N cycle

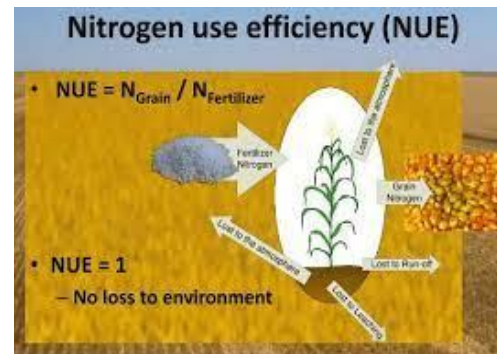
- ▶ Synthetic NH_3 production is a high energy process & generates significant CO_2
- ▶ A significant fraction of N input to cropland is lost to the environment
- ▶ Excess N inputs to soil lead to N_2O emissions and eutrophication in waterbodies

Haber-Bosch Process



- 1% of global energy (0.5% US)
- 1-2% global emissions

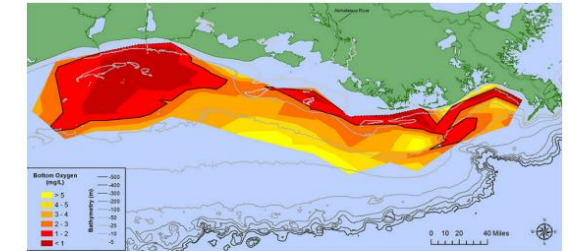
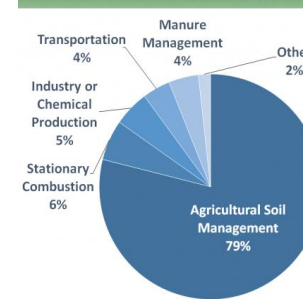
Nitrogen Use Efficiency (NUE)



- Corn <50%
- \$4.2B lost

N_2O emissions and eutrophication

U.S. Nitrous Oxide Emissions, By Source



Map showing the hypoxia area on the Louisiana Gulf of Mexico shelf in 2021

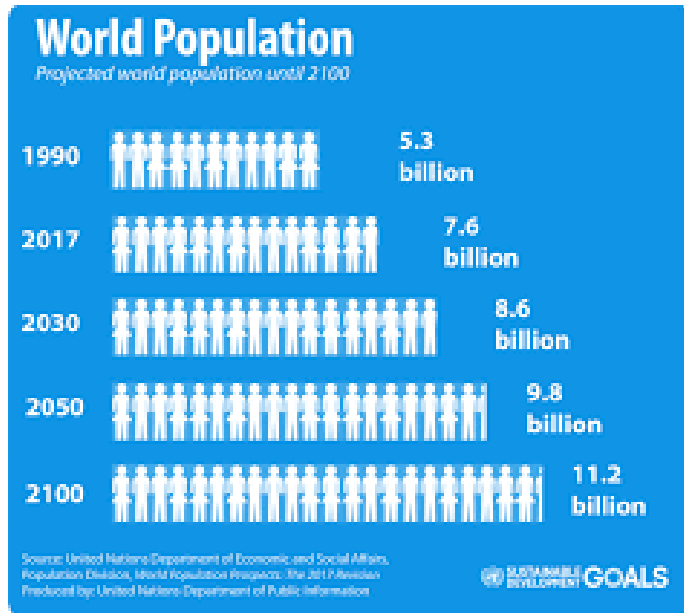
Credit: N. Rabalais, LSU/LUMCON (NOAA 2021)

<https://oceanservice.noaa.gov/facts/deadzone.html>

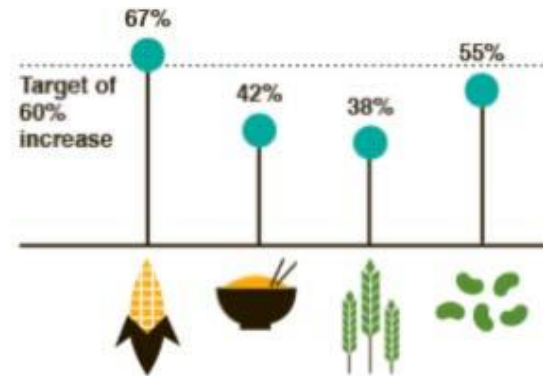
- N_2O GWP is 273x CO_2 at 100-year time horizon
- Ag N_2O emission 4% of total GHGs as CO_2e
- ~27% of N input to soils is lost to waterbodies
- Creates coastal dead zones

These challenges will only get more difficult!

Food



Yields of maize, rice, wheat, and soybean all need to **INCREASE BY 60%**, by 2050 to meet demand but current growth in yield are falling short of the target.



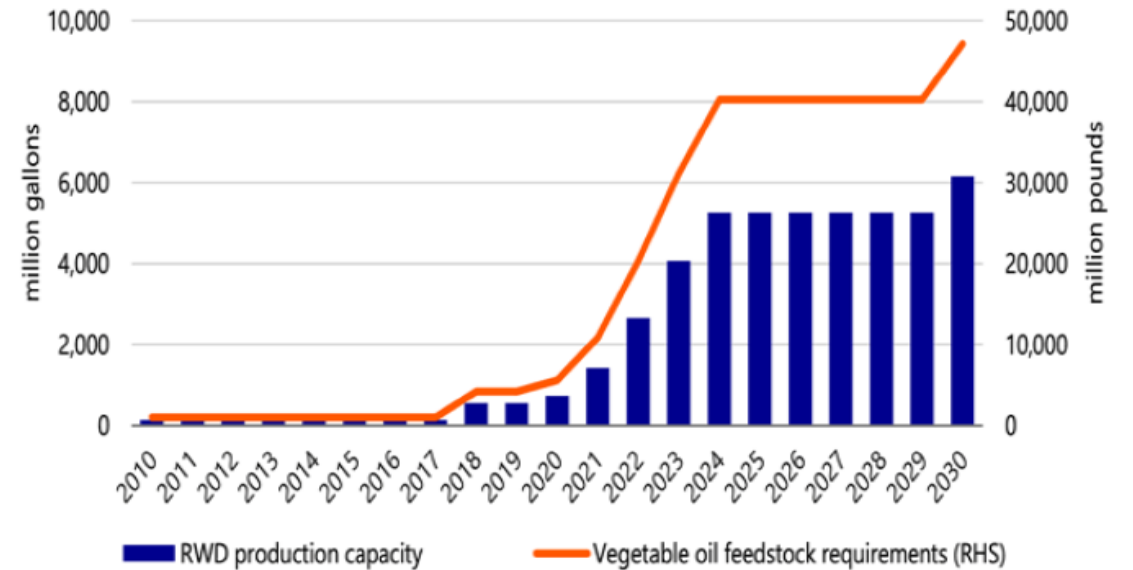
Source: Ray et al., 2013

Big Facts
ccafs.cgiar.org/bigfacts



Fuel

Figure 3: Rapid ramp-up in US renewable diesel production capacity, 2010-2030

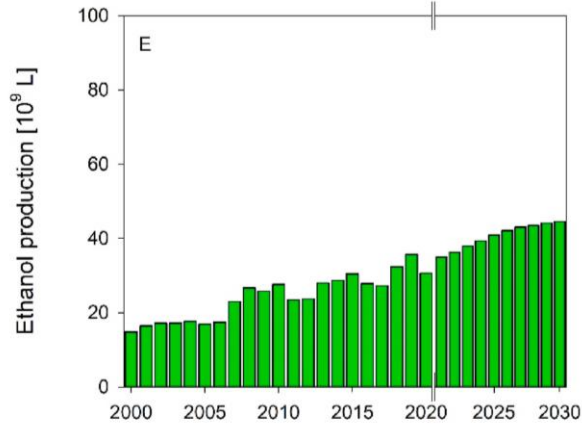


Source: Biodiesel Magazine, Rabobank 2021

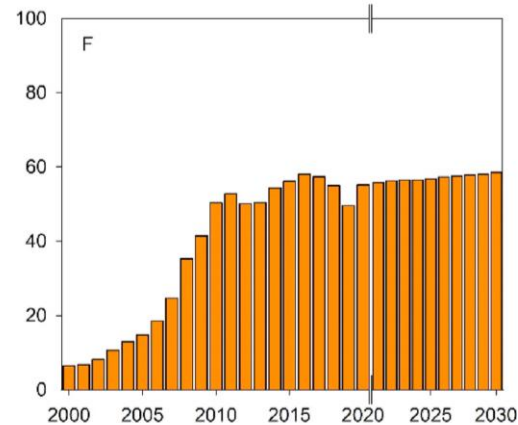
Biofuel production and increased N fertilizer use are correlated

Ethanol production

Brazilian sugarcane

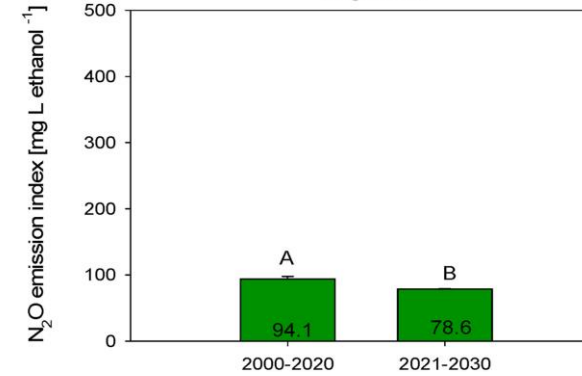


US corn

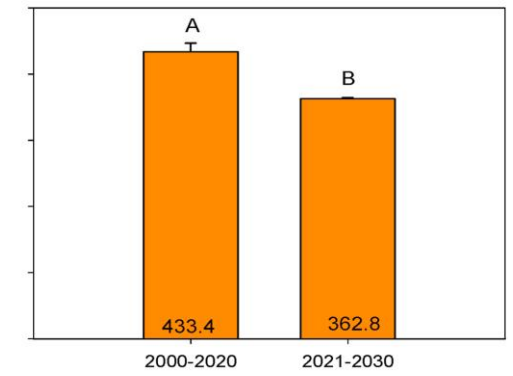


N₂O emissions

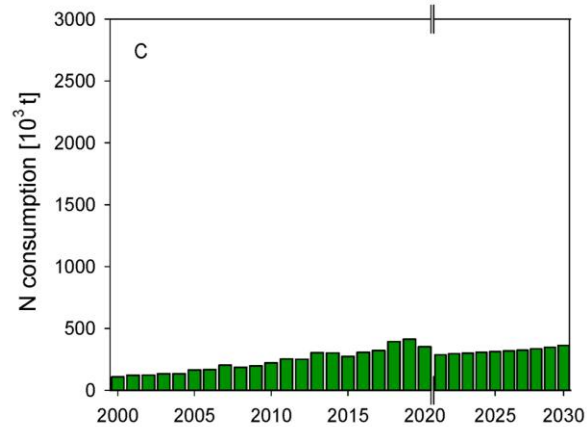
Sugarcane



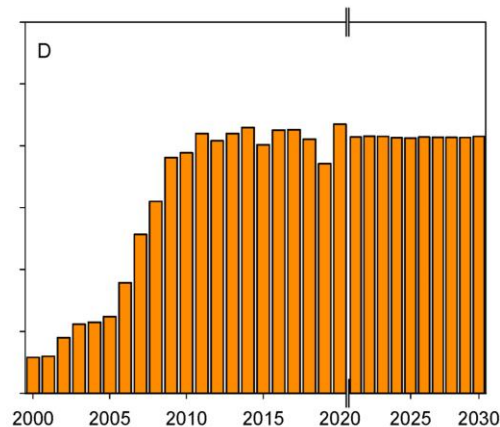
Corn



N consumption [10³ t]



N consumption [10³ t]



FUELING THE FUTURE FOR CLEANER SKIES

Take off with UOP's ethanol to jet (ETJ) process technology. The next generation of renewable fuels.

BENEFITS OF ETJ

- High jet yield output
- Lower CAPEX & OPEX
- Reduced GHG emissions
- Higher profit margins

1. FEEDSTOCK SUPPLY

2. ETHANOL PLANT

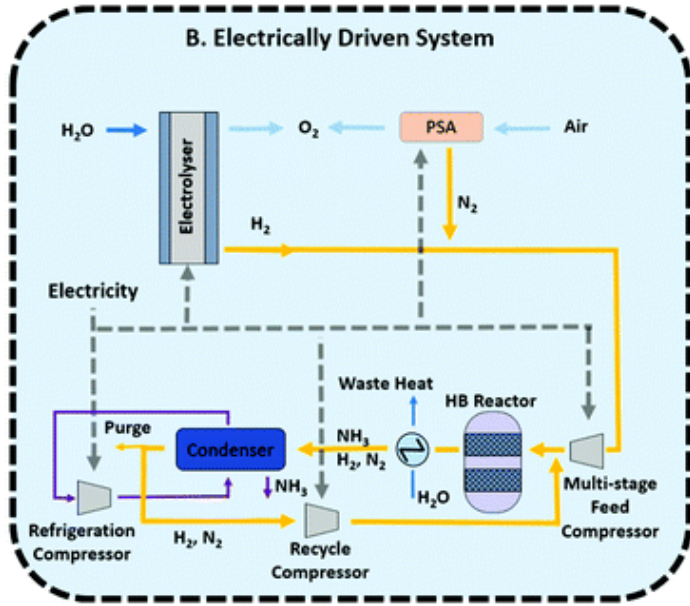
3. ETHANOL TO JET

4. SUSTAINABLE AVIATION FUEL (SAF) BLEND

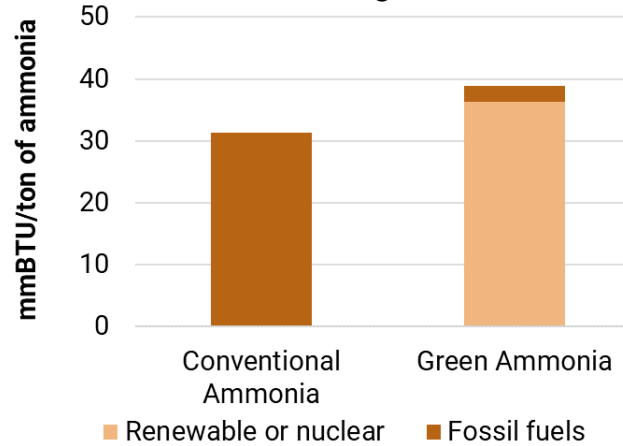
5. SUSTAINABLE AVIATION FUEL (SAF)

Honeywell

Green NH₃ reduces CO₂ emissions in agriculture but not N₂O

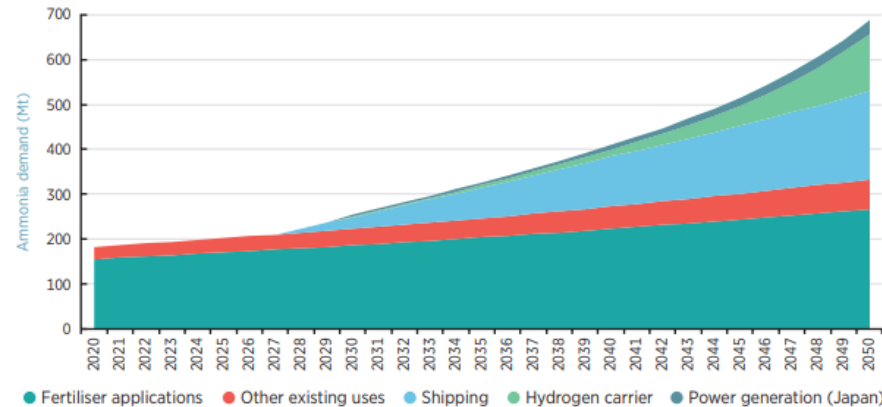


Energy for NH₃ synthesis



GREET 1 2022, Ag Inputs, Table 3

NH₃ in 2050 (1.5°C scenario)



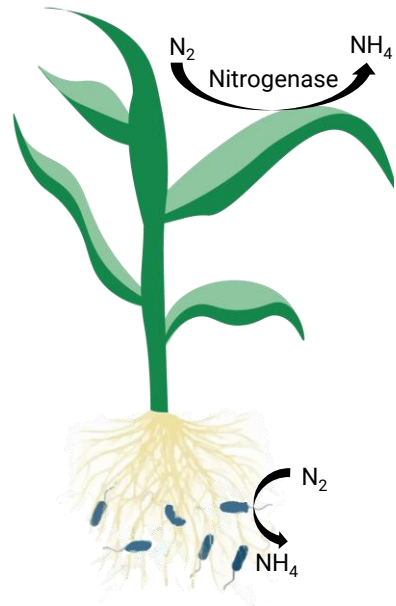
94 million MT of green H₂ needed to produce 80% of projected ammonia demand in 2050 (566 Mt ammonia)

- 44% of projected global H₂ in 2050 (Wood MacKenzie)

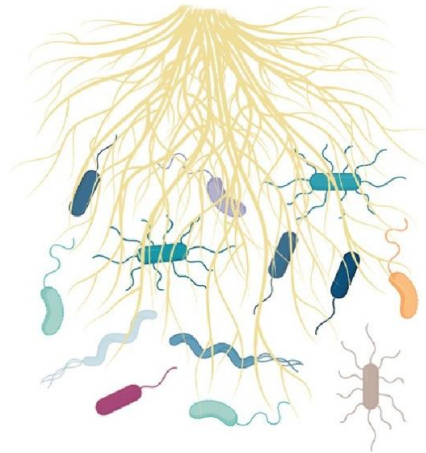
Adapted from: *Energy Environmental Science*, 2020, 13, 331

Biology can address the challenges of the N cycle: N inputs

Engineer nitrogen fixing crops, and diazotrophs

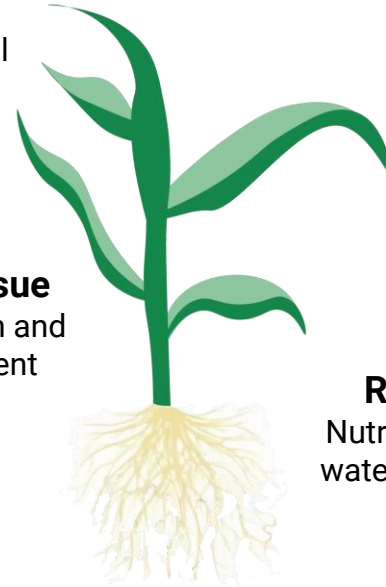


Engineer associative fixation and designed microbiomes



Plant N uptake and metabolism

Leaves
Chlorophyll

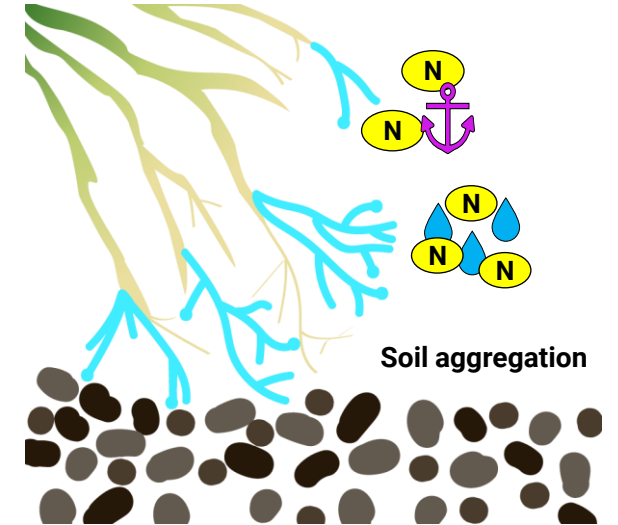


Plant Tissue
Plant growth and development

Grain
Protein

Roots
Nutrient and water uptake

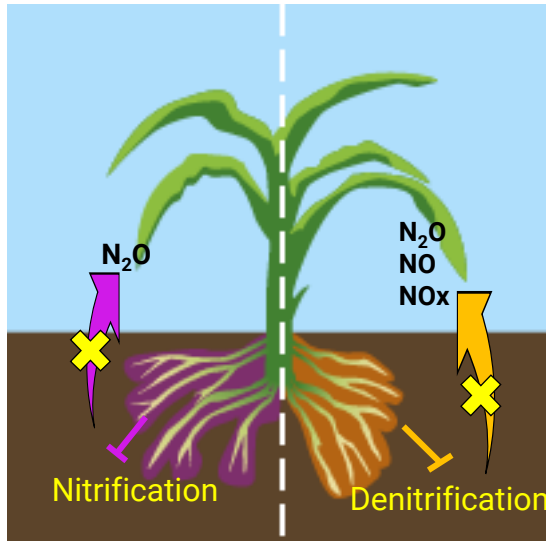
Soil N mineralization



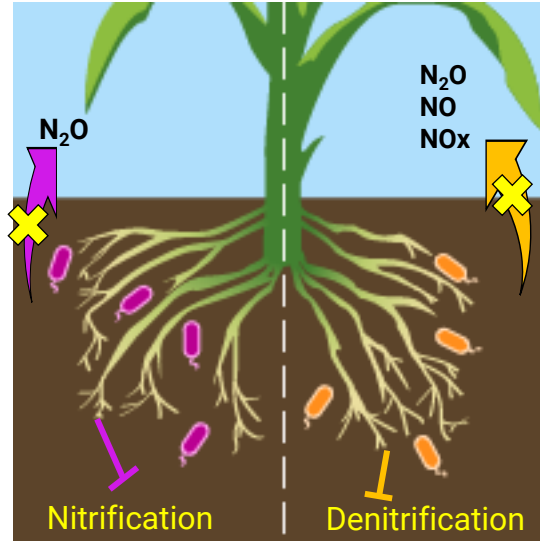
Biology can address the challenges of the N cycle: N₂O emissions

Inhibit N₂O emissions from nitrification or denitrification

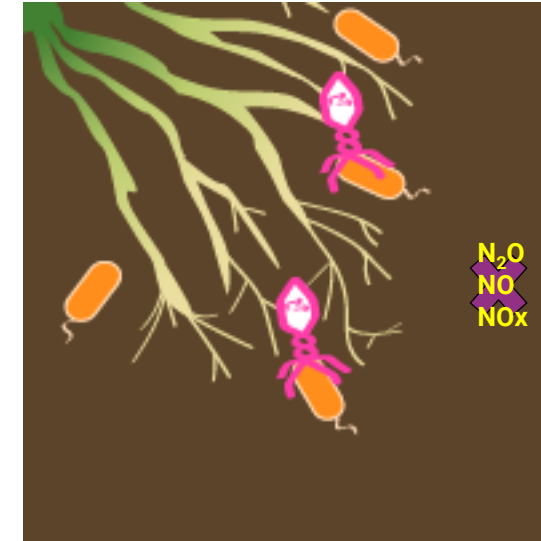
Engineer crops



Engineer microorganisms or design microbiomes



In field gene editing of native soil microbiome



Why ARPA-E: building on established ARPA-E programs

Trait development

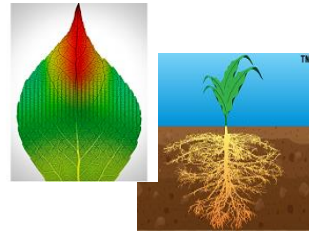
PETRO



Plant metabolic engineering and synthetic biology

Identify improvements

**TERRA
/ ROOTS**



Focus on breeding and high throughput phenotyping of energy sorghum

Enable measurement of GHG outcomes

SMARTFARM



Measuring N₂O emissions from corn and soybeans and developing scalable GHG monitoring technologies

Biofuel crops are exemplary targets for creating a new N cycle

Corn



- 16.1 tons of N/km²/y
- 40% of total US fertilizer; **6 Tg/y N input**
- 94 million acres in US
- 15 billion gallons EtOH production
- EtOH to jet major SAF pathway

Soybeans



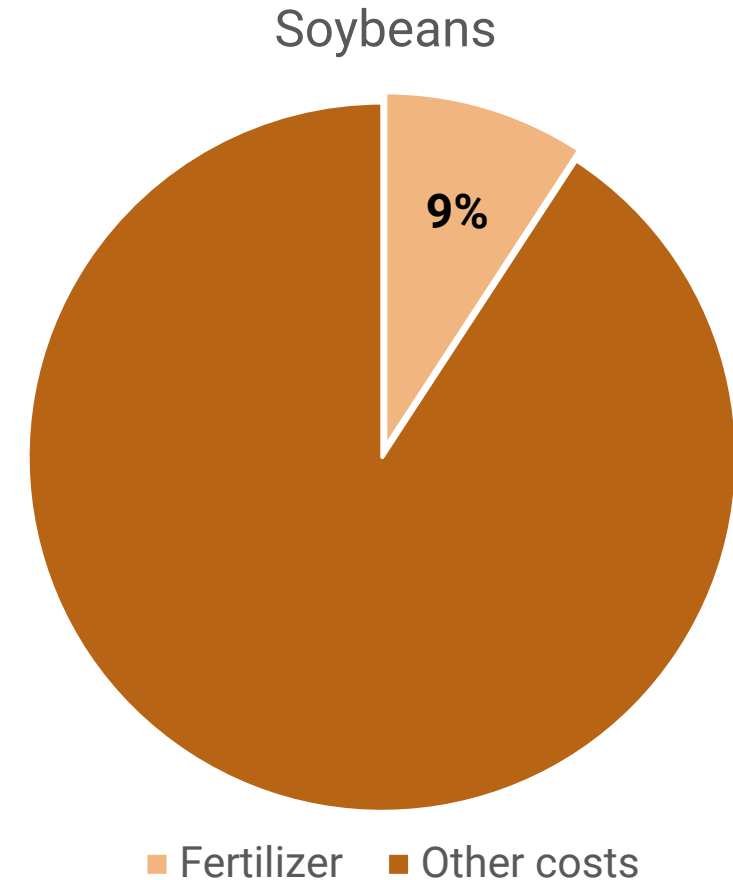
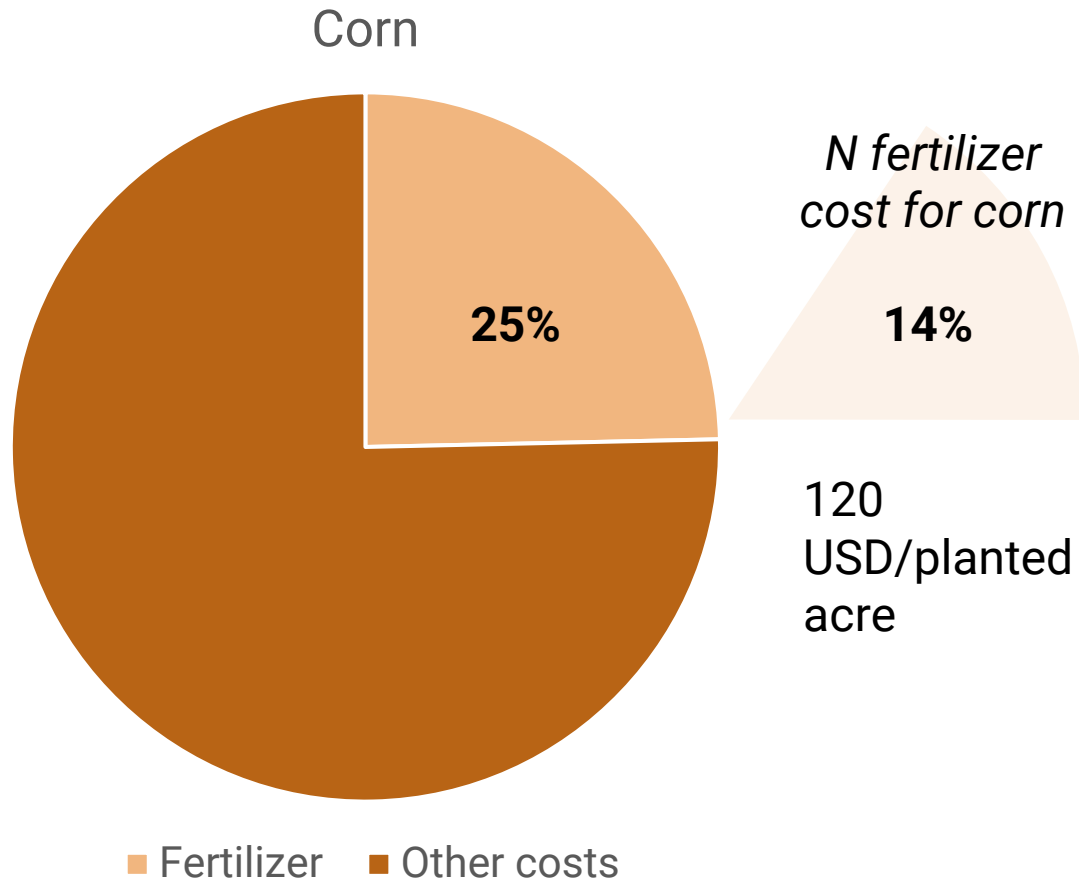
- 0.6 tons of N/km²/y
- N₂-fixing symbiosis with bacteria; **9 Tg/y N input**
- 80 millions acres in US
- 1.5 billion gallons per year biodiesel and renewable diesel
- Hydrotreated oil for SAF

Sorghum



- 7.4 tons of N/km²/y
- Lignocellulosic SAF production: BER focus
- 6.4 million acres
- Potential for carbon storage
- Abiotic stress tolerance

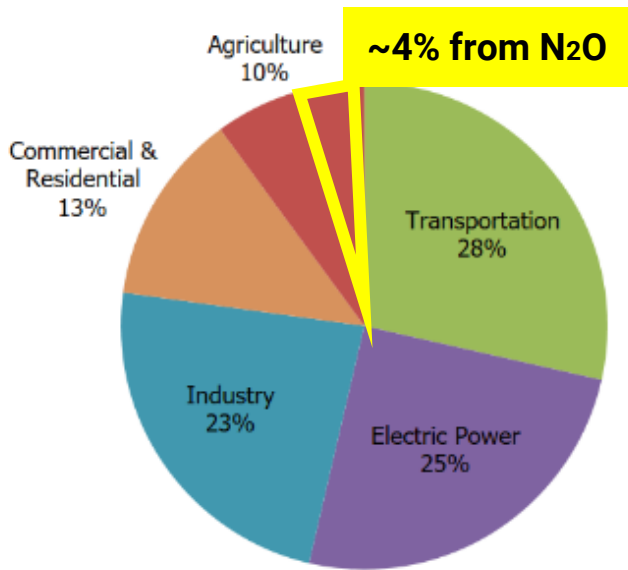
Cost of N fertilizer is significant for corn



Sources: USDA Cost-of-production forecasts for major U.S. field crops, 2022F-2023F, University of Illinois' farmdocDAILY (N fertilizer costs)

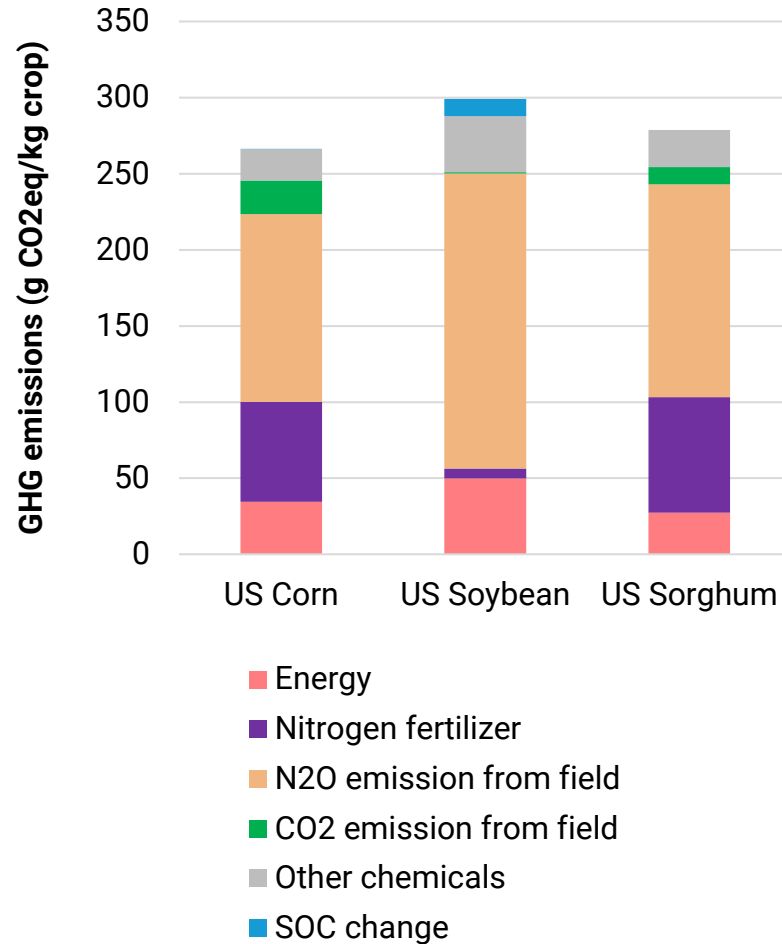
N₂O emissions result from both chemical and biological N inputs

GHG emissions by economic sector (2021)

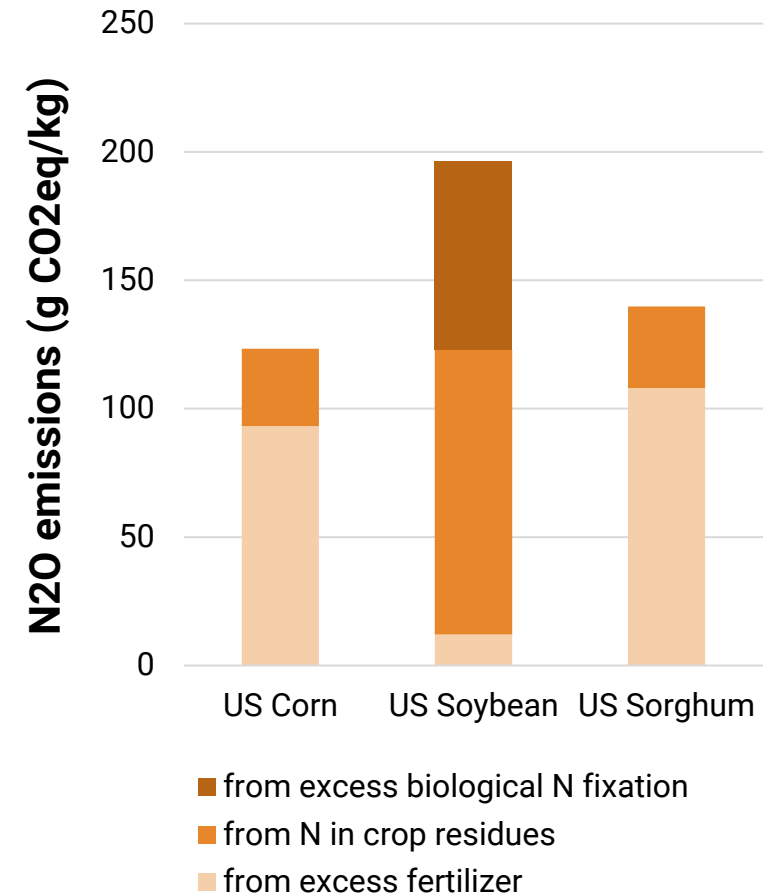


[Sources of Greenhouse Gas Emissions | US EPA](#)

Lifecycle GHG emissions

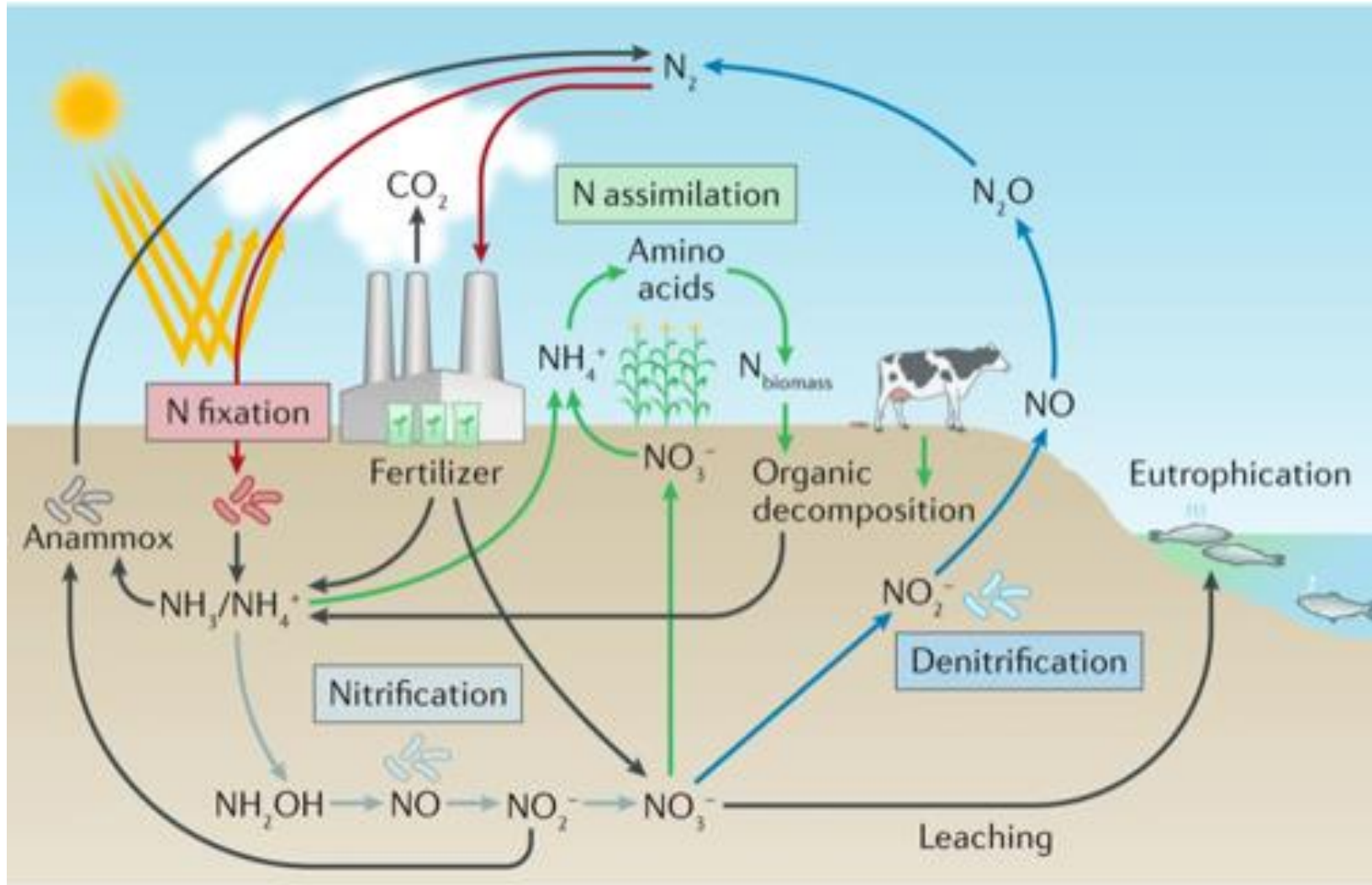


Nitrous oxide emission from field



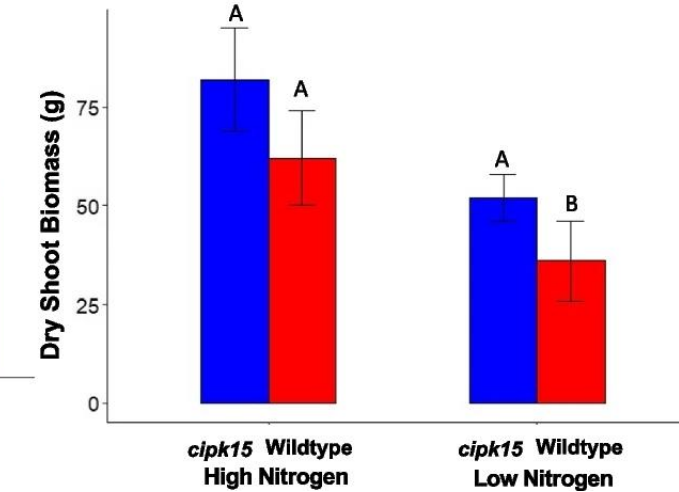
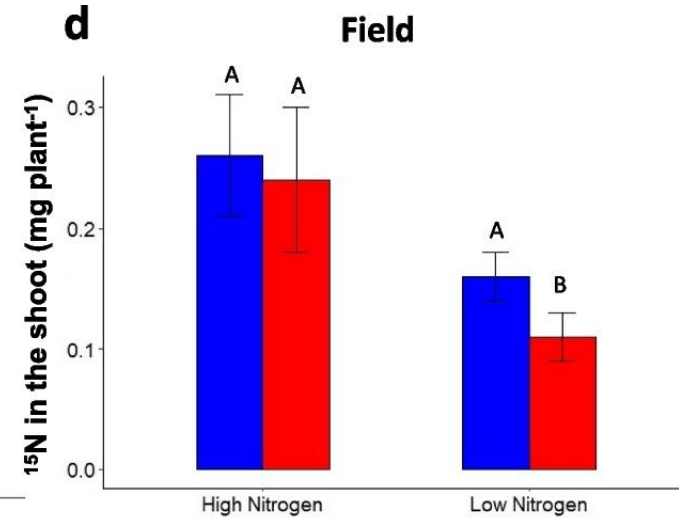
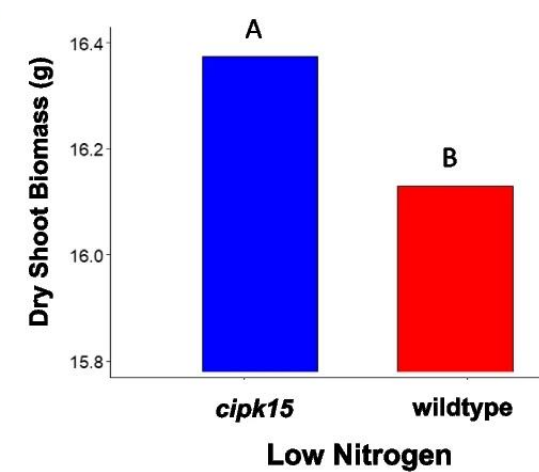
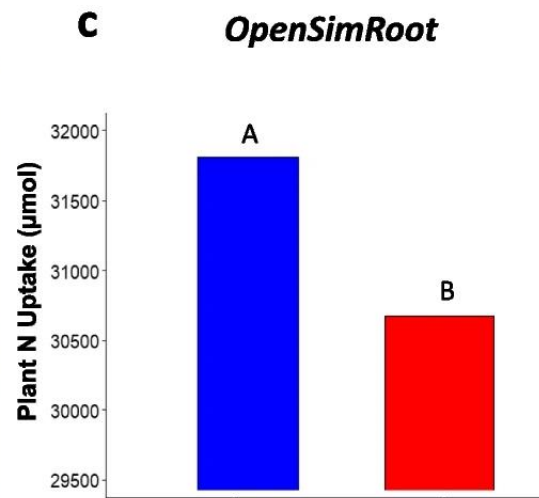
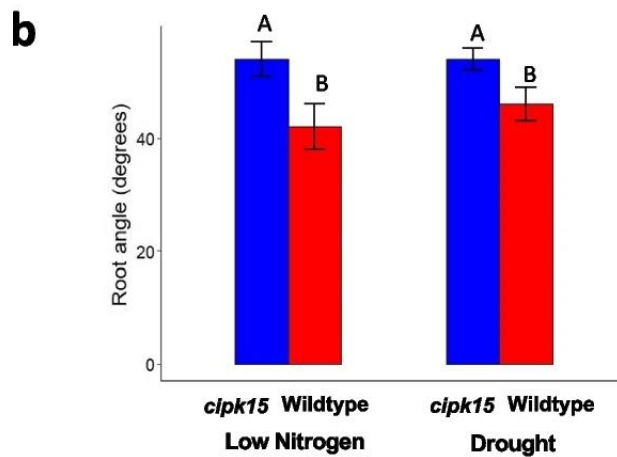
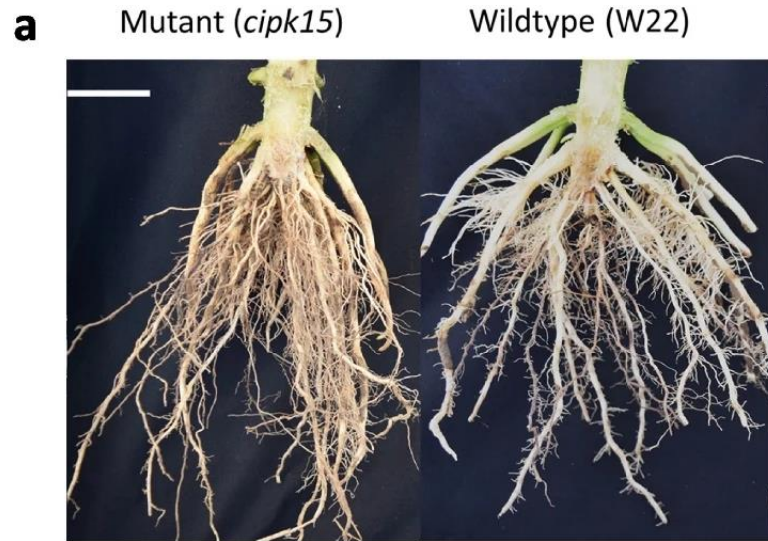
Data from GREET 2022, FD-CIC

Program technical areas



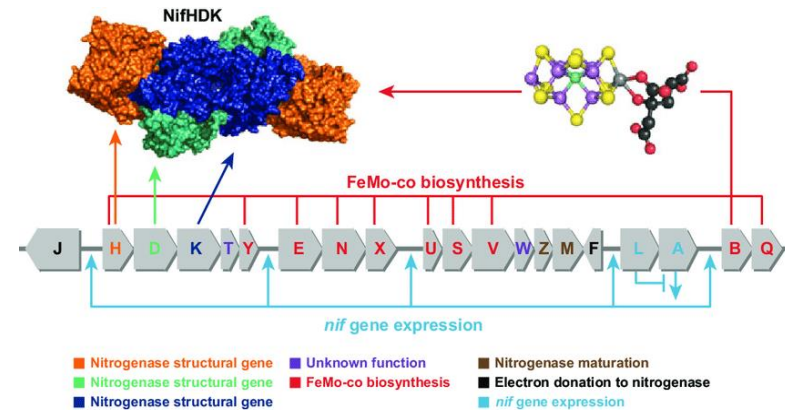
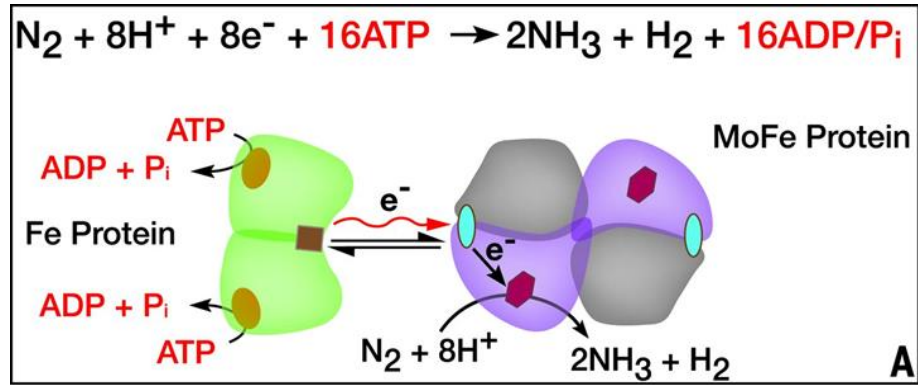
1. Plant NUE
2. Biological nitrogen fixation
3. Soil N mineralization
4. Direct N_2O mitigation

Plant nitrogen use efficiency- can we maintain yield with less N?

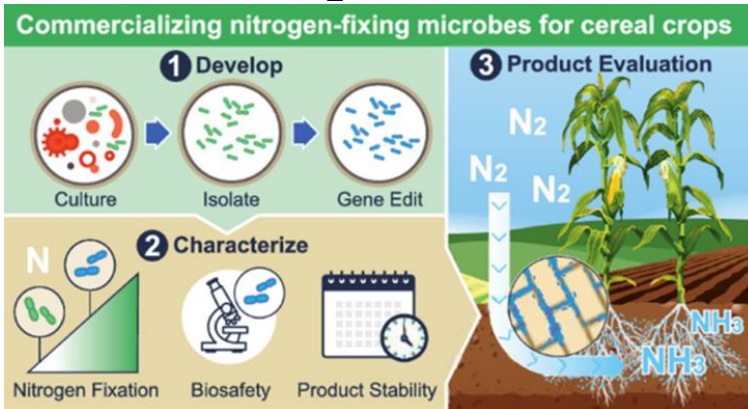


Nitrogen fixation- on roots and in plants

Nitrogenase: a complex enzyme for a complex process



Engineered N₂-fixing isolates



Wen et al. *ACS Synthetic Biology* 10:(2021): 3264–3277

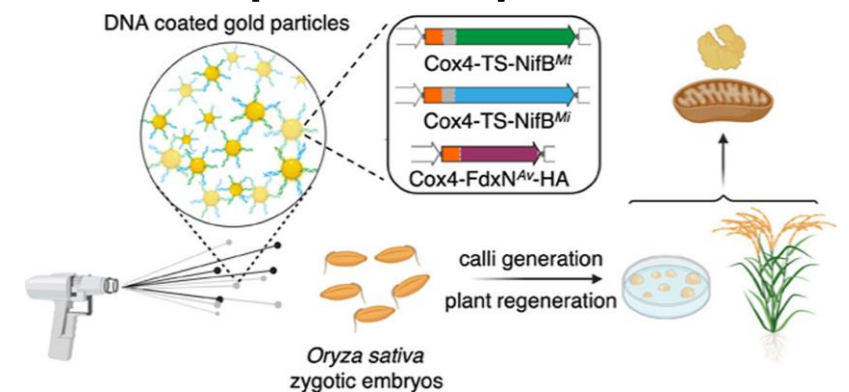
Root-associated microbiomes



Van Dezyne et al. *PLoS Biology* 16 (2018): e2006352

Venado et al. *bioRxiv* 2023.08.05.552127

Expression *in planta*

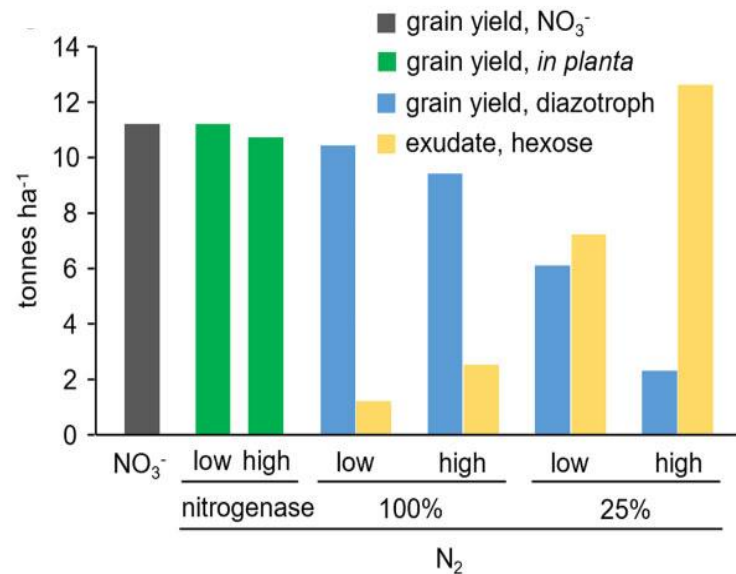


He et al. *ACS Synthetic Biology* 11 (2022): 3028-3036

Is nitrogen fixation enough?

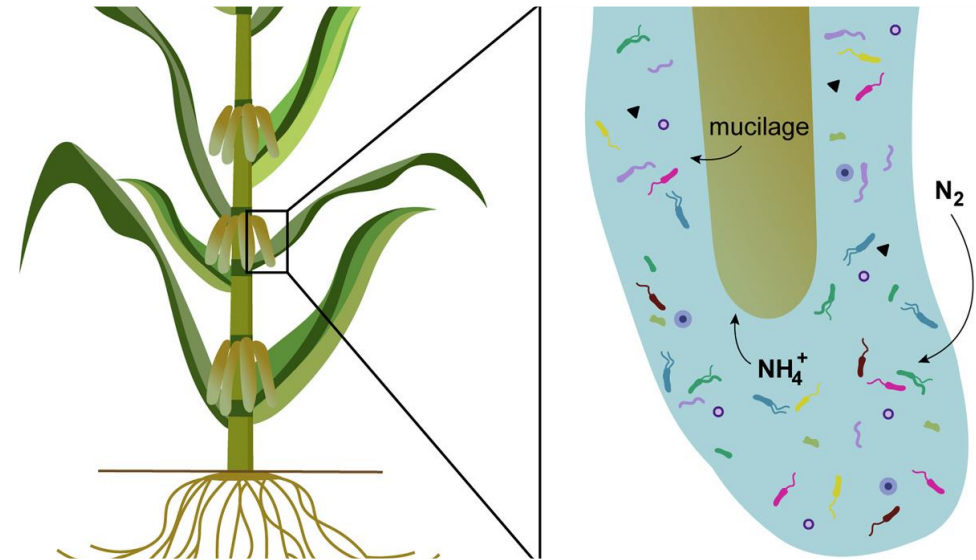
Limited by energy (sugar) from the plant → a BNF/yield trade-off

In planta and free-living: what if you replaced 100% of fertilizer with BNF?
→ Yield loss ~0-80%, depending on scenario



ARPA-E targets: C/N transfer efficiency, respiratory protection

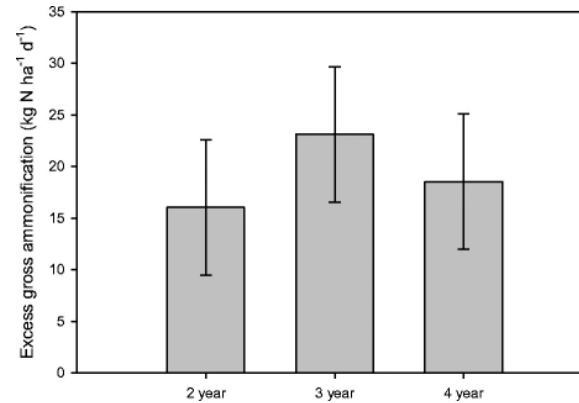
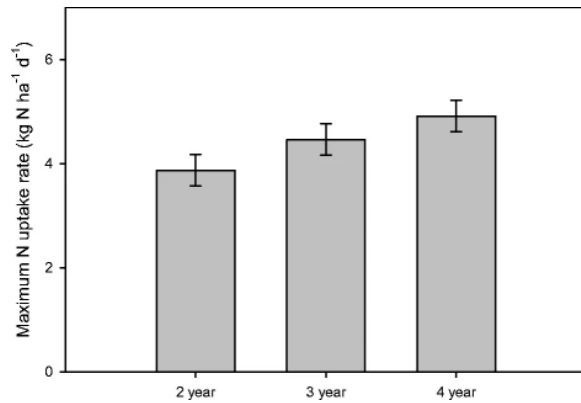
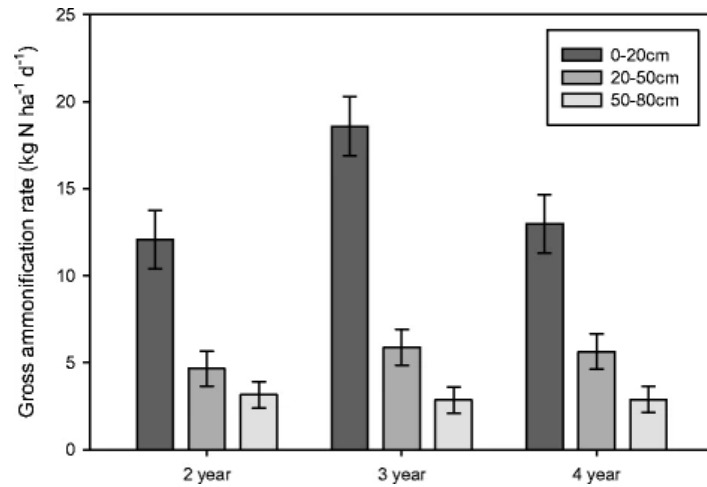
Aerial roots: how much N is fixed by natural aerial root mucilage communities?
→ 15 kg N/ha



ARPA-E targets: mucilage production, microbial NF regulation

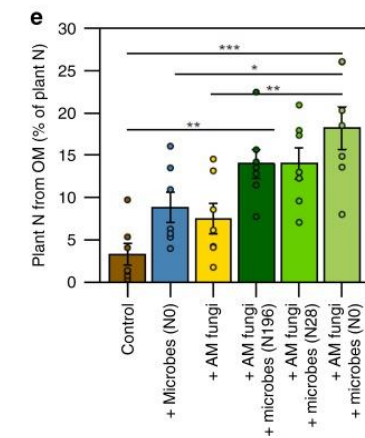
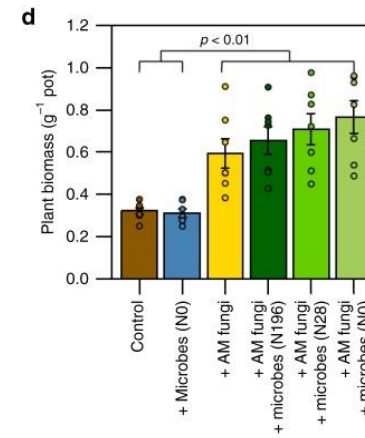
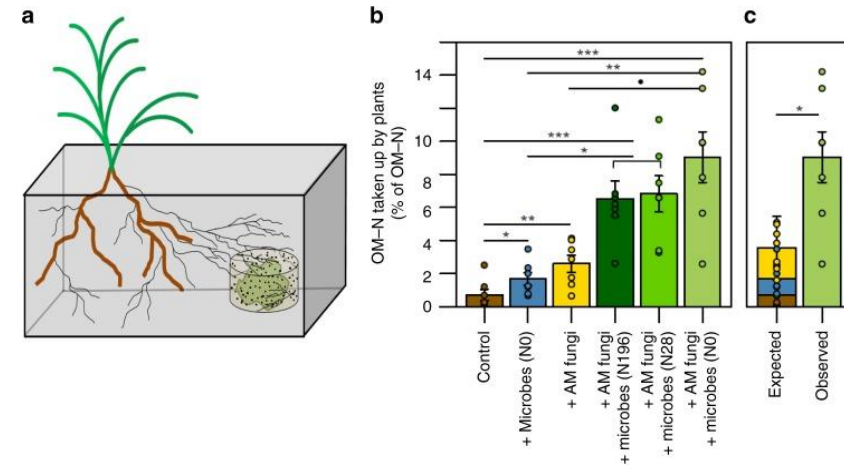
Can soil N mineralization replace synthetic fertilizer?

Gross soil ammonification is greater than plant N uptake



Osterholz et al. *Plant and Soil* 415 (2017): 73-84

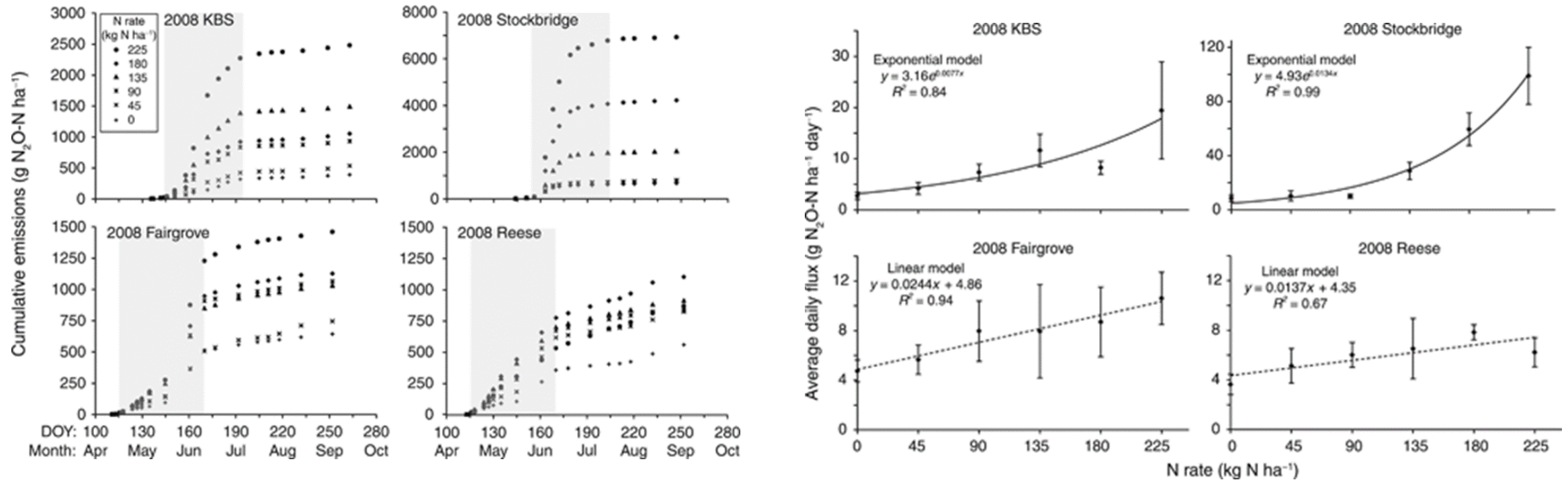
Arbuscular mycorrhizal fungi double N delivered to plant



Hestrin et al. *Communications Biology* 2.1 (2019): 233.

Can reduction in N inputs reduce N₂O emissions?

Positive correlation between applied N and N₂O emissions in corn fields

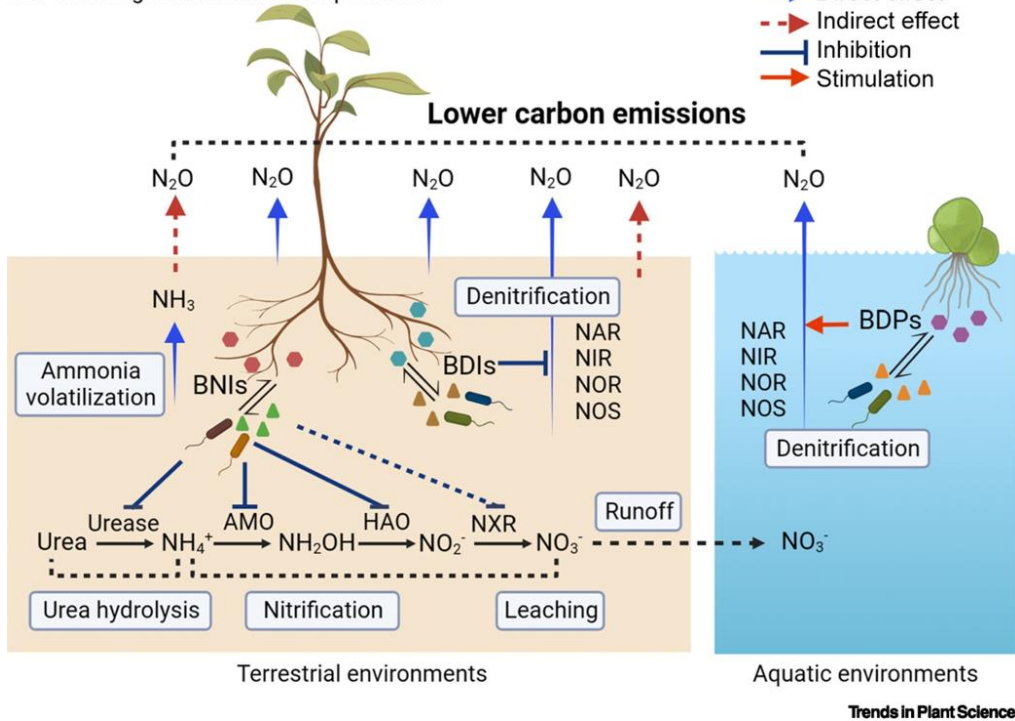


Hoben et al" *Global Change Biology* 17.2 (2011): 1140-1152.

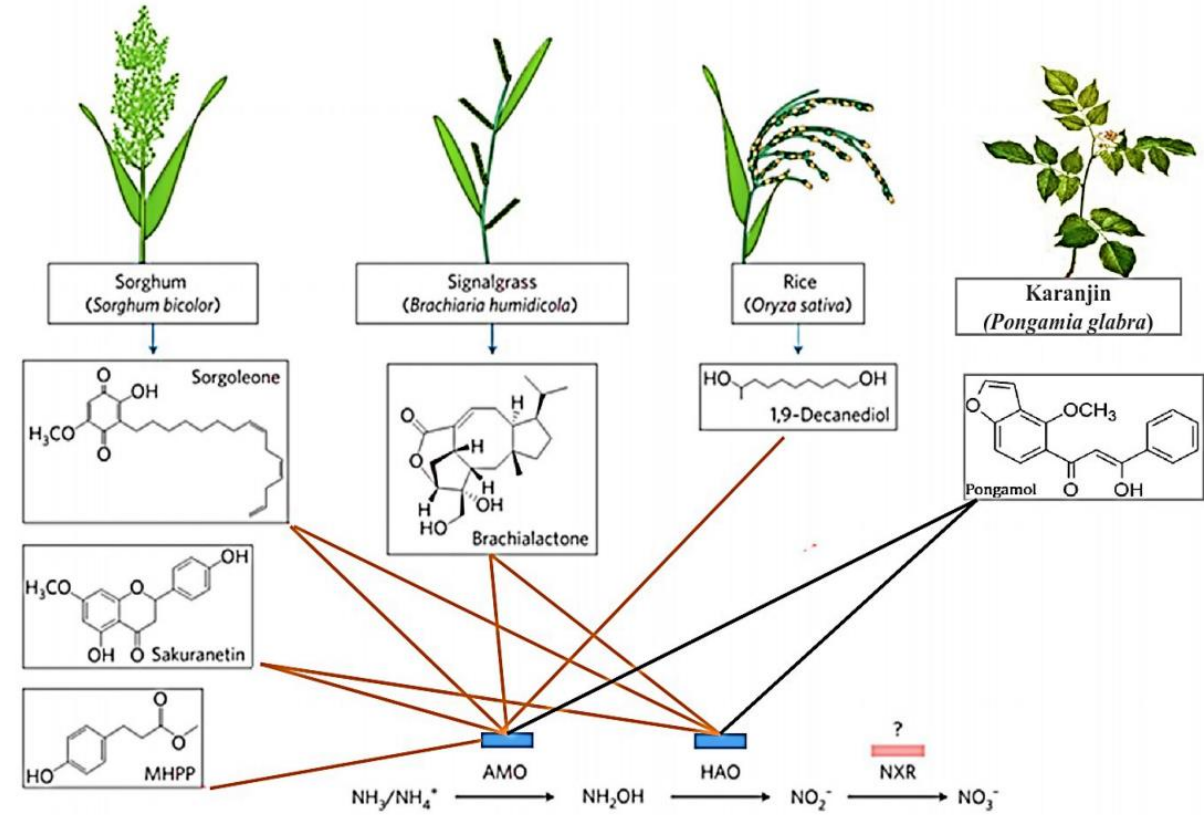
Biological nitrification and denitrification inhibition

BNIs: Biological nitrification inhibitors
 BDIs: Biological denitrification inhibitors
 BDPs: Biological denitrification promoters

- Root exudates
- ▲ Microbial exudates
- Direct effect
- - - Indirect effect
- Inhibition
- Stimulation



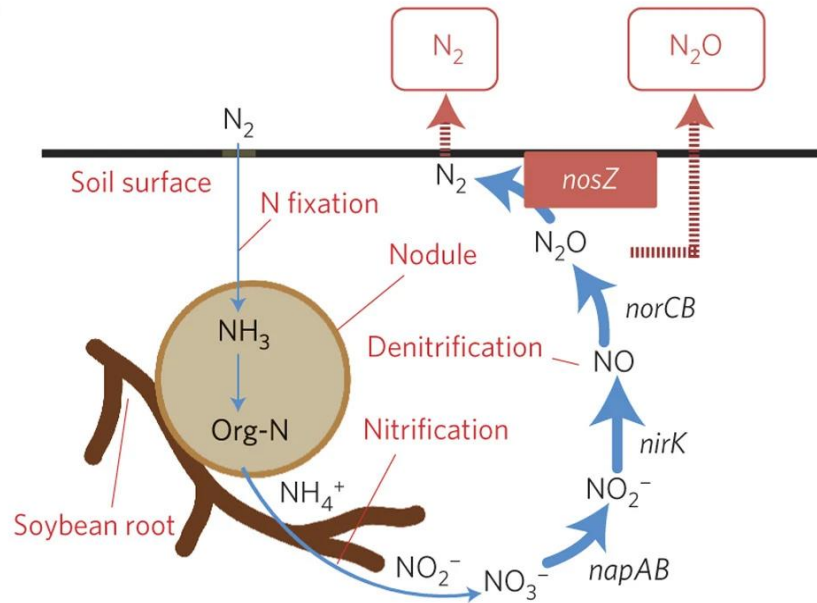
Lu et al. *Trends in Plant Science* (2023).



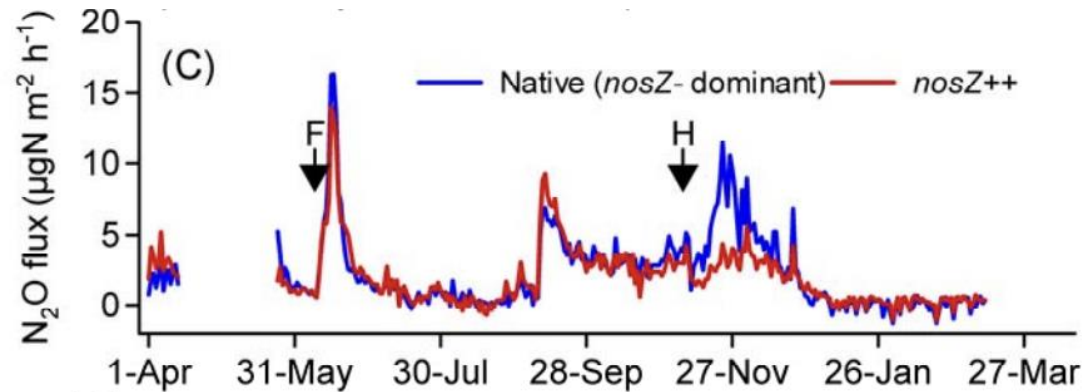
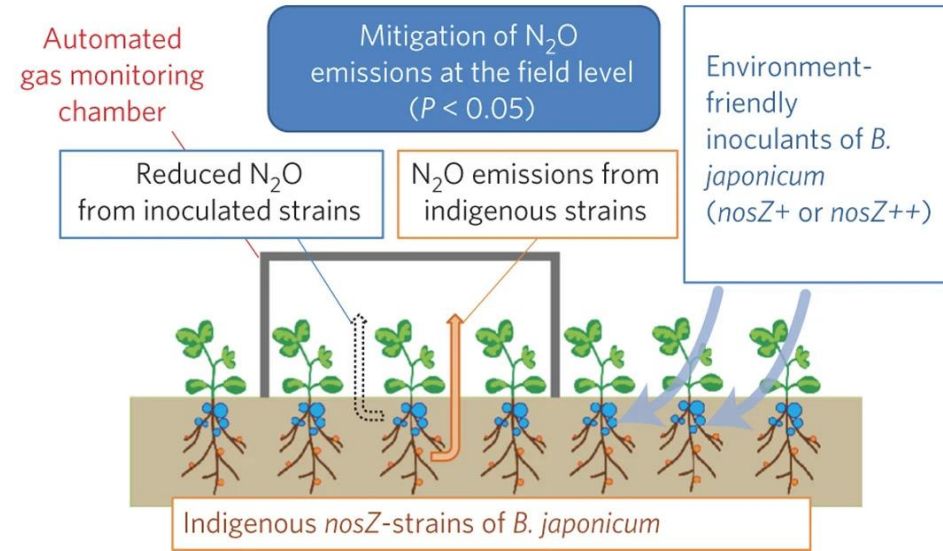
Saud, Shah, Depeng Wang, and Shah Fahad. " *Frontiers in Plant Science* 13 (2022): 854195.

Altering N₂O emission directly in the soil in soybeans

a

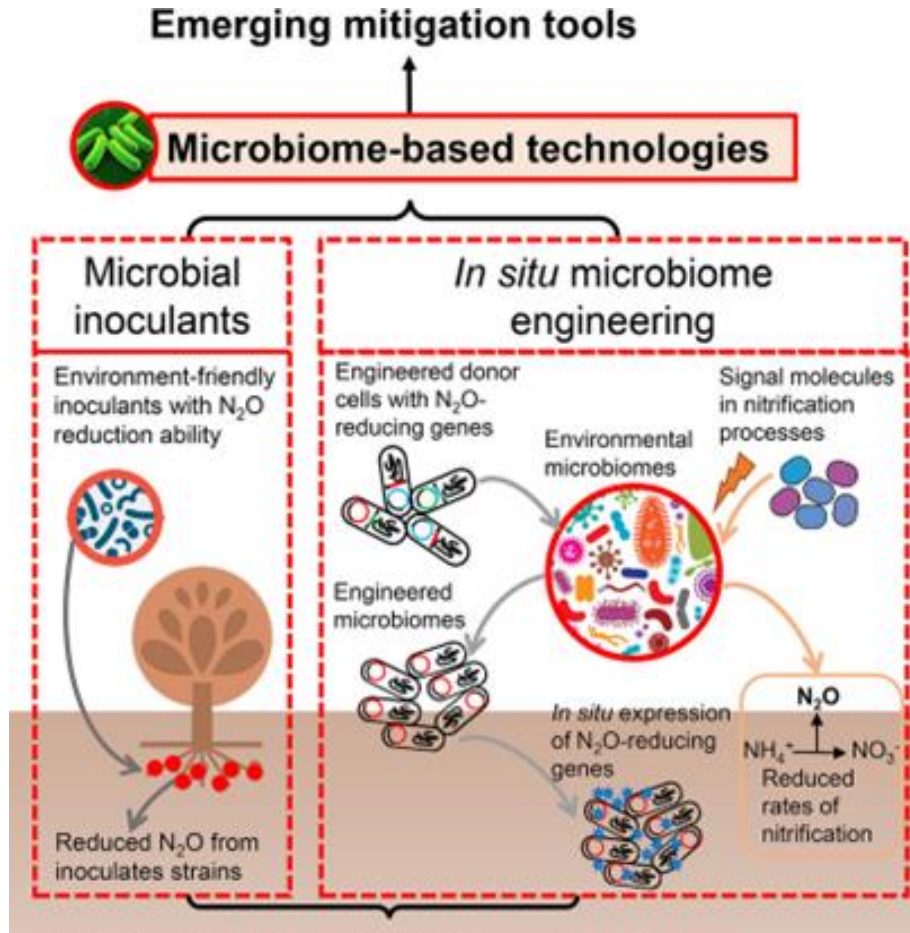


b



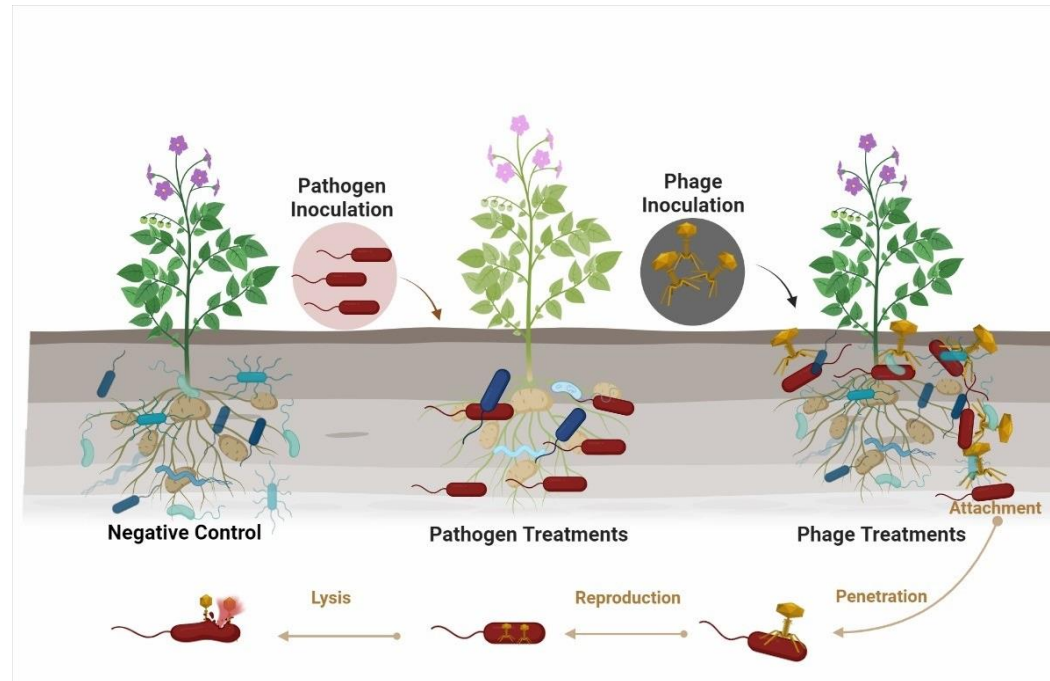
Itakura et al. *Nature Climate Change* 3.3 (2013): 208-212

Engineering the soil microbiome to mitigate N₂O



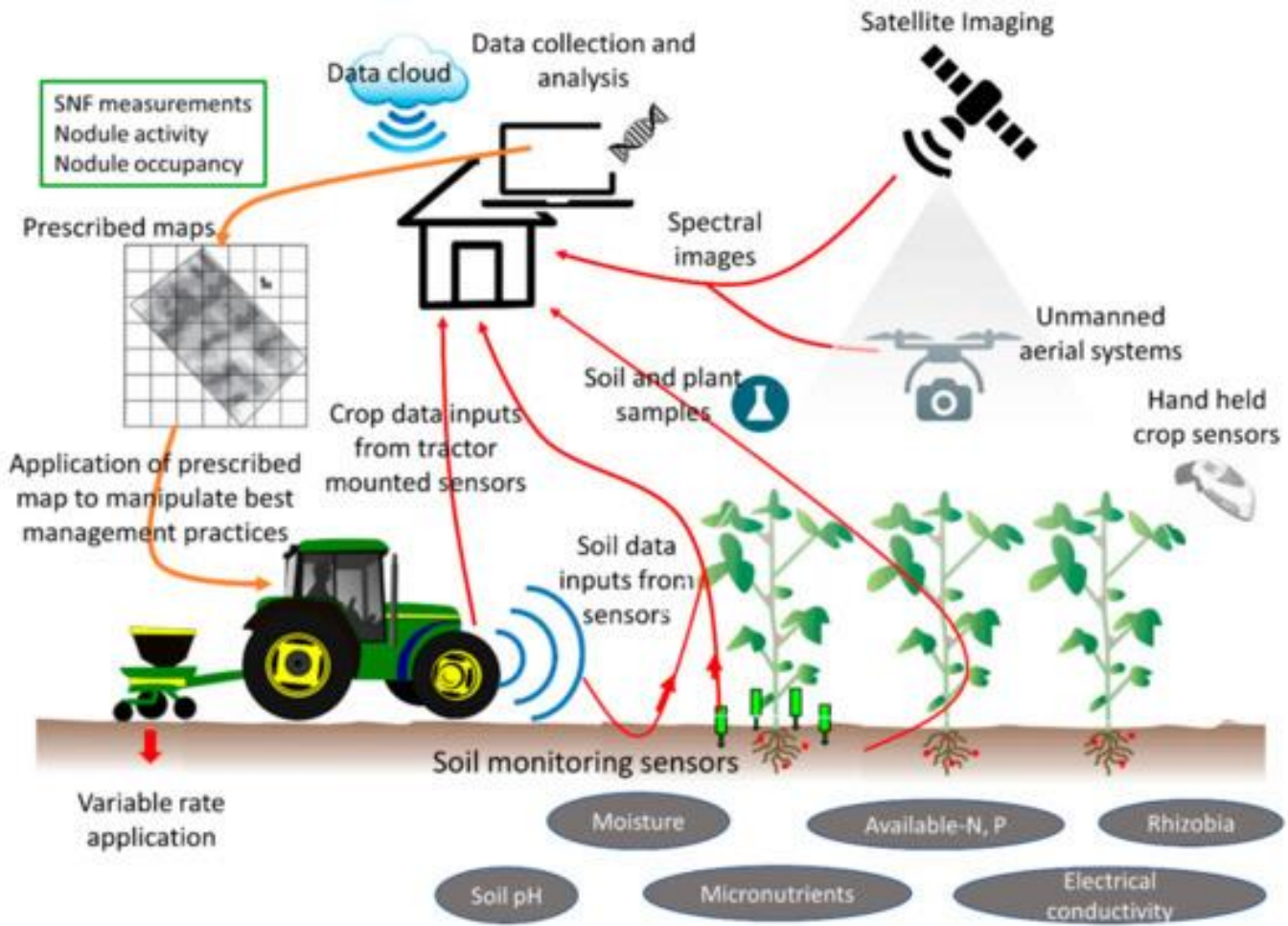
Hu, Hang-Wei, Ji-Zheng He, and Brajesh K. Singh. *Microbial Biotechnology* 10.5 (2017): 1226-1231.

Phage therapy for the N cycling microbiome



Mousa et al. *Antibiotics* 11.8 (2022): 1117.

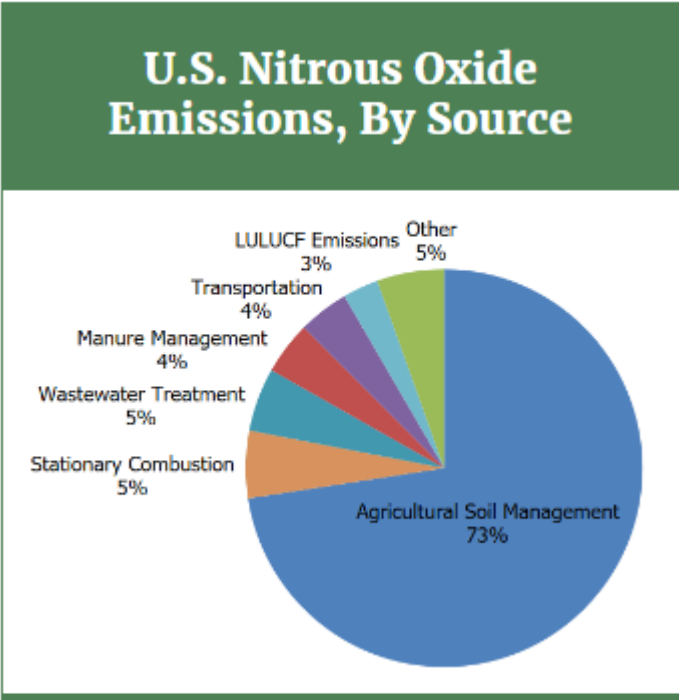
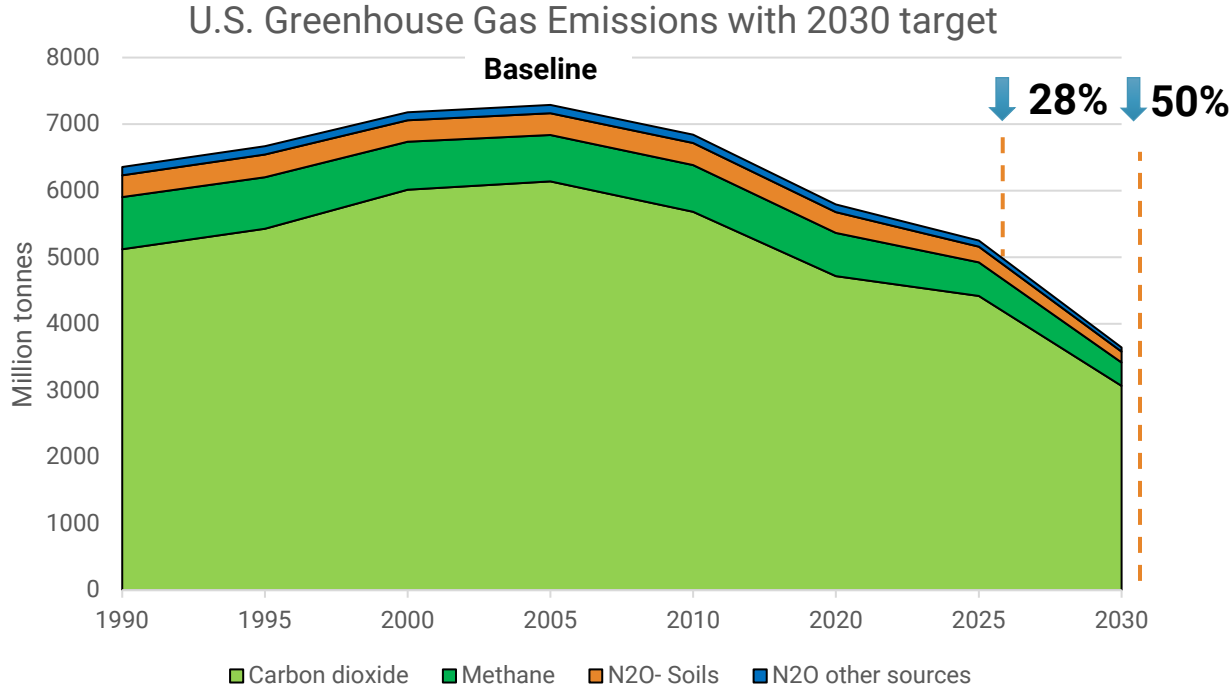
Combining biological approaches with precision agriculture



Thilakarathna, Malinda S., and Manish N. Raizada. *Agronomy* 8.5 (2018): 78.

Establishing program metrics: 50% N₂O emissions reduction

- **Paris agreement:**
 - Reduce GHG emissions by ~50% below 2005 base year levels by 2030 (U.S. nationally determined contribution)
 - Mitigating other GHGs will become increasingly important
 - 73% of N₂O emitted originates from agricultural soil

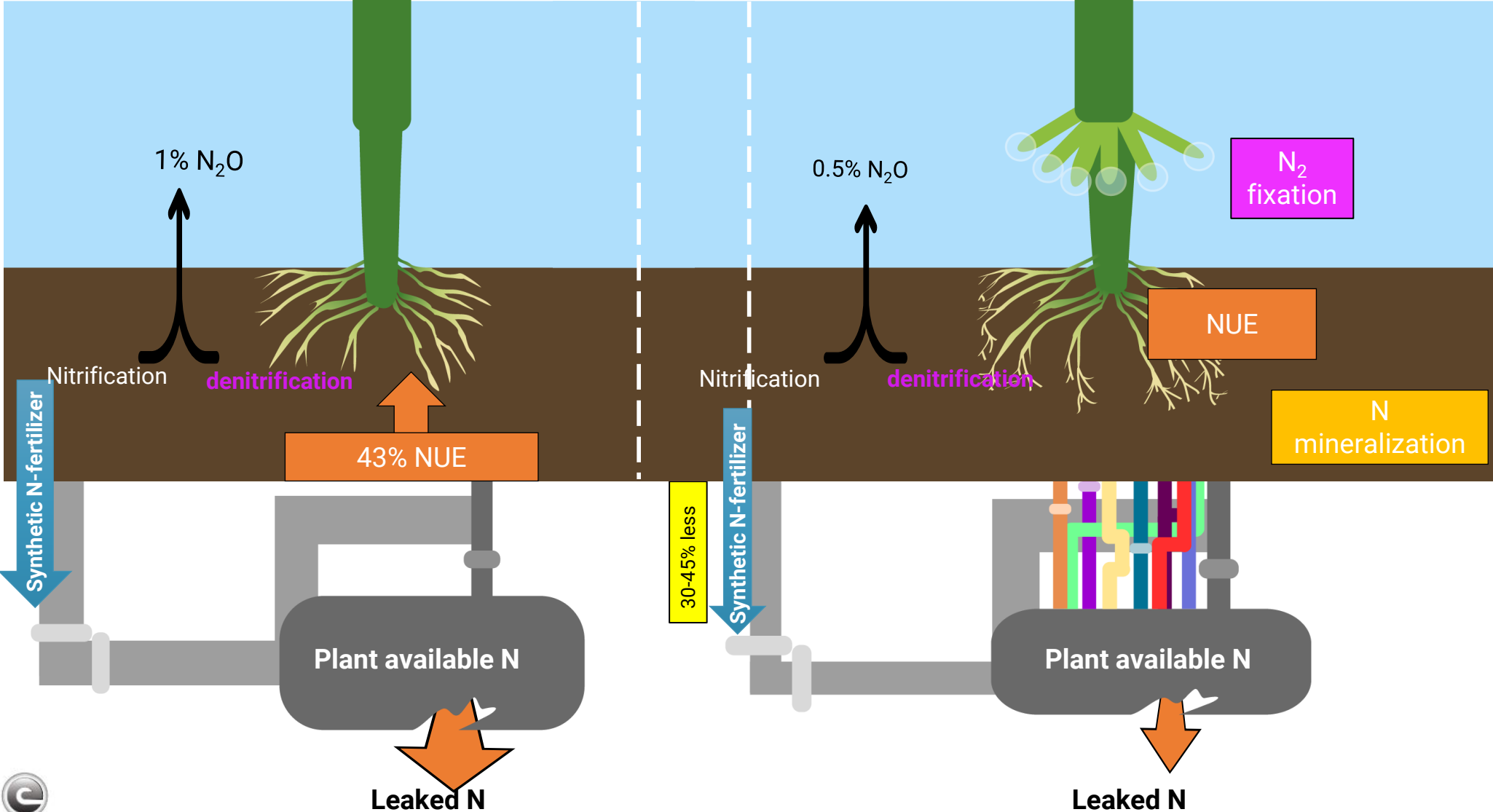


Data from EPA (2021). [Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2021](#).

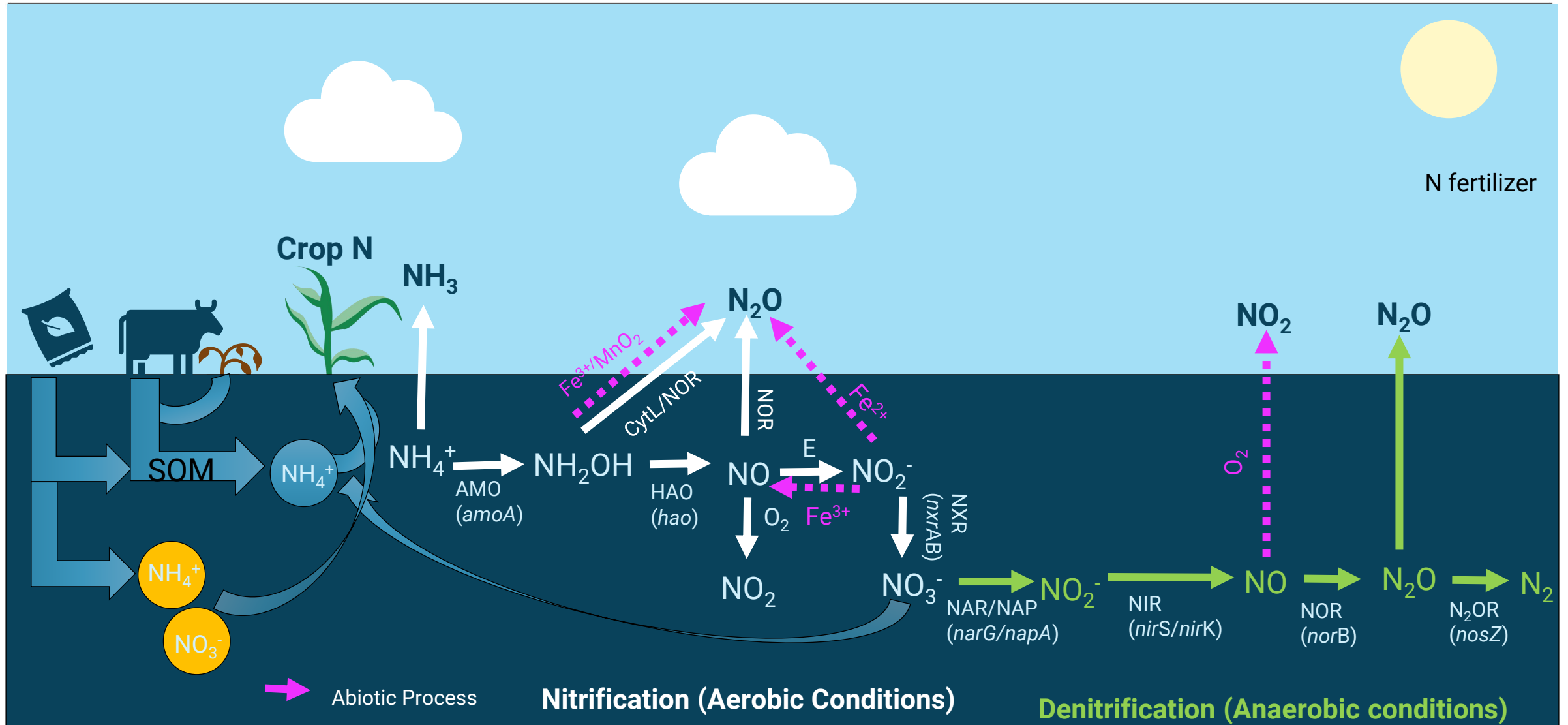
Year	CO ₂ (Mt)	CH ₄ (Mt)	Total N ₂ O(Mt)	N ₂ O Soils (Mt)
2005	6137.6	697.5	453.3	330.9
2025	3848.7	502.2	326.4	238.3
2030	2764.4	348.7	226.7	165.5

Data from EPA Climate Change Indicators: U.S. Greenhouse Gas Emissions (2021) 26

N₂O emission reduction: reduce synthetic N fertilizer application



N₂O emission reduction: direct N₂O mitigation



Speakers

- ▶ Chris Voigt, Massachusetts Institute of Technology
Future Low-Energy Sources of Nitrogen for Agriculture
- ▶ Ed Buckler, USDA ARS/Cornell University
Create a Sustainable Food System for Nitrogen: Moving Synthetic Nitrogen from the Field to Barn
- ▶ Wendy Yang, University of Illinois-Champaign-Urbana
Manipulating Microbes to Mitigate Soil Nitrous Oxide Emissions from Bioenergy Cropping Systems
- ▶ Jean-Michel Ane, University of Wisconsin-Madison
Nitrogen Fixation on Aerial Roots of Sorghum for Sustainable Bioenergy Production

Workshop agenda

Tuesday, November 14, 2023

Time	Event
8:00 – 9:00 AM	Registration and Breakfast Room: Lucerne Level Foyer
9:00 – 9:15 AM	Welcome and Introduction to ARPA-E <i>Dr. Jen Shafer, ARPA-E Associate Director of Technology</i> Room: Lucerne I-II
9:15 – 9:45 AM	Introductory Presentation <i>Dr. Steven Singer, ARPA-E Program Director</i> Room: Lucerne I-II
9:45 – 10:15 AM	Future Low-Energy Sources of Nitrogen for Agriculture <i>Dr. Chris Voigt, Massachusetts Institute of Technology</i> Room: Lucerne I-II
10:15 – 10:35 AM	Coffee Break
10:35 – 11:05 AM	Create a Sustainable Food System for Nitrogen: Moving Synthetic Nitrogen from the Field to Barn <i>Dr. Ed Buckler, Cornell University</i> Room: Lucerne I-II
11:05 – 11:25 AM	Manipulating Microbes to Mitigate Soil Nitrous Oxide Emissions from Bioenergy Cropping Systems <i>Dr. Wendy Yang- University of Illinois, Urbana-Champaign</i> Room: Lucerne I-II
11:25 AM – 12:05 PM	Panel discussion: Starting with the End in Mind – Insights to Developing New Technologies by End Users. Room: Lucerne I-II
12:05 – 1:00 PM	Lunch
1:05 – 2:35 PM	Breakout 1: Frontiers in Nitrogen Fixation Room: Lucerne I-II
1:05 – 2:35 PM	Breakout 2: Microbial N₂O Emissions Mitigation Room: Lucerne III
1:05 – 2:35 PM	Breakout 3: Plant pathways towards reducing Nitrogen inputs and N₂O Emissions Room: Alpine 1
2:35 – 3:00 PM	Coffee Break
3:00 – 4:00	SMART FARM N₂O Measurement and Modeling Showcase Room: Lucerne I-II
4:00 – 5:00 PM	One-on-one Meetings with Dr. Steve Singer, Program Director <i>15 minutes per person/group</i> Room: Lucerne III
4:10 – 6:00 PM	Concept Poster Sessions and Networking Reception Room: Alpine II

Workshop agenda

Wednesday, November 15, 2023

Time	Event
8:00 – 9:00 AM	Breakfast and Networking Room: Lucerne Level Foyer
9:00 – 9:05 AM	Day 2 Objectives <i>Dr. Steve Singer, ARPA-E Program Director</i> Room: Lucerne I-II
9:05 – 9:45 AM	<i>Nitrogen Fixation on Aerial Roots of Sorghum for Sustainable Bioenergy Production</i> <i>Dr. Jean Michel-Anne- University of Wisconsin, Madison</i> Room: Lucerne I-II
9:45– 10:30 AM	Panel Discussion: Current Opinion in Ag Tech from Investors, Foundations, Government Agencies and Policy Room: Lucerne I-II
10:30 – 11:00 AM	Coffee Break
11:00 AM – 12:30 PM	<i>Breakout 4: Adoption End-users</i> Room: Lucerne I-II
11:00 – 12:30 PM	<i>Breakout 5: Nitrogen Soil Cycle</i> Room: Lucerne III
11:00 – 12:30 PM	<i>Breakout 6: Plant Microbe Interactions and N-input and N₂O Emissions Reductions</i> Room: Alpine I
12:30 – 1:30 PM	Lunch & <u>Wrap up</u>
1:30 – 3:00 PM	<i>One-on-one Meetings with Dr. Steve Singer, Program Director</i> Room: Lucerne I-II