Course Overview

Introduction
- Overview
- Historical Perspective

Challenges
- Human Health
- Environment

Solutions
- Old Polymers
- Emerging Concepts

Applications
- Food and Ag
- Textiles
- Healthcare
- Materials management

Future
- What do we need?
- How do we get there?

Example Case Study

Dr. Logan Morton

Note: This is not on the test. No need to scribble down notes—this is about learning how to think about these problems. Join in on the conversation! Ask Questions! Contributing in class increases retention by 2-3 times! You're here anyway...speak up!

KULA
BIO

Non-GMO biofertilizer

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Kula Bio

- Introduction: **Why?**
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- Conclusion: **Takeaways**

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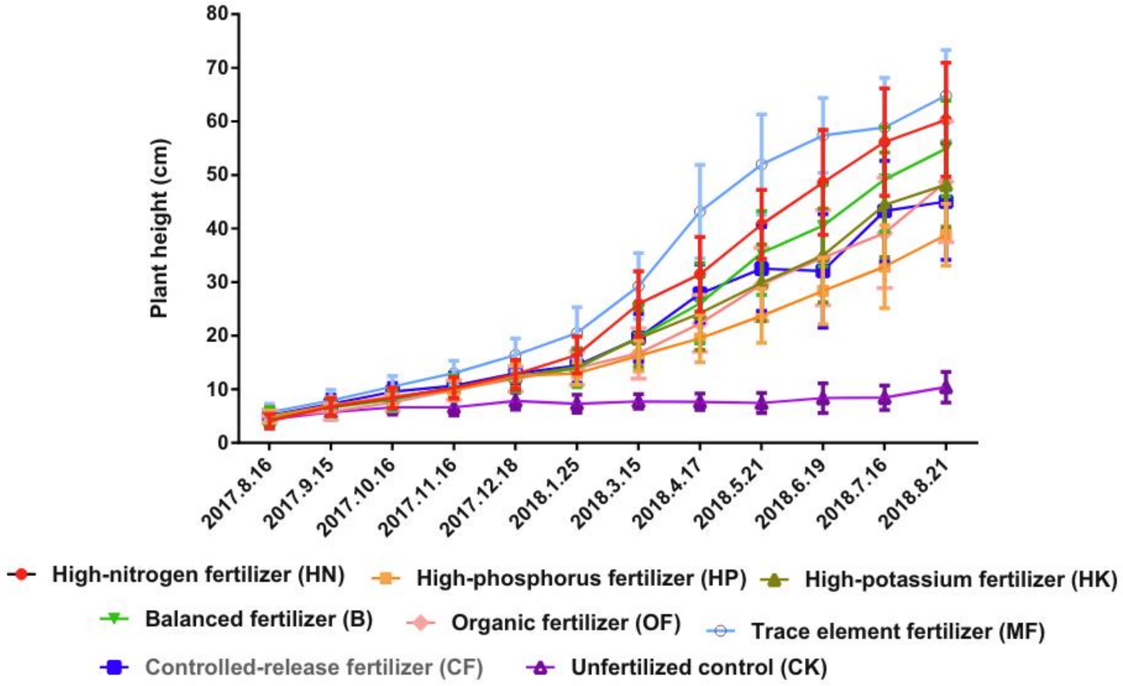
Kula Bio

Agriculture is intimately linked to the environment

Can you think of some ways that agriculture might impact the environment?

Nitrogen is essential in agriculture



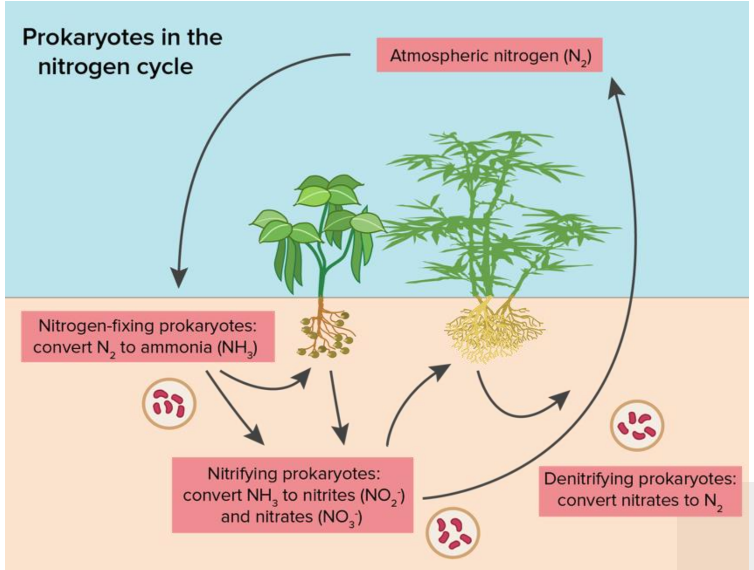
Xu, F., Chu, C. & Xu, Z. Effects of different fertilizer formulas on the growth of loquat rootstocks and stem lignification. *Sci Rep* **10**, 1033 (2020).
Ohyama, T., Minagawa, R et al. (2013). Soybean Seed Production and Nitrogen Nutrition. InTech.
https://www.farmprogress.com/corn/corn-following-rye-needs-shot-of-nitrogen

If only we had access to nearly unlimited nitrogen all around us…

Any ideas?

If only we had access to nearly unlimited nitrogen all around us…

- The solution is in the air!
- Some bacteria naturally sequester nitrogen directly from the air
- We have found other ways, like the Haber-Bosch process



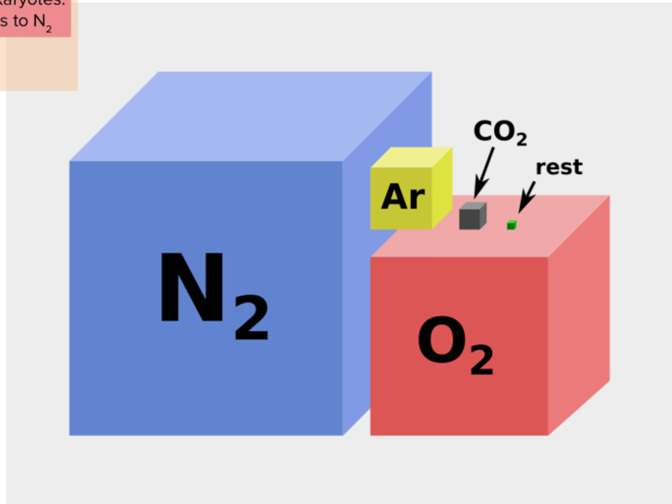


Image credit: modified from *Nitrogen cycle* by Johann Dréo (CC BY-SA 3.0)
Earth's Atmosphere Composition: Nitrogen, Oxygen, Argon and CO2 – Earth How

Producing ammonia from the air-like magic! (or chemistry)

$$N_2 + 3H_2 \leftrightarrow 2NH_3$$

78% of the air

$$CH_4 + H_2O \leftrightarrow CO + H_2$$

Producing ammonia from the air-like magic! (or chemistry)

$$N_2 + 3H_2 \leftrightarrow 2NH_3$$

$$\Delta H = ?$$

Producing ammonia from the air-like magic! (or chemistry)

$$N_2 + 3H_2 \leftrightarrow 2NH_3$$

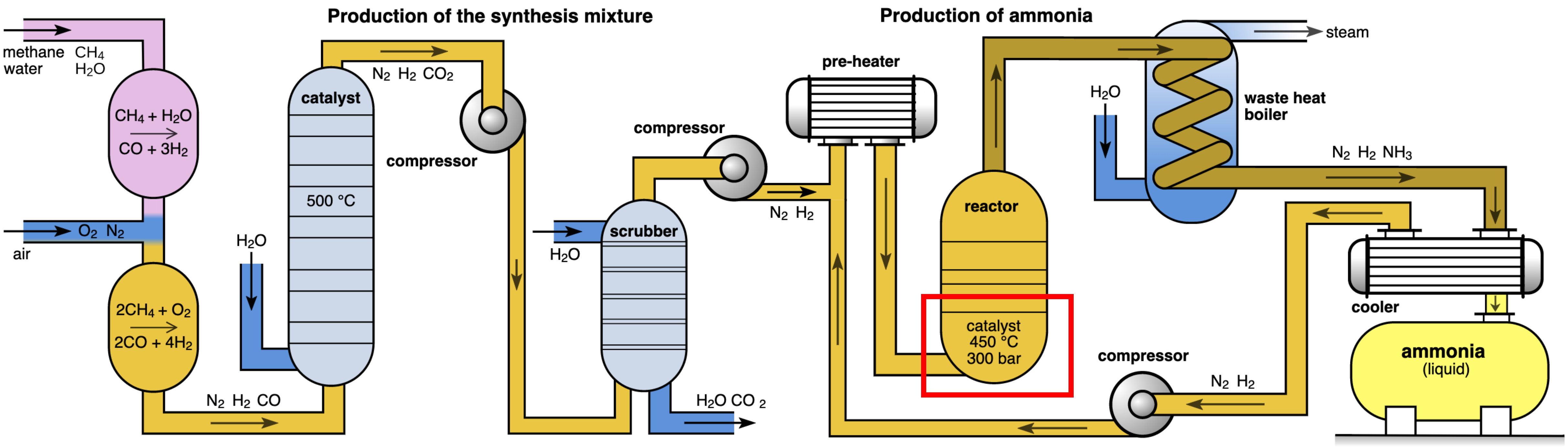
$$\Delta H = \mathbf{-92\ kJ/mol}$$

So what happens if we increase the temperature?

As temperature increases, yield decreases…
So why don't we do this process at low temp?

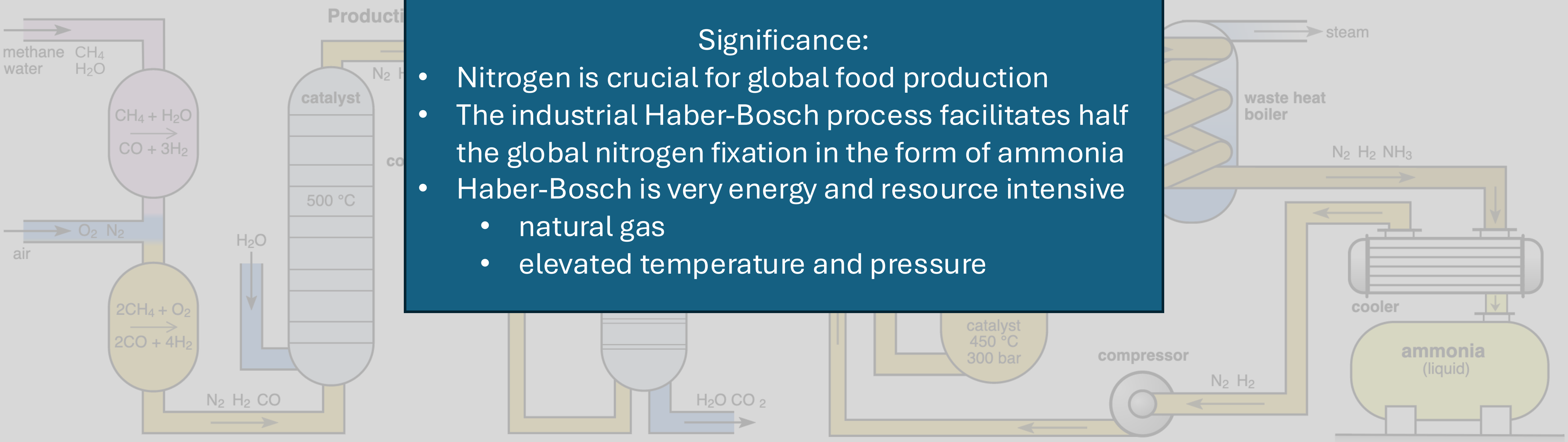
Lower temperatures reduce reaction rates…so we need to compromise (turns out ~450 C is good)
What else might we do to increase this reaction rate?

The Haber-Bosch Process Allows for Synthetic Production of NH_3



Do you notice anything? Why might this not be the best solution long term?

The Haber-Bosch Process Allows for Synthetic Production of NH_3



Significance:

- Nitrogen is crucial for global food production
- The industrial Haber-Bosch process facilitates half the global nitrogen fixation in the form of ammonia
- Haber-Bosch is very energy and resource intensive
  - natural gas
  - elevated temperature and pressure

Do you notice anything? Why might this not be the best solution long term?

Current Fertilizers are Harmful to the Environment

Production

- Synthetic nitrogen is (mostly) from fossil-fuels
- Fertilizer production currently accounts for about 2-3% of the total **global energy consumption**

Use

- Nutrient loss from traditional nitrogen fertilizer into waterways
  1) Loss of valuable nitrogen
  2) Contaminates surface+groundwater
- Eutrophication

Basosi, Riccardo, et al. *Fertilizers: components, uses in agriculture and environmental impacts* (2014): 3-43.

Eutrophication: Nitrogen accumulates unnaturally in bodies of water

- Increased biomass of phytoplankton
- Decreased biodiversity
- New species invasion
- Toxicity
- Fish death
- Deleterious effects on human health

-Infant Methemoglobinemia

(Blue Baby Syndrome)

SCN at Victoria General Hospital in Victoria, BC, Canada. Subject: Amaryllis Kalilah Adoré Nicholson; 2 hours old; d-TGA + VSD; unpalliated, pre-operative, cyanotic.

Kula Bio

OUR IMPACT

We're here to help farmers continue to be good stewards of their land.

Today, synthetic fertilizer causes great harm to the soil and environment. Kula Bio provides a reliable substitute for traditional synthetic nitrogen—one which can help farmers maintain yields and revenue, while adapting to new regulations and consumer demand for sustainable products in the face of climate change.

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Kula Bio

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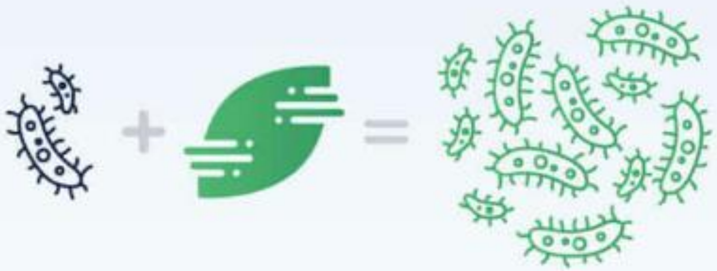
It’s all about the soil

Kula Bio uses beneficial bacteria that have been removing nitrogen from the air and placing it into the soil for millenia. We energize the bacteria without genetic modifications to create Kula-N, a sustainable, organic nitrogen fertilizer for the 21st century.

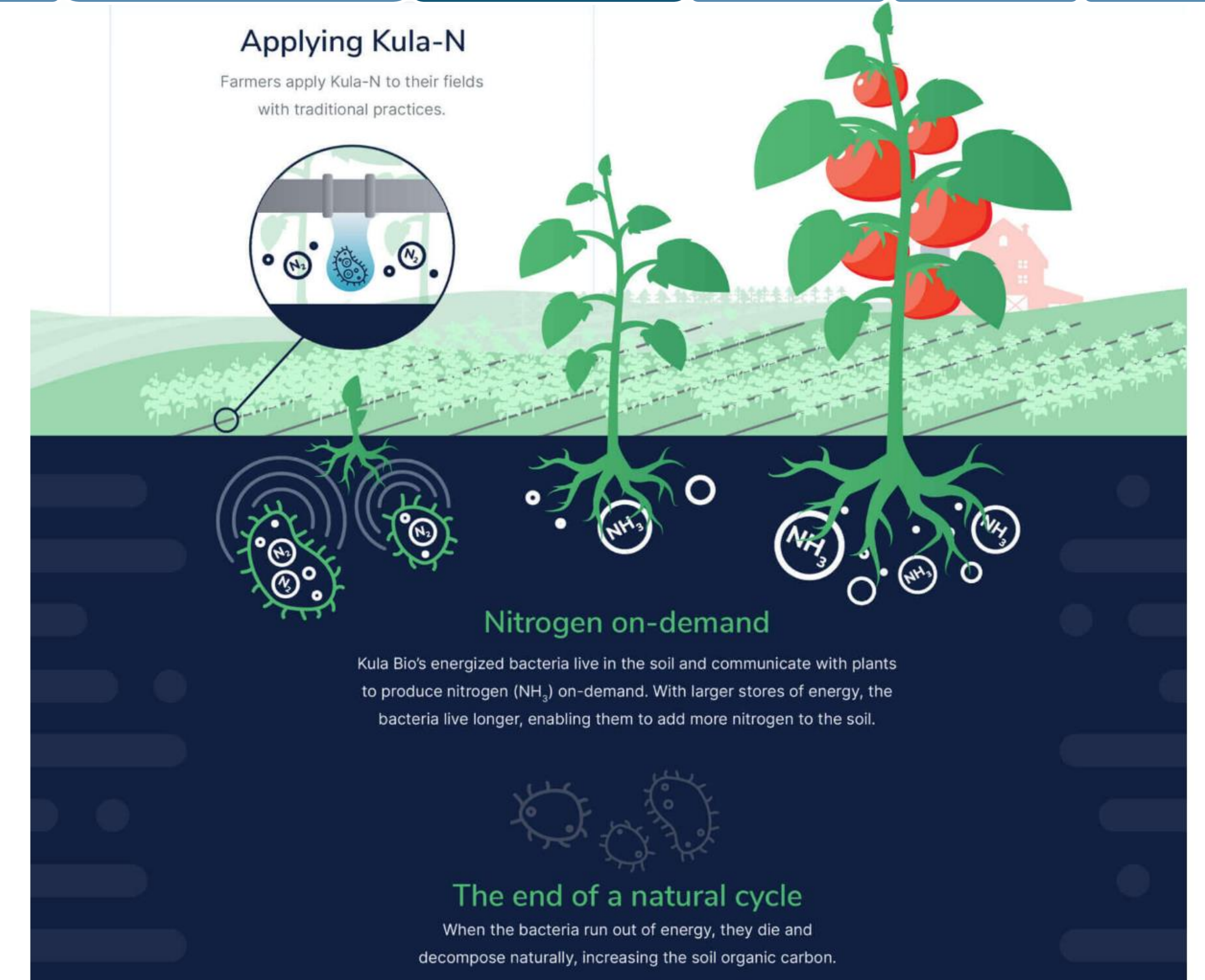


Propagating the bacteria

We increase the density of our non-GMO bacteria in a process that is robust and based on scalable industrial equipment and practices.

Feeding the bacteria

Next, we fortify the bacteria, providing them with a carbon rich energy source. This helps them build up larger than normal stores of energy and nutrients, which is why we call them “energized.”


Applying Kula-N
Farmers apply Kula-N to their fields with traditional practices.
N2
NH3
Nitrogen on-demand
Kula Bio's energized bacteria live in the soil and communicate with plants to produce nitrogen (NH3) on-demand. With larger stores of energy, the bacteria live longer, enabling them to add more nitrogen to the soil.
The end of a natural cycle
When the bacteria run out of energy, they die and decompose naturally, increasing the soil organic carbon.

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Leveraging a Natural Process to Make Nitrogen Fertilizer

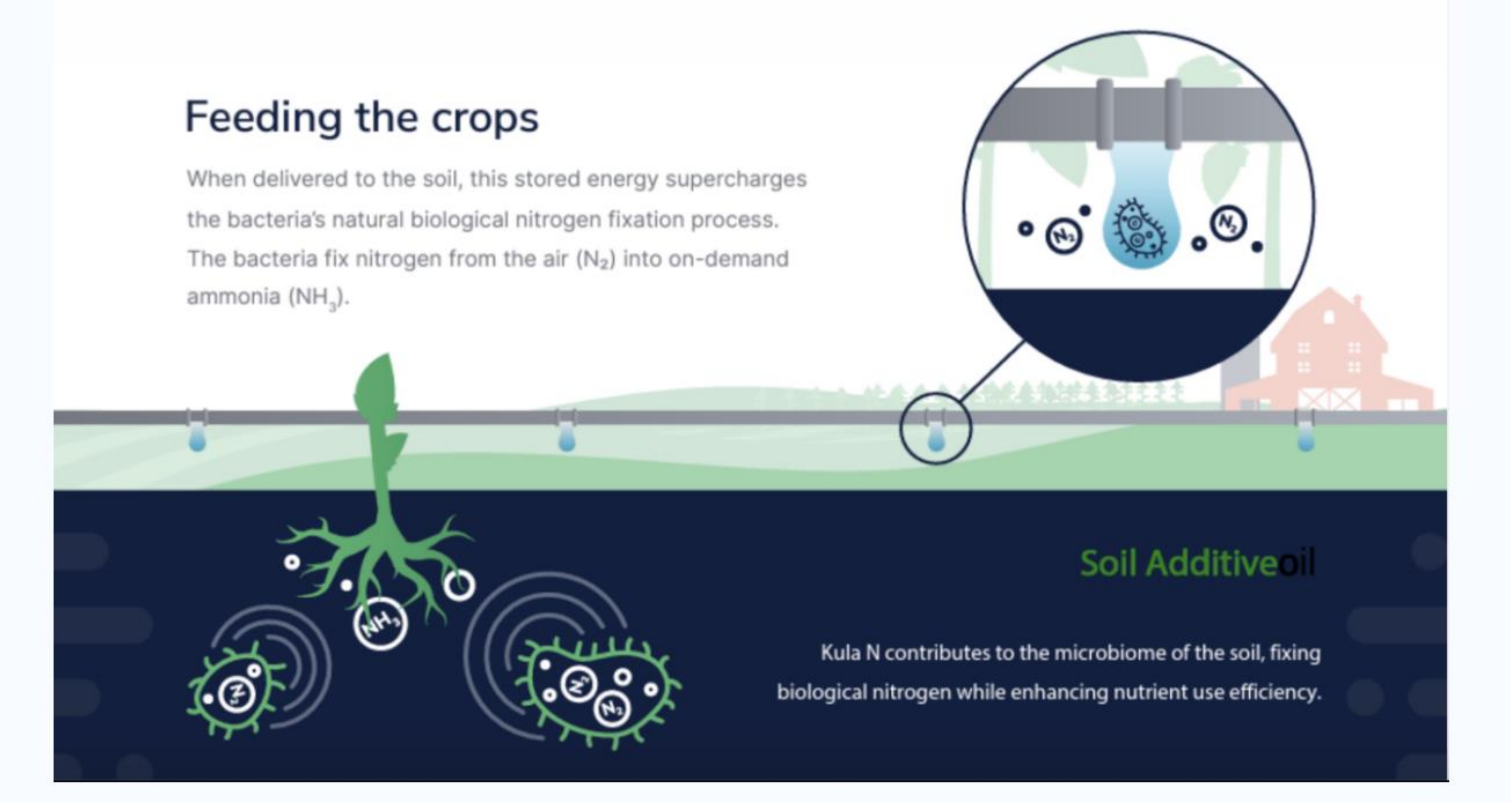
Our technology leverages a specific bacteria's intrinsic ability to fix atmospheric nitrogen in a wide range of soil types. We preserve the bacteria in its natural state to produce Kula-N biofertilizer without genetic modification.

Propagation perfected

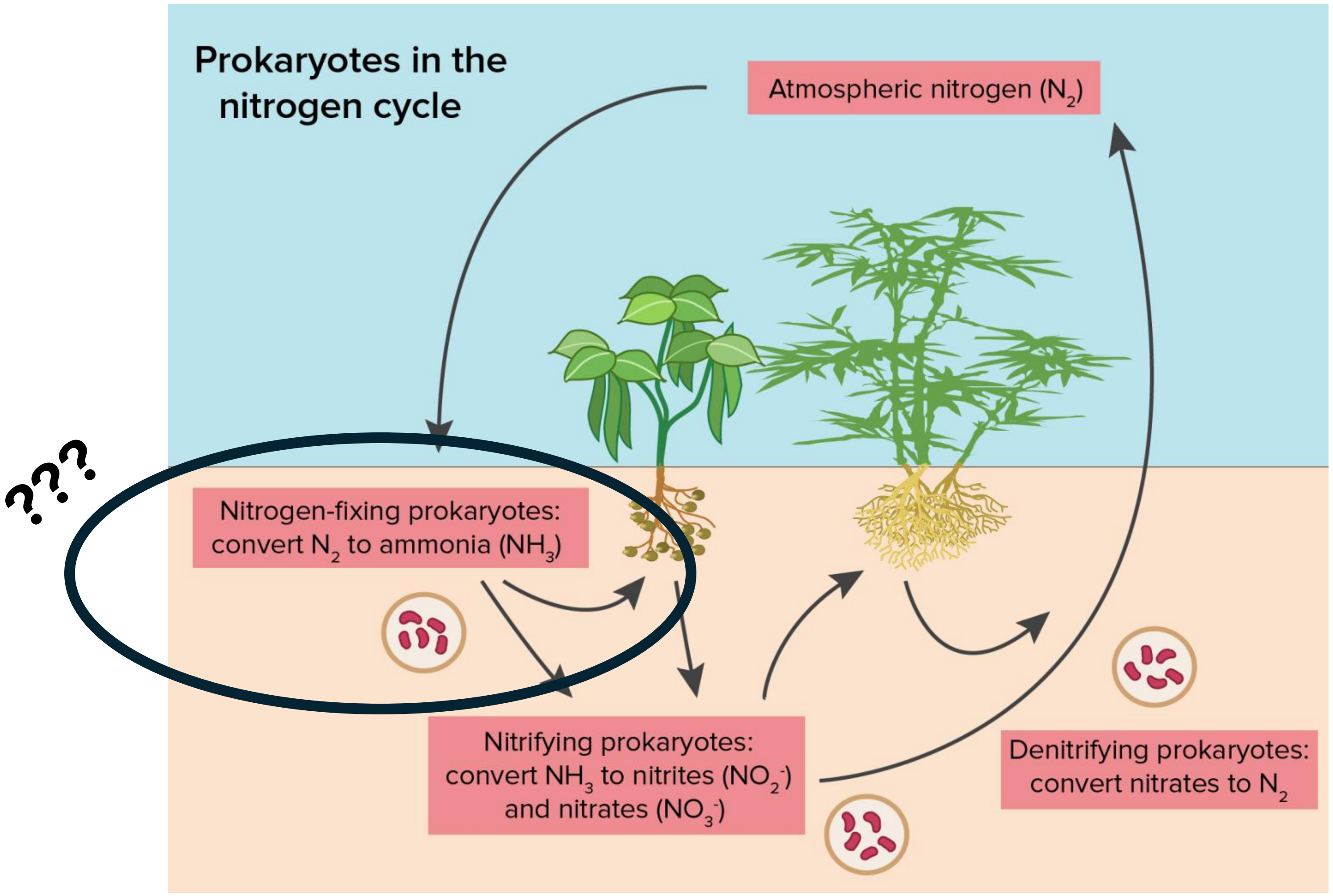
Starting with pure cell banks, we quickly and efficiently produce extremely dense cell cultures.

Energy on board

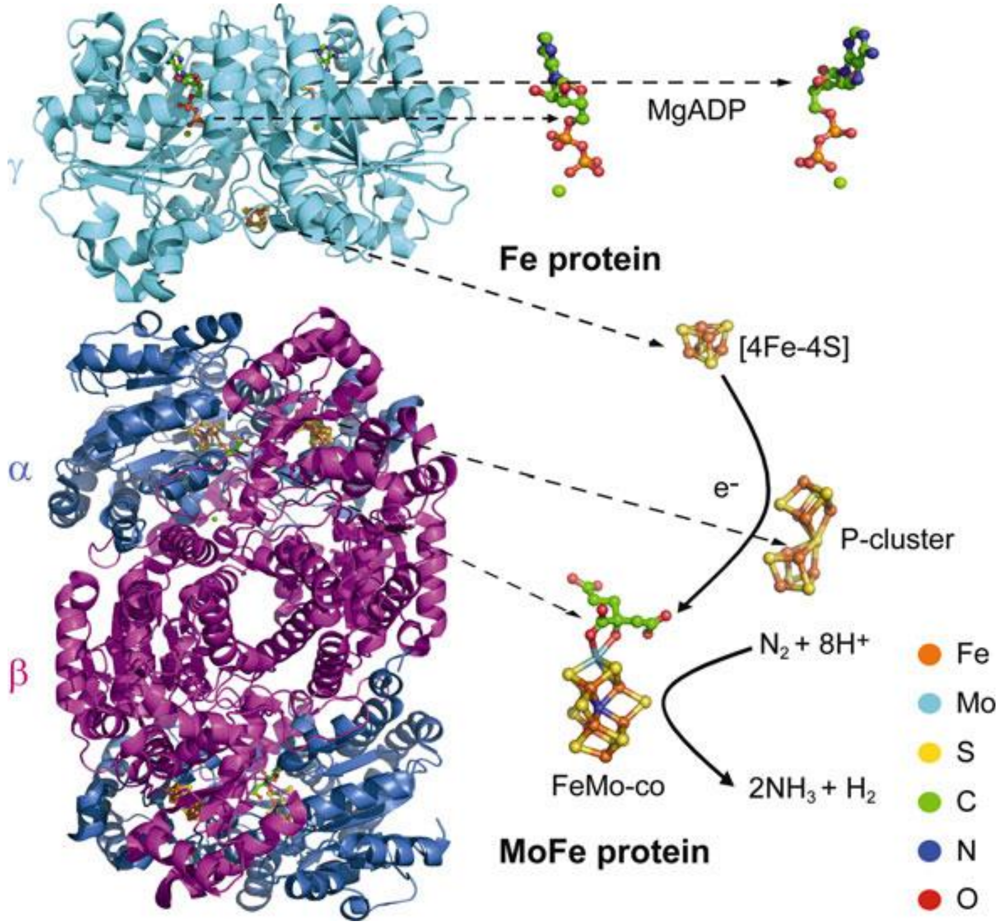
Because biological nitrogen fixation (BNF) is energy intensive and the lack of free energy in the soil inhibits these naturally occurring bacteria from producing meaningful amounts of nitrogen, we designed the perfect system to make the bacteria "fat" with an organic, carbon-rich energy source.


Feeding the crops
When delivered to the soil, this stored energy supercharges the bacteria's natural biological nitrogen fixation process. The bacteria fix nitrogen from the air (N_2) into on-demand ammonia (NH_3).
N_2
N_2
NH_3
N_2
N_2
Soil Additiveoil
Kula N contributes to the microbiome of the soil, fixing biological nitrogen while enhancing nutrient use efficiency.

Let's dig in a little bit deeper...



How do bacteria do it?



- Diazotrophs-bacteria that fix nitrogen
- Reduces N_2 → 2 NH_3
- Catalyzed by a complex metalloenzyme called **nitrogenase**
- Not all nitrogenase is the same
- Most are composed of two components:
  - Large component ($\alpha_2\beta_2$ subunit composition)
  - Small component (γ_2 subunit composition)
- All known nitrogenases contain iron–sulfur clusters in both component proteins
- Shown on the left is the most studied (Mo-dependent) nitrogenase

Yang, Zhi-Yong & Danyal, Karamatullah & Seefeldt, Lance. (2011). Mechanism of Mo-Dependent Nitrogenase. Methods in molecular biology (Clifton, N.J.). 766. 9-29.

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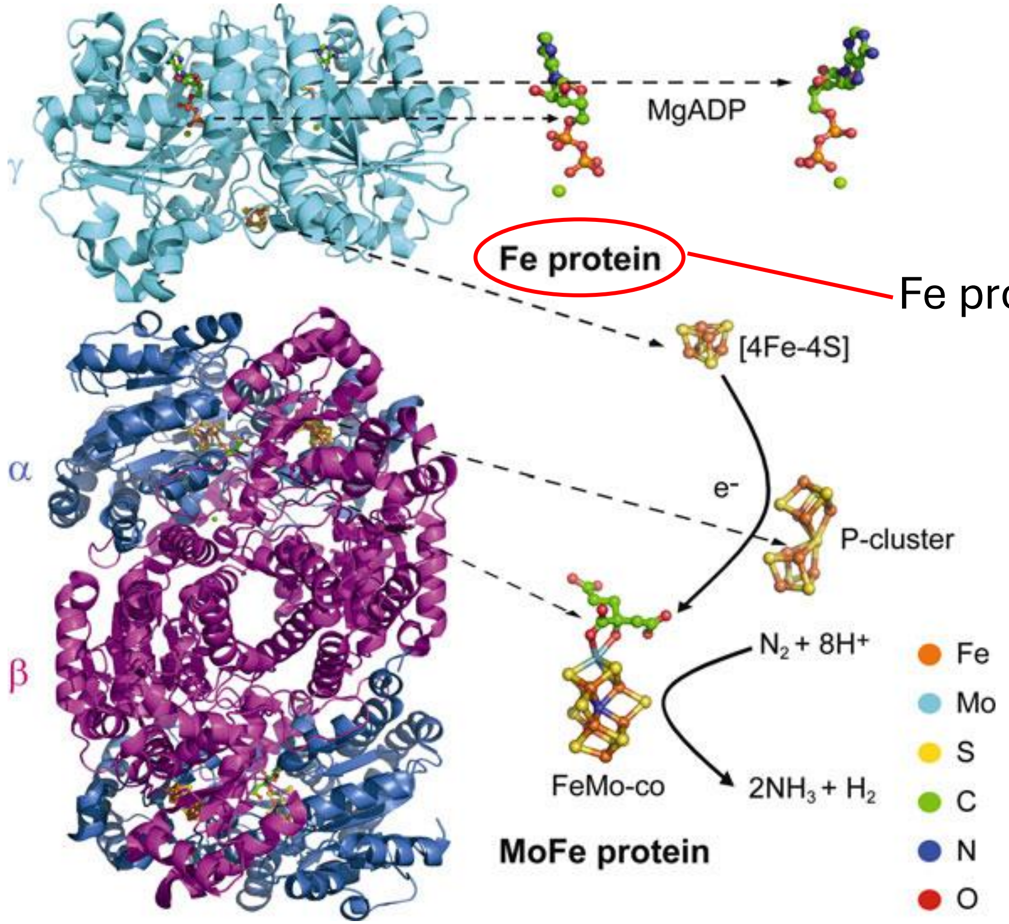

$$N_2 + 8e^- + 16MgATP + 8H^+ \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

Fe protein binds to MoFe protein

Yang, Zhi-Yong & Danyal, Karamatullah & Seefeldt, Lance. (2011). Mechanism of Mo-Dependent Nitrogenase. Methods in molecular biology (Clifton, N.J.). 766. 9-29.

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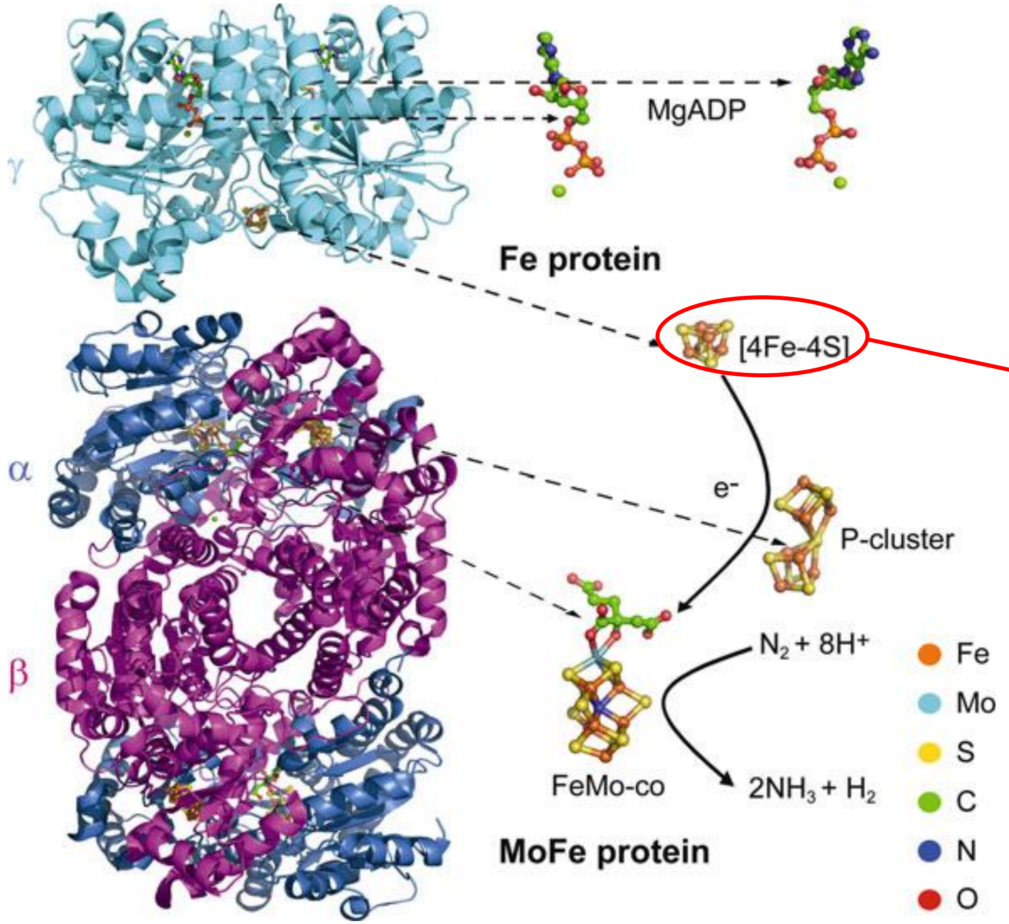

$$N_2 + 8e^- + 16MgATP + 8H^+ \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

This cluster transfers 1 electron to the MoFe protein

Yang, Zhi-Yong & Danyal, Karamatullah & Seefeldt, Lance. (2011). Mechanism of Mo-Dependent Nitrogenase. Methods in molecular biology (Clifton, N.J.). 766. 9-29.

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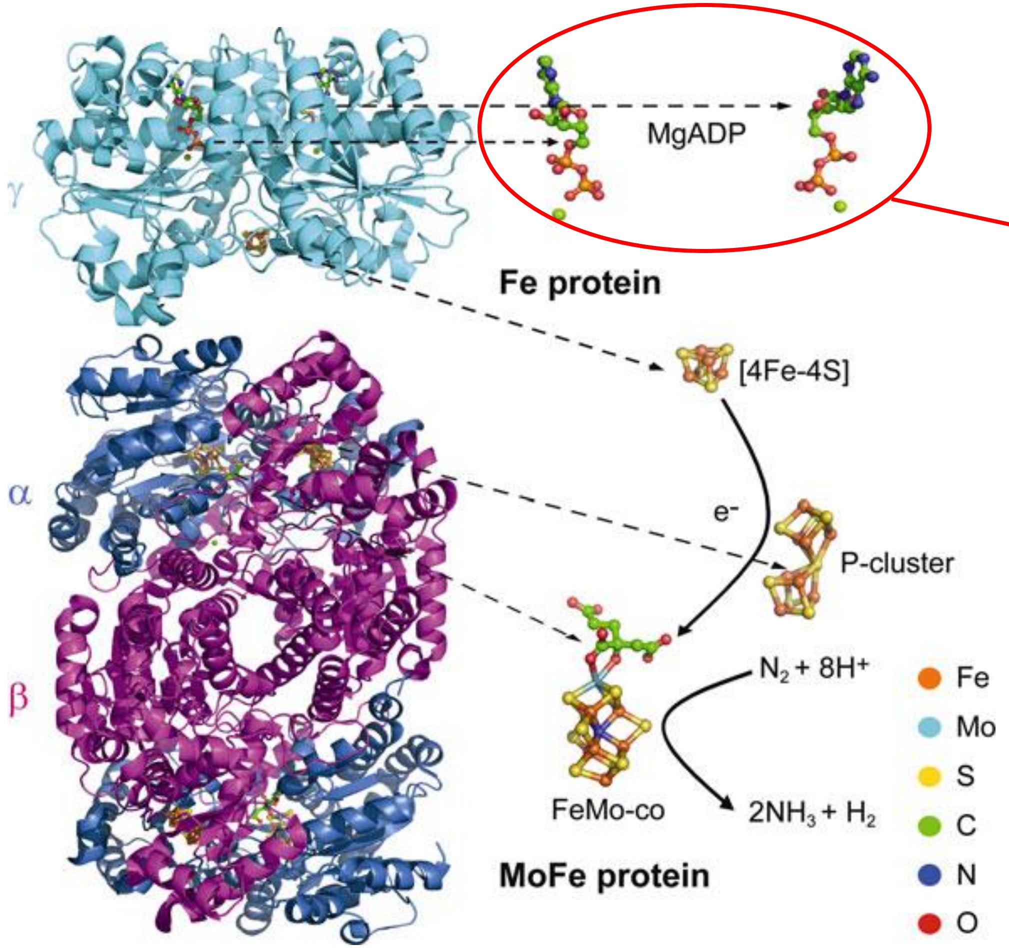

$$N_2 + 8e^- + 16MgATP + 8H^+ \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

2 ATP molecules are hydrolyzed in the transfer of the electron

Yang, Zhi-Yong & Danyal, Karamatullah & Seefeldt, Lance. (2011). Mechanism of Mo-Dependent Nitrogenase. Methods in molecular biology (Clifton, N.J.). 766. 9-29.

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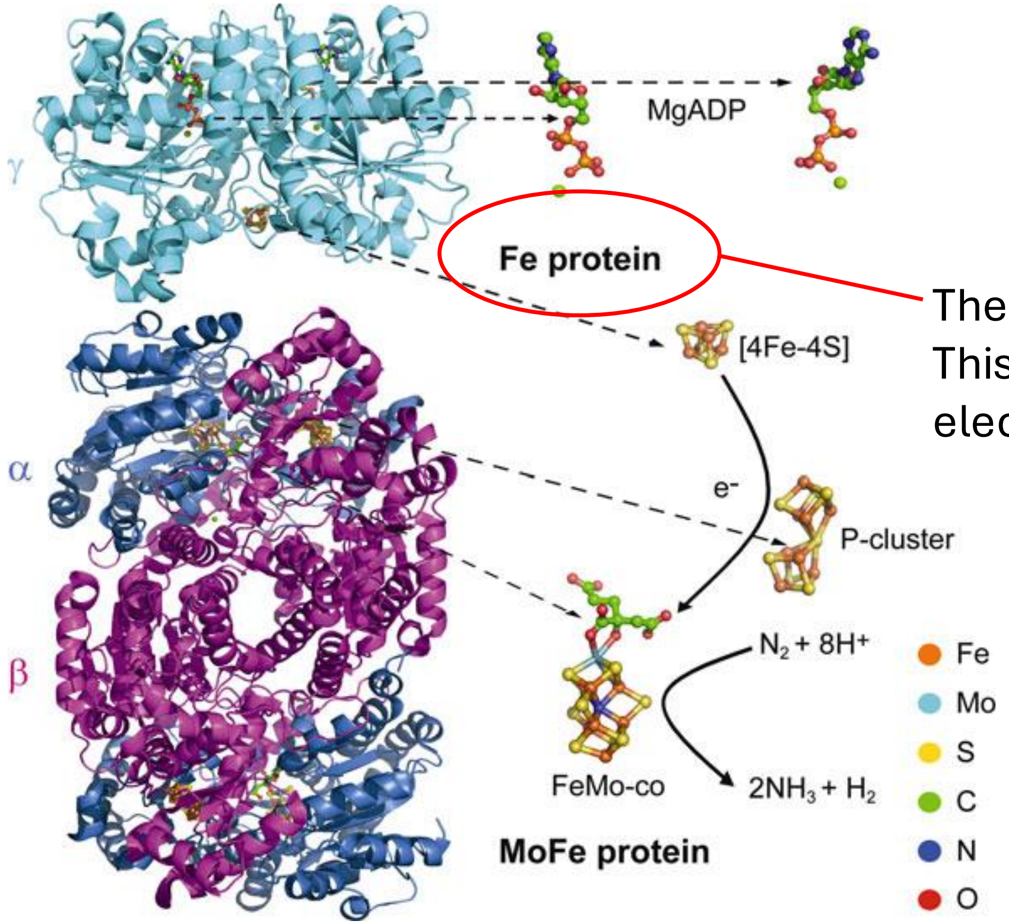

$$N_2 + 8e^- + 16MgATP + 8H^+ \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

The Fe protein disengages, to be replaced by another
This must repeat a minimum of **8 times** to transfer 8 electrons

Yang, Zhi-Yong & Danyal, Karamatullah & Seefeldt, Lance. (2011). Mechanism of Mo-Dependent Nitrogenase. Methods in molecular biology (Clifton, N.J.). 766. 9-29.

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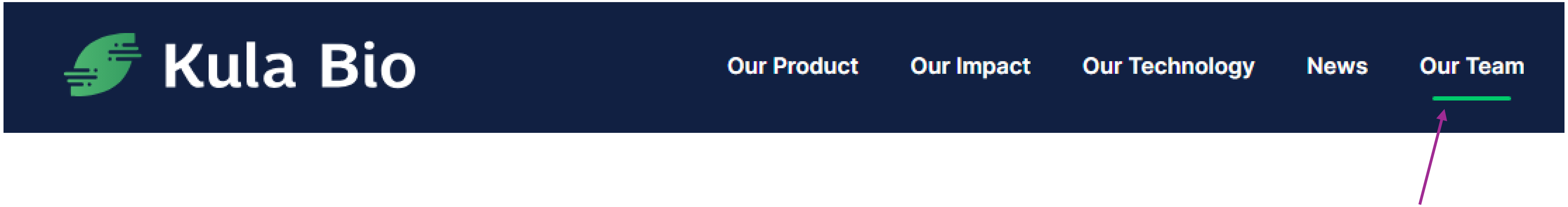
Let's do some research



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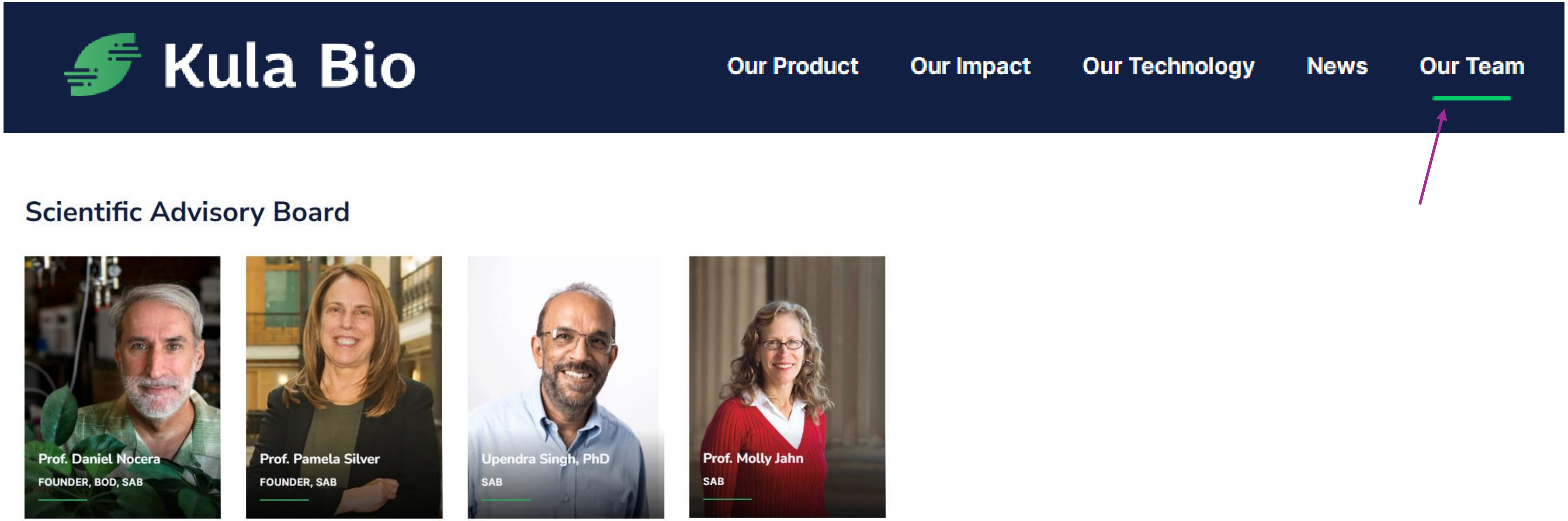

https://www.pnas.org/doi/abs/10.1073/pnas.1706371114

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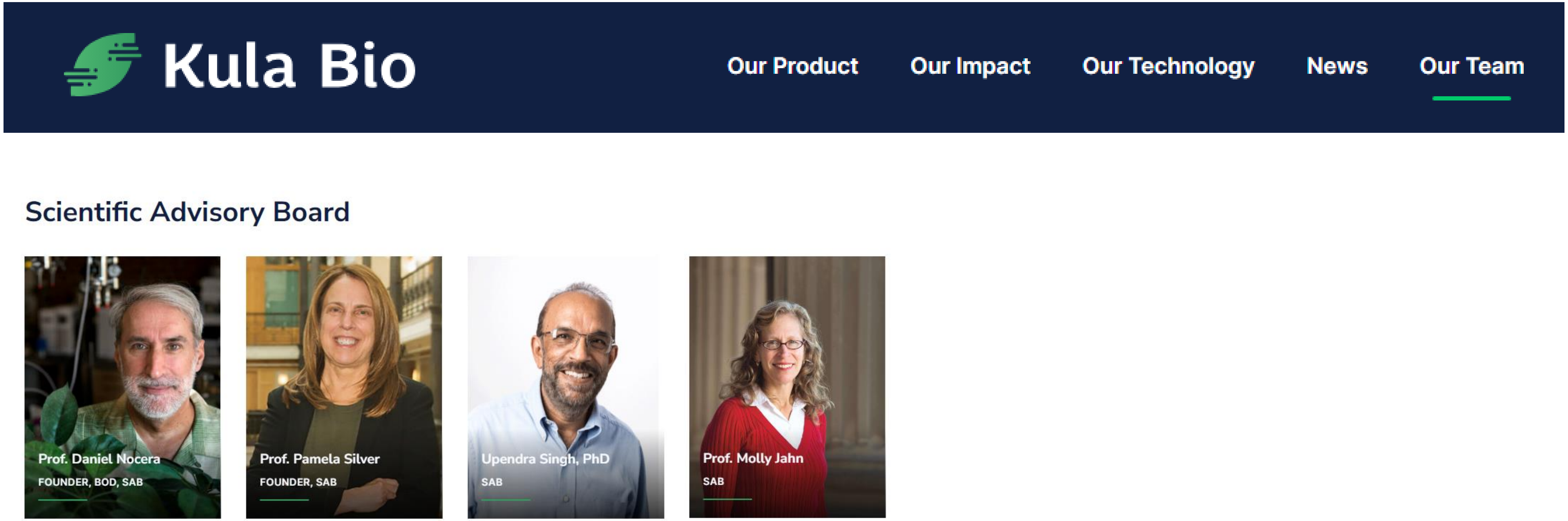

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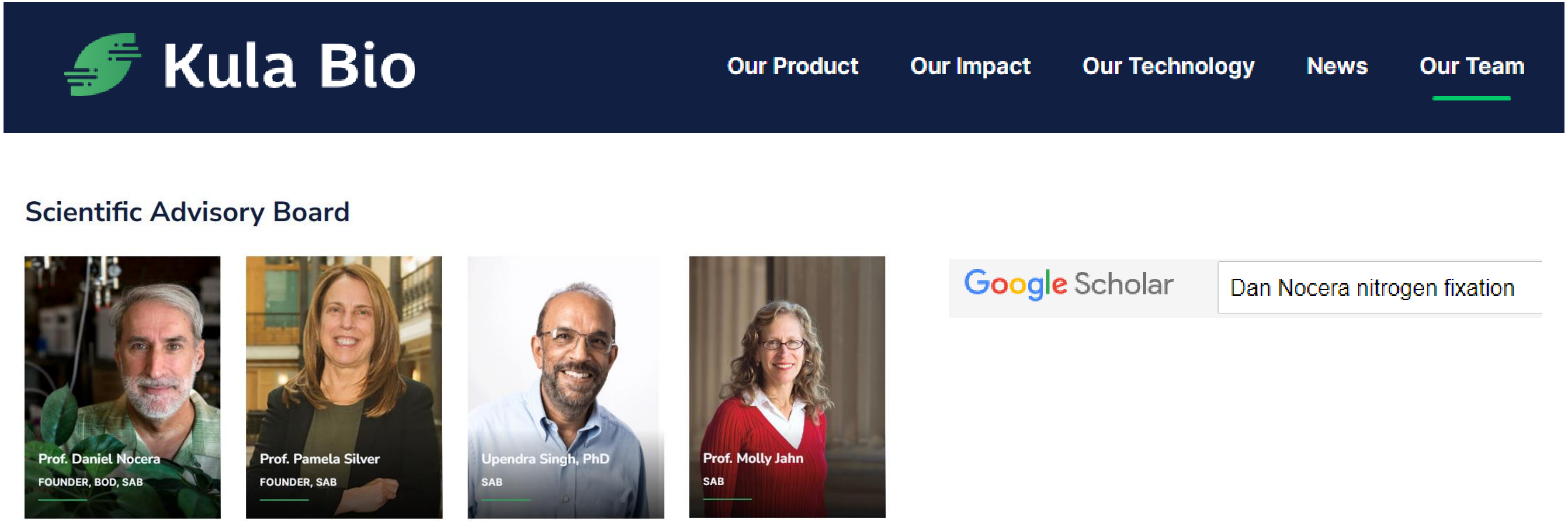

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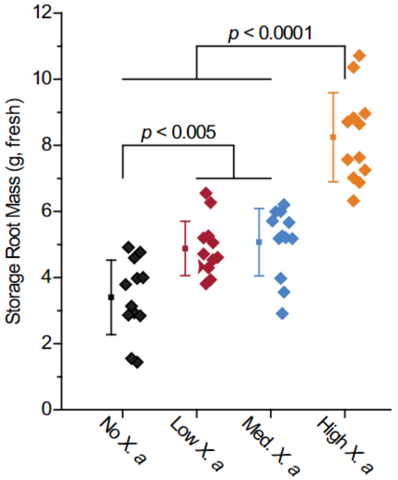

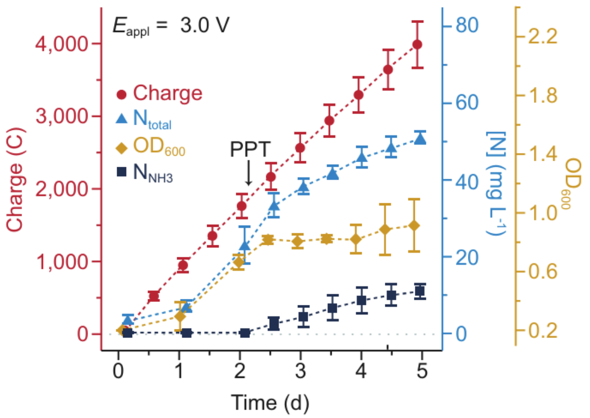

Enhanced Nutrient Efficiency

Nutrient loss from traditional nitrogen into neighboring waterways represents both a waste of expensive fertilizer, as well as a significant impact on the environment. Kula Bio's biofertilizer does better. Our product produces meaningful amounts of nitrogen in the soil, but only when the plant needs it. The result is less chance of run-off or waste.

Sustaining All Crops

Our biofertilizer is crop agnostic and provides the benefits of traditional nitrogen for both conventional and organic farming. We can replace up to 50% of your nitrogen demand. Our Agronomy team can make a recommendation based on your current production practices and yield goals.

Liu C, Sakimoto KK, et al. Proc Natl Acad Sci U S A. 2017 Jun 20;114(25):6450-6455.

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Non-GMO biofertilizer

Kula-N offers all types of farmers a high performance and cost-effective replacement for traditional nitrogen fertilizer.

FAST-ACTING AND EASY TO APPLY

Kula-N retains all of the benefits of traditional nitrogen, including delivering immediate results, but is sustainable and doesn't harm your soil or the environment. It's free of pathogens and easy to apply to your fields through typical irrigation practices.

MEETING CONSUMER DEMAND

Demand for more sustainable food continues to see double digit growth and federal, state and local governments are taking action to push the agricultural sector to limit nutrient losses and be more environmentally responsible. Kula-N is a low run-off solution that is designed to help farmers adapt while maintaining yield and revenue.

Let's Discuss!

Split into groups of 2-4 and think:

1. What does Kula bio do well?
2. What could be improved about their approach?
3. What are some further questions you have?
4. Would you invest in this company? Why or why not?
5. What was your biggest takeaway from this presentation?

Bonus (especially for the grad students out there) What is different about their approach compared to approaches more common in academia?

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Conclusions

- Fertilizer is crucial in feeding billions of humans
- Unfortunately, fertilizer has a significant impact on the environment
  - Eutrophication
  - Energy cost in production
  - Use of fossil fuels
- Kula bio has an interesting and bio-inspired approach-nitrogen fixing bacteria
- Bacteria are more environmentally friendly than current systems of nitrogen fixation

Special thanks to CEO of Kula Bio: Bill Brady!

His recommendations/advice:

1) Get someone involved on the business side early
2) Set clear and ambitious milestones for the project
3) Get in touch with potential buyers early on (see what they're currently using, what are their biggest concerns or issues?)
4) Don't hesitate to take the next step (he had prototypes of his bacteria in the hands of farmers within 6 months of operations, even though it had not been refined yet)
5) Ensure that our technology could slot into existing machinery. Plug and play is essential.
6) Nobody cares about 5% improvement. If it is not substantial it will never be implemented, people prefer the status quo.

Kula Bio

Bill Brady