# Bulk Replacement of Synthetic Nitrogen with Affordable, Consistent, Microbial Fertilizers

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## An Impossible Compromise

Each season, farmers balance conflicting needs: this year's yield against the next four decades of soil productivity; the reliable certainty of synthetic chemicals vs. the diverse benefits of organic practices. Many decisions surround nitrogen (N) fertilizers, which since the advent of the Haber-Bosch process to produce chemical ammonia ($NH_3$), has undeniably boosted global yields with great predictability.

At the same time, its heavy reliance on $CO_2$-emitting fossil fuels makes these synthetic fertilizers a temporary fix for a permanent challenge. Already, the use of synthetic N has reshaped our soils, lowering its natural health and productivity, requiring increasing inputs at a time when farmers are asked to do more with less.[1] This excessive use of synthetic N triggers mass nutrient runoff that contaminates crucial water reserves and habitats, and the concomitant nitrous oxide emissions have further exacerbated agriculture's climate impact. Alternatives often force farmers to make choices between cost, ease-of-use, environmental impact and long-term benefits: an impossible compromise between affordable practices and continued sustainable stewardship of their land.

## The Promise of Biology

The allure of harnessing biological nitrogen fixation (BNF) to convert atmospheric nitrogen ($N_2$) into $NH_3$ directly in the field has flooded the last century with a laundry list of products. The responsive feedback loop between plant, microbe and soil helps to create on-demand synthesis of $NH_3$ that greatly reduces nutrient runoff, increases nutrient-use efficiency, boosts soil health, and minimizes many of the $CO_2$ emission sources in fertilizer manufacturing and use. But challenges persist:

## Solving the Symbiosis Energy Tax

The deceptive simplicity of symbiotic BNF, found naturally in legumes and soybeans, obscures the difficulty of forcing microbes to form new, mutualistic relationships. Though uncommon in most crops today, phylogenetic analyses[2] have revealed that many common ancestor plants once engaged in such partnerships, working with bacteria living inside the plant's roots themselves to exchange plant sugars for N fertilizer. And yet, natural selection has forced most plants to abandon that strategy. Why? Nitrogen fixation is a huge energetic cost, and a bad deal for most plants, asking the plant to spend an average of 6.5 lb carbon (C)/lb N, roughly a 10% loss in biomass C.[3] Energetically, the biochemical equation for BNF necessitates 16 ATP/$N_2$, among the most energetically expensive biological reactions. In most conventional farming systems, this energetic cost is paid for by fossil fuels in the Haber-Bosch process, at an average energy input of 37 GJ/metric ton of $NH_3$,[4] translating to more than 970,000 calories/acre of energy at a modest rate of 100 lb N/acre. It's no surprise that synthetic $NH_3$ production is the most costly industrial chemical process in the world,[4] requiring the most energy and emitting the most $CO_2$. The massive energy burden explains why attempts to re-engineer symbiotic BNF into modern crops have not been able to supply significant amounts of N (< 20% of N demand).[5]

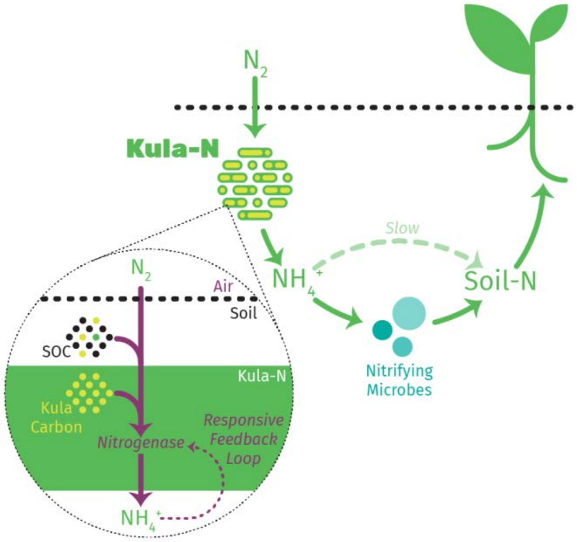

**Fig. 1: Kula-N Mode of Action.** Soil organic carbon (SOC) and intracellularly stored Kula Carbon supply the high energy requirement for fixing $N_2$ into $NH_3/NH_4^+$. A plant-/soil-responsive feedback loop mediates the amount of N delivered to the soil, working in with native nitrifying microbes to speciate $NH_4^+$ into plant available Soil-N.

**The Kula Solution:**

At Kula Bio, we're focused on C and energy. Rather than leeching off the plant's photosynthetic C, we utilize non-symbiotic, associative BNF[6] that taps into the soil organic carbon (SOC) pool for energy. An average soil with a modest 1% SOC translates to nearly 18,000 lb C/acre, requiring only 3-4% of that total pool, compared to the >10% of biomass C required for symbiotic BNF.

But that alone is not enough. To reach commercial yields, more energy is required, particularly in the face of depleting global SOC pools due to farming practices and climate change,[7] Kula Bio augments the energy available to our microbes through a range of renewable, non-agriculturally derived C sources. This approach provides a

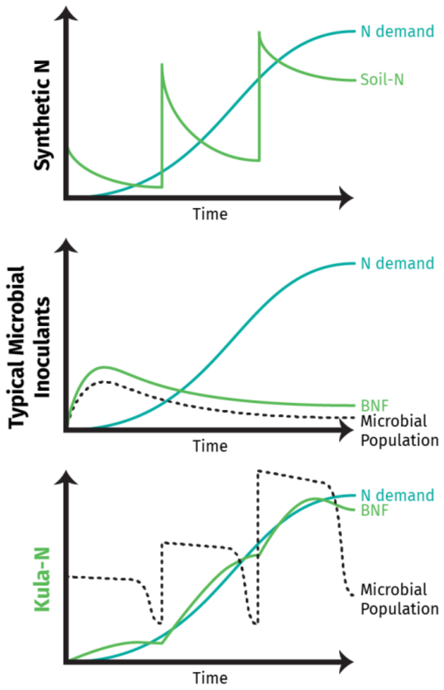

Fig. 2: Kinetic Comparison of N-Fertilizers. Synthetic N applied at intervals spikes soil-N concentration to meet the variable N demand. Typical inoculants applied pre-season decay over time, delivering marginal quantities of N. Kula-N applied at intervals to maintain the robust, stable microbial population, dynamically adjusting biological N fixation (BNF) to meet crop demand. Qualitative graphs for illustrative purposes only.

new pathway for energy and C to enter the soils, rather than plant-derived biomass and crop residues that suffers the same taxing bottleneck as symbiotic BNF.

Our proprietary fermentation process enhances the microbe's naturally-occurring ability to store this additional C and energy as Kula Carbon, an intracellular C storage compound that delivers a supplemental energy source to the soil. These fortified microbes consistently replace 50-80% of the synthetic N applied in controlled field trials and commercial operations (from 150 to >200 lb N/acre each planting).

## Taming the High Variability of Biology

The diversity of organic practices is both its blessing and its curse: microbial inoculants demonstrate widely ranging performance from year to year, farm to farm, and even within a single field. Many are often characterized by "win rates", or the probability the product has any net positive effect. This metric encapsulates the difficulty of an inoculant 1) surviving through application to the soil, 2) finding suitable SOC substrates, 3) outcompeting the native soil microbiome, 4) replicating in the soil and 5) surviving the season to provide significant BNF.[8] Failures at any of these critical steps will lead to unpredictable and often underwhelming performance. For most microbes grown in the laboratory, either engineered or evolved beyond their native context, they struggle to survive the lengthy storage and distribution of centralized industrial manufacturing, often requiring expensive additives and stabilizers. Once in the soil, the high variability of the SOC pool doesn't guarantee that these foreign inoculants can find their preferred type of C source, nor that they can compete with the highly adapted and evolved native soil microbiome for these precious resources.[9]

As most microbial inoculants need to replicate in the soil, their application has to account for the imprecise rate of microbial growth. To complicate management practices even further, organic amendments such as compost or manures, have their own variable rate of breaking down into the building block nutrients driving microbial replication. They also introduce a host of other microbes competing for the same nutrient pool. Thus, farmers must grapple with the uncertainty of simply when to use a new product.

As insurance, new products ask farmers to adopt new practices, often applying products pre-season with additional equipment, or utilizing expensive seed coatings to ensure plant-microbe compatibility, from which point the biological product runs on auto-pilot. This "hit-or-miss" approach to microbial products is incompatible with the adaptive, dynamic nature of farming, requiring methodical and precise mid-season changes to account for the variability of the environment.

**The Kula Solution:**

Our first microbial N strain, Kula-N, is a natural microbe with a dedicated source of Kula Carbon. As a natural, wild-type microbe, it is already evolved to thrive within a diverse range of soils and climates. The Kula Carbon fortification enables robust, consistent performance by directly tackling the issues that have plagued most agricultural biologics. The on-board supply of Kula Carbon acts as a backup energy source to help Kula-N survive the transition from our fermenters all the way to the field, having evolved as a natural defense mechanism against all forms of environmental stresses (temperature, osmotic shock, desiccation, etc.) Once in the field, this Kula Carbon guarantees that our microbe immediately has its preferred C source, even in soils with depleted or inaccessible SOC pools, and in the face of steep microbe-microbe competition. As an intracellular C source, our microbe doesn't have to compete with other microbes, gaining first priority to this prized resource.

Our microbes are a bulk addition of the BNF biochemical machinery to the soil: active nitrogenase enzymes to perform BNF, contained inside live bacteria to maintain and produce more enzymes and regulate on-demand $NH_3$ synthesis, and Kula Carbon to supply the energy). This means they don't have to replicate or activate for full efficacy. They're immediately ready to go, allowing them to be applied with the same timing and dynamic precision as conventional synthetic fertilizers as a drop-in replacement.

## Lowering the Cost of Biotechnology

New biotech products take significant amounts of time and money to develop, test, and manufacture, the cost of which gets passed to the farmer. Particular approaches, such as engineering symbiotic BNF microbes or screening millions of strains for beneficial features, requires extensive R&D pipelines to reengineer new overly-specific microbes for each cultivar and each geographic region, similar to the breeding of proprietary GMO seeds.

The production of such microbial products relies on traditional, centralized, high-capital cost infrastructure inherited from the pharmaceutical industry, with price-tags to match.[10]

**The Kula Solution:**

To eliminate the need for farmers to compromise on cost and ease-of-use, N fertilization needs new solutions not only in the type of products offered, but how they're produced. Our Kula-N product is a generalist microbe, colonizing the soil rather than the plant itself. This focus on the soil enables our single product to work across a wide portfolio of crop types and cultivars, an ever growing list including: lettuce, corn, tomatoes, strawberries, peppers, cucumbers, brassica, and varieties of each. Farmers use our associative BNF microbes with the same ubiquity that synthetic fertilizers can be applied to any crop, field, or season.

To change how manufacturers produce ag-biologics, Kula Bio has focused on a proprietary bioreactor to grow our robust microbial products at a lower cost. We've pared down the multi-million dollar biopharmaceutical facility model, enabling a decentralized, distributed manufacturing process more like brewing beer and farming algae. By changing the way microbes are grown, we're able to produce living microbial fertilizers closer to the farm, easily scaling from centralized regional manufacturing plants to individual farmer operations.

## Seeing is Believing

Our team of agronomists, soil scientists, microbiologists, and engineers have worked hard to show how a good hypothesis turns into strong, reproducible, robust results in the field. Visit us at www.kulabio.com to see our latest trial results, or contact our technical team to learn more.

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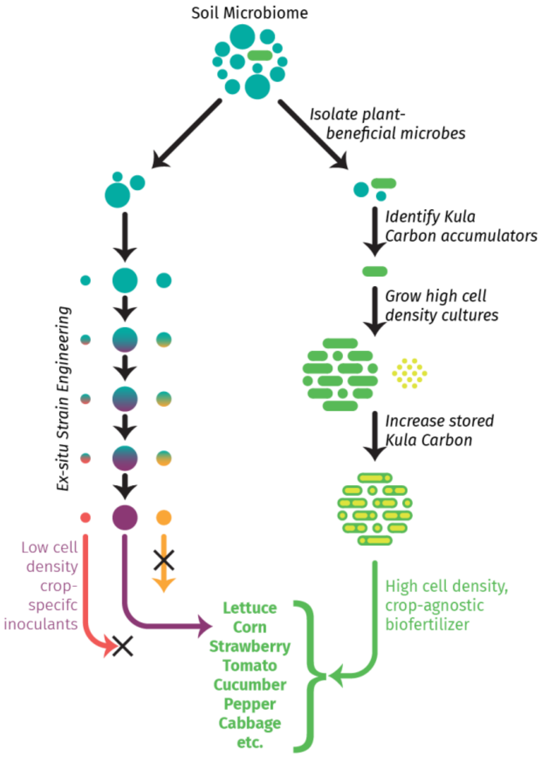

**Fig 3: Kula Bio's Approach.** Traditional microbial inoculants employ engineering techniques that adapt them away from their native soil environment. The result is crop-specific inoculants, grown at low cell densities through traditional bioreactors. Kula Bio has isolated wild-type, natural, non-GMO microbes and grown them to high cell densities while increasing the natural storage of Kula Carbon through proprietary bioreactors. This single product is compatible with a growing list of crops to replace bulk quantities of synthetic N.

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