COMPREHENSIVE ANALYSIS OF NON-SYMBIOTIC NITROGEN FIXING MECHANISMS

UNIVERSITY OF WASHINGTON

STRATEGIC ANALYSIS RESEARCH & TRAINING (START) CENTER

REPORT TO THE BILL & MELINDA GATES FOUNDATION

PRODUCED BY: Gosain S, Manguerra H, Malhotra A, Hossain R, Badshah A.

JUNE 2024

START CENTER
STRATEGIC ANALYSIS, RESEARCH & TRAINING CENTER
Department of Global Health | University of Washington

Photo: Adobe

PROJECT BACKGROUND

The development of inorganic fertilizer has led to exponential growth in global crop production. However, this growth has not been equally distributed; inorganic fertilizers have been largely inaccessible to smallholder farmers in Sub-Saharan Africa, in part due to their cost.

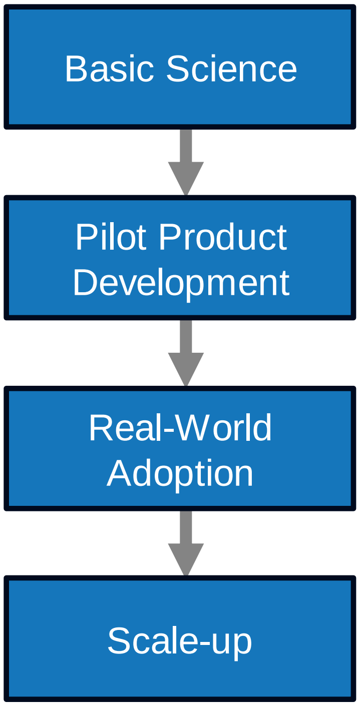
Smallholder farmers manage approximately 80% of the farms in low and lower-middle income countries. These farmers typically own less than two hectares of land and were estimated to comprise half of households experiencing hunger globally. Therefore, alternatives to inorganic fertilizers that increase smallholder farmers' crop production could have a substantial impact on global hunger.

Non-chemical fertilizers could not only increase crop production in Sub-Saharan Africa, but they may also serve as a more environmentally friendly alternative. Excessive use of chemical fertilizers contributes to water pollution and has been associated with poor long-term soil health.

Nitrogen-fixing biofertilizers have had success by harnessing the power of *symbiotic* relationships between rhizobia and legumes. However, these biofertilizers are highly host-specific. Interest is increasing in understanding how **non-symbiotic** nitrogen-fixing microbes could be engineered as biofertilizers for non-legume crops such as cereals or grains.

It is unclear to what extent non-symbiotic nitrogen-fixing microbes are currently used as biofertilizers around the world. Questions also remain around barriers or facilitators to their widespread use.

PROJECT AIMS



This project aimed to summarize information on the potential for underutilized non-symbiotic nitrogen-fixing (NSNF) microbes as biofertilizers. Specifically, our aims were to:

1. Understand the landscape of basic science around NSNF mechanisms
2. Identify barriers, facilitators, and opportunities in the translational process: i) basic science of NSNF, ii) pilot product development, iii) real-world adoption, and iv) scale-up.
3. Identify any existing NSNF products in use.

In particular, we aimed to understand the potential use of these products in Sub-Saharan African contexts. We excluded from this analysis commonly used microbes such as *Azotobacter, Klebsiella*, and *Azospirillum*, as these products have already been translated into commercial products but have not shown wide success.

INFORMATION GATHERING

Rapid Review of Published Literature

- Recommended literature by key informants
- Targeted Keyword search focusing on microbes and crops of interest

Expert Opinions:

- 6 countries, 12 institutions
- 12 experts interviewed across 10 Key Informant Interview (KII) sessions

980 studies imported → 59 duplicates removed

↓

921 studies screened → 849 studies irrelevant

↓

72 selected for full-text screening → 45 studies excluded

↓

27 studies included for data extraction

KEY THEMES FROM LITERATURE REVIEW

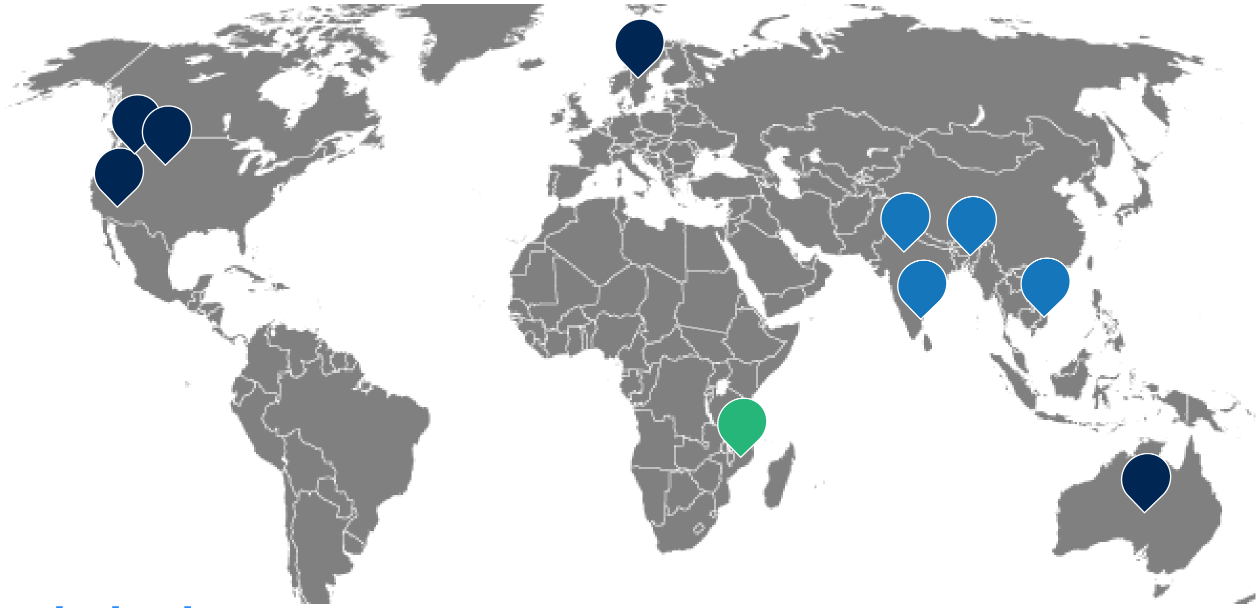
STUDY ID	COUNTRY	MICROBES	PRODUCT	FINDINGS	RELEVANCE
416: Sharma 2021	India	Anabaena–Nostoc consortium, Anabaena–Trichoderma biofilm	Yes	Cyanobacterial inoculation led to a 22–30% increase in soil available nitrogen at the flag leaf stage and a **13–16% higher cob yield** for maize genotype V6 with An-Tr biofilm inoculation, saving 30 kg N ha−1 season−1.	High
15: Alvarez 2023	N/A	Various cyanobacteria	No	Cyanobacterial inoculation in agriculture **catalyzes N2 fixation**, decomposes organic wastes, detoxifies heavy metals, and produces bioactive compounds for plant growth. Specifically, in rice paddy fields, cyanobacteria can contribute **~20–30 kg ha−1 of fixed nitrogen.**	Moderate
977: Dhar 2007	India	BGA	Yes	BGA biofertilizers developed at Indian Agricultural Research Institute (IARI), New Delhi, revealed that **BGA can provide 25-30 kg N/ha/season and increase paddy crop yield by up to 30%.**	Moderate
975: Kumar 2017	India	Diazotrophs	No	Continuous application of high doses of inorganic nitrogenous fertilizers (60 & 80 kg N ha−1 year−1) limits the frequency and diversity of rhizospheric diazotrophs in the long-term aromatic rice-rice cropping system.	Low
972: Nayak 2004	India	BGA, Azolla	Yes	Biofertilizer treatments significantly affected N fixation (measured as ARA) and chlorophyll content, with the highest ARA values in samples from the N30 + BGA + Azolla treatment.	High

KEY CHARACTERISTICS OF IDENTIFIED STUDIES

CHARACT-ERISTIC	SPECIFIC CHARACTERISTIC	NUMBER (%*)	SOURCES (COVIDENCE STUDY ID)
Geography	Low/middle income	15 (56%)	341, 387, 416, 533, 624, 649, 683, 961, 963, 964, 970, 972, 975, 977, 978
	High income	3 (11%)	805, 955, 957
	No geographic focus	9 (33%)	15, 43, 52, 109, 761, 841, 954, 956, 979
Bacteria	Cyanobacteria/ BGA	6 (22%)	15, 955, 963, 964, 972, 977
	Nostoc	5 (19%)	341, 954, 955, 963, 970
	Azolla, Anabaena	9 (33%)	387, 416, 649, 954, 961, 963, 964, 970. 972
	Other	14(52%)	43, 52, 109, 533, 624, 683, 761, 805, 841, 956, 957, 975, 978, 979
Crops	Maize	6 (22%)	416, 683, 841, 955, 956, 957
	Wheat	2 (7%)	970, 977
	Rice	12 (44%)	15, 387, 649, 954, 961, 963, 964, 970, 972, 975, 977, 978
	Other/not applicable	9 (33%)	43, 52, 109, 341, 533, 624, 761, 805, 979
Yield	Yes (as outcome)	5 (19%)	533, 955, 961, 963, 977
	No	22 (81%)	15, 43, 52, 109, 341, 387, 416, 624, 649, 683, 761, 805, 841, 954, 956, 957, 964, 970, 972, 975, 978, 979
Product Focus	Yes (discussion of product)	5 (19%)	416, 683, 963, 972, 977
	No	22 (81%)	15, 43, 52, 109, 341, 387, 533, 624, 649, 761, 805, 841, 954, 955, 956, 957, 961, 964, 970, 975, 978, 979
Setting	Lab	2 (7%)	683, 956
	Field	9 (33%)	387, 416, 955, 956, 961, 963, 964, 970, 972
	Not applicable	17 (63%)	15, 43, 52, 109, 341, 533, 624, 649, 761, 805, 841, 954, 957, 975, 977, 978, 979

**Total may exceed 100% since some papers cover multiple characteristics*

GEOGRAPHICAL CONTEXT



5 Global North

4 Asian Context

1 African Context

Limitations: Only one key informant had significant experience in African contexts. Furthermore, although this KII's program of research is in Mozambique, their home institution is in Portugal. We had received recommendations for other researchers in African contexts but were unable to secure interviews during the time frame of this project. We recommend further outreach to researchers in African contexts to ensure the validity of our findings.

Finding: Informants' perceptions differed substantially by geographical context

In the **Global North**, key informants agreed that more basic science research is needed before NSNF microbes could be translated into successful products. Although all knew of pilot products that had been developed, all expressed skepticism about the quality of those products. The interviewees mentioned limited proven success in field testing. Real world adoption has been limited, and widespread scale-up of these products was not ongoing.

Key informants from **Asian contexts, particularly India,** reported being farther along in the translation process. Informants in Asia were more aware of the use of historical use of NSNF pilot products in other Asian countries, such as India and the Philippines. Many products are available, with agricultural extension work ongoing. More focus is on distribution and scale-up challenges rather than basic research.

The informant from **African contexts** indicated little research on NSNF microbes originated from Sub-Saharan Africa. However, they saw high potential in the Miombo woodlands as a source for native, underutilized microbes.

Key:
- ✓ - Ongoing
- ⚠ - Early Stage
- X - Inactive
- ? - Unknown

	Basic Science	Pilot Products	Real-world Adoption	Scale-up
Global North	⚠ - ✓	⚠ - ✓	⚠	X
Asian Context	✓	✓	⚠ - ✓	⚠
African Context	⚠ - ✓	? - X	? - X	? - X

STAGE 1: BASIC SCIENCE

BARRIERS

Lack of in-field trials

- The most commonly cited barrier by the key informants was the lack of on-farm or in-field experimental trials. The majority of published experimental evidence on the effectiveness of NSNF microbes are from controlled laboratory settings.

"What the large research community struggles with is when we measure these rates in the field, we often don't know who is active. There has been a bit of progress made more recently. We often have a total rate, and we know who in the soil has a nitrogen-fixing gene, but that doesn't tell us who is actually doing the work." - Key informant 1 (Global North)

" (I am) not convinced there is quantitative data in experimental systems that suggests it is worthwhile to invest (in these products)." - Key informant 2 (Global North)

- Two informants from the Asian context (whose institutions have developed NSNF pilot products) reported data from in-field trials, stating that their products were able to provide 20-30 kg of nitrogen per hectare and increased yield by 10-12%.

Unclear impact of variable soil and atmospheric conditions on N-fixation

- More basic science research is needed to understand how variable soil and atmospheric conditions affect nitrogen fixation. It is unclear how these microbes would survive environmental stresses.
- A Global North informant mentioned concerns about denitrification, which is the conversion of nitrogen back to its gaseous form. In Europe, it is common to add a product to the soil that reduces denitrification. Furthermore, they noted that temperature increases this process.

Conversely, one informant from Asian contexts reported that their products have been tried across a large number of different ecologies. *"We've even done it in the hills where the vegetables and orchards are. Even in that condition they work very well.... We have tested all over India." - Key informant 3 (Asian Context)*

"That's why these microbes [in the Miombo woodlands], the indigenous, native microbes, are so unusual. Because they are fully adapted to a set of environmental extremes." - Key informant 4 (African context)

Microbial competition

- Informants expressed concern that a microbe newly introduced to a microbial community might not survive.
- One Global North informant mentioned that more diverse and complex microbial communities tend to be more stable.
- The mixed microbe approach has had success in Asian contexts: *"If one grows less, the other will grow more. They take care of one another." - Key informant 3 (Asian context)*

BARRIERS

Slow microbial growth

- Microbial research was perceived as "slow and patient" research. This was seen as less attractive to researchers seeking funding.

"Cyanobacteria are not fast growing, especially the ones that we use as a biofertilizer, because these are all filamentous organisms. For a bacteria, [generation time] would be 20 minutes. But for a cyanobacterium, it would be between 48-96 hours. Because these are photosynthetic organisms, they have an additional set of genes that have metabolic activities to take care of. So when they double, it's a longer process."
- Key informant 3 (Asian context)

Human resources and infrastructure

- A key informant from the African context noted that soil laboratories are generally available in Sub-Saharan African countries but issues may arise when seeking certified labs. They did not feel it was an insurmountable barrier.

Referring to human resources in Sub-Saharan Africa: *"You might lack a critical mass...normally people that are able to perform this work are too busy with other commitments." - Key informant 4 (African context)*

- The informant said biopiracy is a large concern: *"Many people from other countries do the work and take resources, which is not quite fair." - Key informant 4 (African context)*
- They emphasized the importance of building capacity for the next generation of researchers from Sub-Saharan Africa.

Unclear mechanisms of association with plant rhizosphere

- More research is needed to understand how free-living microbes move towards plant roots, as *"they don't have much intention to move to the root system." - Key informant 5 (Asian context)*

- Free-living microbes fix nitrogen for themselves, so it is still unknown why additional nitrogen is available for the plants.

"What we don't know is why [free-living nitrogen fixers provide nitrogen for plants]. Microbes are short lived, so maybe they are just dying. But maybe there is some sort of association with the root. If we know that, that helps us better predict how available that nitrogen is, how quickly it can get to the plant. So, if they're only giving it up to some plants, that's going to be useful to know." - Key Informant 1 (Global North)

FACILITATORS

Rich soil in African contexts

- Several researchers praised the soil condition in Sub-Saharan Africa and noted it is rich in carbon.

"The top of African countries are very rich in organic carbon. They are quite good. The countries that are close to the Nile River, that is a potential area. I have classmates working in the sugar industries in Africa, and they used to tell me the soil is so rich, rich in organic carbon there. We can introduce this free-living biofertilizer." - Key informant 5 (Asian context)

"You also have very rich soils and very good soils in Sub-Saharan Africa. It's not all dry and acidic. When you the higher altitudes, you have very high levels of organic matter." - Key Informant 4 (African context)

"We could also develop the infrastructure there because they have very beautiful soil. The soil is so wonderful there." - Key Informant 3 (Asian context)

- The Miombo woodlands, which covers 2.4 million sq. km in SSA, has rich soil with microbes that have been uncharacterized.

Cross-disciplinary research

- Expertise from different fields will accelerate basic science in this area: *"This research needs to be addressed by a team with agronomic, genetic, and biochemical expertise." - Key Informant 2 (Global North)*

- Some of the open basic science questions are limited by technology. However, one informant believed the required technologies are available in other fields. *"I think the technology exists or we have something close but would take some thoughtful people who work with some of those cutting-edge technologies to figure out how to that work." - Key Informant 1 (Global North)*

FACILITATORS

Promising Microbes and Microbe Characteristics

- Characteristics of promising microbes include: 1) Broad symbiotic competencies; 2) Can be grown in a reasonable manner; 3) Uncomplex morphology.
- Existing products from one informant's institute *"basically all have Anabaena in them." - Key informant 3 (Asian context)*
- One product contains a combination of three non-cyanobacteria (including *Providencia* and *Chronobacter*). It was noted to grow much faster than cyanobacteria.
- The following list of promising free living bacteria was provided by a key informant: *Achromobacter, Arthrobacter, Azoarcus, Azomonas, Bacillus, Beijerinckia, Clostridium, Derxia gummosa, Desulfovibrio, Kosakonia, Paenibacillus, Pantoea, Priestia, Rhodopseudomonas, Rhodospirillum, Sphingomonas, Streptomyces.* This list of microbes all produce polysaccharides and improve the soil. Several are able to store water or produce a 'gum-like' substance that binds to the soil and creates a small microenvironment. *"[This microenvironment] leads to an increase in soil diversity and is a unique way of increasing biological activity. In that sense, the free-living organisms are much better than your plant-associated microorganisms." - Key informant 5 (Asian context)*
- Stem-nodulating bacteria such as *Frankia* were perceived by one informant to not have shown sufficient mechanistic requirements to associate well with maize/rice.

OPPORTUNITIES

Incentivize an organized, experimental system of on-farm trials

"Research needs to be supported to test these microbes in soil and field conditions. Funds for research to test the effectiveness and what can be done to improve effectiveness -- that has to underpin any program to promote the use of these biologicals in a new country or a new place." - Key Informant 6 (Global North)

"The best approach may be to find experimental systems and then focus intently on those systems, whether it is a cereal grain, an aquatic plant, a shrubber or a tree.... We don't have a consistent system. Somebody works with this, somebody works with that, and they don't quantify it. You can't compare them. So it really needs to be organized and produced, approached systematically." - Key Informant 2 (Global North)

OPPORTUNITIES

Funding mechanisms that allow for slow, high-risk research

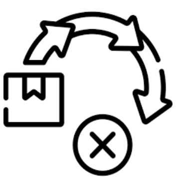
- Increasing funding, particularly for research that is slower-paced but high-risk / high-reward, was seen as a potential opportunity. Researchers noted that microbial research has become less popular.

"Working with microbes that fix nitrogen is a very old story nowadays. It is almost 150 years old. Nowadays, most research is focusing on genome and molecular biology, meta-genome.... whenever your new friend comes, everyone will follow it. They want very good, high-impact publications and so on." - Key Informant 5 (Asian context)

"Normally the resources are limited [in African research contexts], and these resources are applied to food. So sometimes these potentialities are neglected." - Key Informant 4 (African context)

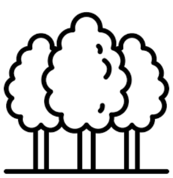
"It's going to take a backer with patience to discover this kind of thing." - Key informant 7 (Global North)

"Whether there's the incentive and the resources to do that with cyanobacteria is the real question." - Key Informant 2 (Global North)



Influence basic science researchers to think ahead to product development

"A lot of the work that has been done has been in sort of an exploratory science framework. But if the goal is to address this particular problem, maybe ask some folks to work together to focus their research direction into that particular problem." - Key Informant 1 (Global North)



Bioprospecting the Miombo woodlands

- An informant stated that the Miombo woodlands, a multi-country ecosystem in Sub-Saharan Africa, is under-explored. They believe systematic and coordinated surveying of the Miombo woodlands and similar ecosystems in SSA will yield microbes that well fit for translation into bioinoculants for the SSA context.

"The key message is that, according to my experience, the potential is enormous. The world will gain from coordinated action on surveying and prospecting these native ecosystems." - Key Informant 4 (African context)

BARRIERS

Skepticism around importing products based on non-native microbes

- Expert consensus was that indigenous microbes are much more likely to be successfully translated into products. One informant whose institute has developed products believed a two-pronged approach could be tested, in which indigenous microbes were characterized for product development but existing products from other contexts could also be tried.

"The belief that a microbe developed in one place will work in all other places...that would have been a bit more like 20 years ago. But now we know, it's well-established that the diversity and the composition of any group of microbes that we are interested in, including these, can vary geographically because of the different soil and environmental factors."
– Key Informant 6 (Global North)

Quality control of products

- Informants noted that products can experience degradation over time, particularly since it is difficult to store the products at the required temperature. One informant reported that a European group developing NSNF products for commercial use said, *"We know these products are alive in the container when they leave the assembly line, but after they leave the facility, we don't know what happens." – Key Informant 7 (Global North)*

Mixed concerns about toxicity

- Four experts mentioned the need to check for pathogenicity, but half said the chance of harm was low.

"Anytime you're putting something on an edible product, you have to be very careful, and cyanobacteria do produce toxins. Most of the toxins they produce are not lethal, and it takes a high amount for lethality to occur." – Key Informant 2 (Global North)

Regulatory Challenges

- New products often experience challenges with regulatory environments.

"The government will ask why you want to replace current fertilizers with this new fertilizer. Will have to respond to questions around safety." – Key Informant 5 (Asian context)

- However, the regulatory context was seen as less of a barrier in the United States.

"There's not a chemical company on the planet that isn't looking at this because to get a new product through the EPA costs tens of millions of dollars, maybe up to a hundred million dollars right now... But there's no regulations, as far as I know, on any of these organisms. So I think that is why they're pursuing. There's a possibility to make some money. There's very little R&D. They don't have to put anything through the EPA. And the consequences of something happening later on are probably pretty slim because these are all natural organisms." - Key Informant 7 (Global North)

STAGE 2: PILOT PRODUCT DEVELOPMENT

FACILITATORS

Existing models from Asian countries
Long history of developing NSNF pilot products in Asian context (India, Philippines). Significant experimentation has already been completed that can be replicated in other contexts

Renewed Government Interest
Governments in Asian contexts (India, Philippines) have reinstated research into NSNF biofertilizers in the last decade.

OPPORTUNITIES

Navigating Regulatory Challenges
Opportunities exist to assist researchers and implementers in overcoming regulatory challenges in developing pilot products

Model of Developing Products (Experience from Asian Context)

1. Isolation & Characterization
Process of screening and identification takes time and trained personnel, but is not a major barrier.

2. Mixing cultures
Cultures of promising microbes are mixed to understand how they relate to one another. Looking for a combination that is stable and able to compensate for one another.

3. Carrier experimentation
Carriers need to be easily accessible, inexpensive, lightweight, able to store water, and have stable storage. This process can take time and experimentation. At one institute, their two main carriers are clay-based and a 1:1 ratio of patty straw and vermiculite

4. Production optimization
Optimize growth in large-scale production facilities (e.g. how long do the microbes take to grow, quantification of growth)

5. Commercialization
Noted as the *"main hurdle so far"*. Product needs to be distributed to other companies with capacity to replicate the product at large scale and high quality

BARRIERS

Lack of quantitative data

- Lack of experimental data that demonstrate that NSNF products work in fields and in what context.

"That research is certainly required especially if you want to take these products to small holders, where they will not have a large, 4,000 hectare farm in US or Australia where they could probably afford to do testing themselves." - Key informant 6 (Global North)

- A review of NSNF products' impact on corn yield in the United States found that 51/53 of the experimental field trials did not have a significant effect.

Hesitancy to adopt unfamiliar products over chemical fertilizers

- Chemical fertilizers, particularly in Asia, are highly prevalent. Farmers who are familiar with using chemical fertilizers like urea are usually hesitant to try alternatives. Local governments may also be hesitant to recommend new products over current fertilizers.

- An informant in India said that village leaders will need to be engaged to overcome farmer hesitancy.

"I feel [addressing hesitancy] has to come at the level of the village. Here we have a panchayat, each village has a person who is in charge. That person has to stay while you give it out and let them try it out." - Key informant 3 (Asian context)

Low yield from NSNF products

- NSNF products provide substantially less yield than chemical fertilizers, which makes the products less appealing.

"Only 8% of farmers are using biofertilizers. In Tamil Nadu only 24% of farmers are using biofertilizers. We have to go a long way to satisfy the farmers. Because once a farmer applies urea, the next day he can see the greenness of the plant. Within 24 hours, he can observe the difference. But our microbes are not so super, super powerful. They don't have super powers. They do it very slowly, and the farmer could not sense that it is slowly improving." - Key informant 5 (Asian context)

- Two informants emphasized the importance of a holistic approach to improving smallholder farmers' yield. Both expressed skepticism that NSNF products could stand alone.

"One thing to understand - asymbiotic fixers will never be able to supply all the nitrogen that a crop needs because they are not connected with the plant themselves. There are no feedback mechanisms." - Key informant 7 (Global North)

BARRIERS

In-field testing kits not available

- Technologies are available in research laboratories to measure the presence of NSNF microbes and test if they are still alive. However, these tools are not available to farmers or industry representatives to test if the product is still viable, either while in the container or in fields.

"These techniques at present are available to researchers in sophisticated labs. So, development of tools and such techniques could be made available and for use by industry people. Low-tech, low-cost techniques and development of those tools will help overcome the barrier of maintaining quality control." - Key informant 6 (Global North)

"[During a meeting with an industry representative], a tech rep said, 'I wish there was some kind of a kit or something that we could use in order to figure out if these things are alive and functioning in the soil.' "- Key informant 7 (Global North)

FACILITATORS

Historical Success in Certain Regions

- Past successes in South and Southeast Asia can facilitate renewed interest and application in other regions.

Willingness of Younger Farmers

- Younger farmers show more interest and openness to trying new products

Joint Distribution of Bioinoculants with Seeds or Chemical fertilizers

- In India, researchers have had success with offering both seeds and NSNF fertilizers together to habituate the farmers into using the biofertilizers.

- An informant in India recommended an "integrated management" strategy. In this strategy, both chemical fertilizers and biofertilizers are given to farmers. Over time, the proportion of chemical fertilizer is reduced relative to the proportion of biofertilizers. This gradual process leads farmers to accept the biofertilizers.

OPPORTUNITIES

Invest in Implementation Science

- Invest in rigorous research into implementation strategies for delivering NSNF products to users

Incentives for Distribution

- Providing incentives for the distribution of biofertilizers can encourage wider adoption among farmers.

- For an informant whose institute disseminates products for real-world adoption, incentivization was seen as the most important strategy for influencing farmers' adoption of NSNF products.

Development of Testing Kits

- The development of testing kits can empower farmers to assess the quality and effectiveness of biofertilizers, which can increase their trust and willingness to adopt these products.

FINDINGS

STAGE 4: SCALE-UP

BARRIERS

Need for Training and Regulation for Entrepreneurs

Ensuring that entrepreneurs are properly trained and regulated is critical to maintaining the quality of biofertilizers as they move from lab to market. Without this, the risk of low-quality products reaching farmers increases.

"We always teach the farmers that the soil will provide nutrients to the plant, and if you provide these chemicals, the plant will grow; but they need to sustain their soil health for the longer term. That missing link is there. Still, the farmers don't know why they should they maintain soil health. They say they are applying urea and getting good yield - why add organic amendments? We used to tell them it will be useful for your sons and not you. But still we cannot reach them." - Key informant 5 (Asian context)

Potential Loss of Farmer Trust

Encountering low-quality biofertilizers could cause farmers to lose trust in these products, which could hinder broader adoption and scaling efforts.

Protection of Intellectual Property

Protecting intellectual property rights and ensuring proper technology transfer are crucial to incentivize investment in biofertilizer technologies.

"In Sub-Saharan Africa, not all countries have implemented international treaties for the protection of biodiversity and intellectual property on the use of these genetic resources... Technically [this research] is not difficult. Legally, it might be tricky." – Key informant 4 (African context)

FACILITATORS

Industrial-Level Production Feasibility

The feasibility of industrial-level production of biofertilizers is a significant facilitator, indicating that scaling up from lab production to large-scale manufacturing is achievable.

"Industrial-level production is possible. While production in the lab is done in large flasks, large-scale production is possible with snaked tubes that have access to a great deal of light." - Key informant 3 (Asian context)

Decentralized Lab Networks

Establishing a network of decentralized labs can enhance regulation, ensure quality control, and streamline distribution to entrepreneurs wishing to commercialize the labs' NSNF products.

CONCLUSION

There is untapped potential of underutilized non-symbiotic nitrogen-fixing microbes, especially in native ecosystems. These diverse microbes could promote plant growth and nutrient cycling, offering promising bio-based solutions.

Using indigenous microbial strains that are already adapted to local environments is key. Microbes from local soils are better suited for developing biofertilizers for those contexts.

A major challenge is the translation of research findings into commercial products. Quality control and regulatory support are needed to ensure biofertilizers perform effectively across different field conditions. Inconsistent field performance and low farmer awareness remain significant obstacles, even with promising NSNF products.

Pilot studies and field trials are necessary to validate the real-world benefits and scalability of non-symbiotic nitrogen-fixing microbes across diverse agricultural settings. These trials demonstrate the advantages of using these biofertilizers.

NEXT STEPS

Establish a Comprehensive Research and Development Program: A comprehensive R&D program focused on characterizing microbial strains in Sub-Saharan African ecosystems could accelerate development of effective NSNF products.

Pilot Studies and Field Trials: Investments in field trials and pilot studies would validate the efficacy of NSNF products outside of controlled settings. With field trials, products can be tested in diverse environmental conditions.

Education & Capacity Building: Educating farmers about the importance of long-term soil health is a key strategy for ensuring the use of NSNF biofertilizers. Capacity building of local laboratories, scientists, and researchers should be emphasized when developing products for Sub-Saharan African contexts.

Regulatory Support and Quality Control: Collaborating with local governments to establish regulatory frameworks will help ensure the quality and safety of NSNF biofertilizers, providing farmers with reliable products they can trust.

In summary, the use of NSNF biofertilizers can be advanced by a focus on native microbial strains, rigorous field trials, establishment of regulatory support, and capacity building. These steps could improve crop yields for small-holder farmers and contribute to sustainable agricultural development.

Covidence Study ID	
979	Berthrong ST, Yeager CM, Gallegos-Graves L, *et al.* Nitrogen Fertilization Has a Stronger Effect on Soil Nitrogen-Fixing Bacterial Communities than Elevated Atmospheric CO_2. *Appl Environ Microbiol* 2014; 80: 3103–12.
978	The role of blue-green algae in nitrogen fixation in rice-fields. *Proc R Soc Lond B* 1939; 127: 121–39.
977	Dhar DW, Prasanna R, Singh BV. Comparative Performance of Three Carrier Based Blue Green Algal Biofertilizers for Sustainable Rice Cultivation. *Journal of Sustainable Agriculture* 2007; 30: 41–50.
975	Kumar U, Panneerselvam P, Govindasamy V, *et al.* Long-term aromatic rice cultivation effect on frequency and diversity of diazotrophs in its rhizosphere. *Ecological Engineering* 2017; 101: 227–36.
972	Nayak S, Prasanna R, Pabby A, Dominic TK, Singh PK. Effect of urea, blue green algae and Azolla on nitrogen fixation and chlorophyll accumulation in soil under rice. *Biology and Fertility of Soils* 2004; 40: 67–72.
970	Prasanna R, Jaiswal P, Nayak S, Sood A, Kaushik BD. Cyanobacterial diversity in the rhizosphere of rice and its ecological significance. *Indian J Microbiol* 2009; 49: 89–97
964	Song X, Zhang J, Peng C, Li D. Replacing nitrogen fertilizer with nitrogen-fixing cyanobacteria reduced nitrogen leaching in red soil paddy fields. *Agriculture, Ecosystems & Environment* 2021; 312: 107320
963	Purwani J, Pratiwi E, Sipahutar IA, Husnain. The effect of different species of cyanobacteria on the rice yield and nitrogen use efficiency under different levels of nitrogen fertilizer on Alluvial West Java. *IOP Conf Ser: Earth Environ Sci* 2021; 648: 012196.
961	Zhang J, Song X, Wei H, Zhou W, Peng C, Li D. Effect of substituting nitrogen fertilizer with nitrogen-fixing cyanobacteria on yield in a double-rice cropping system in southern China. *J Appl Phycol* 2021; 33: 2221–32
957	Van Deynze A, Zamora P, Delaux P-M, *et al.* Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS Biol* 2018; 16: e2006352.
956	Bloch SE, Clark R, Gottlieb SS, *et al.* Biological nitrogen fixation in maize: optimizing nitrogenase expression in a root-associated diazotroph. *Journal of Experimental Botany* 2020; 71: 4591–603.
955	Solomon W, Mutum L, Rakszegi M, Janda T, Molnár Z. Harnessing the Synergy of the Cyanobacteria-Plant Growth Promoting Bacteria for Improved Maize (Zea mays) Growth and Soil Health. *Sustainability* 2023; 15: 16660.
954	A. A, Hemida M, Ohyam T. Nitrogen Fixing Cyanobacteria: Future Prospect. In: Ohyama T, ed. Advances in Biology and Ecology of Nitrogen Fixation. InTech, 2014. DOI:10.5772/56995.
841	Li X, Rui J, Xiong J, *et al.* Functional Potential of Soil Microbial Communities in the Maize Rhizosphere. *PLoS ONE* 2014; 9: e112609.
805	Jiménez-Ríos L, Torrado A, González-Pimentel JL, *et al.* Emerging nitrogen-fixing cyanobacteria for sustainable cotton cultivation. *Science of The Total Environment* 2024; 924: 171533.
761	Lu J, Yang F, Wang S, Ma H, Liang J, Chen Y. Co-existence of Rhizobia and Diverse Non-rhizobial Bacteria in the Rhizosphere and Nodules of Dalbergia odorifera Seedlings Inoculated with Bradyrhizobium elkanii, Rhizobium multihospitium–Like and Burkholderia pyrrocinia–Like Strains. *Front Microbiol* 2017; 8: 2255.
683	Sharma V, Prasanna R, Hossain F, *et al.* Priming maize seeds with cyanobacteria enhances seed vigour and plant growth in elite maize inbreds. *3 Biotech* 2020; 10: 154.
649	Marzouk SH, Tindwa HJ, Amuri NA, Semoka JM. An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. *Heliyon* 2023; 9: e13040.
624	Hu W, Wang X, Wang X, *et al.* Enhancement of nitrogen fixation and diazotrophs by long-term polychlorinated biphenyl contamination in paddy soil. *Journal of Hazardous Materials* 2023; 446: 130697.
533	Bidyarani N, Prasanna R, Babu S, Hossain F, Saxena AK. Enhancement of plant growth and yields in Chickpea (Cicer arietinum L.) through novel cyanobacterial and biofilmed inoculants. *Microbiological Research* 2016; 188–189: 97–105.
416	Sharma V, Prasanna R, Hossain F, *et al.* Cyanobacterial inoculation as resource conserving options for improving the soil nutrient availability and growth of maize genotypes. *Arch Microbiol* 2021; 203: 2393–409.
387	Priya H, Dhar DW, Singh R, *et al.* Co-cultivation Approach to Decipher the Influence of Nitrogen-Fixing Cyanobacterium on Growth and N Uptake in Rice Crop. *Curr Microbiol* 2022; 79: 53.
341	Karaushu EV, Lazebnaya IV, Kravzova TR, *et al.* Biochemical and Molecular Phylogenetic Study of Agriculturally Useful Association of a Nitrogen-Fixing Cyanobacterium and Nodule *Sinorhizobium* with *Medicago sativa* L. *BioMed Research International* 2015; 2015: 1–16.
109	Manantsoa FF, Rakotoarisoa MF, Chaintreuil C, *et al.* Occurrence and diversity of stem nodulation in Aeschynomene and Sesbania legumes from wetlands of Madagascar. *Sci Rep* 2024; 14: 5024.
52	Sadvakasova AK, Kossalbayev BD, Token AI, *et al.* Influence of Mo and Fe on Photosynthetic and Nitrogenase Activities of Nitrogen-Fixing Cyanobacteria under Nitrogen Starvation. *Cells* 2022; 11: 904
43	Rezasoltani S, Champagne P. An integrated approach for the phycoremediation of Pb(II) and the production of biofertilizer using nitrogen-fixing cyanobacteria. *Journal of Hazardous Materials* 2023; 445: 130448.
15	Álvarez C, Jiménez-Ríos L, Iniesta-Pallarés M, *et al.* Symbiosis between cyanobacteria and plants: from molecular studies to agronomic applications. *Journal of Experimental Botany* 2023; 74: 6145–57.

OTHER REFERENCES IN THE REPORT

1. Franzen D, Camberato, J, Nafziger E, *et al.* Performance of Selected Commercially Available Asymbiotic N-fixing Products in the North Central Region. 2023; published online April. https://www.ndsu.edu/fileadmin/snrs/Files/SF2080_Performance_of_Selected_N-fixing_Products.pdf (accessed March 5, 2024).

2. Nakei MD, Venkataramana PB, Ndakidemi PA. Assessment of the soil suitability for soybean growth and the prospect biofertilizers use in selected areas of Tanzania. *T* 2023; **3**: 0–0.

3. Sanchez PA, Swaminathan MS. Cutting World Hunger in Half. *Science* 2005; **307**: 357–9.

4. Lowder SK, Sánchez MV, Bertini R. Which farms feed the world and has farmland become more concentrated? *World Development* 2021; **142**: 105455.