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Biofertilizer Impacts on Soybean [*Glycine max (L.)]* Cultivation, Humid Tropics | Biological Nitrogen Fixation, Yield, Soil Health and Smart Agriculture Framework

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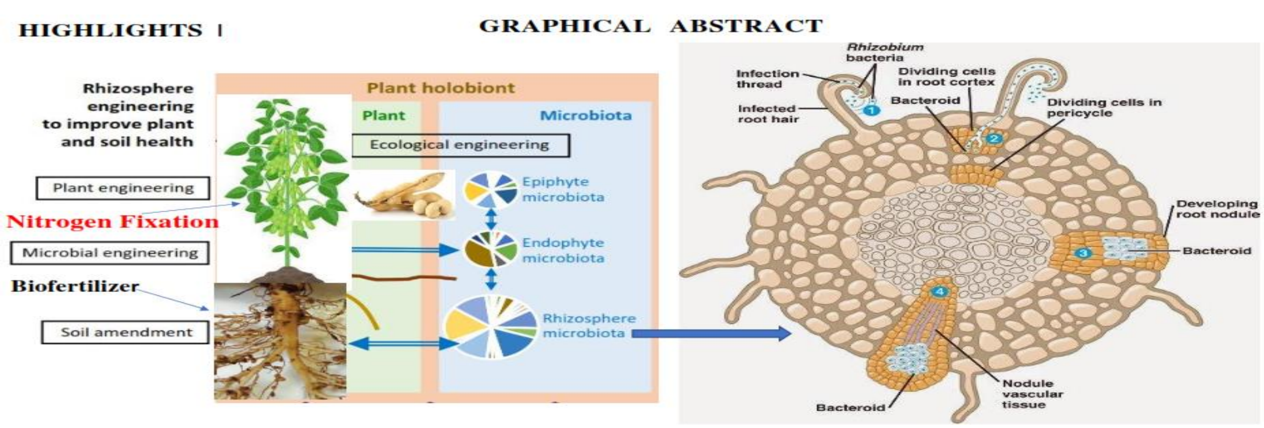

ABSTRACT | *Biological nitrogen fixation (BNF) soybean cultivar TGx 1440 -1E cultivation for grain yields and soil microbiome with different soil amendments in humid tropics in the late cropping season of 2010, Abeokuta, Nigeria, at Latitude 70 121 N and Longitude 30 251 E in randomized complete block design (RCBD) replicated three times. Treatments application includes: agro-waste recycled to biofertilizer in anaerobic biodigester with two biofertilizer formulations (GF1 and GF2), sunshine fertilizer (SF) and chemical fertilizer (NPK 20:10:10). Soybean vegetative growth parameters, nodulation, amount of nitrogen fixed, yield and yield components were determined at 8 weeks after planting (WAP). GF2 had significantly ($P < 0.05$) higher number of leaves. GF1 had significantly ($P < 0.05$) higher number of pods and seed weight/plant with lower biological nitrogen fixation, compared to other treatments. Soybean cultivars breeders should integrate biofertilizer into seed development programme, that bypass the naturalized soil rhizobia and nodulate only with highly effective inoculant strains under environmental stress, improved soil resilience for climate mitigation with rhizosphere-microbial interactions to manage soybean cyst nematode (SCN). Smart agriculture framework developed impacts on trans-disciplinary approach, soyabean cultivation nitrogen use efficiency (NUE), remote access to agriculture data in real-time, crop development, supply chain management, proftability and biofertilizer varietal characteristics.*

KEYWORDS | Soyabean, Biofertilizer, Biological nitogen fixation, Soil Health, Nodulation, Rhizobium, Sensors, Wicked Problems, Agriculture 5.0, Rhizosphere engineering, Microbiome.

INTRODUCTION

Nigeria is the largest soybeans non-genetically modified organisms (non-GMO) producers in Sub-Saharan Africa (SSA) with less than 500,000 metric tons, compared to 8,000-10,000 metric tons for maize and sorghum 4,000 - 5,000 metric tons cultivation. Soybean crop well-suited for Nigeria weather and ecology, disease and pest tolerant and low farm inputs in production with rotational crop (with corn, sorghum and cotton).The Nigeria Soybean yield 1.0 ton/ha compared to Argentine/Brazil (2.5-3.0 ton/ha and USA (4.5 tons/ha). Alguacil *et al.*,[14] reported animal waste when mixed with chemical fertilizer584 improves the soil microbes, aggregate stability, N, P, K, carbon sequestration and pH compared to urea. Animal waste (cattle and poultry manure) increased organic matter, Nitrogen, pH, microbial biomass and soil fauna in sandy soil. [486] Organic matter significantly impacts on the formation of soil aggregates, maintenance of soil structure, fertility and water holding capacity in soils ecosystem [611,304] and degraded soil of semi-arid region.[293] Nitrogen increases carbon sequestration rate of crop residues than un-amended soil.[409] Biofertilizer applied to agricultural soil can transform current carbon-neutral status of soils to a carbon sink.[93] The field research objectives are:

i. Agro-waste recycling to biofertilizer production using anaerobic biodigester that will simulate biological nitrogen fixation (BNF) in soyabean cultivation and improved soil microbiome;
ii. Soybean cultivar (TGx 1440–1E) cultivation with biofertilizer, chemical fertilizer (NPK 20:10:10) and sunshine fertilizer in humid tropics in the late cropping season of 2010, Abeokuta, Nigeria, at Latitude 70 121 N and Longitude 30 251 E in three replicates.
iii. The formulation of biofertilizer inoculants strains able to fix N_2 and non-rhizobial inoculants that increase root growth and improve rhizosphere uptake efficiency and reduce any abiotic or biotic constraints on crop growth and development.
iv. Develop a trans-disciplinary framework that transform agriculture 5.0 and integrate academic-industry -institutions collaboration for agriculture development, and;
v. Smart agriculture framework to capture soybean field trial data in real-time for improve crop production and management.

Principle of Root/Rhizosphere Management and Architecture |

The relationship between amount of fertilizer added and the crop yield called law of diminishing increments by Mitscherlich equation,[399] "decreasing increments law", where the successive nutrient supply results in decreasing increments of productivity. Achieving high nutrient use efficiency and high crop productivity has become a challenge with increased global demand for food, depletion of natural resources, and deterioration of environmental conditions.[99, 612,98] Plant roots can not only highly regulate morphological traits to adapt to soil environmental conditions, but also significantly modify rhizosphere processes through their physiological activities, particularly the exudation of organic acids, phosphatases, and some signalling substances, proton release, and redox changes.[237, 690] The root-induced rhizosphere processes not only determine mobilization and acquisition of soil nutrients but also the microbial dynamics, nutrient use efficiency by crops, and profoundly influence crop production and sustainability.[690] Application of biofertilizer in Soybean cultivation can manipulate the root growth and rhizosphere processes to provide an effective approach to improve nutrient use efficiency, biological nitrogen fixation and crop productivity simultaneously. The efficiency of root and rhizosphere processes can be enhanced with intensity of soil nutrient supply with slow release biofertilizer. However, overuse of fertilizers may lead to high concentrations of nutrients in the rhizosphere, resulting in inhibition of root

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growth and rhizosphere processes.[334, 392, 69] Maximizing the efficiency of the root/rhizosphere in nutrient

mobilization and acquisition (Figure 1)[353] by crop demands spatially and temporally at an optimal level of nutrient supply in the rhizosphere r requires synchronizing root-zone nutrient supply. The main strategies of root/rhizosphere management are: (1) manipulating root growth in terms of both morphological and physiological traits; (2) intensifying rhizosphere processes in terms of acidification and carboxylate exudation; and (3) synchronizing root-zone nutrient supply with crop demand by integrated soil–crop system management.[690, 106]

For decades globally, green agriculture revolution uses of insecticides, fungicides and pesticides to increase the productivity which has negative impacts of biodiversity with the demand for alternative soil amendment called biofertilizer (cultures of microorganisms packed in a carrier material). Biofertilizer contain live or latent cells of efficient strains of phosphate solubilizing, biocontrol microbes,[140,595] nitrogen fixing or cellulolytic microorganisms used for the application to seeds, soil or composting areas [197,71] which are helpful for the availability of nutrients that can be easily assimilated by plants and improving soil fertility by fixing atmospheric nitrogen and promote root growth by producing hormones, antimetabolites, soil mineralization and decomposition of nutrients.[311, 76]

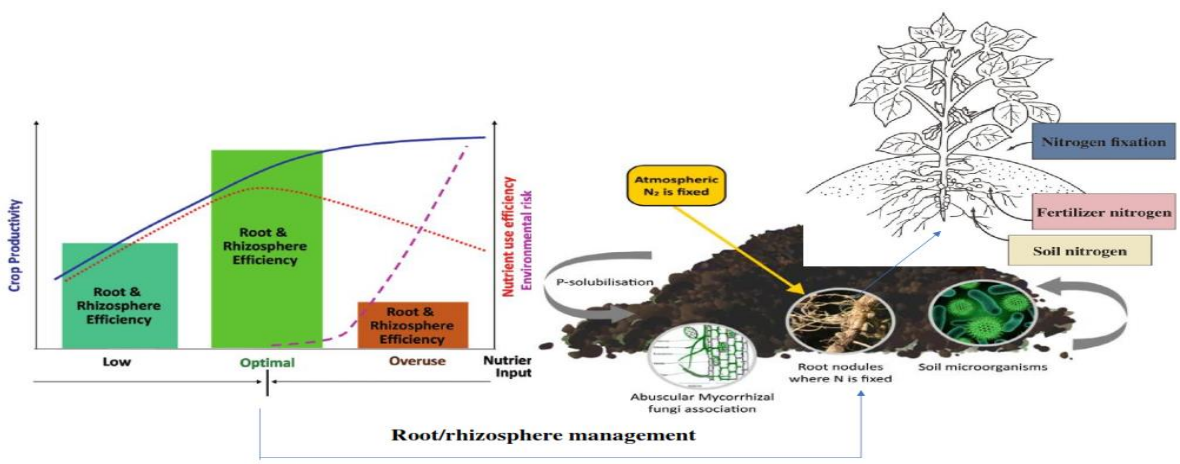

Figure 1 | The efficiency of the root/ rhizosphere management can be regulated to an optimum status by controlling nutrient input where there is a strong root system and efficient rhizosphere processes in increasing nutrient acquisition and crop production and adapted from Evert-Jan and Aniek.

Biofertilizer are cost-effective and can be used as a supplement to chemical fertilizers.Microorganisms like bacteria, fungi and blue-green algae [372] are used as biofertilizer. Biofertilizer have paramount significance in sustaining agricultural productivity and healthy environment [71] and are characterized into various categories like: nitrogen fixing biofertilizer, phosphate solubilizing biofertilizer, phosphate mobilizing, biofertilizer for micro-nutrients and plant prowth promoting biofertilizer. Rhizobium *spp*. is the nitrogen fixing bacteria formed in the roots of leguminous and some nonleguminous plants.[213] Plants show high plasticity in root growth and development in response to varied environmental conditions.[239. 354] In Figure 1, root size can be determined by soil nutrient concentrations whereas root distribution and proliferation are highly dependent on the localized supply of nutrients in the soil. It was reported that

more aerenchyma tissues in the cortex of the roots can also be an important trait that contributes to efficient Nitrogen (N) uptake with lower carbon input in root growth.[472 ,409] Thus, a deeper root with more aerenchyma tissues is important for efficient capture of soil resources. This root architecture may also be efficient in the uptake of deep water. Root response can be induced by nutrient supply intensity on the whole as well as spatiotemporal resource variation, especially availability of the water and nutrients.[132,409,44.] Thus, higher phosphorus availability was found in surface soil strata, a shallow root system with enhanced adventitious roots is relevantly important for crops to absorb phosphorus,[354] Figure 19.

Maximizing Rhizosphere Efficiency |
The rhizosphere efficiency can be enhanced through optimizing nutrient supply. [353] Rhizosphere processes reflect dynamic changes in rhizosphere biology and chemistry for the interactions between plants and soils and are bottlenecks controlling nutrient transformation, availability, and efficient use by plants (Figure 1).This approach can modify rhizosphere processes and efficiency by regulating root development and thus carboxylate exudation, proton release, and acid phosphatase activity in the interface between roots and the soil. Rhizosphere acidification can enhance P mobilization and acquisition from soil by plants.[237, 690]The form of N supply, to a great extent, controls the uptake ratio of cations and anions and thus influences root apoplastic pH and rhizosphere pH. Ammonium supply can induce release of protons from roots and thus cause rhizosphere acidification but nitrate supply can induce hydroxyl secretion by roots and thus cause rhizosphere alkalinisation. The success of an introduced inoculant also depends on the quality of the inoculant [505, 511] wherein critical for successful nodulation are the number of viable rhizobia per unit of inoculant and the number of introduced rhizobia that result in root infection (Figure 18). The mobility of rhizobia in soil is limited under real field conditions, inoculation methods must ensure that sufficient rhizobia are present around the seeds for successful nodulation.[193] Soybean demand for Nitrogen is determined by the yield potential of a crop in a given environment (Figure 2). If the N demand of soybean can be matched by the indigenous rhizobia population, inoculation with even efficient rhizobia strains may not show any improvement in yield. [661,591, 605 , 606] The highest probability of meeting the goals for agricultural systems occurs when science is applied to define a suite of practices from which farmers can select, using adaptive management (Figure 5). This will implies application of fertilizer best management practices (BMP) called 4R addressing the right fertilizer source, at the right rate, the right time, and in the right place [502] which provide the foundation for a science-based framework to achieve sustainable on-farm nutrient management practices. The definition of the normative “right” is provided by the principles of sustainable development: optimizing the sustainability performance of agriculture, using indicators selected by its stakeholders. Universal scientific principles of nutrient cycles, soil fertility, and plant nutrition manifest themselves in specific management practices that vary with climate, soils, access to technology, local economies, and culture.

Plant Growth Promotion (PGP) by Endophytic Bacteria |
After water, nitrogen is the major limiting compound for crop production. Direct PGP mediated by

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endophytes bacteria is mostly based on providing essential nutrients to plants and production and/or regulation of phytohormones.[679,188,366] Many plants can obtain nitrogen through a process

known as BNF (biological nitrogen fixation). Nitrogen fixation is regulated by the concentration of oxygen and the availability of nitrogen. BNF by legumes is based on a symbiosis with root-nodule nitrogen-fixing bacteria [251] while other agriculturally important plants such as maize, rice, sugar cane [547] and wheat can benefit from the association with diverse endophytic diazotrophs (Figure 3).The best studied endophytic diazotrophs include members of *Azoarcus*, *Burkholderia*, *Gluconobacter*, *Herbaspirillum* and *Klebsiella*.[265] The ability of endophytic diazotrophs to fix N_2 *in planta* (Figure 20) was demonstrated in several studies (monitoring the expression of nitrogenase [185] genes in nitrogen-fixing cells at the endophytic stage[155,509,673] and by isotope analysis.[547] Research reports affirmed that plants can get up to 70% of the required nitrogen through BNF mediated by endophytic diazotrophs.[26]

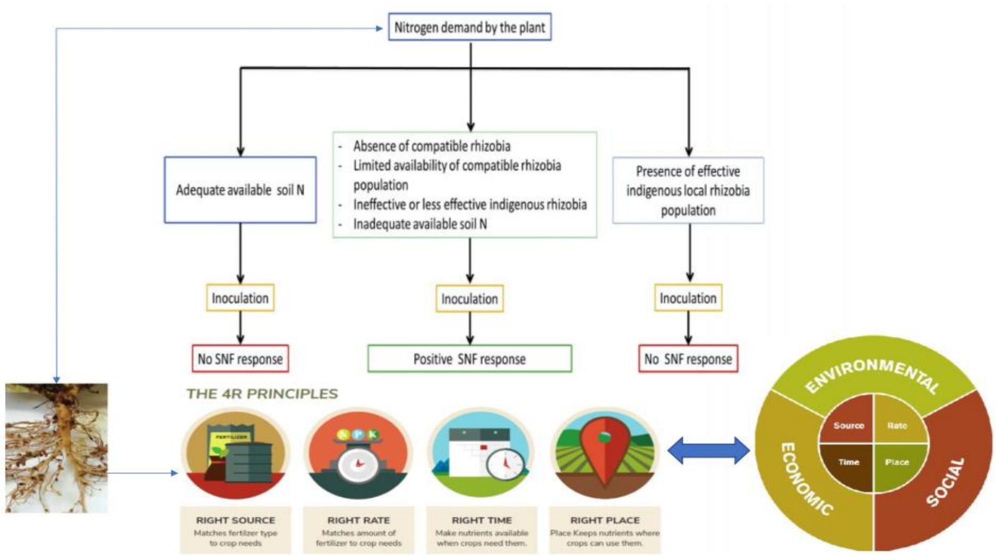

Figure 2 | 4R Robert [502] and Precision Agriculture for optimizing the symbiotic nitrogen fixation (SNF) response of legumes to inoculation with rhizobia and adapted from Raizada and Thilakarathna. [478 , 592]

The principal role of IAA (indole-3-acetic acid) in PGP was confirmed only for rhizobacteria, using mutational studies.[461,580] N starvation can also derepress the biosynthesis of the plant hormone IAA and was detected in the culture supernatant of *G. diazotrophicus*.[162, 59] This possibility is further supported by the observation that when Nitrogen was not limiting, both wild type *G. diazotrophicus* Pal5 and its *in vitro* production of IAA and its possible involvement in PGP has been reported for many other endophytic bacteria.[206, 514,269,366] Spaepen and Vanderleyden[581] reported microbial production of auxins and its role in the interaction with plants. Many IAA-producing endophytes possess ACC (1-aminocyclopropane-1-carboxylate)-deaminase activity which is involved in lowering the level of plant ethylene.[344] Elevated levels of ethylene caused by some stresses are known to inhibit root elongation and lateral root emergence.[258] Glick[200] report that, bacterial IAA activates ACC-synthase of plants resulting in the production of

ACC, the ethylene precursor. ACC-deaminase activity was described for plant growth-promoting endophytic strains of *Burkholderia* [592,188] *Herbaspirillum* [514] and *Pseudomonas*.[344] Other phytohormones produced by endophytic bacteria include ABA (abscisic acid), [110] cytokinins[548] and GBs (gibberellins).[548]

Inoculants Production |

Production process of the inoculum is key to a final high-quality product because of the direct relationship between the population density of mother culture and the quality of the final products and the inoculum is formed of one strain. The complex relationships among the microorganisms interacting in the rhizosphere has fostered the study on inocula composed of more than one microorganism which have showed promising results both in legumes and non-legume plants.[353,58,587] In legumes, comprised the co-inoculation of rhizobia with *arbuscular mycorrhizal* fungi (AMF) [273, 653] dual inoculation of rhizobium and phosphate solubilizing bacteria [8] an inoculum formed of rhizobium together with a plant growth promoting rhizobacteria (PGPR) and a phosphorous solubilizing bacteria (PSB).

In non-legumes, nutrients uptake comparable to chemically fertilized plants have been reported with dual inoculations involving AMF and free-living N-fixing bacteria [3, 53, 338, 653] also under dry conditions.[34] Better nutrient efficacy was reported also in the case of PSB and KSB mixture inocula.[221, 634] In the formulation of inoculant consortium for biofertilizer production, research reports affirmed that certain bacterial groups appear to associate more frequently with AM fungi or to be inhibited by them by several mechanisms including the fungal release of stimulatory or inhibitory compounds [272, 174, 373, 614, 648] which could result in a higher or limited colonization of the roots, respectively (Figure 22). Granular inoculant (gateway biofertilizer [446]) was formulated for extreme stress environment and soil conditions (Plate 1). Granular inoculant contains plant growth promoting microorganisms (PGPM) consortium with different mechanisms of action of the various microorganisms present, sometimes overlapping also plantprotection mechanisms [633, 634] suitable for Soybean cultivation and cereal. Liquid inoculants, [438] though easier to distribute, have shorter shelf life.[58, 586] Legume biofertilizer containing elicitors of nodulation are already marketed [359,360] but other rhizobial metabolites related to the nodulation process (nod factors) were successful in enhancing the performance of N-fixing bacteria inoculants on soybean (Figure 18).[407, 652]

Soybean Production Challenge |

Soybean is widely grown in the middle belt or the savannah zone of Nigeria [437] but, its production has presently expanded beyond the traditional production areas of the middle belt to cover other Northern and Southern parts of the Nigeria that were otherwise considered unsuitable or marginal for soybean production.[17] The highlighted constraints of Soybean cropping system in Southern parts of the Nigeria are soil related constraints such as low pH, nutrient deficiencies (phosphorus, potassium, molybdenum and sulphur) [333] and toxic levels of some metals like aluminum, iron, and manganese [516] have the potentials to reduce the yield of the crop[566, 254] will requires biofertilizer with board spectrum formulation that could manage the highlighted constraints like gateway biofertilizer (Table 6). Soybean yield has been characterized with high instability within and between species at different sites and among seasons [476, 402, 357, 19] and the use of stable genotypes for high seed yield is an important objective for sustainable soybean production.[13, 688] that was why the soybean cultivar (TGx 1440–1E) was selected for the filed cultivation. Cultivation of Soybean require the knowledge of the genetic variability and the adequate evaluation of breeding materials under different environments with table genotypes are less dependent

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upon good environments to perform well, and this makes their yield more predictable. [113, 119, 129, 46] over a wide range of climatic conditions. Broad adaptation provides stability against the variability inherent in an ecosystem of crop development like soybean with the soil amendment of biofertilizer application impacts of endophytes bacteria. Research reports affirm specific adaptations may provide a significant yield advantage in particular environments.[642] Denis and Gower [138] suggested that plant breeders should

consider genotype environment interaction (GEI) for crop yield. Multi-environment testing makes it possible to identify cultivars that perform consistently from year to year (temporal variability) and those that perform consistently from location to location (spatial variability). Temporal stability is desired by and beneficial to growers, whereas spatial stability is beneficial to seed companies and breeders. [284]

RHIZOSPHERE ENGINEERING

The rhizosphere (which means root, Greek word[236, 225]) is the part of soil which is most affected by the mutual relationship of plant and microbial communities and differentiated from bulk soil,[220] improve the plant nutrients availability and biological activity through plant driven carbon as rhizodeposits.[322] The extent of rhizosphere in the soil can be depended upon the root system and microbial community and climate impacts called “rhizospheric effect R/E”, “rhizospheric effect R/E” can 2 to 20 showed normal range.[657] The plant release compounds and microbial activity are a help to determine the spread of rhizospheric influence in the soil. Rhizosphere can be categories into different layers which spread from plant root to bulk soil.[382] The rhizosphere can be influenced by the root development and root release compounds into the soil (Figure 1).

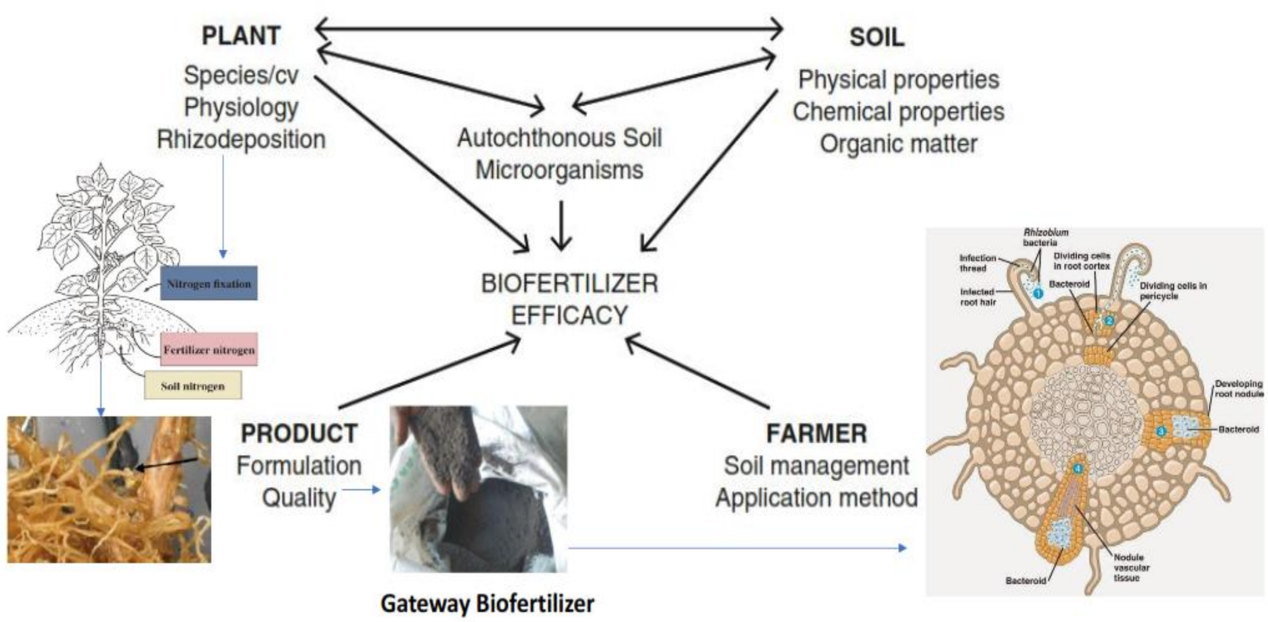

Figure 3 | Determinants of legume crop nutrition, growth and yield and efficacy of biofertilizer, adapted from Malusà *et al.*,[270]

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Rhizodeposits and microbial influences, the rhizosphere divided into three layers that spread from root out layer to adjacent soil.[382] The endorhizosphere are the most intense rhizospheric activity zone at the outer layer of the plant root surface; the rhizoplane is the intermediate zone or actual root-soil interface zone which inner layer directly surrounded to the root including the root epidermis and mucilage and out layer to ectorhizosphere and the ectorhizosphere which is outer most layer of rhizosphere up to bulk soil (Figure 4). The application biofertilizer in soils cultivation seem to be the easiest way of engineering

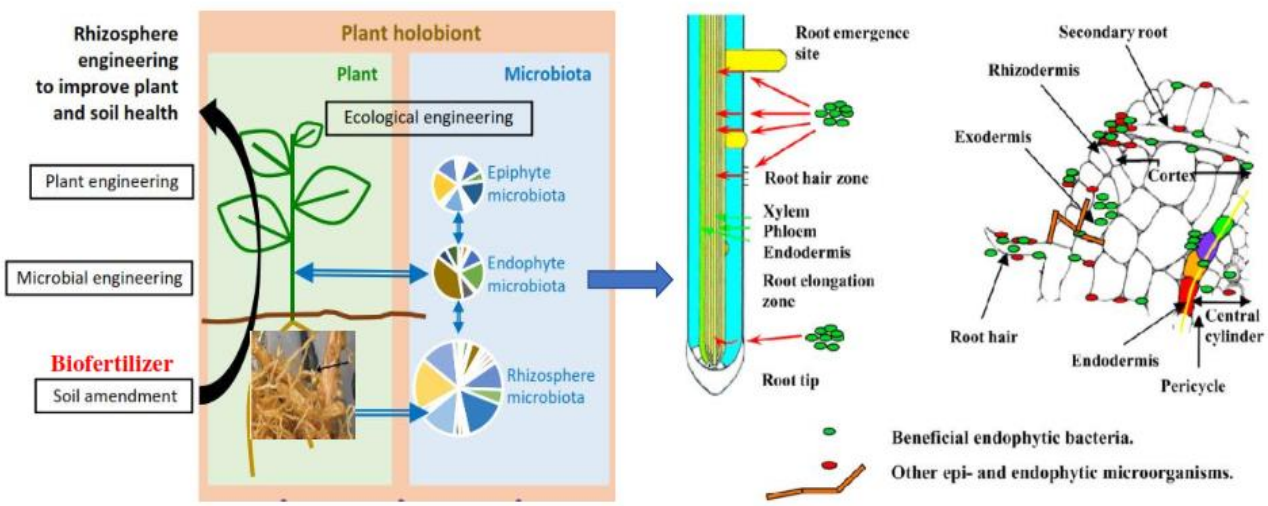

Figure 4 |The plant colonization by endophytic bacteria plays crucial role in the rhizosphere development and adapted from Reinhold-Hurek and Hurek [492] and Compant.[112]

rhizosphere. [139] A vast array of soil amendments is employed for upsurging the plant productivity which also proves to be an important tool for shaping the rhizospheric microbiome (Figure 26). Biofertilizer soil amendment is employed for getting a biased rhizosphere- rhizosphere engineering (Figure 4).

Plants Impact | Rhizosphere Development

In the rhizosphere, plant root plays most active role in designing the soil and rhizospheric environment and plant community assimilates the photosynthates and shifted them toward the root and various plant parts which can be further use for plant physiological and metabolic requirements.[115, 219,322] Rhizosphere community (Figure 26) help to design the plant root system and releasing various low and high molecular weight carbon compounds which are the source of food for microbial communities which influences the rhizosphere biology and signalling. [274] The resultant impacts of the application of biofertilizer to soybean cultivation is to engineer the rhizospheric functions for microbial colonization within root and rhizosphere, properties and amount of root released compounds, plant and microbial interaction, signalling and plant resistance factors [220] and releasing of the various carbon compounds[321] known as rhizodeposits which having various forms of organic substances exudates from the plant root.[274 ,466] In Figure 4, plant colonization by endophytic bacteria plays crucial role in the rhizosphere development and adapted from Reinhold-Hurek and Hurek[492] and Compant.[112] The rhizospheric zone can accelerate the microbial activity in root zone with more exudation of organic compounds from plant communities with the response of various factors as plants, soil and climatic factors.[322] All the rhizospheric biota, their activity, and various rhizospheric processes are affected by the plant root system and their amount of carbon exudates developed within their community.

Microbes Impacts | Rhizosphere Development

Soil more diverse in its biological habitat interacts with plant. Soil is consisted of millions of bacteria, widespread fungal hyphae, number of nematodes, protozoans, earthworms and other arthropods. All the rhizospheric community has oligotrophic in nature so it occurs near the root surface where carbon found in abundance and influences the plant nutrients dynamics through root and microbial activity through

secretion of more variable compounds into the rhizosphere [627]. [465, 322] Microbes release some carbon compounds used by the plant as a nutrient source, biocontrol agent [595] and signaling compounds for soil biotic community. The extent of release of these organic substances can determine the rhizospheric volume because more availability of exudates can create a more diverse and wider rhizospheric activity zone (Figure 3) with biofertilizer is applied during soybean cultivation. The rhizospheric microbial community benefited the soil ecosystem by serving functions of decomposing of organic matter, nutrients availability through solubilization and mobilization, root pests' control and rhizospheric signaling.[274,465] Various pathogenic microbes, denitrifying bacteria, protozoan, and nematodes are deleterious to rhizospheric processes during Soybean cultivation (Plate 6). The rhizospheric microbial community functions and structure have been influenced by soil types and host plant and soil environment conditions anchored by rhizodeposits.[595,218] Soil and seedling inoculation with the biotic community has positively influenced crop performance such as legumes inoculation with *rhizobia species* gave an opportunity to reduce plant external nitrogen demand due to nitrogen fixation [409, 631,100] and mostly vary with soil type, plant type and environmental conditions.[302] Cultivation Soybean with biofertilizer with reduce the agricultural pests. The gateway biofertilizer contents biocontrol agents (*Trichoderma, Pseudomonas*[595] and *Bacillus*) which induce the plant systemic resistance against the pathogenic attack [467] and affirmed by Atungwu.[38] Ryan *et al.,*[518] reviewed that the biotic community help to the production of certain types of the stress hormone, enzymes and another antibiotic which help to plant withstand under various stress conditions. Soil erosion accelerated by unsustainable agricultural activities can break down the soil structure which that negatively coincided with rhizospheric development.[270]

The temperature above and below the optimum temperature can alter the behaviour of plant root exudation and microbial activity.[169] The change in soil water holding capacity might be cause for alteration of soil biology, plant root development, physiology of exudation, microbial mobilization and activity.[220] Soil pH and heavy metal influence the plant and microbial physiology through soil acidification and redox reactions which can be altering the rhizospheric processes.[479] Biofertilizer improvement of the soil microbiota can help to improve plant productivity and provide environment safety (Table 6) with the resultant impacts to change soil pH, increase the nutrients availability to plant especially N, P, Ca, Mg, Fe and Zn.[382] The healthy soil biology can also be improved through supply of organic residues, crop residue management, apply compost/ manure, reduced tillage, minimum compaction, minimum use of pesticides along with growing cover crop or rotate the crop or intercrop for synergistic rhizosphere shaping.[332, 700 ,469, 67]

Rhizospheric Biota Management Holobiont Approach |

Soybean research breeding community should focus on improving the rhizospheric biodiversity with targeted functioning for crop plants [408] since plants and soil biota can shape the rhizosphere in

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collaborations [83] fulfilling the requirements of agricultural sustainability.[101] For example, root exudation and carbon allocation into rhizosphere have the source of energy for root symbionts.[643] Biofertilizer application will enhance carbon excreting crops in the rhizospheric biota and their activity with specified plant microbiome providing an opportunity for altering plant features, suppression of diseases [385] and plant flowering time.[454] For example, a *Bacillus species* genetically altered for nitrogen fixation mechanism for production of higher concentrations of plant hormones.[298] A combined three-strain consortium such as *Bacillus spp., Pseudomonas, Rhizobium* or *Bradyrhizobium* are improved nitrogen fixers could provide great opportunity of a diverse and complex natural rhizospheric biological functioning.[6] The reduction in denitrification and Nitrogen losses from the soil through decreasing microbial activity by plants can improve the nutrient use efficiency (NUE).[96,568] The integrated development of plant and their rhizospheric microbiome (Figure 2) can be an important step toward rhizospheric exploitation for better plant nutrient use efficiency.[52] Water use efficiency (WUE) and root nutrient uptake will increase the crop yields in the changing climate.[6] Colonization of the plant's interior by bacteria generally starts with their establishment in the rhizosphere extensively reviewed by Lugtenberg *et al.* [350] and Lugtenberg and Kamilova.[351] A number of mutational studies showed that attachment of bacterial cells to the root is a crucial step for subsequent endophytic establishment in the root surfaces [147] maize depends on LPS (liposaccharide).[47] A similar study showed that EPS (exopolysaccharide) is necessary for rhizoplane and endosphere colonization of rice plants by *Gluconacetobacter diazotrophicus.*[388,493]

Nitrogen Fixation

Nitrogen (N) constitutes about 2% of the total dry matter of a plant and is essentially required for plant growth, the synthesis of nucleic acid, proteins, and enzymes.[50] N deficiency leads to reduced growth, yellowing of leaves, and reduced branching in legumes. The dinitrogen gas (N_2) that represents about 80% of the atmosphere is not accessible to plants. Plants can only take up soil-available N in the form of ammonia and nitrates through their roots (Figure 18). The ammonium form is directly assimilated into amino acids and stimulates root branching to increase the surface for the uptake of nutrients as well as results in higher amino acid, chlorophyll contents, sugar, starch and the nitrate has to be converted into ammonium before it can be used.[62] Nitrate improves the uptake of more nutrients through lateral root elongation and has a more direct effect on different signalling pathways.[429] The process in which inert N_2 gas is converted to a metabolically tractable form in the soil is called nitrogen fixation (Figure 18). The manufacturing of synthetic N fertilizer through the industrial process is an expensive process as it needs six times more energy than required to produce either phosphorus (P) or potassium (K) fertilizers.[79] Crop yield has been significantly decreased due to the poverty of farmers who are unable to apply costly synthetic fertilizer demands for crops cultivation. Biological nitrogen fixation (BNF), the process in which elemental nitrogen is converted to ammonia by bacteria is an alternative source of N for plants (Figure 22). These nitrogen-fixing bacteria are ubiquitous in nature and function under different environmental conditions.

The input of nitrogen into soil through BNF ranges from 0 to 60 kg /ha year [491] with an estimated contribution of 175 million metric tons annually covering 70% of all annual fixed nitrogen on the Earth.[342]There are generally two categories of nitrogen-fixing microorganisms; (a) symbiotic and (b) non-symbiotic bacteria. The most common symbiotic N_2 fixing bacteria able to infect legumes include

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Rhizobium, *Bradyrhizobium*, *Ensifer*, *Azorhizobium*, and *Mesorhizobium*, etc. while *Frankia* nodulates non-leguminous trees and shrubs. These rhizobia infect the root hair, stimulate root hair curling, and leads to the formation of infection thread (Figure 4). The bacterial cells enter the plant cells through infection threads (Figure 22) and result in the formation of a nodule wherein the rhizobia reside and fix nitrogen for plants.[23] The non-symbiotic diazotrophic bacteria include *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Enterobacter*, *Mitsuaria*, *Pseudomonas*, etc. which fix atmospheric nitrogen in the free-living form.[213] The nitrogen-fixing bacteria improve the soil NH^{+} concentrations, rhizobacterial population levels, soil nitrogenase activity [185] as well as the growth and N uptake in plants.[379]

Table 1. Comparative analysis of microorganisms as single inoculant and efficiency (%) when co-inoculated in the soil in absence or presence of biofertilizer in stimulating soil fertility and structural parameters.

No.	Microorganisms	N	P	K	OM	AF/GP	AS	References
	Single inoculant							
1	Bacillus megaterium	√	√	√	√	√	√	Ortiz *et al*., 442
	Bacillus thuringiensis						√	ARATI Biopesticide ® 26
	Pseudomonas Putida						√	Patten and Glick 461
2	Bacillus megaterium	√	√	√				
	Arbuscular mycorrhizal fungi							Ashrafi *et al.*, 36
3	Bacillus megaterium	√	√	√	√	√	√	Mengual *et al.*, 390
	Enterobacter spp						√	Sheng and He, 552
4	Rhizophagus irregularis					√	√	Leifheit *et al.*, 327
	Natural soil microbes	√	√	√	√	√	√	Soni and Sharma, 576
5	Piriformospora indica	√	√					Kumar *et al*., 310
	Pseudomonas R81						√	Patten and Glick, 461
	Co-inoculant							
1	Bacillus megaterium + AM fungi + compost	1	-3	2				Wang *et al*., 654 Bai *et a* l., 44
2	Azospirillum brasilense + Pantoea dispersa + organic olive residue	13	-29	67	8			Mengual *et a* l.,381
3	Ps Putida + AM Fungi (Rhizophagus intraradices)		12	248				Ortiz *et al*., 442
4	Bacillus megaterium + AM fungi	7	-42	16				Zheng *et al.*, 697
5	Azospirillum brasilense + Pantoea dispersa	8	133	84	1			Mengual *et al*, 391
6	Rhizophagus irregularis + natural soil microbes						1	Leifheit *et al.*, 327
7	Piriformospora indica + Pseudomonas R81	21	29	12				Zhang and Smith, 689
8	OTAI AG and OBD-Plus (+)							Otaiku *et al.*, 447, 448; Otaiku *et al*, 449
	Single/co-inoculant + Organic fertilizer							
1	Bacillus megaterium + AM fungi + compost	1	−3	2				Wang, 650
2	Azospirillum brasilense + Pantoea dispersa + organic olive residue	13	−29	67	8			Mengual *et al.*, 391
3	Bacillus megaterium + sugar beet residue	13	25	14	−6	−14		Walpola and Arunakumara, 647
4	Bacillus thuringiensis + sugar beet residue	18	42	−21	0	42		Mengual *et al*., 390
5	Enterobacters + sugar beet residue	19	24	−3	−6	61		Mengual *et al.*, 390

N.B |
N = Nitrogen, P = phosphorous, K = potassium, OM = organic matter, AF = aggregate formation,
GP = glomalin protein, AS = aggregate stability. N/A Positive effect of inoculant.

Similarly, the application of biofertilizer produced from rhizobia *Bradyrhizobium* strains) and free-living PGPR (*Streptomyces griseoflavus*) improved the growth and yield of several plants such as cowpea, and soybean.[242]BNF is carried out by a nitrogenase complex that consists of two components (i) dinitrogenase reductase (an iron protein) which provides high reducing power electrons and (ii) dinitrogenase which uses the electrons to reduce nitrogen to ammonia and has a metal cofactor. [185]There are three different forms of nitrogenase classified based on metal cofactor. (a) Mo-nitrogenase whose cofactor contains molybdenum, (b) V-nitrogenase which contains a prosthetic group with vanadium, and (c) Fe-nitrogenase contains only iron. Symbiotic as well as free-living diazotrophs use *nif* genes for the fixation which include structural genes that are involved in the biosynthesis of iron-molybdenum cofactor, iron protein activation, donation of electrons, and regulatory genes required for the functioning of the enzyme.[185] The

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nif genes are found in the form of 20-24 kb cluster with seven operons that encode 20 different proteins in diazotrophs.[199]

The amino acid sequence of *nifH* is highly conserved and generally used to study the evolution of nitrogen-fixing bacteria and widely used to analyze the diversity of diazotrophs in the soil. [184] Investigation of diazotrophs community structure using metagenomics based on nitrogenase sequences identified a key group of diazotrophs including *Anaeromyxobacter, Azoarcus, Bradyrhizobium, Frankia, Geobacter, Nostoc*, and *Pelobacter* based on *nifH* phylogeny[421] can be evaluated using sequence analysis of the marker gene *nifH.*[398] Besides the ability of PGPR to fix N_2 to ammonia, they also have a great impact on nitrogen nutrition of plant by increasing the uptake of nitrate (NO_3) [62] and by stimulating NO_3^- transport systems

or indirectly as a consequence of stimulated lateral root development (Figure13). It has been reported that two putative nitrate transporter genes, that is., NRT2.5 and NRT2.6 appeared to be strongly upregulated in response to the inoculation of *Phyllobacterium brassicacearum* in *Arabidopsis thaliana.*[291] Plants can produce nitrification-inhibiting compounds which are known as biological nitrification inhibitors (BNIs) and significant increase in plant biomass, chlorophyll content, and nutrient uptake.[87,62]The exposure of pasture grasses, sorghum, and wheat to more NH^+ showed an increase in the synthesis and release of BNIs. Application of BNI promoted the root branching along with increased nutrient uptake providing a dual strategy to enhance fertilizer efficiencies.[339]

Microbial Inocula |

Microbial inocula are one of the valuable bio-resources that could be helpful in restoring degraded lands and the significant reasons why broad spectrum inoculants are inserted into the agrowastes biodegraded in biodigester for the production of the biofertilizer applied to the soybean cultivation. The reasons for lower response than co-inoculation could be that single microbial inoculum is not likely to be active as it faces competition of resources from indigenous microorganisms in order to survive in soil environment. The mixture of bacterial or fungal inocula may create synergistic effects while organic manures can meet their nutrient demands to make them successful under field conditions (Table 1). These associative interactions could be successful and may play a crucial role in restoring the productivity of degraded soils with the lacking evidence to support these hypotheses (Figure 14). [563] Singh [562] proposed that successful restoration of degraded soil using microbes required a combined knowledge on microbiology, ecology, biochemical mechanisms and field engineering (Figure 3). Research reports affirmed the use combination of bacteria or fungi inocula with organic amendments to reinstate parameters of degraded soils. [383, 390, 391, 698] Leifheit *et al.*[327] used fungal inoculum with organic residues to increase soil aggregation and their stability in pot experiment while Mengual *et al*., [390, 391] found increase in soil P availability, total N and other microbiological and biochemical parameters with co-application of bacterial inocula (Figure 27). Hydrolytic enzymes released by soil inoculants are main drivers of carbon, N and P cycling hence they play a key role in hastening the nutrients cycling in soils for plant growth.[84, 85]

Cyanobacteria fix atmosphere N_2 in degraded soils and release extracellular polysaccharides, which are metabolized by the associated soil microorganisms influence soil fertility, decrease soil pathogens and therefore improve crop growth.[562] Biological N_2 fixing bacteria encourage the growth and persistence of other soil microbial groups in the rhizosphere by providing Nitrogen and bacteria exude extracellular polysaccharides that promote soil aggregations.[135,546] Bacterial strains mobilize the fixed or unavailable P, K and Fe in the soil including phyto-hormones (Auxin/Indole Acetic Acid) which improve plant defense against various pathogens**.**[350, 205] These inocula also produce aminocyclopropane-1-carboxylate deaminase that promotes the root elongation (Figure 14), shoot growth, and enhances rhizobial nodulation as well as N, P and K uptake in various crops.[199,205] Plant growth promoting bacteria (PGPB) induce systemic resistance and produce antifungal metabolites (HCN, phenazines, pyrrolnitrin,2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin) and also biocontrol agent in various diseases and environmental stresses.[73, 205] The formulation of gateway biofertilizer (Table 2) contains ectomycorrhizal and AM fungi, which increase the soil nutrient and water transport through soil exploration by their hyphal network/pipelines and production of organic acids to mobilize the fixed nutrients.[22, 54,403, 86] AM Fungi can mobilize N, P, K, Fe and other nutrients in the soil and transfer these nutrients to the host plants [570] through translocation process by hyphal network. AM fungi can reduce N and P losses [35] through leaching and N_2O emission [68] and enhanced nutrient interception of AM fungi rooting systems. Due to these activities, microbial inocula drive nutrient cycling and at the same time also determined whether these nutrients are made available to plants. By doing so, these microbial inocula can achieve satisfactory results in the restoration of degraded soil. Seneviratne *et al*. [545] affirms that application of biofilm based fertilizers developed from N_2 fixer bacteria increased N_2 fixation and soil organic carbon within few months in tropics which leads to sustainability of agro-ecosystem and environment.

THEORETICAL FRAMEWORK - WICKED PROBLEM

What is Wicked Problems? |

Wicked problem are multidimensional challenges that are difficult to resolve due to incomplete or contradictory information, differing views on the nature of the problem, or complex interactions with other issues like soil amendments, climate change, agriculture etc as a ‘wicked problem. What makes a problem a wicked problem are: significant conflict over the values at stake in and the very definition of the problem at hand, and the absence of any institution, structure or process that provides a natural social or political location in which the problem can be nominated for attention, sized up in a process of deliberation and design, and used as the platform for directing coordinated action across many different independent organizations. The notion of wicked problems is an approach to understanding the dynamics of a major proposed change with multiple and conflicting inputs and multiple possible outcomes, all of which play over time against, or occasionally with, each other.[445] Wicked problems occur at the interface of human/environmental interaction and are characterized by the fact that solutions create a ‘plethora of new problems,[273 ,531] and the failure to reach an agreement on the desired outcome further exacerbates the original wicked problem, therefore transforming it into a ‘super-wicked’ problem.[325 329, 330] The values of any problems, are in the continuum and spatial to improve things on as many dimensions as possible. Wicked problem has two traits, uncertainty and complexity, all encapsulated in the typology of agriculture.[445] Scholars on frameworks (Otaiku [444] ; Folke[178]; Walker and Salt,[644]) argue that, change does not necessarily occur in a linear fashion. There are discontinuities and tipping points. Crises can promote innovation in complex systems. Rather than just sustaining what is, it is important to develop the capacity

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to bounce back after adversity (resilience) and to adapt to change (soil resilience impacts of biofertilizer application on soybean cultivation) and the co-evolutionary framework for managing disease-suppressive soils.[300] Adaptation and resilience are undoubtedly needed given the extent of the impact of human practices in agriculture. The participation of local populations is necessary for sustainability changes to occur, and this can be done more effectively from the bottom up than imposing changes decided at the top of the power structure (stakeholders). Values are in the continuum for any defined problems and thrives in chaos and methodologies across many different human activities (framework) encapsulate with Figure 5.

Spatial PolySingularity | Metaphor

Spatial polysingularity theory is post-modernism impacts of human mind creation with spatial objectivation beyond intended nor foresaw consequences in cyberspace-time continuum or non-linear dynamics framework for problem solving (Figure 1). Wicked problems solution should be 'adaptive management' [273] that involves feedback loops defined by the problems contexts -'specific situation, that it addresses', rather than the 'disciplines' involved incorporating knowledge from those who move knowledge to action. It is integrated with collaboration for multidisciplinary decision value creation framework [273] construct that encapsulate drawbacks of "wickedness theory" affirmed by Termeer *et al.,*[603]

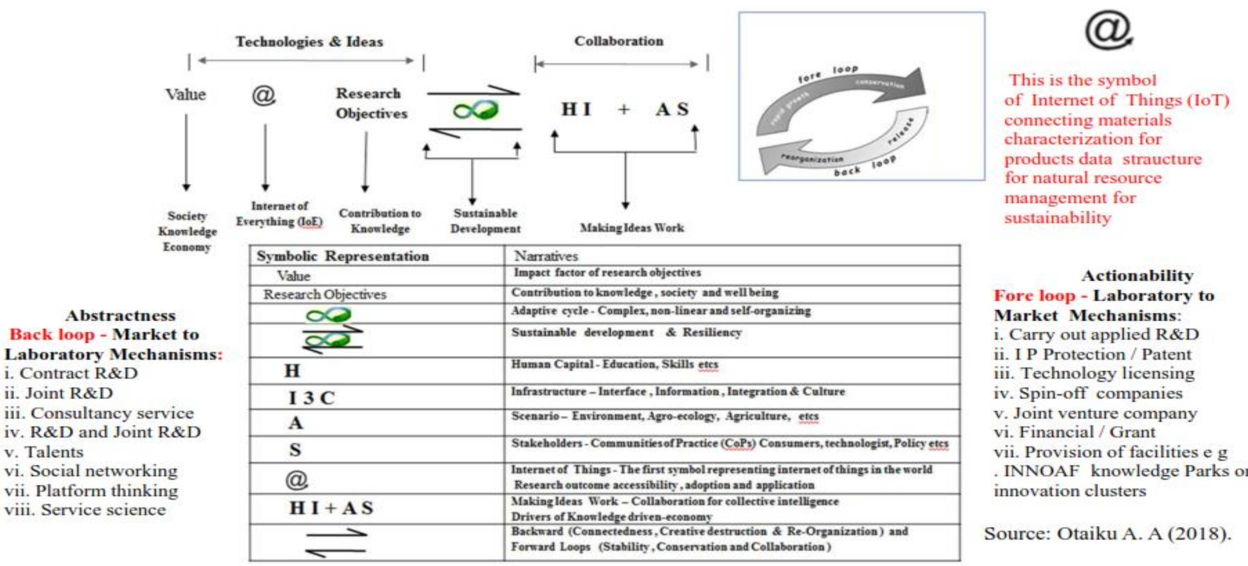

Figure 5 | The framework [444] "Where = Technologies and Ideas" and "how = Collaboration" called Spatial polysingularity, the construct provide a means for integration of descriptive, narrative elements, qualitative and quantitative information object of research via internrt of things (IoTs) powered by artificial intelligence.[662]

What Is a Metaphor?

A metaphor is defined as a word or phrase applied to an object or concept that it does not literally denote in order to make a comparison with the other object or concept under consideration.[443] Metaphors are especially useful in understanding concepts in social science and theory development. [245] Scholars and practitioners rely on metaphors to gain a deeper understanding of various domains.[406] Metaphors can be used to perform a variety of analyses [459] as they can facilitate and further our understanding [111] trigger new avenues for analysis, and surface new relationships.

Research Wicked Problems Construct | Spatial Polysingularity

Ahlfors *et al.*,[7] asserted that, instruction should be a process of "extracting the appropriate concept from a concrete situation. A more systemic and holistic approach should replace the reductionist, disciplinary worldview of academic research [184] into the trans-disciplinary research approach oriented to solve wicked problems, comprising phases of problem identification, structuring, investigation, and bringing results to fruition. [216] Many of tomorrow's breakthroughs will occur at the intersections of diverse disciplines as

accentuated by the 'spatial polysingularity' framework define as 'things only make sense in context, determined by its fundamental parts.[273] Spatial polysingularity is a recursive function of divide and conquer construct, determined by its simplest inputs (retrospective thinking). A function is a process that turns an input into an output so that, we can construct outputs (value). Spatial polysingularity philosophy analyses the problem solved by the sub-problems of various scales using holism and reductionism. Holism - The whole is greater than the sum of its parts; [503] reductionism - a whole can be understood completely if [we] understand its parts, and the nature of their sum.[503] Recursion is the idea that the behaviour of something is determined by its fundamental parts. [470,331,543,574] Spatial polysingularity application to a problem must have three distinct properties:

i. It must be possible to decompose the original problem into simpler instances of the problem.
ii. Once each of these simpler sub-problems has been solves, it must be possible to combine these solutions to produce a solution to the original problem.
iii. As the large problem is broken down into successively fewer complex ones, those sub-problems must eventually become so simple that they can be solved without further subdivision.

This is essentially the same as one of the fundamental principles of science: If we can predict how something behaves in all experimental setups, then we know what it is. So long as we believe that a function (value from the context) is what it does. The variable that controls the termination condition is called loop value (depends on the solution of the previous problem). Value, not result, drives competitiveness for innovation adaptive cycle of development (Figure 5). Value based management makes strategy happen by developing close links between strategy, operation, innovation with shareholder value as the principal measure of success or failure. Conceptual and theoretical framework are the cognitive tools needed to make assertions and supporting knowledge claims (Figure 5) and guide the profession toward action.[507] Researchers engaged in pragmatic problem solving and product development placed a higher premium on viability, workability, and impact, while contributions seeking algorithmic models of complex phenomena were associated with simplicity, predictive power, and parsimony.[301]

Scenarios can catalyse and guide appropriate action today for a sustainability transition. [597]
The collective activity of individuals and their modifications [651] to the environment are responsible for intelligence using the internet of things (IoTs conceptual framework to drive methodology and rigor in inquiries (Figure 7). A theoretical framework usually precedes the conceptual framework and includes a

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general representation of the investigated topic. Theoretical (Figure 5) and conceptual (Figure 6) frameworks are both part of the methodological toolbox for spatial polysingularity. Together, they are referred to as analytical framework. Analytical framework = theoretical + conceptual framework (secondary data review, analysis plan, methodology, tools).

The Spatial Polysingularity Construct
Analytics |

a. Value (research outcomes) of talented people (human capital) goes beyond predefined tasks: building brands, relationships, products, reputations, and other intangibles (high value).
b. Collaboration - It refers to the capacity of human communities to evolve towards higher order complexity and harmony, through such innovation mechanisms as differentiation and integration, competition and collaboration. The collective activities of individuals and their modifications to the environment are responsible for intelligence.
c. Technologies and ideas -Sustainability of research outcomes are vital to any methodologies of research or techniques in products development because all knowledge development is in the 'continuum', the first principle of wicked problems and what (Niggli *et al.*, [425] called concept of eco-functional intensification.
d. Spatial polysingularity thrives on chaos and methodology to solve the wicked problem, like soil amendments, soybean cultivation with ecological agriculture etc. Collaborate with the **Stakeholders** and the use various brainstorming techniques to build on each other's ideas (Figure 7). **Human Capital** [Brainstorm to reach to quantity and encourage wild ideas. In fact, it is important to move beyond current understanding of the problem views and solutions]. Use **Scenarios** [Prototypes to represent the problems in a catchy way which can fuel ideas generation]. Take feedback on each solution that have been built and continuously iterate it until you find an optimal solution. Because these problems are difficult to define and address, a visual explanation of the problem can also help to gain a wider view of the problem. To achieve a visual understanding, several methods and tools [**Infrastructure]** can be used for example develop stakeholder's map, customer-journey map, and rapid personas, application of internet, internet of things (IoTs), blockchain and artificial intelligence for agriculture. Thinking outside the box to expect different results, stakeholders need concrete reasons,, definitely a new paradigm able to explain, objectively, the complexity of the problem [163,177] suggest that organizations need both to learn (develop insights, knowledge, and associations of past actions) and to adapt (make incremental adjustments where required). Stakeholders resort to what Rittel and Webber [501] called 'good enough' measures that seem neither right nor wrong, and which do not necessarily result in any quantitative change to the problem (Figures 5 and 6).

Research Objective | Biowaste Recycle to Biofertilizer (Figure 5).
Scenario (A) | Soybean biofertilizer field application and cultivation.
Infrastructure (I) | Anaerobic Digester (design, fabrication and development)
Visit: YouTube https://www.youtube.com/watch?v=pG2ODAx3ICYBiowaste Recycle to Biofertilizer | Gateway fertilizer Plant, Abeokuta, Nigeria.

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Human Capital (H) | Ayodele A. Otaiku Ph.D Thesis (Researcher).
Stakeholders (S) | Farmers, Academic researchers, Beneficiaries and others.

The key ways in which scientists and ecological modellers can contribute to the search for solutions to wicked problems in collaboration with stakeholders are described. Modelling is identified as a tool that scientists can bring into the deliberative process to facilitate dialogue and evidence-based decision-making within a stakeholder forum (Figure 5). Any solution to a wicked problem like agriculture (soil amendment like biofertilizer) [445] will significantly affect a wide range of stakeholders, and cannot be separated from human ethics, values and social equity. Experience with participatory approaches that include stakeholder's shows that ecological metabolomics modelling [522] can lead to applied outcomes that may inform environmental management and policy, thus helping to solve wicked problems. The division of responsibility is essential, especially when addressing a problem like agriculture (farm inputs), with many choices, perspectives, needs, and alternatives to consider.

Research Objective | Biowaste Recycle to Biofertilizer (Figure 5).
Scenario (A) | Soybean biofertilizer field application and cultivation.
Infrastructure (I) | Anaerobic Digester (design, fabrication and development) visit:
You Tube https://www.youtube.com/watch?v=pG2ODAx3ICY
Biowaste Recycle to Biofertilizer | Gateway fertilizer Plant, Abeokuta, Nigeria.
Human Capital (H) | Ayodele A. Otaiku Ph.D thesis (Researcher).
Stakeholders (S) | Farmers, Academic researchers, Beneficiaries and others.

Human Capital |
Building on the potential of the human mind to learn and to expand its diversity of thought and imaginative processes, those looking to solve the cognitive processing shortcomings that perpetuate wicked problems, including, formulation of biofertilizer, climate change, also seek to create new models for learning, capable of breaking down the barriers and biases that prohibit freely shared understanding and develop frame for smart agriculture. Figure 5 framework, seek new models to use specific functionalities of human thought, like reflexivity, to improve learning capacity, in order to more fully 'reflect upon our actions, intentions, and motives' while, at the same time, noting the fallibility of the human mind in matters of egocentrism and related traits, in order to avoid error and enhance cognitive processing. Knowledge clusters have the advantage of stimulating and levelling social interaction and personal exchanges of information and innovation, which constitute another means of observation and learning.[471,450,330,167] At the organizational level, many organizations seek out knowledge clusters in order to develop their competitive advantage in today's business environment with the understanding that, at present, wealth accumulation most often comes from knowledge rather than from manufactured items [602,375] and affirmed by learning theorists, is that previous models based on linear rationality do not represent the optimal approach to a complex or even wicked problem.[343] We postulate that innovation, comes most frequently from the human mind at moments of pressure, emotion, and imaginative play (Figure 6). The role and use of heuristics, skill development, and sense-making or interpretation, such as the use of scenario-planning or strategic mapping, are vital in responding to a wicked problem like development of smart agriculture framework.

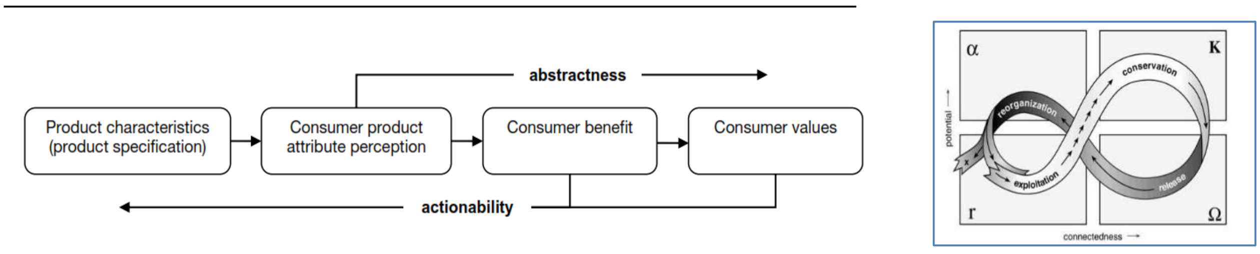

Figure 6 | Actionability (forward arrow for collaboration) and abstractness (backward arrow for research objective) of the spatial polysingularity construct, adapted from Van Kleef. [628]The cycle of sustainability [644] is the adaptive cycle symbol (right).

Figure 7 | Human capital context for the research design called research-to-value/outcome.

Scenario (A) | Field Case Study - Soybean cultivation
Modern scenario methods are well-suited to these tasks (factors limiting biological nitrogen fixation in soybean listed, Table 9). These tasks can help to organize scientific insight into an integrated framework, gauge emerging risks, and challenge the imagination. They can provide a means for integration of descriptive and narrative elements, and qualitative and quantitative information (Figure 8). The IoTs (internet of things) communication with non-scientific audiences, and can engage diverse stakeholders as actors in scenario design and refinement (Figure 5). Though their subject is the future, scenarios can catalyse and guide appropriate action today for a sustainability transition [597] like application of biofertilizer field application and evaluation of soybean cultivation with focus on the development of a for smart agriculture (Figure 23) for regenerative agriculture (Figure 27).

MATERIALS AND METHODS

BIOFERTILIZER PRODUCTION

Biofertilizer Production Machines |

Dryer - An open-air dryer designed and fabricated for the biofertilizer production. The dryer is used twice in production, both at the wet phase of the production and after the digestion period.

Biodigester -The anaerobic digester (designed and developed) are cubical concrete designs (Plates 5 and 6) for biodegradation with the aid of microbial inoculants (Table 2).The digested organic materials releases methane gas (hollow bored on the digesters to enable the passage of methane gas) and reduces the organic products to a digestible size through biodegradation digesters to enable the passage of methane gas) and reduces the organic products to a digestible size through biodegradation.[446]

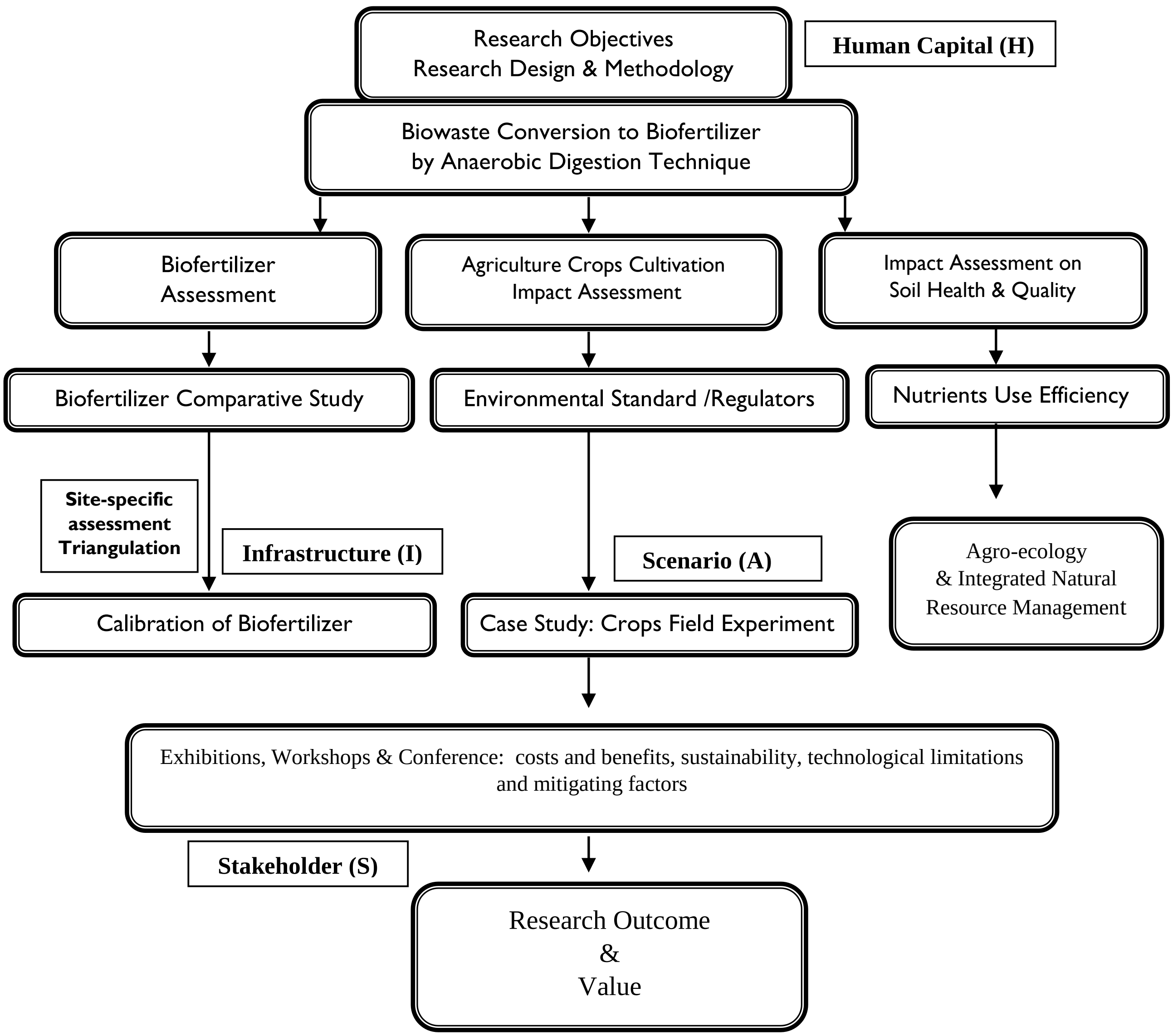

Figure 8 | Schematic for the spatial polysingularity construct for execution of the research objective and these accentuates the critical thresholds of 12 pillars of Global Competitive Indexes by Taranenko, [601]

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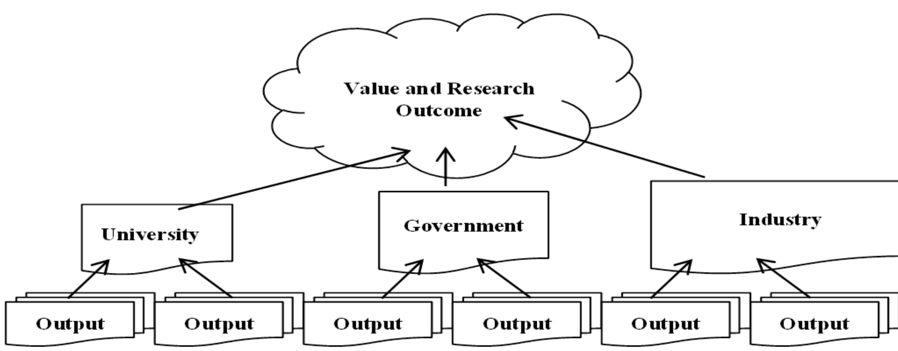
Smasher -This machine cuts the materials into smaller pieces to enable it mix thoroughly in the mixer. The slasher is designed to run mechanically and powered by electricity.
Mixer -This machine mixes the biowaste material (s) poured into mixed with the OBD Plus® and OTAII AG ® microbial inoculants (Plate 3). The machine is referred to as sigma mixer and powered by electricity. Classification of carrier materials for the production of biofertilizer (Table 3).
Granulator / Grinder - Improved hammer mill machine pulverizes the biodegraded organic material into granules. This machine is designed as hammer mill and powered by electricity.

Shaker -This is a machine designed and developed for sieving granules of the organic material into desired sizes. This machine has a lot of perforated small holes at the feeder stock to enhance the shaking processes (Plate 1).
Sealer - The hand sealing machine is used to seal the biofertilizer bags after bagging the product which marks the end of production processes, before it is transferred to the store.
Biowaste Materials |
The animal waste -The animal wastes (poultry, cow dung, pig) are used in the production of biofertilizer with no heavy metals or pollutant.



Internal and External Validity	Knowledge Parks	Analytics Blueprints	Training	Patents Intellectual Property	Agro-Corridors Shea Bioeconomy
Ecological Grazing Management	Crowdsourcing Agriculture Aviation	Collaboration Food Security	Wealth Creation	Commercialization Innovation ecosystem	PC3R Technology
Critical Thinking	Smart Farming Consulting	Techniques	Workshops	Innovation Academy	Exhibitions
Intelligence	Conferences Publications	Surveillance	Accreditation	Markets and Brands Development	Products Development

Figure 9 | The construct value of research outcome from the theoretical trans-disciplinary research framework.

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Farm product and waste -The farm wastes (cassava peel, beans shell, yam peels, potato peels), shea cake and wood ash.

Biofertilizer Production Processes |
Microbial inoculant (Table 2) formulation protocol adapted (Figure 11) mixed with the slashed dried agro-wastes which is then loaded into the anaerobic biodigester (Plate 1) . Microbial inoculant a powdery material (plant extracts carrier) impregnated with strains of micro-organisms capable of rapidly breaking down organic matter (Figure 13) into nutrient-rich-biofertilizer within four (4) weeks (Plate 1). Biofertilizer bag in 25 kg and inclusive with micronutrients (Plate 1). The first production stage is to ensure that the biowaste are dried through the open air drier.

Depending on the size of the materials they may be required to run through a slasher to cut them into smaller pieces and hence; if the size is considerable small after drying, they are transferred to the mixer where microbial inoculants will be applied and ensure thorough mixing to enable good and adequate biodegradation of the biowaste materials. Having mixed the product thoroughly in a mixer, they are transferred to the anaerobic biodigesters for biodegradation (four weeks). The biodegradation processes reduce size and loss of weight of the organic waste's material by a factor of 3:1. After digestion period (biodegradation) there is high tendency for the product to become wet again and therefore require a re-drying under control heat (hot air) because not to kill the microbial inoculant. Then, the products are off loaded form the biodigester to the open-air dryer for the second drying (reduce biofertilizer moisture to 10%). After the second drying, the products are transferred to the slasher for granulization and pulverization powdery substance or to a very small grain size. This process leads to sifting through the use of an electric shaker to sieve the product to remove impurities. Bagging is the last manufacturing process after shaker process because the product is collected from the shaker to the bagging unit, where they are bagged and sealed (Plate 1)

Microbial Inoculant Development |
Biofertilizer (microbial-based fertilizer) are considered to be crucial components of sustainable agriculture (Figure 3), with long lasting effects on soil fertility.[367, 55, 565] The term biofertilizer (Table 1) can be defined as formulations comprised of living microbial cells, either a single strain or multiple strains (mixed or consortium), that promote plant growth by increasing nutrient availability and acquisition [497] term itself has evolved over the last 30 years receiving many interpretations.[158] Macik *et al* [361] asserted that the greatest misconception occurs when including microbial inoculants with other beneficial applications (biopesticides and phytostimulators) as biofertilizer. Likewise, plant growth-promoting bacteria or rhizobacteria (PGPB/PGPR) and biofertilizer should not be considered an interchangeable term, since not all PGPB/PGPR are biofertilizer.[497] Nonetheless, it is worth mentioning that biofertilizer can also provide other direct and indirect benefits for plant growth, such as phytostimulation, abiotic stress tolerance and biocontrol.[173,555] Multi-omics technologies (Figure 11) enhanced the understanding of the complexity of microbiomes, characterization, the structure and function of microbial communities,[287] soil microbial communities and their influence on plant nutrient acquisition and other PGP traits.[519, 617, 618] Microbial consortia inoculation (Table 2) was used for the production of gateway biofertilizer (Plate1).

The negative effects of AMF on nodule development or non-significant effects on crop yield was reported. [26,387] Despite the promising beneficial effects of developing biofertilizer consisting of microbial consortia, it is unknown how these inoculants would establish across a range of agricultural field settings [175] defined as a wicked problem in agriculture because even if inoculated microbes colonize their new environment initially, their persistence over time is not guaranteed. Our approaches to develop suitable bioinoculants at commercial scale from screening potential candidate microorganisms, designing the inoculant and adaptation of formulation protocol (Figure11) based on host soil micro-organism.

Microbiological Analysis |

Bacteria Characterization and Identification |

Sample Preparation for Microbiological Analysis

Morphological characteristics of bacterial isolates were examined by Gram's staining according to the method by Salle.[525] Biochemical tests, including triple sugar iron agar test, Methyl Red (MR) test, utilization of citrate, nitrate reduction test, gelatin agar test, and starch hydrolysis test were studied by Cruickshanrk *et al.,*[122,105] The bacterial and fungal isolates were characterized based on their cultural, biochemical properties and microscopic appearances as described by Cheesbrough.[104] Various biochemical tests were performed on isolates for characterization according to Burgy's manual books.

Morphological, Physiological and Biochemical Characterization

There are great potential for use of PGPB as biofertilizing agents for a wide variety of crop plants in a wide range of climatic and edaphic conditions. Plant growth-promoting bacteria (PGPB) are of great agronomic importance. There is widespread distribution of PGPB that flourish in different geographical habitats.

Isolation of Bacteria

The collection, transportation and conservation of the samples, the methodologies proposed by Cline [108] and Sosa [579] were used. The beneficial effect of rhizobacteria lies on different mechanisms, such as: production of growth promoting substances, siderophores and antibiotics; as well as resistance induction in the plant and nitrogen fixation.[616] Consequently, the isolation of diazotrophic bacteria, their identification through reliable methods and the evaluation of their capacity as plant growth promoters, Single biofertilizer slurry consisting of 1g of biofertilizer and 9 mL of 10 mM phosphate buffer (pH 6.8) was used as a common inoculum source for most probable number (MPN) assays. One-gram root pieces were homogenized in 10 mL of sterile water and serial dilutions were prepared. These dilutions were used to inoculate N-free combined carbon medium and N-free malate medium and incubated at 30 ± 1 °C. The vials showing bacterial growth and acetylene reduction activity were used to inoculate plates of the same solid media to obtain pure colonies.The bacterial isolates were grown in Luria Bertani medium with shaking at 30 ± 1 °C [671] and studied for mean generation time [217] and Gram reaction.[639] The morphological and cultural characteristics of the bacterial strains were studied by light microscopy.[202] Total aerobic bacteria, total Coliform bacteria and *E. coli* were analyzed in surface plate method using TSA (Tryptic Soy Agar) and Sorbitol MacConkey Agar medium (oxoid, UK) followed by biochemical tests.[207] Other bacteria including *Bacillus sp., Pseudomonas sp., Azotobacter sp., Rhizobium sp., Klebsiella sp*. were identified using selective agar medium followed by API immunoassay analysis.[9] Phosphate solubilizing fungi and Phosphobacteria was identified using surface plate method on

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Pikovskaya's agar (PVK) and yeast malt agar plate. In addition, total fungal count, and Nitrogen fixing fungi, was determined Sabouraud Dextrose Agar (SDA) in surface plate method followed by microscopy.[506] Also, *Salmonella sp.*, and *Shigella sp.*, was detected using surface plate methods on *Salmonella-Shigella* (SS) agar (Oxoid, UK) followed by biochemical test. Phosphate estimations were done by the ascorbate method.[20] Iron was estimated by the bipyridyl method.[483] Phosphate-solubilizing microorganisms (PSMs) may play an important role in developing sustainable phosphate fertilizer systems [590,499] ubiquitous in soil and the plant rhizosphere. Various microorganisms have been isolated which solubilize phosphate in pure culture conditions, mainly by decreasing the pH of the medium, either by proton extrusion and/or by secretion of organic acids.309 Bacterial abundance was determined using the MPN method.[224]

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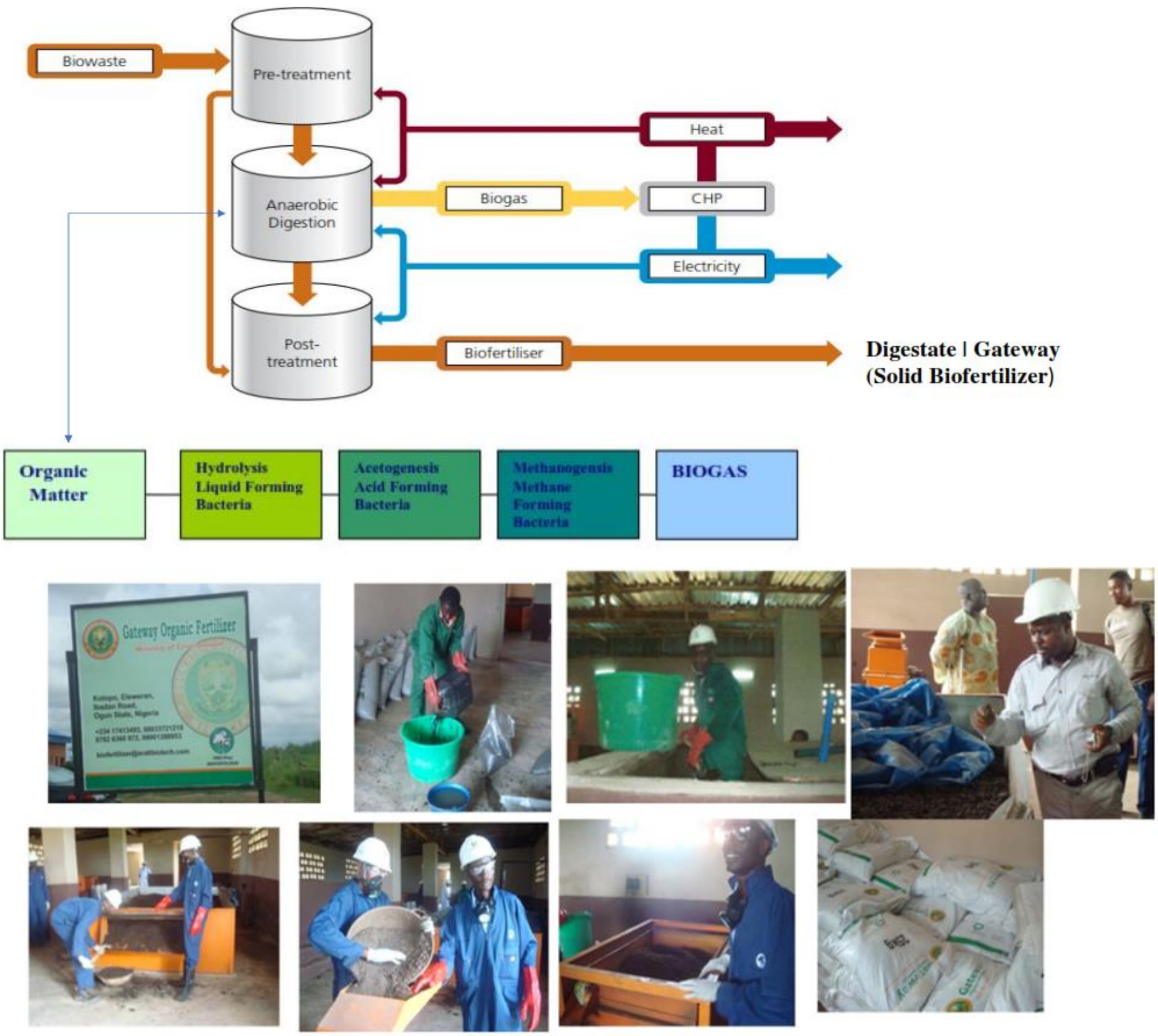

Figure 10 | Intrastructure - Anaerobic digestion is a multi-stage process of biowaste recycle to biofertilizer. Gateway Biofertilizer Plant, Turkey Project, Nigeria | https://www.academia.edu/video/jEepAj
Bio-waste Conversion to Biofertilizer Production | https://www.academia.edu/video/lBboEl
Visit: YOU Tube | https://www.youtube.com/watch?v=pG2ODAx3ICY

Plate 1 | Gateway biofertilizer [446] production machine Model OTAIKU Y2K09 designed, fabricated and developed at Gateway Biofertilizer Plant, Kotopo, Eleweran, Ibadan Road, Abeokuta, Ogun state, Nigeria produced Gateway biofertilizer (GF1) and Gateway biofertilizer (GF2) and see Table 6.

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Detection Methods |

Detection of IAA Producing Microorganisms

Indole-3-Acetic Acid (IAA) production by agricultural microbes was determined using Salkowski's method [156] and optical density (OD) of the test solution was measured at 530 nm by a UV spectrophotometer (Shimadzu, CPS-240A, Japan).[401, 647] Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil.[533] Some bacteria need longer period for optimum IAA production.[355]

Fungi |

Sample collection

100g of the soil were taken and packed into labeled sterilized bottles.[186, 78] The leave and fruit samples were collected by cotton swabs from the leaves and fruits of different plant families, and were placed in sterile plastic bags.[489, 576]

Isolation of Fungi

The soil fungi were isolated by the soil dilution method. One gram of the soil sample was suspended in 10 mL of sterile distilled water to make serial dilutions (10^{-1} to 10^{-5}). One mL of each dilution was placed on Potato Dextrose Agar (PDA) containing 1 % streptomycin. The plates were incubated at 28˚C in the dark. The plates were observed for one week.[186,490] The leaves and fruit samples were placed and shaken in flasks filled with 100 mL of distilled water, then (0.2mL) of the sample was taken from the flasks and transferred into PDA medium with *streptomycin.* The cultures were incubated at room temperature in an incubator for three to five days. The fungal colonies were observed, and the pure cultures were maintained. [186, 267, 262]

Macroscopic and Microscopic Examination of Isolated Fungi

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton bluestained slide mounted with a small portion of the mycelium.[186]

SOIL TESTS FOR MACRO AND MICRONUTRIENTS

There are about 20 nutrients required for plant health. Three of them, carbon, hydrogen, and oxygen (C, H and O) are considered part of the protoplasm, and the remainder are considered to be mineral elements. When fresh plant material is dried down, the dry matter remaining will be roughly 10% to 20% of the fresh weight. More than 90% of the dry weight will consist of carbon, hydrogen, and oxygen. If only 10% to 20% let's say 15% of a plant's fresh weight is dry matter and all but 10% of the dry matter is represented by carbon, hydrogen, and oxygen, it follows that all the other mineral elements that make up the plant account for only 1.5% of the dry weight ($0.15 \times 0.10 = 0.015$). Three main elements are nitrogen, phosphorus, and potassium (N, P, K) and are required in abundance. They must be readily available through soil medium or fertilizer. The secondary elements are sulphur, calcium, and magnesium (S, Ca, Mg). Soil pH has a significant effect on nutrient availability. High pH (>7.5) greatly limits the solubility of many elements (Zn, Cu, Mn, Fe), while low soil pH can lead to deficiencies of P or Ca and toxicities of Al, Fe or Mn. Similarly, low soil temperature, poor aeration, or the presence of a hardpan can limit the plant's ability to obtain nutrients by limiting root growth and health. In general, determination of soil pH can aid in the diagnosis of nutrient deficiencies. Soil pH affects the availability of mineral nutrients. Low pH (<5.5) may result in deficiencies of Ca, Mg, P or Mo and perhaps excesses

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of Mn, Fe or Al. High pH (>7.5) may immobilize Mn, Zn, Fe or Cu, making them unavailable to the

plant up to 4 ppm Boron (B) in the soil without adverse impacts on yield. The long- term effect of excess soil boron (>4 ppm) may be reduced photosynthesis from leaf necrosis that can affect 50% of the total area.

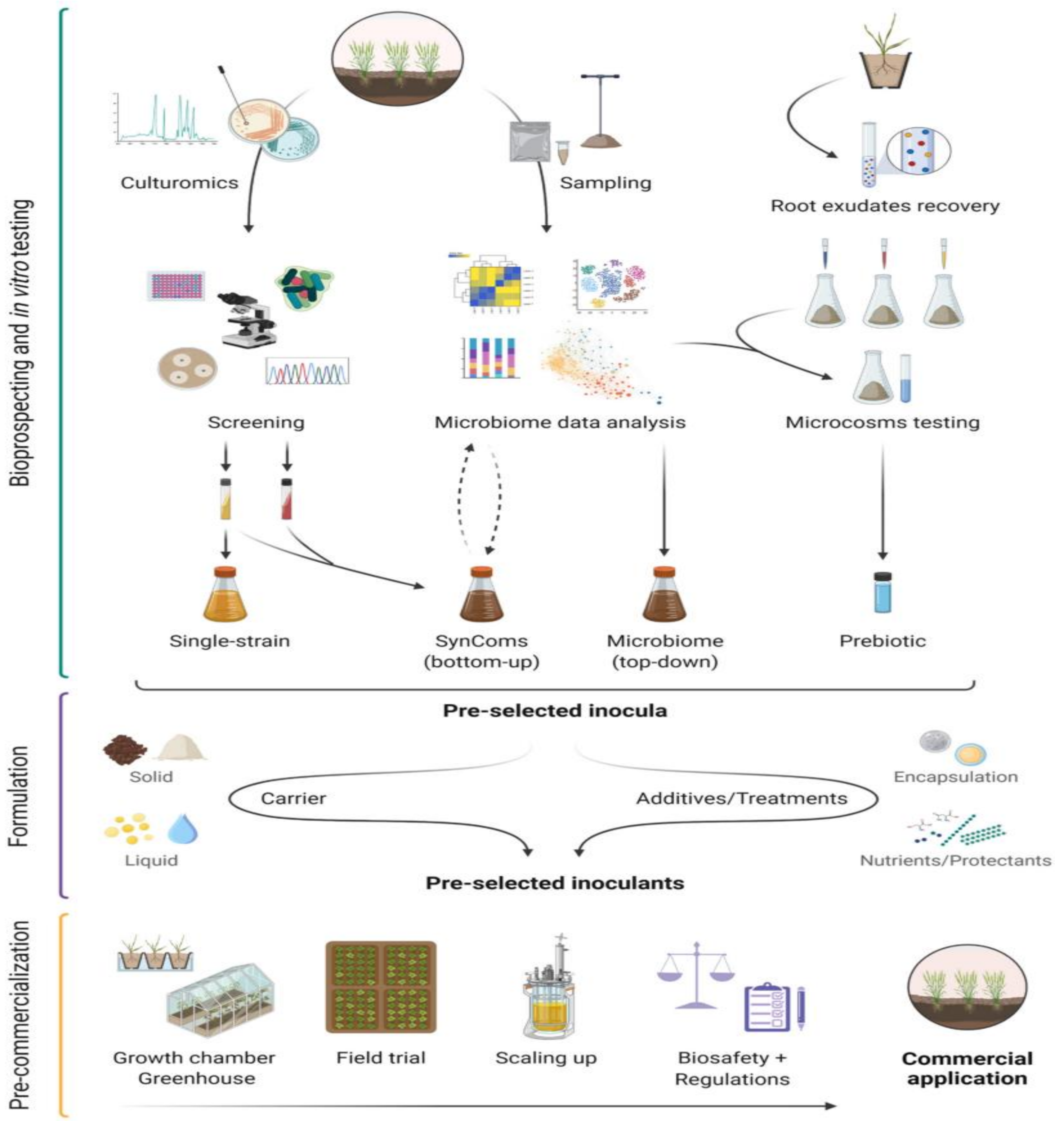

Figure 11 | Envision to Lunch (Figure 12) biofertilizer development workflow from potential candidate microorganisms to commercial applications with four novel approaches: single-strain inoculant obtained using emerging culture-based methods (culturomics), synthetic microbial communities (SynComs) obtained from a bottom-up approach, whole microbiomes recovered from natural or engineered ecosystems (top-down approach), and prebiotics obtained from root exudates [125] and adapted from BioRender (https://biorender.com/)

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Characterisation of location and experimental site
A field experiment was conducted at the research field of Gateway Organic Fertilizer Plant, Eleweran, Abeokuta, Ogun state, Nigeria Latitude 7^0 12^1 N and Longitude 3^0 25^1 E in the late cropping season of 2010 (Plate 1). The collected soil samples were air-dried and sieved through 2mm sieve. Particle size distribution was determined using the hydrometer method.[78]

Analytical Methods
The textural class of the site was determined using the USDA textural triangle. Soil pH was determined in water with the aid of pH meter using 1:2 soil: solution ratio.[381] Organic matter (Figure 13) was determined by using chromic acid digestion method.[646] Organic carbon was determined using Walkley and Black's method as described by van Reeuwijk.[629] Total nitrogen was determined using macro Kjeldahl method.[261] Available phosphorus was extracted using Bray-1 method [439] and read with spectrophotometer. Exchangeable cations were determined using ammonium acetate (NH_4 OA) buffer.[102] Boron test use some modification of the hot-water extraction procedure originally developed by Berger and Truog (1939) although the Mehlich 3 extractant is receiving more interest.[558] Soil tests for cations Mg and K^+ [209] and Ca[48] typically estimate the quantity of water-soluble and exchangeable forms by replacing the cations on the soil's exchange site with a counter ion such as Na^+ (Morgan) or NH_4^+ (Modified Morgan and Mehlich 3). Sodium and potassium were determined with the aid of flame photometer while Calcium and Mg were determined using Atomic Absorption Spectro -photometer (AAS). The available iron, zinc, copper and manganese were determined by atomic absorption flame photometer after extracting the soil with Diethylene Triamine Penta Acetic acid (DTPA) as described by Lindsay and Norvell.[336]

Despite its success on the human microbiota, multiple combinations in culturomics (that is., various growth media, culturing conditions, atmospheres, and times of incubation) have yet to be extended and developed for the soil and plant- associated microbiome. Sarhan *et al*. [532] suggested a "plant-tailored culturomic technique" that combines culturomics with plant-based media (Figure 11) used containing nutrients of animal origin (that is, nutrient agar, R2A, and LB) to isolate plant associated microbes, whereas plant materials or dehydrated juices powders should be used instead. In fact, P-solubilizing *Bacillus circulans* and N_2- fixing *Azospirillum brasilense* have been successfully grown on plant-only-based culture media.[675, 404] Thus, ensuring inoculant survival and function.[502, 617] Microbial consortia (Table 2) can consists of two or more strains that are either closely [317, 271] or distantly [480, 157] related that provide an overall additive or synergistic biofertilization effect. One of the most common applications is the co-inoculation of rhizobia and AMF on legumes, as a number of studies report a synergistic effect on plant growth promotion and classificsation of carrier materials for the production of biofertilizer (Table 3). [663.36, 281] In addition, online platforms such as KOMODO (Known Media Database, http://komodo.modelseed.org) that includes >18,000 strain–media combinations and >3,300 media variants/compound concentrations can be used as a guide for developing suitable lab media for growing microorganisms.[431] Therefore, new culturing methods to discover novel isolates with biotechnological applications are key for biofertilizer development (Figure 11).

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Biofilms consist of associated microorganisms, either from a single or multiple species, adhering to the biotic or abiotic surfaces in a self-produced matrix of extracellular polymeric substances (EPS).[481] This matrix provides the structure and protection by which microbes have the ability to chemically link with each other by quorum sensing (QS) and work as one unit.[641] In soils, microbial communities such as bacteria and fungi (Table 3) can form biofilms on abiotic surfaces such as ore (minerals), water-air interfaces, and dead organisms.[494] Biofilmed biofertilizer (BFBFs) (biofertilizer containing microbial communities capable of forming biofilms) have emerged as a new inoculant strategy to improve biofertilizer efficiency and sustain soil fertility.[458]

The idea behind BFBFs is that biofilm formation will create a more suitable environment for biofertilizer to compete with resident organisms and to cope with the heterogeneity of biotic and abiotic factors in soil [625] where, several studies have shown that biofilmed biofertilizer augmented P-solubilization,[596] N_2 fixation [649] siderophore production [498] and Zn solubilisation.[622] Kopyci´nska *et al*., [305] studies highlighted the role of biofilm formation, by exopolysaccharides (EPS) production, in Rhizobium leguminosarum during Zn stress where the EPS-deficient *R. leguminosarum* mutants were more sensitive to Zn exposure, whereas cell viability and root attachment were significantly higher in EPS producing strains. Multi-species biofilms were more resilient in comparison to single-species biofilms. [649, 635] Natural rhizobacterial biofilms are often in mixed communities with interspecies interactions (Table 2 and Figure 22). This assembly is usually more advantageous than single planktonic cells, with optimal and maximal use of nutrients and resources [422] like the fungal–bacterial biofilms have been shown to enhance nutrient uptake and environmental stress tolerance compared to mono - or mixed-cultures of no biofilm-forming microorganisms.[227] Inspired by the concept of personalized diagnosis in medicine, Schlaeppi and Bulgarelli [538] proposed a similar strategy in agricultural systems and adapted as eco-strategy (Figure 5) consists on customizing tools such as microbial inoculants into farming practices (Tables 1 and 2 respectively) as the protocol for the smart agriculture framework development. Bell *et al.*,[65] affirmed a customizable field-scale microbial inoculant that, with appropriate implementation, could have long-lasting effects (Figure 26) and could be adopted by farmers in humid tropics. In Sub-Sahara Africa considering that soil conditions might change dramatically over short distances [464] product development strategies of one formulation applied for all fields" seems unrealistic (Figure 21). One strategy is the on-farm production of *mycorrhizae*-based inoculants in which studies have shown their effects on potato [148, 201] and eggplant [148] growth and nutrition.

Table 2 | Biofertilizer Production Inoculants Composition/Information on ingredients: CFU Count per ml … > 6 x 10^9

S/N	Micro-organisms	GF1 - Micro-organisms Charaterization	GF2 - Micro-orgamisms Charaterization	References
	Microbial Inoculants Commercial	OBD-Plus (+)	OTAI AG	Otaiku *et al*., 447 ; 448 , Otaiku *et al*., 449
1	Nitrogen-Fixing Bacteria	Azotobacter spp	Azotobacter spp	Sangeeth *et al*., 526 ; Zheng *et al*., 697
		Rhizobium spp	Azospirillium spp	Panwar,and Singh 2000 455
		Micro-organisms Bacillus spp	Clostridium spp	Diep and Hieu,145 ; Minamisawa *et al*., 397
		Cyanobacteria spp	Bacillus spp	Badr *et al*. 43 ; Han *et al*. 222
		Azospirillium spp	Esherichia spp ; Cyanobacteria	Pettigrew, 462
2	Fe (iii)- reducing Bacteria	Desulfuromonas acetoxidans	Geobacter metallireducens	Lovley *et al*., 349
		Shewanella putrefaciens	Desulfuromonas acetoxidans	Devereux *et a* l., 141 ; Babauta *et al.*, 42
		Geobacter sulfurreducens	Geobacter spp	Babauta *et al*., 42
3	Arbuscular Mycorrhiza fungi	Glomus spp	Glomus spp	Schweiger *et al*., 542 ; Tiwari, 613
		Mycorrhiza (AMF)	Mycorrhiza (AMF)	Thompson, 609
4	Phosphate Solubilizing Fungi	Aspergillus niger	Aspergillus niger	Wang *et al*., 651 ; Zeroual *et al.*, 686
		Aspergillus terreus	Aspergillus terreus	Mehrvarz *et al*.,384
		Actinomycetes spp	Actinomycetes spp	Adesemoye *et al*., 3
			Penicillium spp	Barea *et a* l., 53
5	Potassium Solubilizing Bacteria	Pseudomonas putida	Pseudomonas putida	Han and Lee , 221 ; Vassilev *et al.* 634
		Enterobacter hormaechei	Bacillus mucilaginous	Sheng and He , 552
6	Phosphate Solubilizing Bacteria	Rhizobium spp	Rhizobium spp	Boraste *et al* ., 76
		Pseudomonas fluorescens	Agrobacterium spp	Idriss *et al.*, 253 ; Khan *et al*., 296
			Paenibacillus glucanolyticus	Richardson *et al*., ; 500 ; Sangeeth *et al*., 526
7	Cyanobacteria	Blue-green algae	Blue-green algae	Mandal *et al.*, 372 ; Singh, 562
8	Sulphur Oxidising Bacteria	Thiobacillus thioxidans, Xanthobacter	Bacillus, Pseudomonas, Streptomyces	Santra *et al*., 529
9	Sulphuroxidation fungi	Fusarium, Aspergillus	Penicillium , Aspergillus niger	Grayston *et al.*,212 ; Zhen *et al*., 696
10	Sulphur Reducing Bacteria (SRB)	Desulfovibrio desulfurican	Desulfomonile spp.	Jones *et al.*,275 ; DeWeerd *et al*.,143 ; Cravo-Laureau *et al*.,116

GF1 = Gateway Biofertilizer | GF2 = Gateway Biofertilizer

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Table 3 | Classification of carrier materials for the production of biofertilizer.

N/S	**Categories of Carrier Material**	**Carrier Materials**	**References**
1	Natural materials	Peat, lignite, coal, clay, and organic soil.	Paliya *et al.* ,452 ; Shravani,556
2	Inert materials	Talc, vermiculite, perlite kaolin,bentonite, silicate,	Saravanakumar *et al.*, 530
		rock phosphate, calcium sulphate and zeolite.	
3	Synthetic polymers	Polyacrylamide, polystyrene, and polyurethane.	Herrmann and Lesueur , 235
4	Natural polymers	Xanthan gum, carrageenan, agar agar, and agarose.	Thirumal *et al.*, 608
5	Organic materials	Charcoal, biochar, composts, farmyard manure, sawdust, maize straw,	Roychowdhury *et al* , 515 Wang *et al* ., 650
		vermicompost, cow dung, corn cob, and wheat husk.	Hassan *et al.* , 226 ; Rodrigues *et al.* , 504
6	Agro-industry by-product	Sludge ash, jagerry.	Paliya *et al.* , 453 **;** Shravani , 556
7	Biowaste (Agriculture)	Gateway biofertilizer (animal dung and wood ash based + inocula) .	Otaiku, 446 ; Soretire *et al* .,578 ; Onyenaliand Olowe , 441
8	Biowaste (Agriculture)	OBD-Biofertilizer ((animal dung and wood ash based + inocula) .	Otaiku *et al.*, 447; 448 ; Otaiku *et al.* ,449 ; Wang , 650

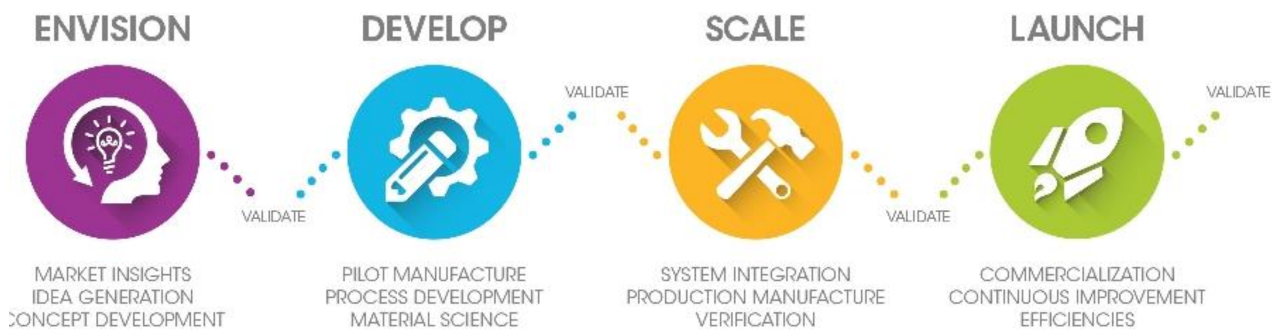

Figure 12 | DEVELOP - Conversion of microbial inoculants into biofertilizer https://www.academia.edu/video/jEepAj (anaerobic biodigester production, Plate 1) following the initial stages (ENVISION) of bioprospecting and *in vitro* testing, selected inocula and/or prebiotics require a proper formulation to

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ensure shelf life and protection. SCALE | Field study finally, product pre-commercialization steps include *in planta* trials under controlled (growth chamber/greenhouse) and uncontrolled (field) conditions (Plates 2 and 3), Production scale-up to a commercial scale, proper biosafety screening tests (example, toxicity and pathogenicity), and compliance with existing regulations and adapted from BioRender (https://biorender.com/).

Table 4 | Factors limiting biological nitrogen fixation in soybean cultivation.

N/S	**Factors**	**Effect**	**Effect References**	**Recommendations**
1	High soil temperature	Reduces the survival of rhizobia	Munevar and Wollum, 411	Surface mulching; placement
		in soil and inhibits nodulation and N2 -fixation.	Michiels *et al*., 394	of inoculum in deeper soil layers; select heat-tolerant strains.
2	Soil moisture	Reduces rhizobial numbers, limits migration of rhizobia,	Hunt *et al*., 246	Optimization of soil moisture;
		reduces nodulation and Nitrogen fixation.	Smith Read,570	select moisture-stress tolerant strains.
3	Soil acidity	Reduces the survival of rhizobia in soil,	Zahran, 685	Use of acid-tolerant legume
		inhibits nodulation and	Giller, 193	cultivars and rhizobia; liming
		N2 - fixation and leads to P fixation.	De Freitas *et al*., 131	of soil to pH at which Al and
		Increases aluminum toxicity and calcium deficiency.	Singh *et al*., 565.	Mn are no longer toxic.
4	P deficiency	Inhibits nodulation, N2 -fixation and rhizobial growth.	Gates and Muller, 191	Addition of P fertilizers,
			Cassman *et al*., 97	Amelioration of soil acidity, inoculation with effective
			Shridhar, 557	mycorrhiza, and selection of P-efficient cultivars.
5	Salt stress	Reduces nodule formation, respiration	Tu, 623 ; Delgado *et al*.,137	Select salt-tolerant strains.
		and nitrogenous activity.	Shiraiwa *et al*., 554	
6	High soil N level	Inhibits root infection, nodule development	Abdel-Wahab *et al*., **2**	Breed cultivars which are less sensitive to mineral Nitrogen.
		and nitrogenous activity.	Imsande, 256 ; Arreseigor *et al*., 32	
7	Herbicides, fungicides	Inhibits rhizobial growth; reduces	Schisler *et al*., 535	Test the particular rhizobial inoculum and
	and insecticides	nodulation and Nitrogen -fixation; deforms	Mallik and Tesfai, 368	its behavior in respect of the product used before application;
		root hairs and inhibits plant growth.	Isol and Yoshida, 257	separate placement of rhizobia and fungicides.
8	Competition from native organisms	Suppression of inoculation by native rhizobia.	Dowlig and Broughton, 150	Targeted research.

Locally produced inoculants (Table 2) often have low costs and are applied shortly after production, without the need of shipping and storage.[149] Yet, it is important to consider how these products will be feasible or cost-effective on a global scale (Figure 21). A good starting point could be establishing an optimal range for biofertilizer performance, in which the inoculants would be introduced to conditions best

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resembling the soils they were isolated from. Here, different formulations can be designed for particular soils and (or) plant-root systems with the incorporation of certain aspects of precision farming (Figure 23). Thus, identifying areas in a particular field that might be more suitable to one formulation or another (Table 3). *P. putida* has been previously studied for their ability to solubilize phosphate and thus promote growth of leguminous.[51]Multiple lines of evidence show that root exudates 125 could be used as compounds to stimulate the growth of beneficial microbiota, rather than introducing microbes by inoculation (Figure 22).

A similar approach was proposed by Arif *et al.,*[30] affirmed that particular soil amendments could act as "prebiotics" to promote microbial functions (Figure 26). Qiu *et al.* [475] affirmed that synthesized compounds can be added to crops to attract or favour particular microbes (Table 1) these "plant prebiotics" could be used in combination with microbial inoculants to enhance biofertilizer efficiency (Figure 14). By acting as signalling molecules, these compounds could potentially attract introduced microbes to the rhizosphere, thus giving them an advantage over other microorganisms for early colonization (Figure 4). Biofertilizer formulations (powders and granules, Table 3) are challenging for non-sporulating bacteria, as desiccation disrupts cell membranes, causing cell death and overall loss of viability during rehydration and can lead to major setbacks for product commercialization (Figure 12).

SOYBEAN FIELD CROP CULTIVATION

Soybean yield potential (Plates 2 and 3) has been defined as the maximum yield of a crop cultivar grown in an environment to which it is adapted, with nutrients and water non-limiting, and pests and diseases effectively controlled.[165] Maximizing legume (biomass) and seed yield within the constraints imposed by agronomic management and the environment because larger biomass crops require more Nitrogen (N), N_2 fixation will be increased as biomass yield is increased. This approach assumes a capacity for N_2 fixation sufficient to satisfy increased N demand of larger plants (Table 4). Soybean reviewed reports [266, 231] much of the breeding has been conducted in moderate to high N soils, breeding for symbiotic nitrate tolerance [232, 230, 575] althousgh plants were grown in low N soils for matching plant genotypes and rhizobia for selective [117] and indiscriminate (promiscuous) nodulation[307] was reported. Optimizing legume nodulation through specific nodulation traits (mass and duration), and depending on the circumstances, for promiscuous or selective nodulation [142, 307,117] were reported. Continued improvements in the effectiveness of legume inoculants and the matching of strains with host genotypes should be research [82] using Figure 5 framework for the scope to select rhizobia for specific environmental niches, [273] that is, acid tolerant strains for acidic soils.[240, 247]

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The future soybean field trials should consider host strain specificity important and to identify highly effective combinations of host cultivar and rhizobial strain.[416,417,419,18] An extension of this strategy (Table 2) is to develop cultivars of soybean that bypass the naturalized soil rhizobia and nodulate only with highly effective inoculant strains.[117] To avoid the need for inoculation, scientists in the soybean breeding program at the International Institute of Tropical Agriculture (IITA) sought to exploit locally used cultivars, such as Malayan and Orba, grown by small farmers in Nigeria for more than 30 years with low inputs and without inoculation.[420] IITA hoped to establish soybean as a widely grown crop in west Africa in which promiscuous nodulation was combined with the yield, quality and disease resistance traits of improved United States cultivars resulted to N_2 fixation and yield may be limited by then low effectiveness of the naturalised soil *bradyrhizobia.* [315] Vasilas and Fuhrmann [632] showed that Forrest soybean nodulated with highly effective strain USDA 122 fixed 29% more N_2 and produced 31% more grain than plants nodulated with the naturalised soil bradyrhizobia.

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Design and Treatments

A field trial of 4 x 3 factorial treatment structure was laid out in Randomized Complete Block Design (RCBD) replicated three times. Three sources of organic fertilizers (Gateway biofertilizer 1 (GF1), Gateway biofertilizer 2 (GF2) and Sunshine fertilizer (SF)) at four application rates (0, 5, 10 and 15) t/ha and NPK 20:10:10 as a check (30kg N/ha) were applied on soybean cultivar (TGx 1440 - 1E). GF1 consisted of cassava peels and rumen content with OBD Plus inoculants and GF2 had Swine waste, cow dung and wood ash with OTAI AG inoculants (Table 6, Plate 1) while Sunshine consisted only of poultry manure [macronutrient composition 3.5g/hg (Nitrogen); P(%) 1.0; K(%) 1.2].The land was cleared and stumped manually. It was ploughed and harrowed before planting. The net plot size was 2m x 2m, with two seeds planted per hole. Biofertilizers were incorporated into the soil 2 weeks before planting at the rate of 0, 5, 10, and 15 t/ha, with basal application of inorganic fertilizer. Soybean variety TGX 1440-1E was planted by seed drilling with a spacing of 0.5 m between rows. Weeding was done manually two weeks after planting, and all other cultural practices were observed apart from the treatments applied.

Sampling and Data Collection

Pre-planting soil sample was collected randomly with soil auger on each plot, this was air dried and passed through 2 mm sieve. Data were collected at 8 weeks after planting (WAP) on the number of nodules per plant, nodules dry weight, plant height, number of leaves per plant, shoot and root dry weight.

Table 5. Soil profile of soybean field trial.

Plate 2 | Biofertilizer applied to soybean cultivation.

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Parameter	Values
pH	6.4
Sand %	81.2
Silt %	12.8
Total Nitrogen (g kg-1)	1.2
Available P(mg kg-1)	6.28
Exchangeable Ca (Cmol kg-1)	11.35
Na (Cmol kg-1)	1.42
Mg (Cmol kg-1)	0.81
K (Cmol kg-1)	0.8
% Base Saturation	99.79

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA), General Linear Mixed Model (GLMM), with model parameters evaluated using Restricted Maximum Likelihood (REML) algorithm. Significant means were separated using Duncan's Multiple Range Test at 95 % confidence level. The statistical package used for the analysis was SAS.[534] Reproductive growth parameters (number of pods/plant, pod weight/ plant and seed weight/plant) were also determined following standard protocol. Nitrogen fixed was determined at 8 WAP by ureide assay by Herridge and People.[233]

Plate 3 | Soy bean cultivation, using gateway biofertilizer (GF), Abeokuta, Ogun state, Nigeria.

RESULTS

Growth Response |

Fertilizer sources had no significant ($P > 0.05$) effect on vegetative growth parameters, except plant height at 8 WAP. GF 2 had significantly taller plant, which was not significantly different from GF 1 and SF. This is an indication that omission of phosphorus from soybean nutrition can drastically reduce shoot dry matter yield of soybean as suggested by Bekere *et al* .[64] Significantly, smallest height was observed when CF was applied. Significant ($P < 0.05$) interaction of sources x rates of application was observed on all growth parameters except shoot and root dry weight ($P > 0.05$) at 8 WAP (Table 7). Increasing application rates did not result in any significant difference on the number of leaves for all the biofertilizer sources except GF 2 that had significantly ($P < 0.05$) higher number of leaves (120) at 15 tons/ha application rate (Figure 18). This may be due to microbial population [358] and organic colloids during decomposition [77] and moisture/water relations. Similar trend was observed on the plant height. However, GF1 had a curvilinear response ($P < 0.05$) with the tallest plant (48.2cm) observed at 10 tons /ha application rates.

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Yield Responses |

Biofertilizer sources had a significant ($P < 0.05$) effect on yield components examined at 8 WAP (Figure 15). However, there was no significant ($P > 0.05$) effect of biofertilizer sources on pod weight/plant. GF 1 had significantly ($P < 0.05$) higher number of pods and seed weight/plant. There were no significant differences among other sources on seed weight and number of pods/plants. Significant interaction ($P < 0.05$) of sources x application rates was observed on number of pod and seed weight/plant. No significant interaction ($P > 0.05$) of sources x application rates was observed on pod weight/plant (Table 7). It was observed that increasing application rates for all sources did not lead to any significant differences in the number of pods and seed weight/plant. Significantly higher number pods/plant (131) was observed when there was deficit of GF 1, while significantly least number of pods/plant (58) was observed when SF was applied at rate 10 tons/ha. No significant differences were observed on the seed weight/plant with increasing application rates (Plates 2 and 3).

Nodulation Response |

Soybean plants assimilate the N from three sources, N derived from symbiotic N_2 fixation by root nodules (*Ndfa*), N absorbed from soil mineralized N (*Ndfs*), and N derived from fertilizer when applied (*Ndff*) Ohyama *et al.*, [433] (Figure 14). Fertilizer sources had no significant ($P > 0.05$) effect on nodulation in soybean (Plates 2 and 3, Table 8). Percentage of nitrogen fixed was significantly affected by biofertilizer sources with GF 1 having significantly higher percentage of nitrogen fixed (54.1%), which was not significantly different from GF 2 (52.2%). The results affirmed by Kumaga and Ofori's [308] which states that among various factors that can contribute to soybean success, phosphorus and inoculation had quite prominent effects on nodulation, growth and yield parameters. Significant interaction ($P < 0.05$) of sources x application rates was observed on number of nodules and percentage of nitrogen fixed (Figure 16). No visible pattern was observed with increasing application rates of different sources on the number of nodules. However, increasing application rates of different sources resulted in significant ($P < 0.05$) increase in percentage of nitrogen fixed except SF. Soybean vegetative growth parameters, nodulation, amount of nitrogen fixed, yield and yield components were determined at 8 weeks after planting (WAP), Figure 15.

Fertilizer sources had no significant ($P > 0.05$) effect on growth parameters except on plant height, GF 2 having significantly ($P < 0.05$) taller plant. The results attributed to the fact that there is a synergy result, thereby secreting different organic acids[11] like carboxylic acid and thus lowering the rhizosphere pH [229] in Figure 14. GF 2 had significantly ($P < 0.05$) higher number of leaves and taller ($P < 0.05$) plant with increasing application rate, which was not observed in other sources. Percentage nitrogen fixed increased significantly ($P < 0.05$) with application rates in GF1, which was not observed in others (Figure 17). Conversely, significant ($P < 0.05$) depression was observed on number of pod/plant with increasing application rate in GF1, with no visible pattern in pod weight/plant with increasing application rates. The increasing application rates of GF2 could give comparative performance of soybean as chemical fertilizer in the transitory zone of Nigeria. Soybean has the capacity to form a symbiotic association with *Rhizobium japonicum* and able to fix 20% of the atmospheric nitrogen throughout the world annually.[180] Soybean is a sulphur loving plant and like other oilseed crops, its sulphur requirement is more than that of many other crops for proper growth and yield.

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The response of soybean to sulphur application has been reported by several workers.[418,484] Phosphorus and Sulphur interactions in soils of poor fertility may be more important. Majumdar *et al.* [363] found that combined application of P and S @ 60 kg P_2O_5/ha and 40 kg S/ha respectively increased the number of pods plant/ha. The highest number of pods plant may be due to the fact that, the combined effect of both phosphorus and sulphur had positive effect on the reproductive growth and pod formation of soybean.The present studies (Table 7) are in accordance with the findings of Tomar *et al*.[615] and Majumdar *et al.*,[363] who reported greater increase in grain yield of soybean with combined application of phosphorus and sulphur the highest biological yield of soybean (5.307 t/ha) was recorded with the treatment combination of P_3S_2 (50 kg P/ha+20 kg S/ha.

Pod Number |

GF2 pod number reduction in crop productivity under a condition of limited nutrients energy for pod production similar to Smith *et al.,* [571] Makinde *et al*., [364] and Daramola *et al*., [127] reported that availability of adequate nutrients could improve crop growth and yield parameters, the GF2 phosphorus was too much for the nodulation. Amongst the many factors that can subsidize to the success of soybean, phosphorus has significant implications on growth and yield attributes, Table 6 and affirmed by scholar.[308]

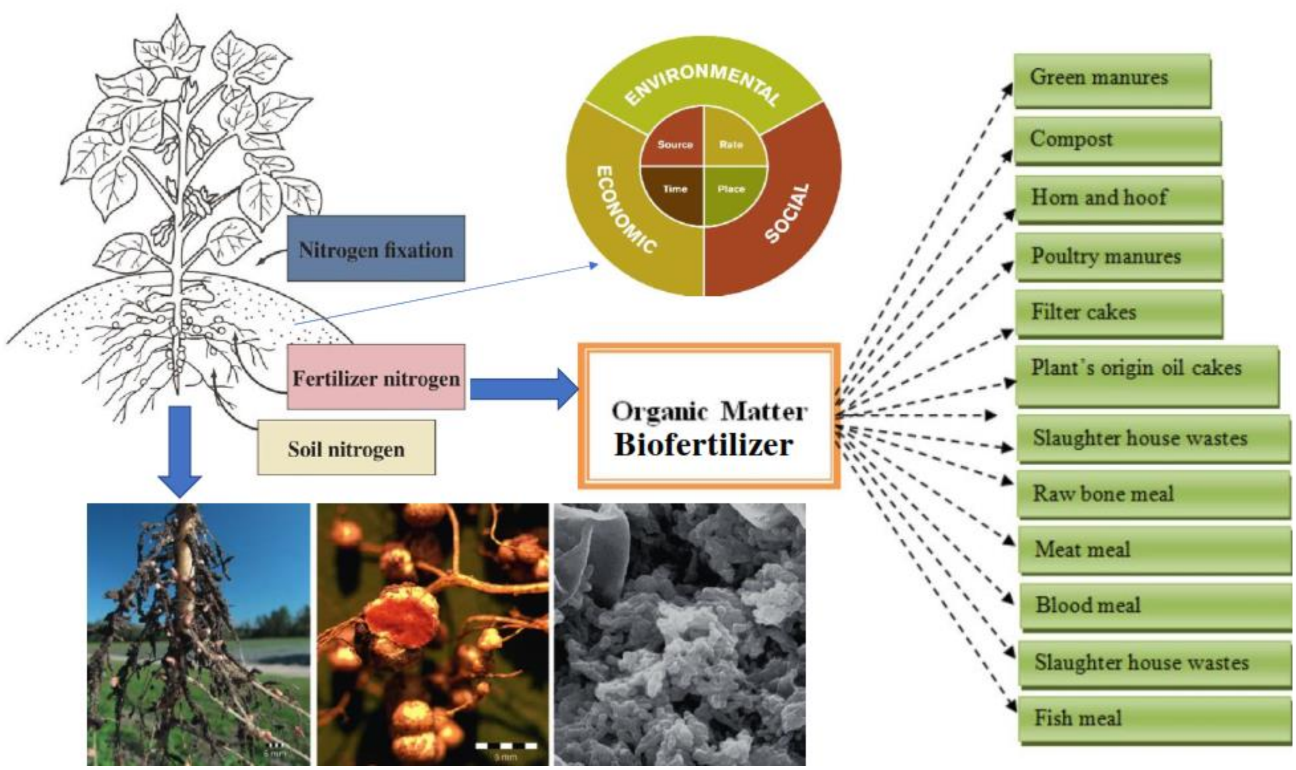

Figure 13 | Ohyama *et al*., [433] adapted three sources of Nitogen assimilated in soybean plants. Organic nitrogen

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available for mineralizationin soil with the nodules on legume roots a close-up shows a few nodules on roots of a soybean plant with one nodule sliced open to expose the red color of its oxygenated leghemoglobin. A scanning electron micrograph shows a single plant cell within a soybean nodule stuffed with the *Bradyrhizobium japonicum* bacteria specific to the symbiosis with soybean and adapted from R. Weil; SEM courtesy of W. J. Brill, University of Wisconsin and Leghari *et al*..[318]

Similar reports affirmed sharp increase in soybean crop yields is partly due to the balanced fertilization and good cultural practices.[400] Whereas, increasing application of phosphorous to 975 kg P_2O_5/ha has no increase in seed productivity, but has an impact on quality of seed.[438,441] GF1 produce the highest pod and similar to the report of Tahir *et al*.,[599] where 94% pod per plant was reported with the application of inoculant + Phosphate + Nitrogen and implies that, the lowest level of P used (30 kg P_2O_5/ha) when combined with inoculant and chemical fertilizer was sufficient to induce increase in soybean pod production in the study area and reducing cost of production. Sharma *et al*.,[551] reported that pods plant of soybean were greatest with 75 kg P/ha and Rhizobium + farm yard manure + phosphate solubilizing bacteria. The use of broad spectrum inoculum (Table 6) is consistent with that of Singh *et al*.,[564] who reported that there was no significant difference between pod yield of non-inoculated and inoculated soybean seeds with the use of *B. japonicum* only. Soybean N_2-fixation has an economic value in terms of the N that it supplies to the plant from the air which otherwise would need to come from soil and/or fertilizer sources. There is also an economic value in the residual benefits for soil N fertility and increased productivity of subsequent crops.[620] In most soils, the savings were equivalent to 40–60 kg N/ha. Duong *et al*., [152] reported that 240 kg N/ha would have been required to produce an equivalent grain yield to the inoculated treatment. The combined application of bacterial inoculant and P fertilizer to soybean and common bean increased biomass production and grain yield compared with the singular use of N and P or (Brady) rhizobial strains. Economic analysis shows that the increase in grain yield with inoculation translated into higher marginal rate of return (MRR) and profitability for soybean and common bean small farms. The emphasis given to *Rhizobium* in biofertilizer research shows its high specificity to only legumes (common bean 47%; lucerne 23%; soybean 14%; desmodium (a leguminous pasture species) 9%; and other minor legumes 7%; in that order) unlike mycorrhiza, that works in 80% of all plants.[432] A wider use of rhizobium inoculants in marginal areas depends on the ability to develop strains which are tolerant to high temperatures, soil acidity, drought and salinity.[432]

DISCUSSION

The broad interest in soybean is largely due to the high protein content of the grains, about 40%, representing an important protein source for human and animal diets. Currently, inoculation with efficient strains allows the soybean plant to obtain its entire N needs from BNF.[247, 249 ,700] Today, concern has been expressed in terms of whether BNF is capable of meeting increased N needs of newly released more productive cultivars as well as if it is capable of allowing maximum grain yields. [638, 650,320,114]

Biofertilizer from Anaerobic Digestion |
Anaerobic digestion (AD) is defined as a waste treatment in which liquor or slurried organic wastes are decomposed biologically under strictly anaerobic conditions (Plates 4 and 5). AD is a natural process in

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which microorganisms (Table 2) break down organic matter (Figure 13), in the absence of oxygen, into biogas [a mixture of carbon dioxide (CO) and methane] and digestate (a nitrogen-rich fertilizer). The biogas can be used directly in engines for Combined Heat and Power (CHP), burned to produce heat, or can be cleaned and used in the same way as natural gas or as a vehicle fuel. The digestate can be used as a renewable fertilizer or soil conditioner (Plates 2 and 3) called biofertilizer (Table 6). The purpose of this pre-treatments is inoculation of agrowaste feedstock because anaerobic digestion is a complex biological process (Figure 10) and its performance is influenced by microbial diversity (Table 2) balanced active inoculum is essential for the possible degradation to be carried out (Plates 4 and 5). Under anaerobic conditions, organic forms of nitrogen (N) are converted into ammonium-N (NH-N), that is, readily available nitrogen. The readily available nitrogen (RAN) content of cattle slurry is typically 50% and pig slurry *c.* 60% of total-N.[24] It might be anticipated that a measurable increase in the proportion of readily available N would occur in these materials, as a result of the digestion process (Figure 10) to nutrient impacts, a number of benefits are claimed to accrue as a result of AD, including a reduced risk of odour nuisance and a reduction in viable pathogenic organisms.[577] Ezigbo [167] reported that, the rate of bio-degradation is affected by the following factors: Moisture content-Bio-digestion occurs faster in the presence of moisture while lack of water makes the survival of degrading organisms difficult; Surface area-this plays an important role in bio-degradation and finer the particles, the faster the digestion rate; pH- bio-digestion occurs best at a medium to slightly acidic pH; temperature: high temperature favours bio-digestion while low temperatures slow it down.

Plate 4 | Anaerobic digester Old design do not biogas produced, 2009, UNDP Project.

https://www.academia.edu/video/jEepAj
Gateway Biofertilizer Plant, Turkey Project, Nigeria.

Plate 5 | Anaerobic digester New design to capture the biogas produced, NESREA Port Harcourt, 2012.

https://www.academia.edu/video/lBboEl
Bio-waste Conversion to Biofertilizer Production.

Recent research results indicate that 55-95% of the N (nitrogen) in animal diets is excreted through faeces and urine.[433] High proportions of P (phosphorus) and K (potassium) in animal diets are also excreted. Animal manures and slurries are therefore rich in plant nutrients. This is also the case for many other types of anaerobic biodigester (AD) feedstock, making digestate a valuable biofertilizer (Figure 10). By making

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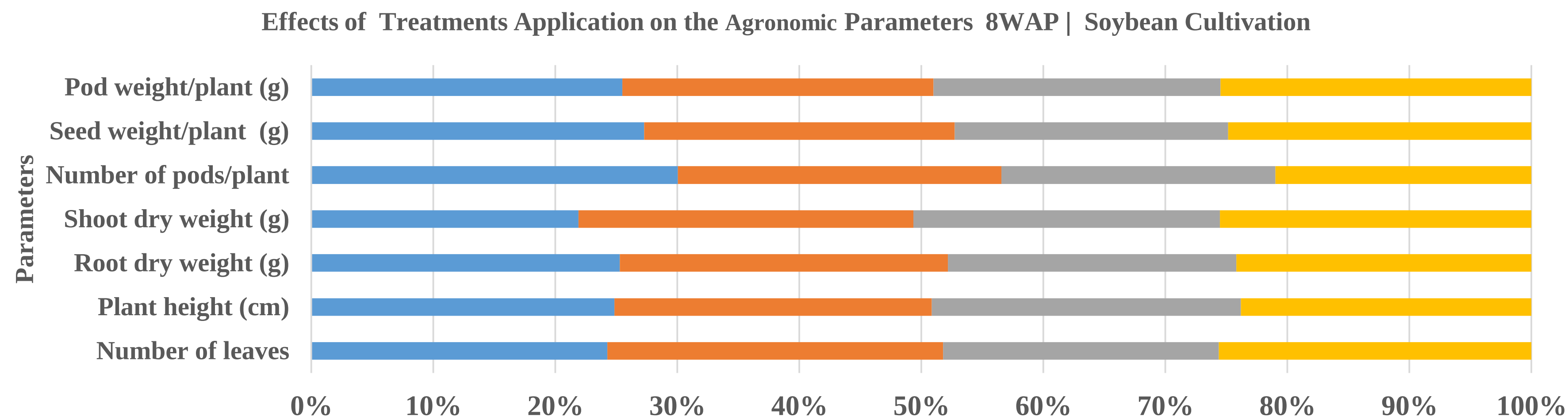
the best possible use of digestate as a biofertilizer, nutrients are returned to the land through natural cycles to replace the input of inorganic fertilizer. Biogas is an extremely useful source of renewable energy, whilst digestate is a highly valuable biofertilizer.[352] Biofertilizer should not be misunderstood for organic fertilizers such as compost, animal manure and plant manure or extracts.[95,371] When the beneficial microbes improve crop accessibility to nutrients [154, 410] or replenish soil nutrients [557, 604] if the overall nutrient condition of crop and soil has been improved, such substances containing the beneficial microorganisms are considered as biofertilizer. [636] The carrier materials (Figure 3) sustain the microbial inoculants and allow the product to be stored for longer period.[76, 486, 487] The beneficial microbes may be rhizospheric; colonising the surface or intercellular spaces of the plant roots, or endophytic (Figures 18 and 26); and, where they colonise the tissue or apoplastic space within the host plants.[213, 370]

S/No	Biofertilizer Formulation	Gateway BioFertilizer 1 (GF1)	Gateway BioFertilizer 2 (GF2)	Micro-organisms Characterization	References
A.	**Biofertilizer Production**	**Batch 2010 GF1**	**Batch 2010 GF2**		
B.	Biowaste Materials	Poultry manure, Cow dung, Cassava peel ,Wood Ash	Poultry manure, Swine waste, Cow dung, Wood ash		Otaiku *et al* .,447; 448 **;** Otaiku *et al* ., 449
C.	Technology : Anaerobic Biodigester	Digestate	Digestate		
D.	Microbial Inoculants :	OBD-Plus	OTAI AG		Otaiku *et al.* ,447 , 448 ; Otaiku *et al.* , 449
E.	Field Crops Trials : External validity	Soybean Cultivar TGx 1440–1E	Soybean Cultivar TGx 1440–1E		
	Institutions | FUAAB	September 2010,	November. 2010		
F.	**Physio-Chemical Analyses**				
	Macronutrients				
1	Nitrogen (N) %	5.79	6.6	Rhizobium spp ; Azospirillum spp	Hungria *et al* ., 248 ; James, 265 ; Kueneman *et al* .,307
2	Phosphorus (P) %	1.3	1.5	Pseudomonas spp. Bacillus spp	De Freitas *et al* . 131 ; Zaidi *et al* . 680 ; Battini *et al* . , 60
3	Potassium (K) %	2.89	2.3	Bacillus mucilaginosus ; B. megaterium	Han and Lee, 221 ; Sheng and He ,552 ; Pettigrew, 462
4	Calcium (Ca)	1.66	3.8	Azospirillum spp.	Giri and Mukerji, 197 ; Khan, 295 ; Cohen *et al* ., 110
5	Magnesium (Mg) PPM	3.24	7.4	Azospirillum spp.	Giri and Mukerji ,197 ; Khan, 295
6	Sulphur (S) PPM	1.97	4.5	Thiobacillus spp. ; Gluconacetobacter spp.	Banerjee *et al* . 49 ; Divito and Sadras, 146 ; Cravo-Laureau *et al* ., 116
7	pH (H20)	6.6	6.8		Graham *et al.* ,208
8	Organic carbon	51.28	52.83		
9	Organic matter	89.74	92.46		
10	Soil organic matter (SOM)	88.2	90.87		
11	C/N	2:01	2:07		
	Micronutrients				
11	Molybdenum (Mo) PPM	1.21	1.25		Parker and Harris, 457
12	Boron (B) PPM	0.009	0.01	Pseudomonas putida	Patten and Glick, 461
13	Copper (Cu) PPM	2.43	2.5	Arbuscular mycorrhizal fungi (AMF) Bacillus spp	Liu *et al.* 340 ; Vlamakis *et al.* , 641
14	Manganese (Mn) PPM	39.62	40.8	Arbuscular mycorrhizal fungi (AMF)	Liu *et al.* .340 ; Singh *et al.* , 565.
15	Zinc (Zn) PPM	45.25	46.6	Acinetobacter spp ;Bacillus spp.	Triveni *et al* ., 622 ; Kohler *et al.* , 303 ; Yazdani and Pirdashti , 699
16	Iron (Fe) PPM	295.25	304.06	Geobacter metallireducens Pseudomonas putida	Lovley *et al* ., 349 ; Park et al . ; 456 ; Sharma *et al* . 550 ; Jones *et al* ., 275
	Physical Properties				
	State	Soild	Soild		
	Specific Gravity	0.018	0.015		
	Moisture content	10.26	10		
	Colour	Brown	Brown		
	Odour	Non			
	Essential Nutrients:				
	PGPR Volatile organic compounds (VOCs)	2, 3 butanediol	2, 3 butanediol	Pseudomonas corrugata	Lugtenberg *et al* ., 351 ; Trivedi and Sa . 621
	Rhizosphere micro-organisms	Volatile organic compounds (VOCs	Volatile organic compounds (VOCs	Bacillus spp. ; Pseudomonas putida	Zaidi *et al.*, 680 ; Diep and Hieu ,145
	Plant hormone	Auxins, Cytokinins (CKs),	Auxins, Cytokinins (CKs),	Bacillus subtilis ; Azospirillum spp	Ali *et al.* 16 ; Ferguson *et al.* ,171
	Phytoremediation practices	Gibberellins (GAs), and Ethylene (ET),	Gibberellins (GAs), and Ethylene (ET),	Acetobacter diazotrophicus,	Elshanshoury, 161
	Mineral imbalance and Salinity	IAA, abscisic acid (ABA) ; Glomalin	IAA, abscisic acid (ABA) ; Glomalin	Trichoderma spp. ; Arbuscular mycorrhizal fungi	Kohler *et al.* . 303 ; Miransari. 397 ; Rodrigues and Rodrigues , 504
	Biotic and abiotic stresses	Mediating interactions between metals	Mediating interactions between metals	Azospirillum lipoferum	Zheng *et al* ., 697
	Rhizobiaceae	Colonization and growth	Colonization and growth	Arbuscular mycorrhizal fungi (AMF) Bacillus spp	Miransari , 397 ; Filion *et al.* , 174
	Rhizobiaceae	Enhanced nodulation, dry weight of nodules,	Enhanced nodulation, dry weight of nodules,	Pseudomonas sp. and Bacillus sp	Graham *et al.*, 208 ; Kyei-Boahen, 117 ; Ricci *et al.*, 498
	Rhizobiaceae	Nitrogen fixation and yield	Nitrogen fixation and yield	Rhizobium spp.	Pulver *et al.* , 474 : Revellin *et al.* , 495
	Rhizobiaceae	Production of phytohormones	Production of phytohormones	Rhizobium spp.	Vance, 630 ; Cruz and Ishii, 112 ; Douds *et al.* , 149 ; Drouin *et al.*, 151
	LOCATION | Gateway Organic Fertiliser Plant, Abeokuta, (Latitude 7 degree 10`N, Longitude 3 degree 15`E) Ogun state, Nigeria				
1	Calculator for Plant nutrient - http://www.endmemo.com/sconvert/perg_kg.php				
2	Organic matter = 1.71 x organic carbon				
3	SOM= organic carbon x 1.72				

Table 6 | Biological and Physio-Chemical Analyses of Composition of Biofertilizer (Gateway) formulation and Production.



	Number of leaves	Plant height (cm)	Root dry weight (g)	Shoot dry weight (g)	Number of pods/plant	Seed weight/plant (g)	Pod weight/plant (g)
GF1	89	44.1	32.6	37.2	103	0.45	2.6
GF2	101	46.2	34.7	46.7	91	0.42	2.6
SF	83	45	30.5	42.7	77	0.37	2.4
CF	94	42.3	31.2	43.4	72	0.41	2.6

Fertilizer Treatments

GF1 GF2 SF CF

Figure 14 | Effect of sources of treatments application on the agronomic parameters of soybean at 8 WAP.

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Means with the same letter in columns are not significantly ($P < 0.05$) different according to Duncan's Multiple Range Test. GF1- Gateway fertilizer 1; GF2- Gateway fertilizer 2; SF- Sunshine fertilizer; CF- Chemical fertilizer; WAP-Weeks after planting.

Table 7 | Interaction of sources x rates of treatments application on agronomic parameters of soybean at 8 WAP.

Treatment	Number of leaves	Plant height (cm)	Root dry weight (g)	Shoot dry weight (g)	Number of pod/plant	Seed weight/plant (g)	Pod weight/plant (g)
GF1 0	74b	44.0abc	26.43	34.09	131a	0.41ab	2.6
GF1 5	97ab	41.2c	34.15	45.69	102abc	0.46ab	2.7
GFI 10	94ab	48.2ab	26.03	35.82	111abc	0.46ab	2.6
GFI 15	89ab	41.9bc	43.66	33.01	68bc	0.46ab	2.7
GF2 0	100ab	46.6abc	37.03	48.58	84abc	0.45ab	2.6
GF2 5	98ab	46.0abc	38.16	53.04	96abc	0.42ab	2.6
GF2 10	87ab	44.4abc	26.66	34.87	105abc	0.42ab	2.5
GF2 15	120a	48.7a	36.95	50.35	76bc	0.40ab	2.5
SF 0	74ab	41.5c	31.11	42.38	72bc	0.38ab	2.3
SF 5	83ab	45.7abc	26.63	38.22	94abc	0.41ab	2.5
SF 10	72ab	45.9abc	26.35	33.91	58c	0.37ab	2.5
SF 15	102ab	45.2abc	38.04	56.27	85abc	0.32b	2.3
CF	98ab	42.4abc	29.21	39.57	79bc	0.50a	2.7
			NS	NS			NS

Means with the same letter in columns are not significantly ($P<0.05$) different according to Duncan's multiple Range Test. GF1- Gateway biofertilizer 1; GF2- Gateway biofertilizer 2; SF- Sunshine fertilizer; CF- Chemical fertilizer; WAP- Weeks after planting.

Table 8 | Effect of sources of treatments application on nodulation and nitrogen fixation at 8 WAP.

Treatment	Number of nodules	Nodules dry weight (g)	Nitrogen fixed (%)
GF1	24	0.29	54.1
GF2	25	0.33	52.2
SF	22	0.22	40.1
CF	16	0.18	39.2
	NS	NS	

Means with the same letter in columns are not significantly ($P < 0.05$) different according to Duncan's Multiple Range Test GF1- Gateway Biofertilizer 1; GF2- Gateway biofertilizer 11; SF- Sunshine fertilizer; CF- Chemical fertilizer; WAP- Weeks after planting.

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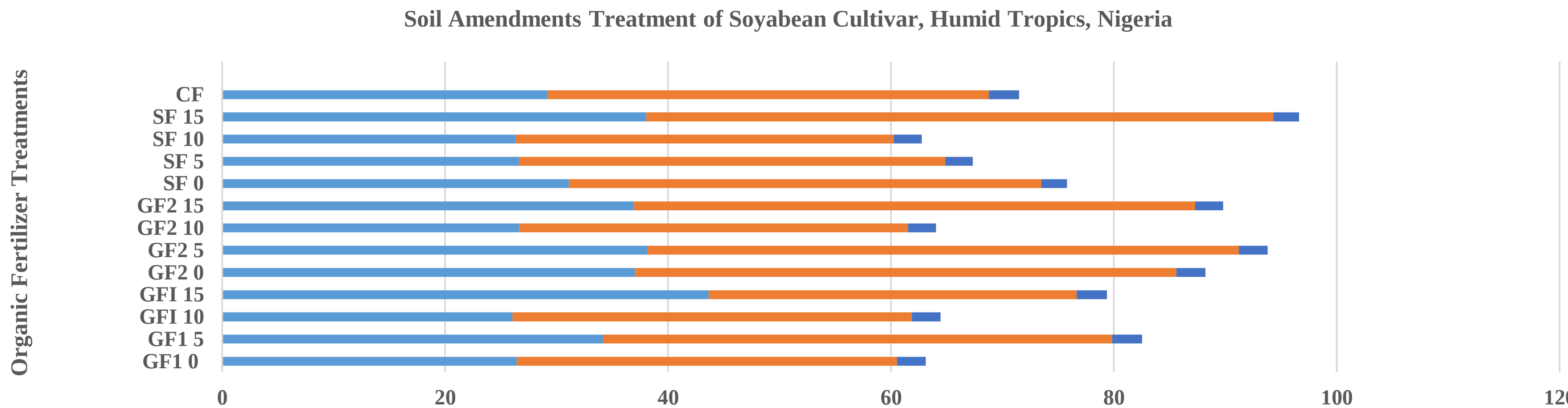

	GF1 0	GF1 5	GFI 10	GFI 15	GF2 0	GF2 5	GF2 10	GF2 15	SF 0	SF 5	SF 10	SF 15	CF	
Root dry weight (g)	26.43	34.15	26.03	43.66	37.03	38.16	26.66	36.95	31.11	26.63	26.35	38.04	29.21	0
Shoot dry weight (g)	34.09	45.69	35.82	33.01	48.58	53.04	34.87	50.35	42.38	38.22	33.91	56.27	39.57	0
Number of pod/plant	0	0	0	0	0	0	0	0	0	0	0	0	0	
Seed weight/plant (g)	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pod weight/plant (g)	2.6	2.7	2.6	2.7	2.6	2.6	2.5	2.5	2.3	2.5	2.5	2.3	2.7	0

Parameters

Root dry weight (g) Shoot dry weight (g) Number of pod/plant Seed weight/plant (g) Pod weight/plant (g)

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Figure 15 | Soybean vegetative growth parameters, nodulation, amount of nitrogen fixed, yield and yield components were determined at 8 weeks after planting.

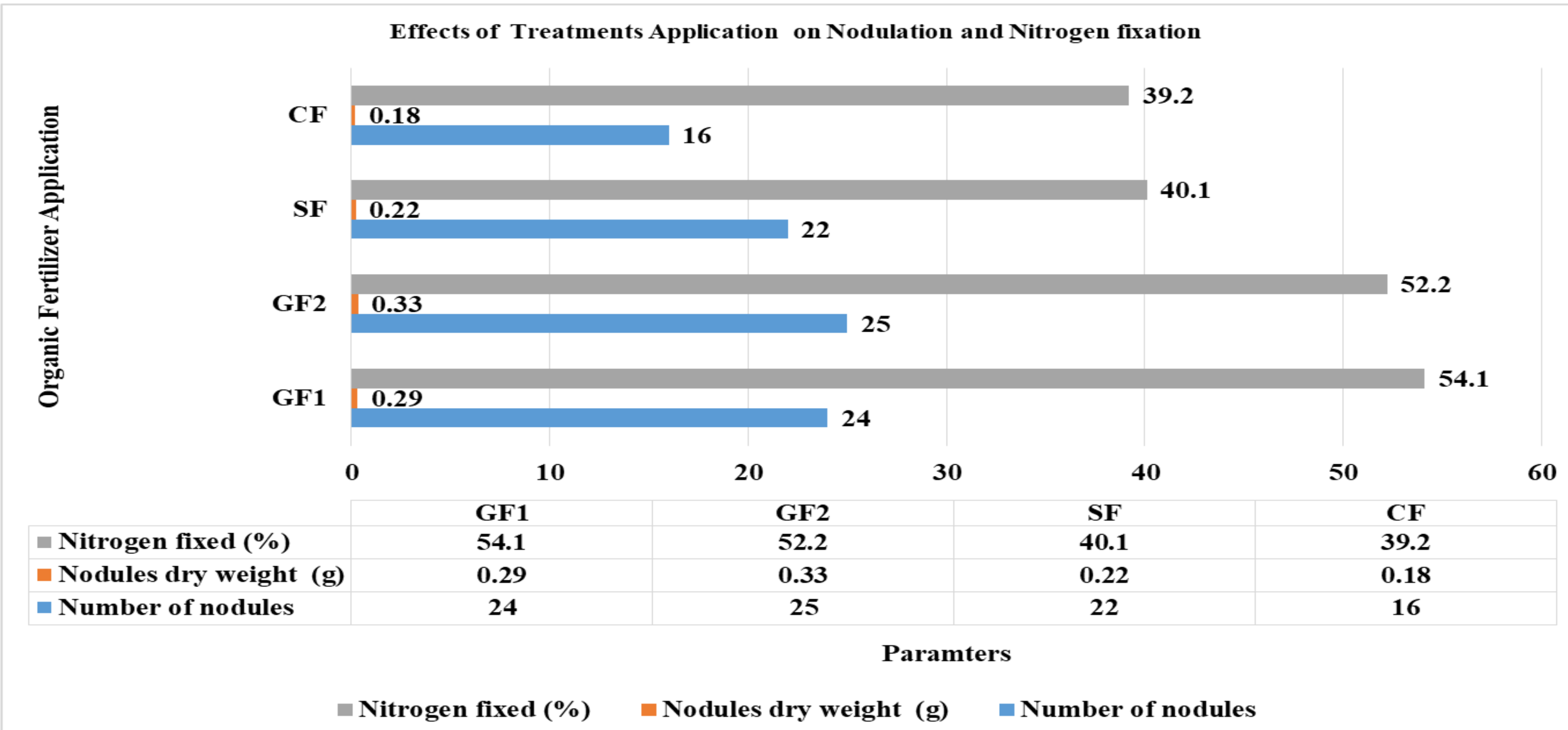

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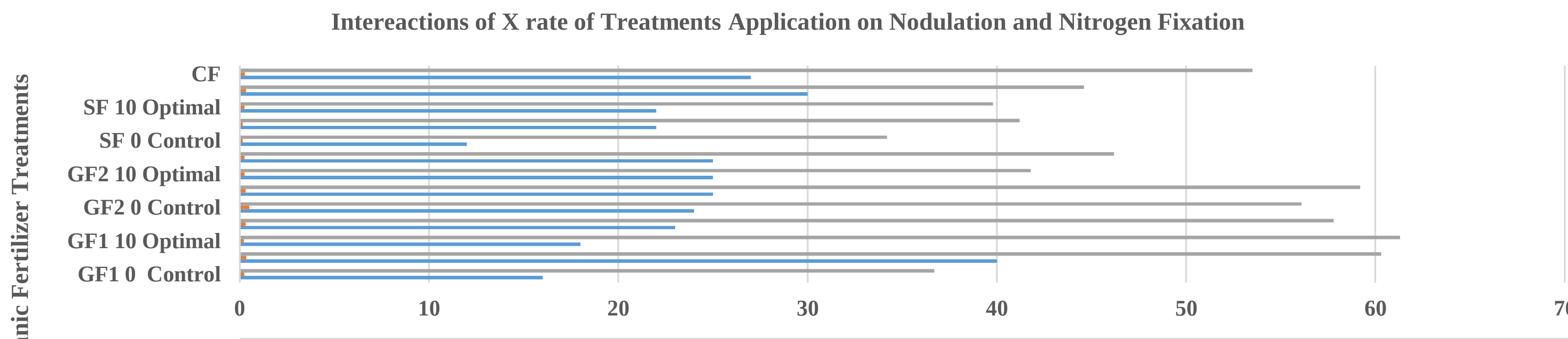
Figure 16 | Effect of sources of treatments application on nodulation of soybean at 8WAP.

BIOLOGICAL NITROGEN FIXATION (BNF) | Soybean cultivar (TGx 1440–1E)

Biological nitrogen fixation (BNF) remains one of the strategies for sustainable production of soybean in the transitory zone of Nigeria especially among the resource poor farmers. Effective BNF could be carried out in soybean if it adequately supplies assimilates to *bradyrhizobium* bacteria, serving as the substrate for organically produced protein, while the bacteria produces reducing agents through oxidative phosphorylation for effective assimilation of atmospheric nitrogen (Plates 2 and 3). A threshold growth of soybean must be attained



	GF1 0 Control	GF1 5 Minimum	GF1 10 Optimal	GF1 15 Overuse	GF2 0 Control	GF2 5 Minimum	GF2 10 Optimal	GF2 15 Overuse	SF 0 Control	SF 5 Minimum	SF 10 Optimal	SF 15 Overuse	CF
Nitrogen fixed (%)	36.7	60.3	61.3	57.8	56.1	59.2	41.8	46.2	34.2	41.2	39.8	44.6	53.5
Nodules dry weight(g)	0.24	0.35	0.23	0.32	0.5	0.32	0.26	0.25	0.16	0.16	0.25	0.33	0.27
Number of nodules	16	40	18	23	24	25	25	25	12	22	22	30	27

Paramters

Nitrogen fixed (%) Nodules dry weight(g) Number of nodules

Figure 17 | Number of nodules and nitrogen fixed (%) at 8 WAP.

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for survival and growth of symbiont (Table 2). This trial corroborated this findings, where application of different sources of fertilizer led to significant growth in soybean, particularly that of GF 2 (Table 7 and Figure 17). O'Hara,[430] reported had indicated nutritional constraints to soybean growth and bacterial activity, especially the essential macronutrients (N, P, K), Figure 20. It was in recognition of this, that a starter

dose of fertilizer is recommended to sustain the growth of soybean prior to the commencement of BNF.When chemical fertilizer is applied (during cultivation) it is ensure that, it is not applied to rhizospheric zone (Figure 18) close to root since it suppresses nodulation factor in soybean.[589] Comparatively higher concentration of nitrogen in GF 1 and GF 2 could have aided the sustenance of soybean growth at the beginning of its phenology (Plates 2 and 3, Figure 20).

Table 9 | Socioeconomic and policy constraints to the use of Rhizobium inoculation technology and possible intervention measures.

	Socioeconomic and policy	**Effect**	**References**	**Recommendations**
1	Limited farmer awareness	Low adoption and use of inoculants	Odame, 432 ; Woomer et al.,*661*	Private sector involvement
	of and access to inoculants	in farming systems	Funtowicz and Ravetz, 183	
2	Poor quality control of inoculants	Low viability of Rhizobium	Odame, 432 ; Walpola and Arunakumara, 647	Cold storage, use of modern technologies,
		inoculants and uncertain per-formance	Van Dam and Bouwmeester, 627	and more research
3	Lack of trained personnel	Limited awareness by farmersof the	Kannaiyan, 285 : Odame, 432	Raise farmer awareness about legume
		existence of BNF (in-cluding inoculants)	Woomer et al*.* , 661 ; Graham, 209	root nodules; familiarize farmers with Rhizobium inoculants
4	Fear over possible human	Limited adoption and use of	Hassani, 227	Involvement of farmers in the process
	and livestock health risks of	*Rhizobium* inoculants to increase	Tilman *et al.,* 612 ; Panke-Buisse *et al.* , 454	of development of inoculants; participa-
	inoculants by farmers	legume productivity	Youssef, 675 ; Philippot *et al* ., 465	tory approach
5	Absence of policy or weak	Forestalls widespread adoption	Macik *et al* ., 361	Include the issue of bio-fertilizers in
	policy support and insufficient	weak development of the	Vassilev *et al.* , 633 ; Pulver *et al* ., 474	governments' effort towards addressing
	biotechnological framework	production and marketing of inoculants	Otaiku 444 : Kinkel, 300	the problems of low and declining soil fertility
6	Limited scientific expertise,	Limited production of inoculants and	Brenner, 81 ; Kamst *et al.* , 283	Linkages between Universities in SSA
	applied BNF brain drain,	low quality inoculants	Walpola and Arunakumara, 647	with those in the North with expertise in
	and poor research funding		Wongphatcharachai,660 ; Cassman,96	Rhizobium science; government policy support

BNF is dependent on nodulation of soybean (Figure 18).[407] Adequate establishment of nodulation would occur if nodulation factor that ensures bacteria-host specificity are not suppressed. Proximity of nitrate in the root growing zone could compromise BNF. The fertility status of the soil with regard to nitrogen availability for this ecological zone together with the sustained releasing property of biofertilizer precluded the adverse effect of nitrogen on nodulation in soybean in this trial (Plate 3). One could assume that environmental factors could have accounted for the non-significant effect of different sources of organic fertilizer. It was reported that growth and survival of bradyrhizobium is dependent on temperature [573] soil pH [208,457] and soil water status. Similar report affirmed our results, trial was established in the late cropping

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season in the lowland. The prevalence of comparatively higher temperature and minimal precipitation could have reduced the activity of

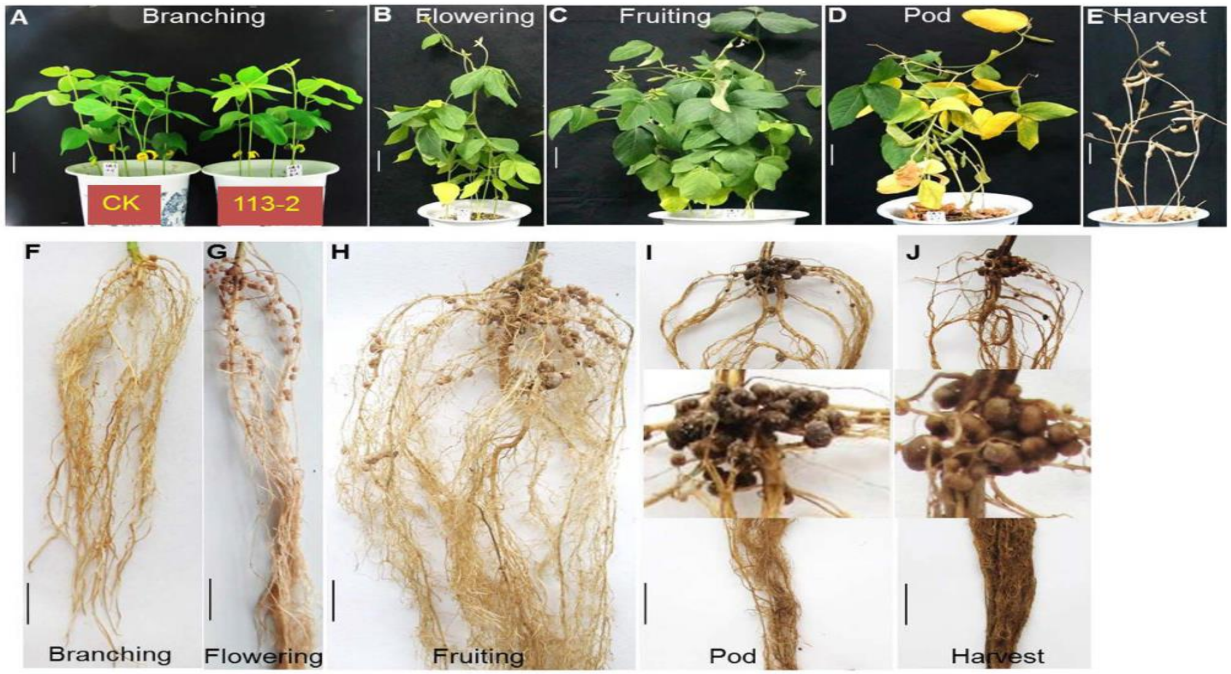

Figure 18 | Symbiotic phenotype features of five important soybean (*Glycine max*) inoculated with *Bradyrhizobium japonicum* strain developmental stages (A - E). Soybean growth at five important developmental stages, including the branching stage, flowering stage, fruiting stage, pod stage and harvest stage. (F- J) Nodulation phenotypes were examined at five developmental stages after inoculation with 113 - 2.(K)q. PCR analysis of the transcript levels of Lbc1 (Glyma 10g34280) at five developmental stages. Bars, 4cm (A, B); 5cm (C - E); 3cm (F - J); d, days and adapted from Yuan *et al*.,

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bradyrhizobium bacteria; a slow growing bacteria [160, 395] and consequently non-significant effect of biofertilizer sources on nodulation. Other factors adduced could be the insufficiency of nitrogen to sustain both bacteria and crop growth, resulting in the growth of soybean at the expense of bacteria growth and its effect on nodulation. The consequence of which might be that BNF might be inadequate to translate to higher grain weight/plant, since assimilates were being partitioned to sustain vegetative growth (Figure 19). Apart from the presumably nutritional and other environmental constraints that could be encountered by *bradyrhizobium* to ensure nitrogen fixation, it was observed that BNF declines with the development of soybean [683] Figure 18. Other option left would be remobilisation of nitrogen from vegetative structures for reproductive growth [524] provided sufficient vegetative growth was observed at the initial stage of soybean growth (Figure 20). Growth response of soybean to GF 2 at increasing application rates could have indicated the presence of other essential nutrients like P and K which were higher in it compared to other nutritional sources, which are necessary for rhizobia growth, nodulation and the metabolic process of nitrogen assimilation (Tables 2 and 6).

Senescence and Remobilization of Nutrients |

Avice *et al*.,[41]; Masclaux-Daubresse *et al*.,[378] research reported that the link between growth and the ageing process is nutritional in nature, by which resources (most of the time, only N is considered) are recycled from obsolete body parts to newly developing structures (Figure 19). Legumes research reports affirmed demonstrate that the N being remobilized from vegetative parts to fill seeds arises from a common N pool translocated throughout the plant.[536] When the N demand cannot be met by uptake from the rhizosphere alone (Figures 18 and 19), N, and potentially its associated nutrients, is withdrawn from older tissues (sequential senescence i.e., acropetal senescence occurring during the vegetative stage) the detailed catabolic pathways involved are not yet fully characterized. The mechanism of autophagy, consisting of the allocation of unnecessary or damaged cytosolic components (such as organelles and macromolecules) for degradation and recycling by the vacuole [393] has been recently characterized during senescence.Such a mechanism might explain how nutrients other than N may also be mobilized, as numerous proteins contain elements such as Zn, Cu, Mn, Fe or N (Figure 20). Scholars affirmed that drought-induced senescence is associated with numerous morphological, physiological and molecular modifications in several species including nutrient remobilization from senescing organs (mainly leaves, and to a much smaller extent nodules in legumes) to young tissues (leaves or seeds), thus compensating for the nutrient uptake deficit that results from low soil water content.[170,561]

The effect of drought specifically on nutrient remobilization from leaves need details research. [653] accelerated senescence under many types of stress, including drought, affects nutrient translocation processes, inducing the remobilization of N from vegetative to reproductive plant parts, and shortening the maturation time, which tends to favour proteins over starch accumulation in cereal grains (Figure 18). Decreased mineral content in seeds can therefore result from reduced root uptake and translocation and/or insufficient remobilization from leaves (Figure 19). In order to discriminate between experiments that used moderate (coupled with maintained yield) or severe drought (associated with a yield reduction) and associated mechanisms (Figure 20, balance between C and mineral nutrients transported to the seeds), In most species (except for soybean), a severe drought leads to an increased N concentration in grains which

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may be the result of a balance in favour of leaf N export relative to C, as opposed to a moderate drought that will less strongly affect photosynthesis.The seed contents of other macronutrients like P, K, Mg, and

sometimes Ca, are reduced most of the time (or remain unaffected) by severe drought (Figure 20) .The situation is more variable for Zn, Fe, Mn or Cu for which seed contents can be decreased or increased by severe or moderate drought, not only as a function of species (Table 2) and the requirements for soybean cultivation (Table 10). In a hypothesis that parallels the one for N content, seed micronutrient contents are a result of a balance between C export from leaves and starch deposition in grains, and micronutrient leaf remobilization (Figure 19).

SOYBEAN PEST MANAGEMENT

Soybean Cyst Nematode (SCN) |

Many farmers don't know their fields are infested with SCN. The effect of SCN on soybean yield is directly related to the numbers of nematodes (Plate 6) feeding on the root system. Seeing adult females on the roots of soybean plants is the quickest and most accurate way to diagnose SCN infestation in the field and resilience in the soil. Hence, the nematode can be managed to minimize SCN reproduction and maximize crop yields with the application of biofertilizer because of its biocontrol properties.[446] Growing non-host crops in rotation with SCN-resistant soybean varieties is the cornerstone for management of SCN. The life cycle of SCN has three major stages: egg, juvenile, and adult will requires slow release fertilizer (gateway biofertilizer) [446] to be able to eradicate SCN with life cycle of 4 weeks soil temperatures at or above 75° F. Atungwu *et al*, [38] reported using gateway organic fertilizer on herbivorous and non-parasitic nematodes associated with *telfairia occidentalis hook.*

Above-ground symptoms

Visible symptoms can include stunted plants, mid-season yellowing, and premature senescence. However, symptoms of an SCN infestation are not always visible above-ground. Injury usually is more severe in light, sandy soils and in dry growing seasons (Plate 6), but it also occurs in heavier soils and growing seasons with average to above-average rainfall. SCN damage is not always confined to smaller areas within a field. When fields are infested with SCN throughout, areas of stunted plants are not obvious.

Below-ground symptoms

Root symptoms of SCN often go unrecognized. It is difficult to recognize if roots are stunted and have fewer nodules unless they are compared to uninfected soybean plants. Symptoms of SCN infection include: dwarfed or stunted roots; fewer nitrogen-fixing nodules and increased susceptibility to other soil-borne plant pathogens. Inoculation with N-fixing bacteria (*Azospirillum* and *Azobacter)* allowed half-rate N-fertilizer application and increased sesame seed yield and oil quality.[438, 441] Similar effects were shown for *Azospirillum vinelandii* inoculated *Brassica carinata* cv. *Peela raya.*[426]

NUTRIENT MANAGEMENT

Nitrogen Use Efficiency (NUE) and Plant-Microbe Interactions

Scholars [204, 326] asserted that NUE for the N harvest index (NHI), defined as N in grain/total N uptake, is an important consideration in cereals. NHI reflects the grain protein content and thus the grain nutritional

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quality.[438, 441] Studies reported on identifying the genetic basis for grain composition showed that breeding progress has been limited by an apparent inverse genetic relationship between grain yield and protein or oil concentration in most cereals [560] oilseed rape [80, 260] It is possible, however, to identify wheat lines that have a higher grain protein content than predicted from the negative regression to grain yield.[278]

It has also been demonstrated that both grain yield and grain protein respond positively to supplemental N fertilizer, and such a paradox suggests that studying the interactive effect of genotype and N availability should provide insights into the genetic and physiological mechanisms that underline the negative yield–protein relationship in the future with the impacts of biofertilizer on Soybean cultivars in humid tropics.

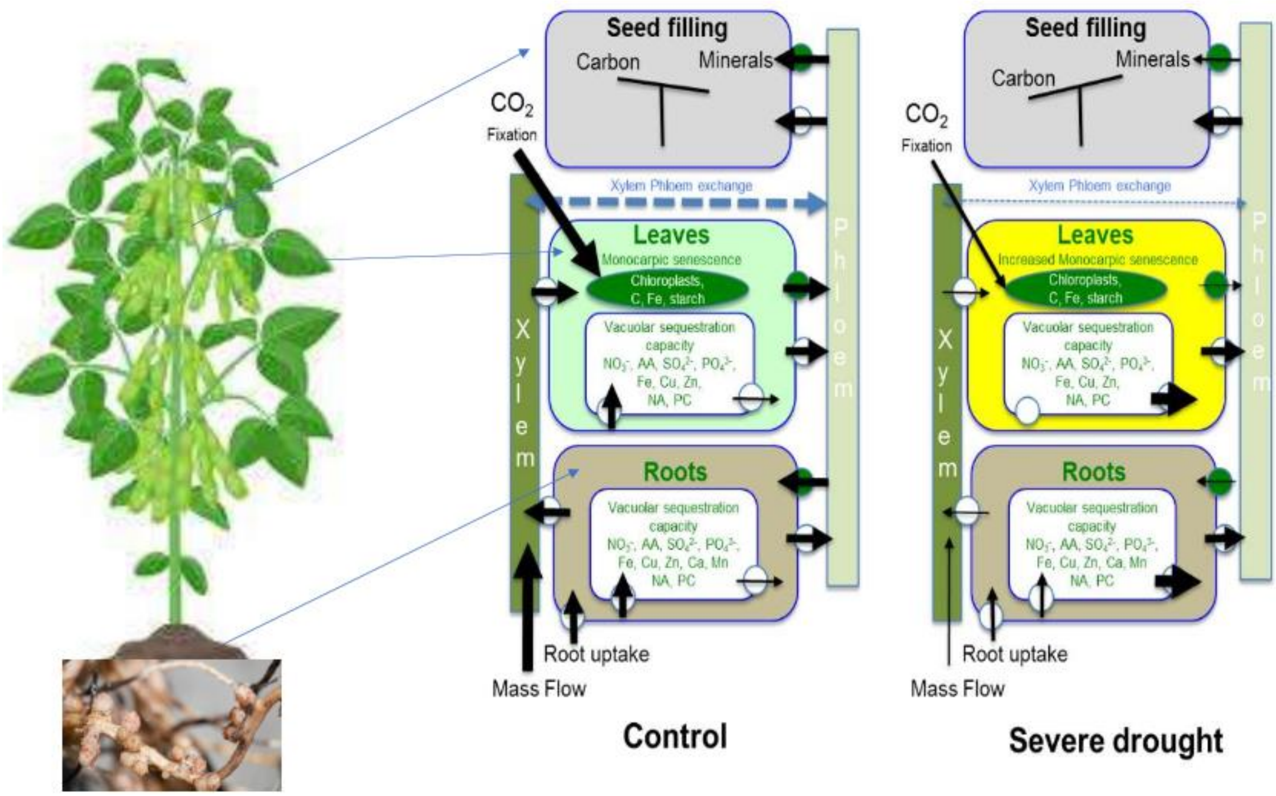

Figure 19 | Proposed model for leaf remobilization of macro and micronutrients towards seed filling under normal conditions or during severe drought illustrating the involvement of vacuolar sequestration capacity, coupled under severe drought with increased monocarpic senescence and reduced photosynthesis. NA: nicotianamide, PC: phytochelatin and adapted from Ourry *et al.*.[451]

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Plate 6 | https://soybean researchinfo.com/soybean -disease/soybean -cyst-nematode-scn/

Another aspect of grain filling in relation to N availability concerns the period before anthesis (Figure 18), which, for example in maize, is known to be critical for translocation of carbon assimilates and kernel set [423] in the N status of the plant around two weeks before anthesis appears to be a determinant for the number of kernels, since it is strongly dependent on the amount of N available during this period of plant development.[66] There is a paucity of data on both the physiological and molecular control of this process in relation to N availability and its translocation during this critical period of ear development.[544]

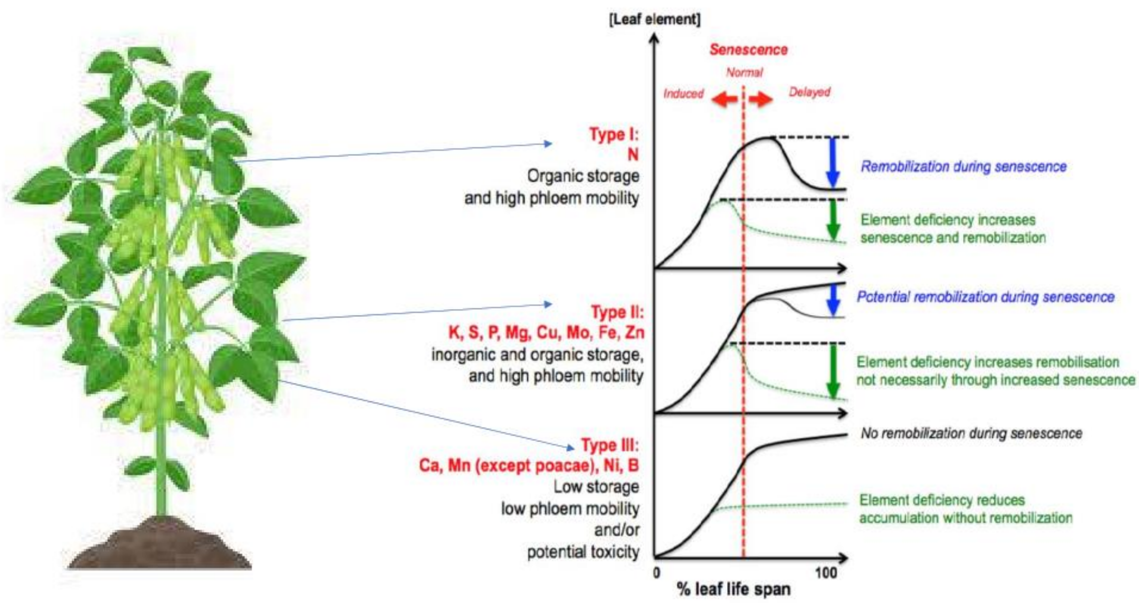

Figure 20 | General patterns of macro and micronutrient contents of leaves resulting from their allocation from root uptake before senescence followed by their remobilisation (net loss) as a function of relative leaf lifespan. According to the macro or micronutrient considered, senescence and/or nutrient deficiency induce high (Type I), variable (Type II) or low remobilization (Type III) from the leaves to the seeds and adapted from Ourry *et al*., [451]

Research on specific microbial strains key contributors to plant nutrition, or about how nutrient availability (Figure 20) affects the composition of the rhizospheric microbiome is an emerging new science.[263] Three mechanisms are usually put forward to explain how microbial activity can boost plant growth: (1) manipulating the hormonal signaling of plants (2) repelling or outcompeting pathogenic microbial strains [386] and (3) increasing the bioavailability of soil-borne nutrients. The growth of soil microbes is usually carbon-limited, so the high amounts of sugars, amino acids, and organic acids that plants deposit into the rhizosphere represent a valuable nutrition source.[45] How can we merge the progress in the individual areas of research to obtain an integrated view? The solution is trans-disciplinary research (Figure 21). Nitrogen-fixing rhizobia, where decades of research have endeavored to define the optimal inoculation practice, searching for the right combination of plant genotypes and rhizobia strains to suit specific climates and soils.[337] Regarding the taxonomy of nitrogen-fixing symbioses, it should be mentioned that nitrogenase genes are present in diverse bacterial *taxa* [214] and that non-leguminous plants have been documented to host N_2-fixing bacterial strains perhaps implying that other plant–microbe combinations (not just legumes and rhizobia) could be similarly optimized to promote nitrogen fixation.[414] Agriculture wicked problems

[445] requires a spatial polysingularity construct (Figure 5), to understand at which temporal and spatial scale the biological system operates. The temporal scale (soybean) is an intrinsic property of each biophysical process and therefore sets specific limits on the methodology and farm technique to apply. The spatial scale (nitrogen fixation) to investigate is chosen by the modeler based on the biological question to address, since we can chose to describe the same microbial community as a single metabolic unit or as a population of individual organisms (polysingularity). These choices are critical since they will determine at the same time the degree of complexity of the model and the requirements for the integration of experimental data[594] the in results of the field trials (Plates 2 and 3).

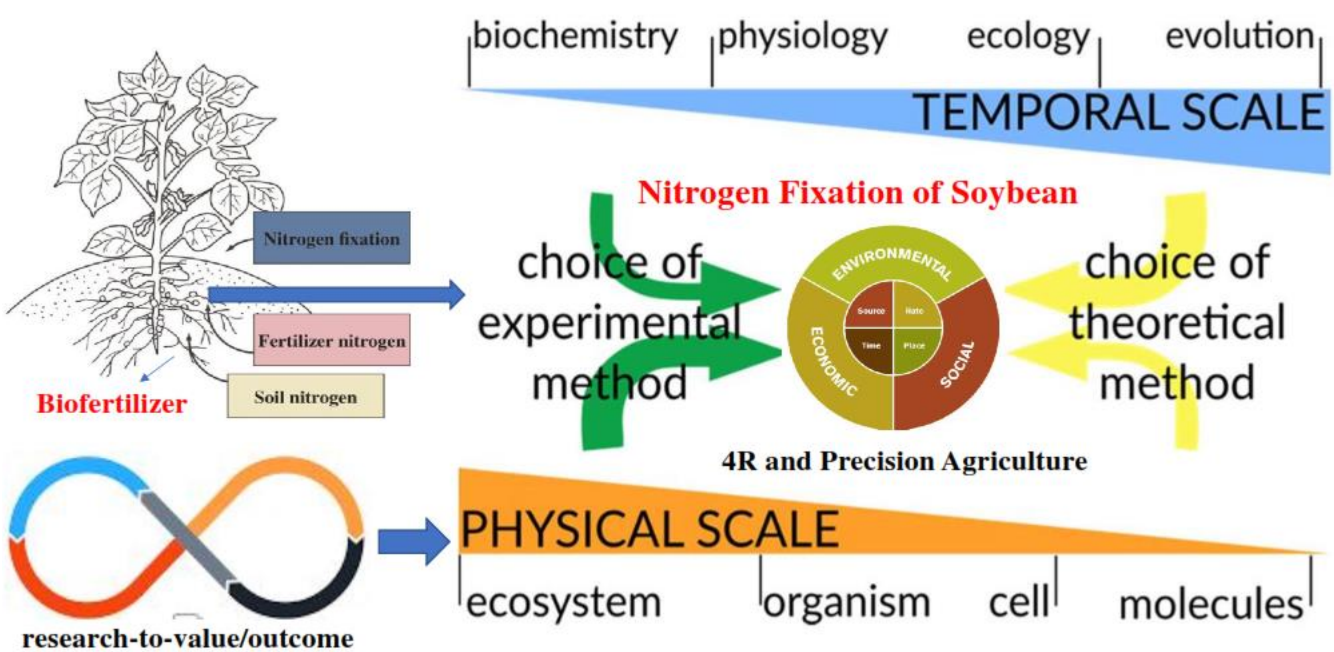

Figure 21 | Ohyama *et al*., [433] adapted three sources of Nitogen assimilated in soybean plants for the case study for combining modeling and experimental approaches (Figure 9). A major goal in biology is to integrate computational predictions with experimental data (Figure 25) to generate predictive models of biological systems. The temporal scale is phenomena under study (soybean), the choice of the physical scale of themodel/experiment is chosen by the scientist (nitrogen fixation). The physical scale will have a strong impact on the choice of experimental technique, while the temporal scale will mostly influence the experimental design (this is represented by thicker/thinner arrows) in trans-disciplinary approach (Figure 5). The experimental and theoretical methods have to be planned together to ensure that the reciprocal results are compatible and can be integrated (Figuire 7). This will allow further improvements (Figure 8) in both experimental design and model development adapted from Jacoby *et al.,* [63]

Photosynthesis and Nitrogen Use Efficiency |
N nutrition drives plant dry matter production through the control of both the leaf area index (LAI) and the amount of N per unit of leaf area called specific leaf N (SLN). There is therefore a tight relationship between N supply, leaf N distribution, and leaf photosynthesis and, as such, an effect on radiation use efficiency (RUE), to optimize light interception depending on N availability in individual plants or in the entire canopy.[190] The photosynthetic NUE (PNUE), which is dependent on the level of CO_2 saturation of

Rubisco, is another factor that needs to be taken into consideration when C_3 or C_4 crop species are studied. At low N availability, C_3 plants have a greater PNUE and NUE than C_4 plants, whereas at high N, the opposite is true.[521] Hence, identifying the regulatory elements controlling the balance between N allocation to maintain photosynthesis and the reallocation of the remobilized N to sink organs such as young developing leaves and seeds in C_3 and C_4 species is of major importance, particularly when N becomes limiting (Figure 19). However, the complexity of the ubiquitous role of the enzyme Rubisco in primary CO_2 assimilation,[365] in the photorespiratory process, and as a storage pool for N needs further investigation to optimize NUE and particularly PNUE under low fertilization input in both C_3 and C_4 species.[521, 324] The physiological impact of plant N accumulation with respect to an increased photosynthetic activity requires critical consideration as a supplemental investment of N in the photosynthetic machinery may be detrimental to the transfer of N to the grain and thus to final yield. The recent finding that the synthesis, turnover, and degradation of Rubisco are subjected to a complex interplay of regulation renews the concept of the importance of N use and recycling by the plant.[238]

Influence of nitrogen nutrition on plant development |
During plants growth, it is well known that N availability influences several developmental processes (Figure 19). According to the species, the number of leaves and their rate of appearance, the number of nodes [572, 362, 520] and the number of tillers [619] are reduced under N-limiting conditions and similar to the soybean trial where GF 1 had significantly ($P < 0.05$) higher number of pods and seed weight/plant with lower biological nitrogen fixation (Figure 17). In both in spring wheat [376] and in rice [362] grain number decreases under N deficiency conditions, a process occurring during the period bracketing anthesis, which is highly dependent on both the intensity and the duration of the N stress (Nitrogen use efficiency, grain composition, and grain filling, Figure 1). The availability of N for yield determination is also important through its direct influence on the sources (leaf area), and consequently the sinks (reproductive organs) accentuated. Hence, Figure 19, the reduction in photosynthesis of the canopy following N starvation is due to the reduction of the leaf area (radiation interception efficiency, RIE), rather than a decrease of RUE.[328]

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Soybean is the most important pulse crop in the world. The magnitude of soybean yield losses due to nutrient deficiency (Figure 20) also varies among the nutrients.[16] Deficiencies of N, P, Fe, B and S may cause soybean yield losses up to 10 %, 29-45 %, 22-90 %, 100 % and 16-30 %, respectively, depending on soil fertility, climate and soybean factors (Appendix 1, Table 10). Yield is also limited by nutrient toxicities, which are more common with micronutrients (Table 6). The broad-spectrum microbial inoculants Table 2 encourage the proliferation of beneficial introduced or indigenous microbial populations that facilitate nutrient uptake (rhizobia and mycorrhiza, Figure 3), promote plant growth directly, or suppress plant pathogens. Biofertilizer applied in the field trials create conditions most beneficial to plant growth by amending the soil, breeding or engineering better plants, by manipulating plant/microorganism interactions (Tables 2 and Table 6 ; Figure 27). Symptoms of nutrient deficiency vary with variety, growing conditions, and plant age. Similar symptoms may be caused by other abiotic or biotic stresses (Table 9). Scholars reported how to identify nutrient disorders observed in soybean.[172,389,118]

Biofertilizer Impacts on Soybean Cultivation

Inoculation of soybean with rhizobia inoculants (Table 2, Plates 2 and 3) helps to improve soybean yield with low stress risk and are cheaper than inorganic N fertilizers [511] Soybean is increasing in importance in many developing countries, where poverty limits fertilizer use (Table 9).[194 , 607] Giller [193]

reported, there are three situations where introduction of rhizobia are necessary to ensure effective nodulation and SNF (Figure 2): (1) in the absence of compatible rhizobia; (2) when there is a low population of compatible rhizobia resulting in slow nodulation; and/or (3) ineffective or less effective indigenous rhizobia than the selected inoculants for a particular legume host variety. Inoculants may be especially required when soybean is introduced into a new geographic area, as compatible rhizobia may not be available in the soil (Figure 3).

Inoculant Formulation |

It was found that a higher percentage of nodules were occupied with inoculant when applied as high titres on seed and soil compared to seed-only treatments or low titres on seed and soil, showing the importance of optimizing the inoculant formation (Table 1) and development (Figure 10). Combined application (Table 2) of different rhizobia strains into a single inoculant has also been shown to improve nodule occupancy compared to individual rhizobia.[436] Significantly ($P < 0.05$) higher number of leaves, plant height, higher number of pods and seed weight/plant was observed by gateway biofertilizer applied because host strain specificity soil rhizobia was considered during the biofertilizer development to nodulate under environmental stress compared other soil amendments.

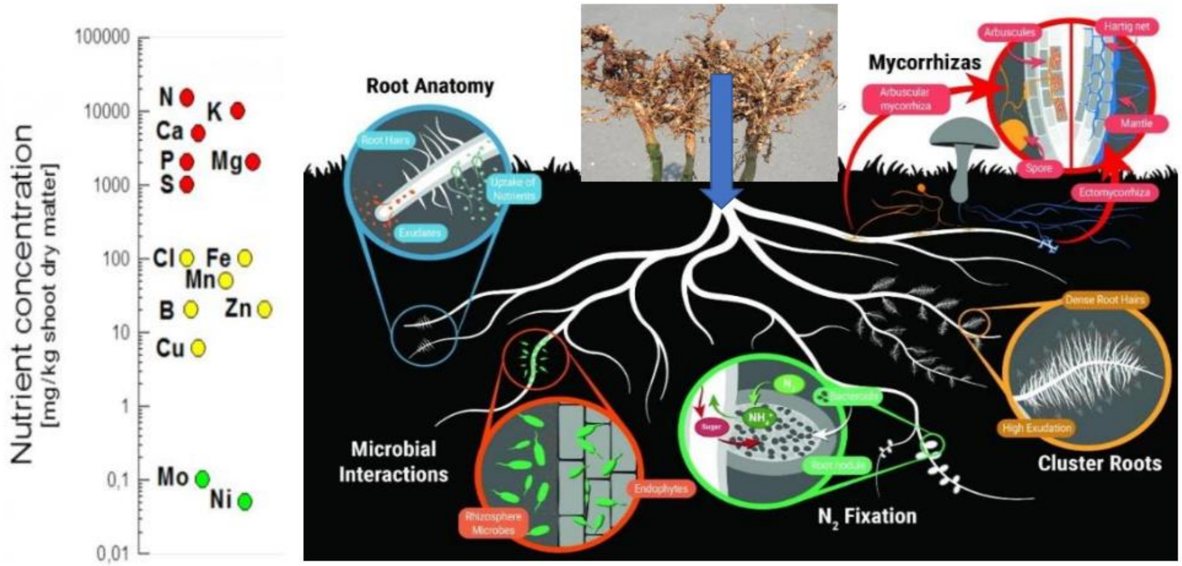

Figure 22 | Soybean co-inoculation of rhizobia and AMF as a number of studies report a synergistic effect on plant growth promotion. [663, 281]

Exploiting Rhizosphere Microbial Cooperation |

The plant and its leaf, root, and endophyte microbiota (Figure 26) make up the plant holobiont.[513] As for other eukaryote hosts, microbiota are major drivers of host evolution by contributing to their growth, behaviour, and adaptation to the environment (Figure 22). Inside the rhizosphere (Figure 26), the soil zone under the influence of plant roots, some root microbiota microorganisms, called plant growth-promoting rhizobacteria (PGPR), promote plant growth by improving plant nutrition (via siderophore production, phosphate solubilization, or nitrogen fixation), by modulating plant hormonal pathways (via auxin production, deamination of 1-aminocyclopropane-1- carboxylate), or by protecting plants against

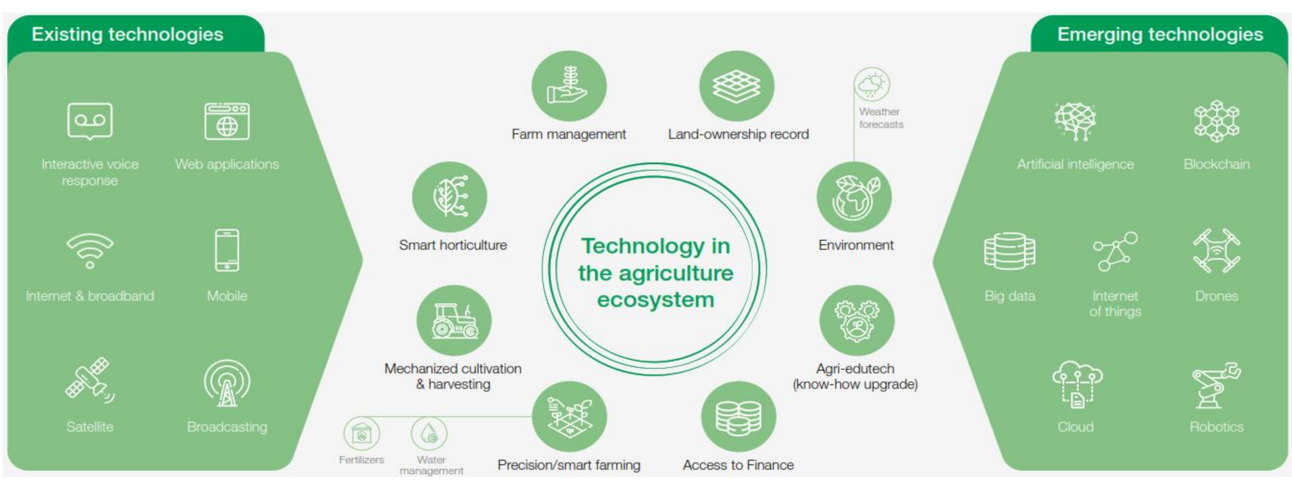

Figure 23 | Internet of things (IoT) - connected smart agriculture sensors enable the IoT and adapted from 2021 World Economic Forum.[662]

pathogens and parasites.[626] PGPR are defined as mycorrhiza-helper bacteria (MHB). In the rhizosphere, microorganisms benefit from 20% to 50% of photosynthetic carbon [220] directly transferred by plant roots or released through mycorrhizal hyphal networks.[279] In exchange for C input into the hyphosphere, *mycorrhizal fungi* (Figure 22) retrieve iron, nitrogen, or phosphate resources from the soil surrounding plant roots.[176, 273] The higher concentration of nitrogen in GF1 and GF 2 could have aided the sustenance of soybean growth at the beginning of its phenology promote plant growth directly, or suppress plant pathogens.

Nodulation and Nitrogen Fixation Correlation |
Scholars studies have reported poor correlation between nodulation parameters compared to grain yield/grain-N content.[248, 528,10,11] For example, in Nigeria, some inoculants significantly affected nodulation and nodule occupancy in soybean , but did not affect grain yield.[528] In Brazil, Hungria *et al.* [248] demonstrated that rhizobia inoculants affected grain yield, but did not influence nodulation parameters (nodule number, nodule dry weight and nodule occupancy). In southern Spain study, several introduced strains resulted in very low nodule occupancy (1-18%) under field conditions compared to indigenous *Bradyrhizobium,* yet surprisingly nodulation and N yield traits showed significant improvements.[10] In the field study conducted in Ethiopia, Argaw [29] found that nodule number and nodule dry weight of soybean were highly correlated with grain yield, especially with late maturing genotypes. In Nigeria soybean cultivar (TGx 1440–1E) trial where GF2 had significantly taller plant, which was not significantly different from GF 1 and SF. This is an indication that omission of phosphorus from soybean nutrition can drastically reduce shoot dry matter yield of soybean as suggested by Bekere *et al* .[64]

Stress Impact |
Nodulation is a luxury for a stressed soybean plant (Table 10). Nodulation is reduced in plants experiencing stress (Figure 18). [407] This likely helps the plant to conserve its resources for combating the stress and for all-important seed development. To date, a number of stress-related factors (Table 9) have been found to inhibit nodule formation locally in the root, including ethylene, salicylic acid and various reactive oxygen species.[171] Acidic soil conditions also reduce nodulation, with low pH also causing elevated soil Al^{3+} levels that negatively affect root growth.

Inoculants Adapted to the Local Environment |
Research studies suggested that locally adapted rhizobia strains are capable of performing better under environmental stress conditions (low/high temperature) [306] compared to introduced rhizobia [691, 477] Zhang *et al.* [691] showed that strains selected from a northern adapted climate were more effective under cool conditions, a finding that shows the benefits of testing strains under similar environmental conditions as their origin. In Iran, local rhizobia originally isolated from a high temperature environment resulted in greater soybean yield traits under hot field conditions compared to inoculants isolated from a moderate temperature environment. [477] Identification of thermotolerant soybean rhizobia strains are especially important in Okereke *et al*. [435] semi-arid regions, which have high soil and air temperatures, stresses that lead to poor nodulation and SNF. [247] Selection of adapted rhizobia strains (Table 2) for various environmental stress factors will enable higher SNF (Figure 2) and grain yield in soybean compared to the currently available inoculants.[247]

Effect of Soybean Genotype |

Several studies from around the world demonstrate that soybean survive after introduction to soil, nodulate plants under various soil conditions, and be compatible with farmer practices.[241] The N2Africa project which works across a diversity of field sites has observed that survival of rhizobia in inoculants depends on the carrier material (Table 3) as well the type of rhizobia strain (Table 1). Several studies from around the world demonstrate that sub-sequent to inoculation, traits such as nodulation, nodule occupancy, SNF and soybean yield can be affected by the soybean genotype [474, 126, 434, 10, 374] and strain-by genotype interactions.[488, 29, 699] Field study conducted in Nigeria, local soybean cultivars did not respond significantly to inoculants from the United States compared to U.S. bred cultivars [474] and the locally bred cultivars were more promiscuous than the U.S. bred cultivars in this study, plants were nodulated with local soil rhizobia strains rather than the foreign inoculants. Southern Spain study, the nodule occupancy of several introduced strains was dependent on the soybean genotype, again highlighting the importance of considering the host genotype for efficient SNF.[10] Zimmer *et al*. [699] reported that protein content and protein yield of soybean were significantly affected by inoculant-by-host genotype interactions and affirmed by a report on oil seed research with liquid biofertilizer with inoculant.[438, 441, 640]

Soil pH |

Youseif *et al.*,[674] reported that at least some soybean rhizobia can tolerate a wide range of soil pH conditions ranging from pH 5-11. Soil pH is a major factor that drives Br*adyrhizobium* survival.[195] During nodule occupancy by fast growing soybean rhizobia was greater under moderate pH conditions (6.8 -7.9) compared to acidic pH (5.1-5.4). [250, 667, 10] Based on rhizobia collected from nodules in Egypt, it was found that even some fast growing rhizobia can tolerate low soil pH (pH 4).[675] Soil pH favours or inhibits the distribution or population of soil rhizobia, which is highly dependent on the species.[335, 4, 660]

Soybean -compatible rhizobia in Soil fertility
(N, P, K) |

Soil N concentrations were shown to be significant factors affecting the survival, abundance, and diversity of soybean rhizobia in the soil case study (Table 5). Available soil N is shown to have either positive/negative or neutral effects on survival, abundance, and diversity of soybean inodulating rhizobia in soil.[666, 660] Nitrogen fertilization may not have significant effects on soybean rhizobia diversity (based on the Shannon diversity index) when a soil contains a limited rhizobia population and diversity.[234] Our meta-analysis of the soybean literature clearly showed that inoculants only succeeded when at least minimal soil N was present. Available soil P has been reported to be one of the possible determinants of the geographic distribution of soybean rhizobia.[223] Based on canonical correspondence analysis (CCA) of 12 soybean nodulating rhizobia groups in China, it was observed that available P in soil influenced soybean rhizobia diversity compared to the available N, potassium, and organic matter.[335] Scholar's studies reported, available soil P was shown to have a slight influence on the distribution of soil rhizobia.[666] Available soil K is shown to have a weak impact on soybean rhizobia diversity and abundance in soil.[335] Yan *et al*.,[666] and affirmed by the biofertilizer formulation for the soybean field application (Plates 2,and 3 and Table 6).

Application of Fungicides, Herbicides and Insecticides |
Insecticides, herbicides, and fungicides can have negative effects on soybean rhizobia in soil. Based on 122 rhizobia strains tested for tolerance against different agrochemicals, it was observed that rhizobia were least tolerant to fungicides, followed by herbicides and then insecticides.[151] Application of pesticides can have negative effects on soil rhizobia diversity, their activity, and plant-microbial interactions.[179, 252] Seed treatment [318] with fungicides is a common practice, but it can negatively affect soil rhizobia populations in soybean fields, resulting in low SNF and yield reduction.[495, 91] The negative effects of fungicide-treated seeds on rhizobia were even greater in fields where rhizobia were newly introduced. The type of fungicide used has also been shown to be important. Studies reported, that the fungicide Captan had less influence on native soybean rhizobia compared to Carbendazim, when applied as seed treatments.[288]

Carbendazim is a biochemically specific inhibitor and persists in soil compared to Captan. A study reported, that seeds treated with Mancozeb reduced the survival of *B. japonicum* on seeds compared to a mixture of Carbendazim and Thiram fungicides, resulting in poor nodulation.[377] Quinolate Pro (carbendazim and oxine copper), Vitavax 200 FF (carboxin and thiram), and Monceren (pencycuron) were also shown to be compatible with soybean seed inoculation, whereas Germipro UFB (carbendazim and iprodione), Apron 35J (metalaxyl), and Tachigaren (hymexazol) negatively affected soybean rhizobia survival and nodulation [495] and thus were not compatible with soybean seed inoculation. Similarly, nodulation and nodule activity were also influenced by the type of pesticide used.[368, 678] The concentration of fungicides is a critical factor as seeds treated with fungicides at high concentrations negatively affect rhizobia and SNF.[368] In a study reported, reduced nodulation and nitrogenase activity was reported above 100 $mgml^{-}$ of Thiram as a seed treatment.[74] Hence, special attention has to be paid when soybean seeds are treated with different fungicides in order to minimize negative effects on nodulation, SNF and the soil rhizobia population (Figure 2). Interestingly, it was found that some fungicides, which were applied as either a seed treatment (thiophanate-methyl) [319] or foliar application (pyraclostrobin) [276] increased the number and activity of soybean nodules.

Research Framework | Smart Agriculture
Spatial polysingularity theory is the impacts of human mind creation with spatial objectivation (actualization of the subject or materialize of values and judgments) beyond intended nor foresaw consequences in cyberspace-time continuum or non-linear dynamics for value.
It is the dynamic process leading to improved outcomes and constant improvement on the subject via spatialization (Figures 5 and 23).

Scenario |
Design of a sensor network [687] which connects agriculture and internet of things (IoT) can be established among agricultural experts, farmers and crops regardless of their geographic locations [356] and this system offers considerable reliability, interoperability, low cost, management and monitoring and control system model based on IoT (Figures 23 and 25). IoTs, with its real-time, accurate and shared characteristics, will bring great changes to the agricultural supply chain and provide a critical technology for establishing a smooth flow of agricultural logistics.[664] Sensors and Radio Frequency Identification (RFID) chips aids to recognize the diseases occurred in plants and crops. RFID tags send the EPC (information) to the

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reader and are shared across the internet (Figure 25). The farmer or scientist can access this information from a remote place and take necessary actions (Figure 24). Automatically, crops can be protected from coming diseases.[134] Some of the most outstanding technologies (Figure 23) that are combined with IoT to develop agricultural solutions are wireless sensor networks, cloud computing, middleware systems, and mobile applications.[159, 523] The trans-disciplinary research construct (spatial polysingularity) encapsulate the IoT-based agro-industrial and environmental applications (Figure 25) and value streams namely execution, collaboration , converged technologies, monitoring, control, logistics, prediction and actionability for valuecreation in agriculture illumination Agriculture 6.0 [artificial intelligence and regenerative agriculture].Tzounis *et al.* [624] presented IoT technologies and their utility in agriculture, as well as their value to future farmers. The scenarios that can be studies instantly and real time are:

Fertilization - The most commonly used fertilizers in agriculture contain the three primary plant nutrients: nitrogen, phosphorus, and potassium. Smart fertilization system (Figure 24) based on IoT and artificial intelligence (AI) and designed the NPK sensor to integrate the colorimetric mechanism by using Light Dependent Resistor (LDR) and Light Emitting Diodes (LEDs based on fuzzy rule-based system to analyze measured data (Figure 25) and to determine proportions of N, P, and K in the soil (Figure 20).

Pest Control - Sensors can collect data automatically, such as the presence of a pest, or a trap trigger that indicates that a pest has been captured [677] and proposed an intelligent high-resolution model for pest detection. Results showed that the proposed method greatly improved the recall rate, reaching 202.06%.

Herbicides application - Arakeri *et al.,*[27]reported a system based on IoT, image processing, and machine learning to identify weeds and to selectively spray the right amount of herbicides. Self-driving farm robot [676] uses lasers to kill 100,000 weeds an hour, saving land and farmers from toxic herbicides. But not weeding will cost half crop, killing profitability.

Monitoring and Evaluation - Kurihara *et al.,*[314] reported the technical details and functionality of the UAV based hyper-spectral imaging system and high quality hyperspectral imaging dataset. In the field monitoring, sensors in the field collect data and transmit them to the processing centre, which uses the

corresponding software applications to analyze the operating data (Figure 24) using GPS controlled robot system for remote monitoring and control of field data and field activities.There is no single universal architecture of the IoT applications, and different researchers have proposed various architectures (Figure 25)[36] and agricultural IoT-based applications.[282, 313, 415, 624]

Human Capital |

The average age of farmers in the last decades has been alarmingly increasing around 58 years old in the USA and Europe, 60 years in sub-Saharan Africa, or 63 years in Japan [164] and about to change now with European policies, for example, are being set to support a generational renewal, facilitating access to initial investment, loans, business advice, and training.[164] A generational renewal in a rural development context goes beyond a reduction in the average age of farmers; now empowering a new generation of highly qualified young farmers to bring the full benefits of technology in order to support sustainable

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farming practices.[453] Market analysis report that, the factors that would facilitate the adoption of sustainable farming technologies include better education and training of farmers, sharing of information, easy availability of financial resources, and increasing consumer demand for organic food.[210] Agriculture 5.0, deep training needs [450] to be delivered to users, ideally young farmers eager to learn and apply modern technologies to agriculture and granting a generational renewal still to come.

Infrastructure |

From Figure 5, IoT influences and drives agriculture [624] to generate such a big amount of valuable information (Figure 23).[682] Agricultural robotics vehicles have four main capabilities when performing agricultural tasks: detection, guidance, mapping, and action.[103] Agricultural robotics research covers a wide range of applications, from automated harvesting [57] weed management and control [589] autonomous spraying for pest control [203] environmental conditions monitoring, and animals health helping improve operational reliability while enhancing soil health and productivity. Diedrichs *et al*., [144] established a prediction engine as part of an IoT compatible frost prediction system that gathers environmental data to predict frost events (Figure 25).

Agriculture 5.0 implies the use of robots and some forms of artificial intelligence (AI) for precision agriculture principles and using equipment that involves unmanned operations and autonomous decision support systems.[682] Agriculture is currently developing robotic systems to work in the field and help producers with tedious tasks [61, 69, 549] and robotic applications for agriculture are growing exponentially[549] which others promising solutions for smart farming and profitability where the cost of technology decreases with time, and agricultural robots will be surely implemented in the future as the alternative to bring about higher production [210] where venture capital funding in AI has increased by 450% in the last 5 years. [413] Food and Agriculture Organization of the United Nations (FAO) estimates that, in 2050, there will be a world population of 9.6 billion.[692]

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Stakeholders (S) | Collaborate

One of the best thing about design thinking (Figure 7) is that, it thrives on collaboration (Figure 5). Collaborate with the stakeholders and use various brainstorming techniques to build on each other's ideas (Figure 21). Human Capital [brainstorm to reach to quantity and encourage wild ideas. In fact, it is important to move beyond current understanding of the problem views and solutions]. Use Scenarios [prototypes to represent the problems in a catchy way which can fuel ideas generation and execution for value co-creation].Take feedback on each solution that have been built and continuously iterate it until you find an optimal solution (Figure 6). Agriculture problems are difficult to define [445] and address, a visual explanation of the problem can also help to gain a wider view of the problem (Figure 8). To achieve a visual understanding, several methods and tools [Infrastructure] can be used for example stakeholder's map, customer-journey map, and rapid personas, internet, internet of things (IoTs), Figure 25.

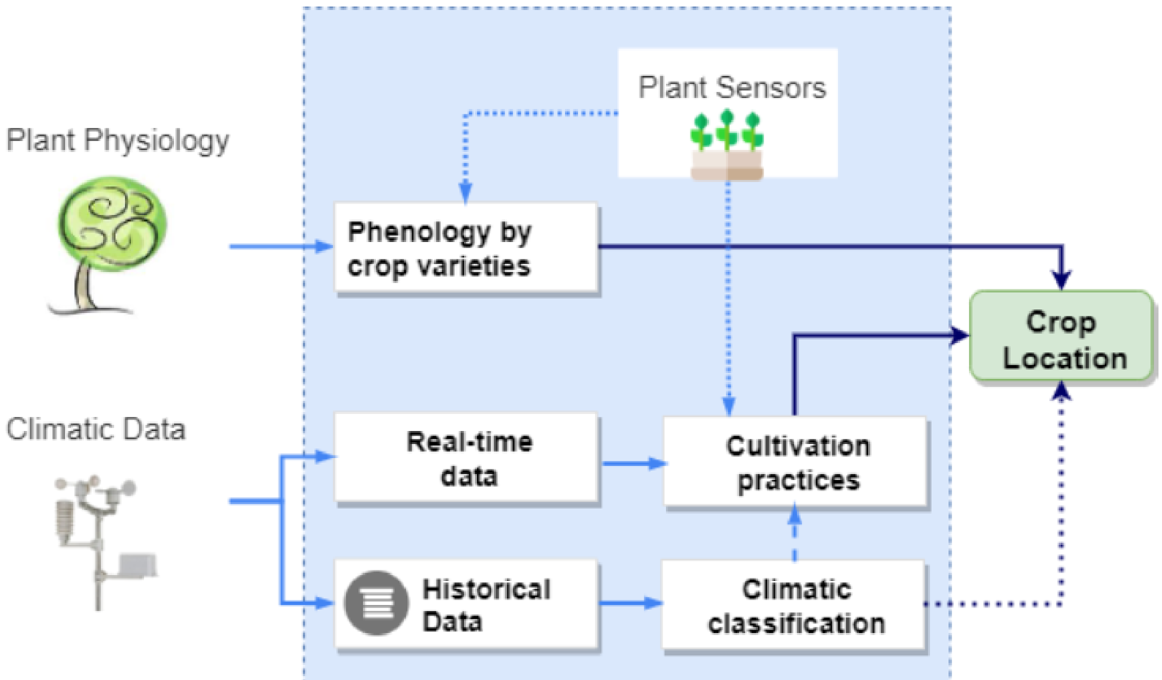

Figure 24 | The architecture for managing a spatial data infrastructure to create, collect, and analyze heterogeneous geospatial value sets from multiple sources and time series using web services accentuate in Figure 5.[346] Demonstrates that there are various possibilities to integrate statistical modeling techniques and spatio-temporal data for area-specific crop management (Figure 2).

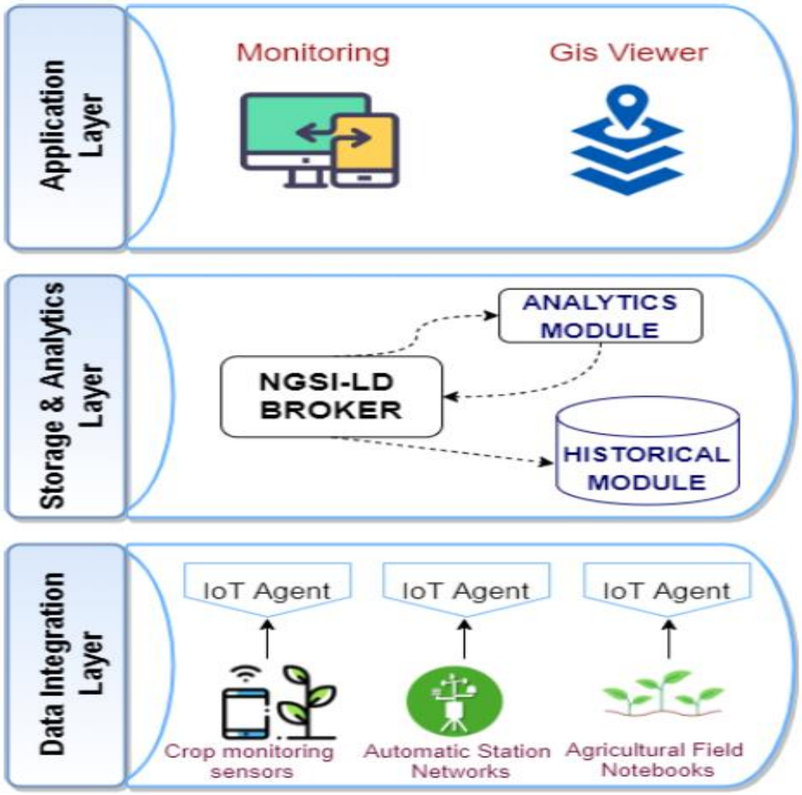

Figure 25 | The architecture of IoT architecture and how to improve decision-making in the agricultural industry and help the sector's digital transformation and adapted from López-Morales *et al.*,[346]

Nitrogen Fixation Soil Microbiome |
Biological nitrogen fixation (BNF) is one of the most important phenomena occurring in nature, only exceeded by photosynthesis.[365, 630] One of the most common limiting factors in plant growth is the availability of organic matter (Figire 13). Predominantly, members of the plant family Leguminosae have evolved with nitrogen fixing bacteria from the family *Rhizobiaceae*. In summary, the plants excrete specific chemical signals to attract the nitrogen fixing bacteria towards their roots. They also give the bacteria access to their roots, allowing them to colonize and reside in the root nodules, where the modified bacteria (asteroid's) can perform nitrogen fixation (Figure 22).The efficiency of BNF depends on climatic factors such as temperature and photoperiod [554] the effectiveness of a given soybean cultivar in fixing atmospheric nitrogen depends on the interaction (Figure 26) between the cultivar's genome and conditions such as soil moisture and soil nutrient availability [583, 277] and the competitiveness of the bacterial strains available (Table 1), relative to indigenous and less effective strains, plus the amount and type of inoculants applied (Table 2), and interactions with other, possibly antagonistic, agrochemicals that are used in crop protection.[90] The selection of an appropriate strain of *B. japonicum* since specific strains can be very specific to soybean cultivar, and subject to influence by specific edaphic factors [243,17] or application of broad spectrum inoculants (Table 2). Soybean meets 50-60% of its nitrogen demand through BNF, but it can provide 100% from Nitrogen fixation. [524] *B. japonicum*, is a gram negative, rod shaped nitrogen fixing member of the rhizobia and is an N_2-fixing symbiont of soybean .[279]

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The first step in rhizobial establishment in plant roots is production of isoflavonoids as plant-to-bacterial signals; the most common in the soybean - *B. japonicum* symbiosis being genestin and diadzein [485] which trigger the nod genes in the bacteria which, in turn, produce Nod factors, that act as return signals to the plants and start the process of root hair curling, leading to nodule formation (Figure 18). The nod-DABCIJ genes, conserved in all nodulating rhizobia [582, 283] are organized as a transcriptional unit and regulated by plant-to-rhizobia signals such isoflavanoids.[92,539,540] Nodulation and subsequent nitrogen fixation are affected by environmental factors. It has been observed that, under sub-optimal root zone temperatures (for soybean 15-17 ºC), pH stress and in the presence of nitrogen, isoflavanoid signal levels are reduced; while high temperature (39 ºC) increases non-specific isoflavanoid production and reduces nod gene activation, thereby affecting nodulation.[44] Seed germination and seedling establishment is enhanced in soybean , common bean, maize, rice, canola, apple and grapes, accompanied by increased photosynthetic rates,[689] foliar application to green-house grown maize resulted in increases in photosynthetic rate, leaf area and dry matter.[297] Soybean is very sensitive to Chlorine, but not greatly affected by Na^+, because of its ability to restrict movement of Na^+ to leaves (Figure 19) and affirmed by Dabuxilatu and Ikeda.[124] Apart from *B. japonicum*, which produces nod factor, other rhizobacteria, such as *Bacillus thuringiensis* NEB17 reside in the rhizosphere of higher plants, [211] forming a phyto-microbiome, much like the human microbiome, now realized to be so important in human health. [299] *Bacillus thuringiensis* NEB17 is symbiotic with *B. japonicum*, produce bacteriocins. *Bacillus* species were first reported to produce bacteriocins in 1976. The low-molecular-weight bacteriocins of gram-positive bacteria have bactericidal activity, mainly against certain other gram-positive bacteria.[598] Bacteriocins are ribosomally produced peptides which affect the growth of related bacterial species. The most studied bacteriocin is colicin, produced by members of the *Enterobacteriaceae.* [473] Due to their commercial importance as natural preservatives and as therapeutic agents against pathogenic bacteria, these antimicrobial peptides have been a major area of scientific research.[598 , 259] Initially, attached to the root-hair tips of soybean plants, rhizobia colonize within the roots and are eventually localized within symbiosomes, surrounded by plant membrane (Figure 26). This symbiotic relationship provides a safe niche and a constant carbon source for the bacteria while the plant derives the benefits of bacterial nitrogen fixation, which allows for the use of readily available nitrogen for plant growth.

Nodulation Effectiveness and Promiscuous Soybean

The inoculation with *bradyrhizobia* is not used by farmers in most soy bean cultivation. Promiscuity defined (i.e. the ability to soybean to nodulate with a wide range of indigenous *bradyrhizobia*) and ecological aspects and on the quantitative limitations of the soil populations concerning whether the restricted range of suitable strains in the soil are sufficient to form ancient nodules on soybean .In the inoculants used for the biofertilizer production electiveness of the strains was addressed (Table 2). Research reports suggests that responses to inoculation are possible in soybean cultivation [440 ,527] in soybean growing areas of the moist Savanna in Nigeria. *B. japonicum* (IRj) strains obtained from soybean, all isolated in 1979 from Ibadan (derived Savanna) in Nigeria. Kueneman *et al.*, [307] reported for selection of promiscuous soybean genotypes, that is, 'the promiscuous soybean crop should be grown by farmers in the growing area without b*radyrhizobial* inoculants" which did not respond either to N fertilizer nor to *bradyrhizobial* inoculation became the line of choice for farmers was reported. The

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soybean genotypes was able to meet its N requirements through the indigenous rhizobial community.

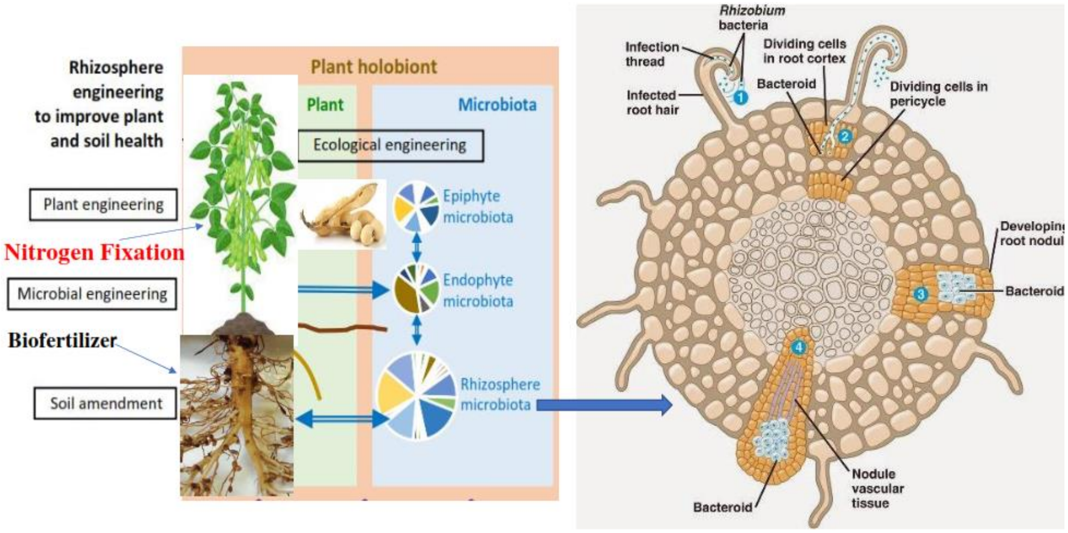

Figure 26 | Rhizobia then invade the root through the hair tip where they induce the formation of an infection thread as shown in This thread is constructed by the root cells and not the bacteria and is formed only in response to infection. The infection thread develops from the inner primary cell wall, which grows inwards in the form of invagination enclosing bacterial cells.

Promiscuity' in the strict sense, only indicates the ability to form nodules with a wide range of bradyrhizobia and does not imply elective nodulation.[307] In IITA (Nigeria) soybean program were healthy in low-N soils and had presumably nodulated effectively with local bradyrhizobia. Observations of nodulation are made afterwards and further selection was made on the basis of nodule mass. The lack of correlation between nodule scores and symbiotic effectiveness is the weakness of the present criteria used by breeders for selection (field data architecture, need to be redefined based on Figure 25 for future research). Eaglesham [153] stated that, it may be safer to rely on effective inoculant strains than to breed for the ability to nodulate with indigenous strains of unknown potential (Table 2). The nitrate considerably reduced root-hair curling of *Medicago sativa* [610] and inhibited root-hair curling and formation of infection threads of soybean.The abortion of infection by N fertilizers could have a direct effect on the formation of root nodules [412] and the nodule number was reduced more after the combined N was added at early stages of nodulation (Figure 19). The inoculant in the study area, biofertilizer formulation has no *Bradyrhizobium japonicum* stains (Table 2 and Table 6, respectively).

Yatazawa and Yoshida [667] found that the inhibitory effect of nitrate on the nodulation of soybean was more severe when applied early during growth season.The reduced nodule number following application of combined N was attributed to a modification of rhizosphere (Figure 22) conditions prior to infection. Nitrate was shown to change the root cell wall composition or to reduce bacterial attachment and invasion to root hairs. [553] These effects consequently reduce nodulation (Figure 18) and affirmed the trial

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study where the percentage nitrogen fixed increased significantly ($P < 0.05$) with application rates in GF1, which was not observed in others (Figure 19). Kwon and Beevers [316] found that in plants which had developed nodules in the absence of nitrate, the addition of 9 mmol/L nitrate led to inhibition of further nodule growth. Gibson and Harper [192] reported that the concentration of nitrate surrounding the roots appeared to be most important in the inhibition of nodule initiation whereas assimilated nitrate appeared to have a greater effect on nodule development. Latimore *et al.,*[323] concluded that Nitrate inhibition of nodule development and impairment of the activity of developed nodules was frequently attributed to photosynthate deprivation of the nodules due to the priority of use of saccharides in Nitrate assimilation and growth. The increase in nodule mass (GF1) observed with low levels of N fertilizer could be attributed to their decreased numbers, that is, fewer nodules with less competition for photosynthates (Plates 2 and 3).

The sensitivity of nodule development (Figures 19 and 22)) to N fertilizers appears to be a general phenomenon in soybean. Abdel-Samae[1] similarly found that application of urea to field-grown soybean had a suppressive effect on the nodule number and dry mass confirm the NPK application in the field study (Tables 7 and 8). Reduction in total nodule mass upon fertilizer application may be attributed to the restriction of primary root nodulation (Figure 18), a result consistent with the report by Dart and Wildon.[128] Da Silva *et al*., [123] concluded that N added at low rates at the time of sowing stimulated nodule mass and, to a lesser extent, the nodule number (Table 8). When N was added after emergence, both method and rate affected nodulation patterns (Figures 15, and 16). Thus, foliar application of N fertilizers was less suppressive to nodulation and nitrogenase activity even at higher N levels than soil treatments.[185] Report by Wolyn *et al*., [659] also indicated that the deleterious effects of N fertilizer on nodulation in secondary roots of common bean and soybean could be alleviated by foliar application. Olowe *et al*.,[458] report similar results on the application of foliar liquid biofertilizer to sunflower cultivation with improved oil seed quality.

Integrated Plant Nutrient Management System |

Secretion, quantitatively and qualitatively, of organic acids by beneficial microorganisms is mainly gene-dependent but could also be influenced by the ecosystem environmental properties.[696] For example, N and C soil content may have a direct impact on the nature of the organic acids produced, the nature of C source could affect the bio-solubilization process (Table 6 and Figure 13), and high C/P ratio seems to increase the production of organic acids (Table 8) while both C/N and N/P may affect the organic acid implication in P solubilization is often attributed to lowering the pH and cations chelating properties.[697, 63] The acidification of microbial cells perimeter leads to the release of P anion by substitution of H C_2C and Ca.[621, 63] In Figure 11, the effect microorganism's development [696, 121] hypothesized that the entophytes closely associated with AMF could be involved in nutrient bioavailability (Figure 20) . Successfully, isolated three endobacetria (*Bacillus sp*., *Bacillus thuringiensis*, and *Paenibacillus rhizospherae*) from *Gigaspora margarita* spores that exhibited multiple PGP properties including P solubilization, ethylene production, nitrogenase activity, and antagonism toward soil-borne patogens. Dual positive effects of AMF and their associative endobacteria with regards to facilitation of P uptake under P-limiting conditions were evidenced by Battini *et al*,[60] In Figure 22, root-nodules are strong P sinks, with nodule P concentrations often exceeding those of roots and shoots also indicates the important role of P in the legume symbiosis processes. [251, 541, 56,428, 510] Other traits related to extensive rooting system and their spatial distribution, hyper-nodulation, root exudates, rhizosphere acidification, and heterogeneity are

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among the most important plant-related belowground traits that contribute to higher nutrient use efficiency (Figure 27).[125]

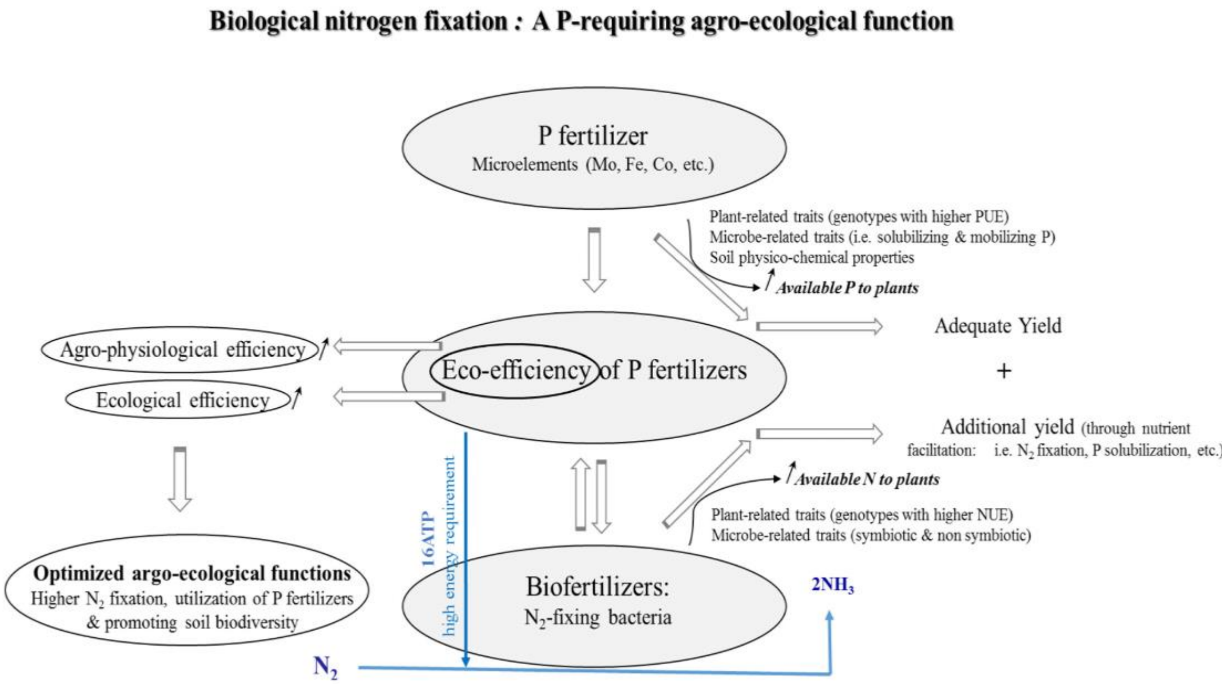

Figure 27 | Conceptual illustration of the relationships between mineral P fertilizers and N_2-fixing bacteria. Biological nitrogen fixation (BNF) is a process for which P is needed in relatively large amounts, especially by legumes for growth, nodulation and grain yield production. Positive interaction between P fertilizers and N_2-fixing bacteria either symbiotic or non-symbiotic (to a lesser extent) would enhance the agronomical eco-efficiency of P fertilizers. Such a positive relationship leading to enhancing use of available P and N would also be attributed to a number of traits related to plants (that is, above and belowground, especially rooting) and microorganisms (i.e., P-solubilizing, phytohormones-producing, and siderophores production, etc.) and adapted from Bargaz *et al*., [55]

Regarding P, these traits may substantially contribute in alleviating the sensitivity of nod factor NF plants to low P availability through ensuring large amount of P-dependent carbon and energy turnover required during the NF process.[541] Exploiting beneficial microbial traits involved in higher P solubilization would positively influence P uptake in addition to multiple advantages attributed to the production of plant growth-promoting substances which could indirectly influence the efficiency of BNF [309] in Figure 22. In Table 2, dual inoculation of soybean plants (Plates 2 and 3) with both a P-solubilizing *(Bacillus*) and NF (*rhizobium*) strains improved symbiotic traits related to growth of nodules and roots, aboveground biomass, total N and grain yield (Table 8) and similar reports were affirmed by scholars.[396, 5] In Table 6, despite positive responses on improved growth, nutrient use efficiency (N and P), and stable yield, all were demonstrated due to microbial application and mineral supply, co-application of multipurpose microbial strains, host plant species, and nutrients sources may generates a highly intricate plant–soil–microbe interactions that need to be profoundly deciphered in order to optimize the agronomical functions [585] that they were designed for (Table 8).

Based on an intercropping cereal–legume study, Tang *et al.* [600] concluded that P fertilization is presumably driving soil microbial communities since it resulted in a higher abundance of bacterial and fungal communities (Figure 26). Research reports shows, the long-term N addition was reported to suppress the mutualistic benefits of the legume–rhizobia associations [94,256, 589, 655] could directly be attributed to decreased rhizobia abundance in soils and reduced selective pressure from legumes to maintain beneficial partners (Figure 22). Generally, it was reported that long-term N rather than P fertilization may decrease significantly the abundance of functional bacterial groups, such as NF bacteria, ammonia oxidizing bacteria, and AMF.[70] This important P uptake (Figure 27) would have relied upon plant-induced changes, especially root growth whose nutrient absorptive capacity could be augmented owing to associated non-symbiotic NF bacteria (such as *Pseudomonas, Azospirillum, Azotobacter, Sinorhizobium, Bacillus, and Glucanobacter,* etc.) with multifunctional abilities (Table 2) other than only improving both N and P nutrition.[455 , 312 , 345, 136]

6.7 | Soil Restoration, Soil Health and Bioremediation

Gateway biofertilizer [446,397] contains broad specrum inoculant consortium (Table 2) that can be used for bioremediation of contamination of soils with heavy metals, such as mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), copper, nickel (Ni) and zinc, or toxic organic compounds, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and other halogenated compounds, decreases crop yield by causing stress-induced ethylene accumulation and reduced nutrient uptake.[296 , 529] Such a pollution represents an important loss of fertile land and a severe threat to human health and sustainable development. Bioremediation mediated by plants via the uptake and/or degradation of pollutants is called phytoremediation. By stimulating seed germination, plant growth and root biomass through the mechanisms discussed above, contamination-tolerant PGP rhizobacteria and endophytes can improve the remediation capacity of plants.[468 , 296] Inoculation with *B. subtilis strain* SJ-101 of *Brassica juncea* growing on Ni-stressed sites resulted in high Nickel concentrations in the plant tissues and an increased plant biomass as a combined effect of bacterial IAA production, solubilization of inorganic phosphate and adsorption of Nickel.[296]

Value |

In Figure 5, Internet of Things (IoT) represents the idea of connecting physical objects (spatial polysingularity) which have sensing, networking and computing capabilities, to other objects and services over the internet connected by different kinds of devices (Figure 24). This leads to big changes in terms of integrations in the existing network and methodologies; ensures that each device is accessible through the Internet. Benefits of IoT applications in agriculture (Figure 25) include improvement in the use efficiency of inputs such as soil, water, fertilizers, pesticides, etc., reduced cost of production, increased profitability, sustainability, food safety, environmental protection.[460] The evolution to Agriculture 5.0 (Figures 5 and 23 respectively) is in the agenda of most major farm equipment makers for the next decade, and therefore of-road equipment manufacturers will play a key role in this move if agricultural robots are considered as the next smarter generation of farm machines (Figure 25) big data has three dimensions: volume, refers to datasets whose size is beyond the ability of typical database

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software tools to capture, store, manage, and analyse information; velocity, refers to the capability to acquire, understand and interpret events as they occur, and variety refers to the different data formats (videos, text, voice) and all encapsulated in Figure 25. Kunisch [313] inserted the concept of veracity, refers to the quality, reliability, and overall confidence of the data (external validity). Valorization is the ability to propagate knowledge, appreciation and innovation [282] and accentuated by Figure 10. In Figure 24, IoT connects all types of objects and devices in both agriculture and the supply chain, huge amounts of data is collected from a wide range of sources including sensors, unmanned aerial vehicle (UAV) , Agricultural Mobile Crowd Sensing (AMCS) reported by Sun *et al.*,.[593]

These data (Figure 26) can be processed, analysed, and used for decision making in real-time.[159] Data analysis is a critical enabler for successfully creating value from these data, and addressing issues such as food security and sustainability.[658] Precision Agriculture (Figure 25), which consist of applying inputs (what is needed) when and where is needed, has become the third wave of the modern agriculture revolution (the first was mechanization and the second the green revolution with its genetic modification [693] The management of farm knowledge systems (Figure 23) due to the availability of larger amounts of data via agriculture technologies to increased net returns and operating profits is the paradigm shift today to maintain the sustainability of farm production. [537] The adoption of these technologies involves uncertainty and trade-offs called spatial polysingularity (Figure 21). Agricultural robotics (Figure 23) vehicles have four main capabilities when performing agricultural tasks: detection, guidance, mapping, and action.[103] Agricultural robotics research covers a wide range of applications, from automated harvesting [57] weed management and control [589] autonomous spraying for pest control [203] environmental conditions monitoring, and animals health helping improve operational reliability while enhancing soil health and productivity.

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What to Learn from Metaphor?

Collaboration (Figure 21) is defined as managing interdependencies and affirmed by scholars [369, 120, 567, 569] and driven by open innovation (Figure 5). Component compatibility and interoperability are important in building and managing wicked problems as an open innovation system.[380, 189, 133] Understanding the necessity of proper alignment and coordination (problems definition) is another point emanating from the new metaphor (solutions to wicked problems [444]) called value (Figure 5) and research outcome (Figure 9). Feedback loops (Figure 6) are a way of ensuring the self-alignment of the system and the knowledge

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flows, having a feedback system is essential.[517] Feedback systems enhance the efficiency and effectiveness of an open innovation network (Figure 6). Metaphor can assist future researchers exploring the topics of the efficiency and effectiveness of open innovation by using analogies domains framework (Figure 5). Within an organization, open innovation schematic (Figure 8) involves the internal exploration of external knowledge and external exploitation of internal knowledge [670] known as stakeholders (Figure 5) and research outcomes (Figure 9).

Biofertilizer Varietal Characteristics
The carrier materials represents the principal portion of inoculants development (Figure 12). Carriers constitute the key for the effective release of the different products, which are formulated (carrier and cells) or unformulated (only cells) biological products can form aggregates that make them more resistant to environmental changes and can represent another type of inoculants (Table 3). The nutrition of bacterial cells applied to plants affects their plant growth promotion capacity by allowing the production of certain enzymes that can trigger plant growth based on their carbon/nutrient ratio (Appendix 1).The materials from which they are made define their effectiveness and the inoculants should be sterile, carriers should be chemically consistent and able to provide enough water holding capacity for microbial growth.[59] A formulated microbial product is a product composed of one or more biological control agents mixed with ingredients that will improve its survival and effectiveness.[535] Plant growth promoting rhizobacteria enhance growth of plants in powder formulations (dry or wet powders) depending on the composition of the powders can be applied directly to the soil, suspended in water or dusted onto seeds (mix the organism and the ingredients with the granules).[681, 684]

Rhizobia act insides the nodules as symbiotic nitrogen fixation, which are symbiotic organs formed on roots of the host plant. The bacteria supply ammonium to plant and the carbon and energy needed for symbiotic nitrogen fixation are from the plant photosynthates. Lodwing *et al.*,[347] found that the metabolic dependence between two symbiotic partners is complex than in single exchange of products of photosynthesis and ammonium. The extended period to maturity in plant inoculated with combined biofertilizers might be caused by the proper conditions provided by biofertilizer which produced plant growth promoting hormones thus increased root‟s absorbency and improved plant growth status then finally prolonged crop maturity [285] and affirmed by Kenndy. [292] Haque *et al.*, [228] reported that increase in nitrogen might the factor to delay phonological stages including crop maturity as nitrogen enhance vegetative growth (Figure 19) and affirmed by Javahey and Rokhzadi [508] who reported prolonged phonological stages due to Nitrogen on sunflower.The maximum number of branches in a field trials might be attributed to the fact that the component bioferilizer such rhizobium fixed atmospheric nitrogen through nodules, hence increases plant height, branches per plant, and number of nodule.[685]

PGPR has the ability to produce phytohormones, organic acids, siderophores, fix atmospheric nitrogen, solubilize phosphate.[33] AMF enhance phosphate nutrition in legumes, which results in plant growth and nitrogen fixation.[109] The results obtained in this investigation are in line with El-Mansi *et al.*,[162] who reported the increase in branches by application of biofertilizers. The significant effect of biofertilizers application on pod length in pea might attributed to the PGPR which enhanced plant growth by synthetizing plant growth promoting hormones,[139] facilitating nutrients uptake from soil [89] or preventing plant diseases. The results obtained are in line with that reported by Rather *et al.* [482] that, significant increase in pod length, number of pods per plant, number of seeds per pod, 100 grain weight of pea

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increased by co-inoculation of rhizobium, *Azotobacter* and PSB. Negi *et al.*, [424] reported that pod length was significantly increased under the influence of biofertilizers.[285] The significant effect of biofertilizer on number of pod per plant might be due to the fact that, rhizobium is nitrogen fixing bacteria (NFB) that can transform inert atmospheric nitrogen to organic compounds.[84] Also, increment in number of pod per plant might be due to the improvement of phosphate uptake and growth in leguminous by AMF. [168] Glick [198] reported maximum number of pods per plant due to PGPR which stimulates growth by fixing atmospheric nitrogen, production of sideropheres which chelate iron and make it available for the plant root, solubilizing phosphorus and secretion of phytohormones. Zhang *et al.*[688] reported an increment of number of pods per plant, number of seeds per pod in two soybean cultivars. Kazemi *et al.*[289] affirmed that, the inoculated seeds of soybean by biofertilizer induced significant number of pods per plant, number of seeds per plant, thousand grain weights and grain yield in soybean . The significant effect of biofertilizer on number of seeds per pod in treated plant might be attributed to provision of needed nitrogen and phosphorus (Table 6 and Figur 17) in the GF1 and GF2 biofertilizer formilation. Similar report was affirmed as the requirement for essential cell division, root and seed formation.[196] Elshanshoury [161] reported increased nutrient uptake like NO_3^- NH_4^{2+}, PO_4^{3+}, K^+ and Fe^{2+} in inoculated plants (Figure 20).

The yield attributes improvement including seed yield due to biofertilizer inoculation might attributed to the increased and balanced nutrients availability (N and P).The significant increase in potassium might be attributed to the fact that when microorganisms cultures are applied to the soil, they enhance organic residues decomposition hence releasing inorganic nutrients which become available for plant uptake.[268] The biofertilizer laboratories results agree with Kaihura *et al.*, [280 ,75 , 587] who reported that farm yard manure increased soil N, P, K and Ca (feedstock for the production of the Biofertilizer, Plate 1). The effect of biofertilizer on available P was significant.[446] The significant increase might be attributed to the fact that organic materials have the ability to cover sesquioxides then reduce P-solubulization and hence, increased P availability in soil solution [72 ,404] and Ipinmoroti *et al.* [255] reported increase of available P due to organic manure use results in higher build-up of N, P, K, Ca, Mg and organic carbon (Figure 20).

CONCLUSIONS AND RECOMMENDATION

Significantly ($P < 0.05$) higher number of leaves, plant height, higher number of pods and seed weight/plant was observed by gateway biofertilizer (GF1 and GF2) applied because host strain specificity soil rhizobia was considered during the biofertilizer development to nodulate under environmental stress compared other soil amendments. Biofertilizer interaction of rhizotrophic microorganisms can improve nutrients uptake in the soil like P, K, Ca, Mg and N and hence increase yields.[463] Smart agriculture framework developed will help provide information about signal cascades and regulatory networks that may occur in plant-microbe interactions that accentuate trans-disciplinary research and affirmed by scholars.[656, 335, 341] Also, easiness and crop performance can always be improved by altering formulation to make soil specific biofertilizer to the user (stakeholder) when regulators lack expertise in the field application and evaluation area (crop scenario), they could tend to delay making a decision or ask for information that sometimes goes beyond what it is needed (re-generative agriculture). Biofertilizer applied to soybean cultivation acts as integrated pest management by promoting natural pest regulation with co-inoculation of *Rhizobium sp. with P. fluorescens* in the Biofertilizer (Tables 2 and 6).

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Among the organic sources for soybean performance GF 1 and GF 2 gave a comparatively higher growth response as a result of the constituent nutritional components that were able to sustain soybean and bradyrhizobia growth at the beginning of the crop phenology, though with no significant effect on its eventual performance. The yield attributes improvement including seed yield due to biofertilizer inoculation might attributed to the increased and balanced nutrients availability (N and P). Rhizobia can affect some plant physiological processes like photosynthesis, nodulation and nitrogen fixation in legumes by stimulating plant dry matter and grain yield. [130 , 286,215] Rhizobia affect soybean physiological processes like photosynthesis, nodulation and nitrogen fixation in legumes by stimulating plant dry matter and grain yield. To ensure sustainable soybean performance through increasing BNF higher application rates of GF 2 could be recommended to meet a short fall in nitrogen deficit as a result of a decline in BNF at the reproductive stage for a higher grain yield. The profitability of soyabean cultivation is the combine use of N_2 fixation by root nodules and absorbed N from roots for the maximum seed yield of soybean.

Conflict of Interest | The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix 1 - Plant growth promotion and biofertilization

Table 10 | Nutrient content of soybean cultivation Mengle [389] and Vitosh [637]

Elements	Content %	Elements	Content ppm
Nitrogen	4.25-5.50	Copper	0.00-30.00
Phosphorus	0.25-0.5	Manganese	20-100
Potassium	1.70-2.50	Zinc	20-50
Calcium	0.35-2.00	Boron	20-55
Magnesium	0.26-1.00	Molybdenum	1.0-5.0
Sulfur	0.15-0.50	Aluminum	<200

Carbon/Nitrogen Ratio |

Residues with wide C: N ratios include hay, straw pine needles, cornstalks, dry leaves, and sawdust.
C: N ratios of > 30:1
Immobilization of soil N will be favored.
C: N ratios of 20:1 to 30:1
Immobilization and mineralization will be nearly equal.
C: N ratios of < 20:1
Favour rapid mineralization of N. Residues with narrow C: N ratios include alfalfa, clover, manures, biosolids, and immature grasses.