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Review

Biofertilizers in Sustainable Crop Production: Linking Rhizosphere Mechanisms, Formulation, and Context-Dependent Field Performance

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Abstract

Biofertilizers are increasingly considered components of resource-efficient nutrient management, but their agronomic performance remains strongly dependent on crop genotype, soil properties, microbial strain, formulation, and management context. This structured critical narrative review evaluates molecular, agronomic, environmental, and implementation evidence on plant growth-promoting rhizobacteria, arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, and emerging nano-enabled microbial formulations. Molecular and omics studies have clarified mechanisms of nutrient cycling, colonization, plant signalling, and rhizosphere restructuring, but these indicators alone do not predict field efficacy. A global meta-analysis reported an average yield increase of 16.2% relative to non-inoculated controls, but responses vary across crops and environments, and reliable mineral-fertilizer substitution remains context-dependent. The evidence favors site-specific deployment within integrated nutrient-management systems and assessment based on yield, nutrient-use efficiency, crop quality, resource-use efficiency, and resilience. Waste-derived substrates, biodegradable carriers, and nano-enabled formulations may improve resource circularity and delivery, but their environmental advantages require life-cycle, stability, safety, and efficacy-based evaluation. Future progress depends on multi-environment validation, robust formulations, predictive multi-omics linked to agronomic outcomes, quality standards, and proportionate regulation.

Keywords: plant growth-promoting microorganisms; integrated nutrient management; nutrient-use efficiency; rhizosphere microbiome; biological nitrogen fixation; arbuscular mycorrhizal fungi; microbial inoculation

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Academic Editor: Soon-Jae Lee

Received: 17 September 2026
Revised: 22 October 2026
Accepted: 26 October 2026
Published: 28 October 2026

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1. Introduction

Global agriculture must sustain crop productivity while reducing environmental degradation and increasing resilience to climatic instability. Synthetic fertilizers have supported major yield gains, but their environmental burden extends from energy-intensive manufacture to nutrient losses after field application. The production, transport, and agricultural use of synthetic nitrogen fertilizers were estimated to generate approximately 1.13 Gt CO_2-equivalent emissions in 2018, corresponding to 2.1% of global greenhouse gas emissions [1]. Inefficient nutrient recovery additionally contributes to nitrate leaching, eutrophication, soil acidification, ammonia volatilization, and disruption of biogeochemical cycles [2–4].

Microbial biofertilizers have therefore gained attention as components of more biologically mediated and resource-efficient nutrient-management systems [5,6].

Terminological precision is essential because biofertilizer, microbial biostimulant, and bio-effector describe overlapping but non-equivalent categories. In this review, a biofertilizer is defined operationally as a formulated product containing viable microorganisms whose principal function is to improve nutrient availability, acquisition, or utilization through processes such as biological nitrogen fixation, phosphorus solubilization or mobilization, and nutrient-related rhizosphere interactions. A microbial plant biostimulant is a broader function-based category that also includes microorganisms improving nutrient-use efficiency, abiotic-stress tolerance, crop quality, or nutrient availability, whereas a bio-effector may encompass both beneficial microorganisms and non-microbial bioactive substances [7–10]. Accordingly, microbial products described as biostimulants or bio-effectors were considered only when they met the nutrient-related operational definition adopted here. Non-microbial extracts, humic substances, and recovered mineral inputs such as biomass ash were not classified as biofertilizers, although they are discussed where they function as complementary inputs, carriers, or components of circular nutrient-management systems.

Biofertilizer performance should be evaluated across diverse crop systems and against outcomes that extend beyond yield alone. Depending on the crop and production system, agronomic relevance may include nutrient-use efficiency, product quality, biomass composition, and processing-related traits. This is particularly important for oilseed, fiber, sugar, starch, rubber, medicinal, and bioenergy crops, for which downstream functionality may be as relevant as biomass production. Crop and food-processing systems can also generate residues suitable for use as fermentation substrates, carrier materials, or complementary recycled nutrient inputs, linking biofertilizer deployment with broader resource-efficiency and circular nutrient-management strategies [11–13]. However, evidence remains uneven across crop groups, with industrial crops generally less consolidated than cereals, grain legumes, and horticultural species [14,15].

Despite strong mechanistic plausibility, field performance remains markedly context-dependent. Meta-analyses report outcomes ranging from substantial yield gains to weak or non-significant responses across microbial groups, crops, soils, fertilization regimes, and environments [6,16]. Response magnitude is strongly conditioned by soil fertility and environmental context, but the direction and strength of these relationships vary among microbial groups and between controlled and field conditions [6,16]. This variability reflects strain–host compatibility, formulation quality, inoculant persistence, competition with resident microbiota, soil physicochemical properties, crop genotype, and management context [11,17,18]. Biofertilizers should therefore be evaluated as site- and management-dependent technologies rather than universal replacements for mineral fertilizers. Advances in genomics, transcriptomics, proteomics, metabolomics, and metagenomics have clarified nutrient-cycling genes, colonization traits, plant signalling responses, and inoculation-induced changes in rhizosphere communities [19–23]. However, detection of a functional gene, metabolite, or microbiome shift does not by itself demonstrate agronomic efficacy. The central translational challenge is to identify molecular indicators that reliably predict microbial persistence, improved nutrient acquisition, reduced fertilizer demand, and reproducible field performance.

Against this background, this critical review integrates mechanistic, agronomic, environmental, technological, and implementation evidence on microbial biofertilizers. It focuses on the gap between molecular potential and field validation, variation in field responses, nutrient-use efficiency, and partial fertilizer substitution. It also examines crop-specific and quality-related outcomes, sustainable formulations, the resource-efficient use

of waste-derived materials, and nano-enabled systems. Complementary agroecosystem practices and economic and regulatory barriers are considered within the broader implementation context. By connecting rhizosphere mechanisms, formulation and delivery constraints, and quantitative field evidence with a comparative assessment of regulatory approaches across five jurisdictions, this review provides an integrated perspective on the conditions governing reliable biofertilizer performance and practical implementation in sustainable crop production. The conceptual relationships among molecular mechanisms, soil microbiome functioning, formulation and resource-efficiency considerations, and agronomic outcomes are summarized in Figure 1.

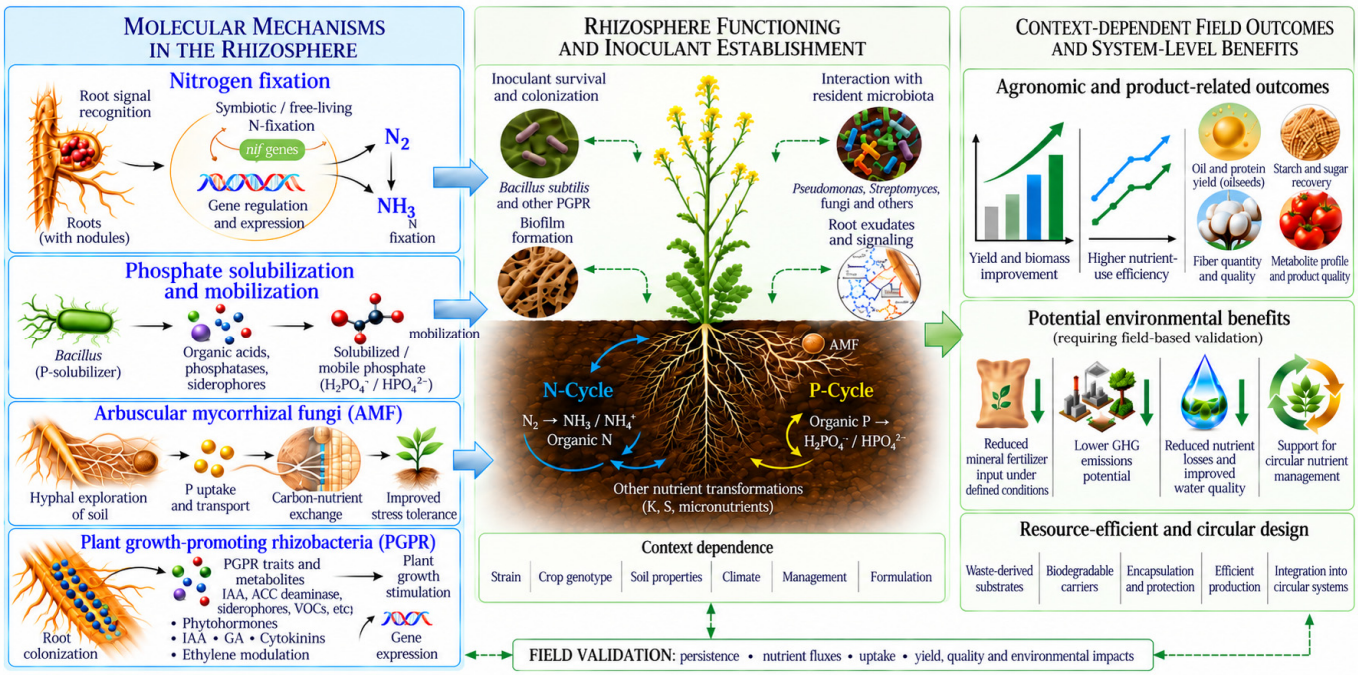


Figure 1. Conceptual framework linking molecular mechanisms of biofertilizer action with soil microbiome functioning, resource-efficient formulation and circular inputs, and agronomic outcomes. The scheme highlights nitrogen fixation, phosphate solubilization, plant growth promotion, and their relevance to productivity, nutrient-use efficiency, reduced synthetic fertilizer use, crop quality, and environmental sustainability. Blue and orange circular arrows represent nitrogen and phosphorus cycling, respectively, while green arrows indicate conceptual links between processes and outcomes. Upward and downward arrows indicate qualitative increases or decreases in the respective parameters.

2. Literature Search Strategy and Evidence Evaluation

This article was developed as a structured critical narrative review aimed at integrating and critically evaluating scientific evidence on microbial biofertilizers, with particular emphasis on rhizosphere mechanisms, agronomic effectiveness, formulation technologies, environmental sustainability, and practical implementation. The literature was identified through targeted thematic searches of Scopus, Web of Science Core Collection, PubMed, and ScienceDirect for publications available up to June 2026. Google Scholar was used for supplementary citation tracking and to identify relevant regulatory documents and international reports. Reference lists of key reviews, meta-analyses, and influential original studies were also examined to identify additional sources, including foundational investigations and studies reporting contrasting findings.

The search strategy covered several interconnected thematic areas, including microbial biofertilizers and plant growth-promoting microorganisms; biological nitrogen fixation and phosphorus mobilization; rhizosphere interactions and molecular mechanisms; crop

productivity and nutrient-use efficiency; microbial formulation, carriers, and delivery systems; and environmental, economic, and regulatory aspects of biofertilizer implementation. Representative search terms included biofertilizer, biofertiliser, microbial inoculant, plant growth-promoting rhizobacteria, arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, rhizosphere microbiome, field performance, nutrient-use efficiency, waste-derived carriers, nano-biofertilizers, and sustainable nutrient management.

To illustrate the thematic search logic, representative Boolean combinations included ("biofertilizer*" OR "biofertiliser*" OR "microbial inoculant*") AND ("field trial*" OR "crop yield" OR "nutrient-use efficiency"); ("arbuscular mycorrhiz*" OR "plant growth-promoting rhizobacteria" OR "nitrogen-fixing bacteria" OR "phosphate-solubiliz*") AND ("rhizosphere" OR "nutrient uptake" OR "field response"); and ("biofertilizer*" OR "biofertiliser*" OR "microbial inoculant*") AND ("formulation" OR "carrier*" OR "shelf life" OR "life cycle assessment" OR "regulation"). These combinations illustrate the thematic scope of the searches rather than archived, database-specific search strings.

Literature selection was guided by scientific relevance, methodological quality, and the strength of evidence supporting the conclusions. The evidence base included peer-reviewed molecular and laboratory investigations, greenhouse experiments, field trials, quantitative meta-analyses, and relevant review articles. Selected regulatory and policy documents were considered where necessary to address implementation and governance. Priority was given to studies reporting clearly characterized microbial strains or formulations, appropriate experimental controls, measurable biological or agronomic outcomes, and sufficient methodological detail. Studies addressing exclusively non-microbial fertilizers, biological control without a plant-nutrition function, conference abstracts, promotional materials, or insufficiently characterized interventions were excluded from the primary evidence base for microbial biofertilizer efficacy. Studies referring to microbial biostimulants or bio-effectors were considered when the investigated products met the operational definition of microbial biofertilizers adopted in this review.

Evidence was critically compared according to microbial group, crop species, soil and environmental conditions, formulation, fertilizer management, and experimental scale. Laboratory and greenhouse studies were used primarily to explain mechanisms of action, whereas field experiments, multi-environment trials, and quantitative syntheses were given greater interpretative weight when assessing agronomic effectiveness and practical applicability. Particular attention was paid to contradictory findings, differences between controlled and field conditions, and the extent to which reported benefits could be attributed specifically to microbial inoculation. Findings from combined microbial and non-microbial treatments were interpreted cautiously when the contribution of the inoculant could not be isolated.

Because the objective was to develop an integrated critical interpretation of heterogeneous evidence rather than an exhaustive systematic inventory, literature selection was not based on a predetermined study quota or numerical relevance score. No new meta-analysis or formal systematic evidence ranking was conducted. Instead, the synthesis focused on the consistency, limitations, and context dependence of available findings, identifying the evidence required to link microbial mechanisms with reproducible field performance.

3. Functional Basis of Biofertilizer Efficacy in Plant–Soil Systems

Biofertilizers influence crop performance through nutrient transformations, plant signalling, and modification of rhizosphere interactions. However, evidence for a mechanism should not be treated as equivalent to evidence of agronomic efficacy. In the nitrogen cycle, inoculation may affect biological nitrogen fixation, mineralization, nitrification, and other

nitrogen transformations, and controlled studies have reported changes in nitrogen-cycling genes and plant-available soil N following biofertilizer application [2,24]. Nevertheless, the detection or increased abundance of *nifH* and other nitrogen-cycle markers has limited standalone predictive value because marker recovery is method-dependent and does not demonstrate the magnitude of N flux, persistence of the inoculant, plant N acquisition, or a reproducible yield response [25]. Molecular signals are therefore most informative when evaluated together with measurements of soil N transformations, plant N accumulation, and field performance.

Phosphorus mobilization provides a clearer example of how mechanistic and agronomic evidence can be linked. Phosphate-solubilizing microorganisms may improve access to poorly available P pools through organic acid production, phosphatase activity, and interactions with complementary rhizosphere organisms [26,27]. Under greenhouse conditions, combinations of phosphate-solubilizing bacteria, humic acids, and arbuscular mycorrhizal fungi altered the soil microbiome and enhanced plant nutrient uptake [28]. However, markers such as *gcd*, *pqqC*, and *pho* genes, as well as in vitro P-solubilization capacity, remain weak predictors of field efficacy unless they are accompanied by inoculant establishment and measurable changes in soil available P and plant uptake. Yahya et al. [4] provided a stronger translational chain by combining microbial characterization and colonization evidence with field measurements of phosphatase activity, soil available P, seed P accumulation, and wheat grain yield under a 20% reduction in di-ammonium phosphate input.

Arbuscular mycorrhizal fungi (AMF) constitute a distinct nutrient-acquisition pathway rather than merely another class of phosphate-solubilizing microorganisms. Following root colonization, fungal arbuscules establish exchange interfaces within cortical cells, while extraradical hyphae explore soil beyond the immediate root depletion zone. Phosphorus taken up by the fungal mycelium can be transferred to the host through a mycorrhizal uptake pathway that operates alongside direct uptake by roots; AMF can also contribute to nitrogen acquisition, although the magnitude of this contribution varies among plant–soil systems [29]. In exchange, the host supplies photosynthetically derived carbon. Consequently, the net outcome depends on the balance between nutrient acquisition and host carbon investment, and a high percentage of root colonization does not necessarily imply improved nutrient status or crop yield [29,30].

The agronomic response to AMF inoculation is strongly context-dependent. Meta-analytic evidence indicates that plant functional group, nutrient limitation, nitrogen fertilization, and soil biotic complexity can alter the direction and magnitude of mycorrhizal responses [31]. In particular, benefits are more plausible when nutrient acquisition is constrained, whereas high soil phosphorus availability, pre-existing compatible mycorrhizal communities, or poorly matched host–fungus combinations may limit the additional value of commercial inoculation. A greenhouse study combining AMF with phosphate-solubilizing bacteria and humic substances reported changes in microbial communities and plant nutrient acquisition [28], but the contribution of AMF cannot be generalized independently of the other components and the controlled experimental setting. Accordingly, field validation should relate inoculant identity and root colonization to plant P and N uptake, soil fertility, nutrient-management regime, and replicated yield responses across environments. Colonization, nutrient transfer, and fertilizer substitution should be treated as separate evidentiary endpoints rather than interchangeable indicators of efficacy [16,29,31].

Indirect effects on plant physiology and the resident microbiome require similar caution. Plant growth-promoting microorganisms can produce indole-3-acetic acid and gibberellins, modify root architecture, and influence pathways related to nutrient transport, photosynthesis, and stress responses [32–35]. Yet phytohormone production in culture or altered plant gene expression does not establish that the same mechanism determines field performance.

For example, the improved photosynthetic traits and chlorophyll-related gene expression reported in rice occurred under combined application of biofertilizer and organic fertilizer, limiting attribution to the microbial component alone [36]. Interpretation of microbiome responses is similarly constrained because rhizosphere community structure is shaped by plant selection, soil conditions, and interactions among resident microorganisms [37,38]. Field studies in soybean and pigeon pea have associated inoculation-induced microbiome restructuring with improved nutrient status and agronomic performance [39,40], but such associations do not by themselves establish causality or long-term ecological stability.

A recent conceptual model proposes that beneficial microbes modulate plant immune thresholds and the growth–defence balance, potentially influencing root colonization and inoculant establishment. This remains a mechanistic hypothesis rather than direct evidence of field efficacy [41]. A meta-analysis of 335 studies found that microbial inoculation increased soil microbial biomass and altered community structure without a general increase in microbial diversity; locally sourced inoculants showed more favorable biomass responses. Microbiome restructuring should therefore not be equated with improved crop performance without agronomic measurements [42].

Omics approaches can therefore clarify modes of action, identify colonization and nutrient-mobilization traits, and explain context-dependent responses, but they should not function as substitutes for efficacy testing [23,43]. Genomic evidence on bacterial adaptation to plants can support the identification of potentially useful colonization traits, although the presence of such traits does not ensure expression or persistence after field application [44]. Predictive value is greatest when time-resolved molecular data are integrated with strain tracking, nutrient pools and fluxes, plant uptake, and multi-environment field outcomes [4,45]. Across crop systems, validation should also consider crop-specific quality and functional traits, including biomass composition and processing-relevant characteristics, rather than yield alone. These relationships are synthesized in Figure 2, which illustrates the evidence chain linking molecular potential and functional activity with inoculant establishment, soil nutrient processes, plant responses, and ultimately field efficacy. The framework emphasizes that predictive value increases only when mechanistic evidence is linked to microbial persistence, nutrient fluxes, plant nutrient acquisition, and reproducible field performance.

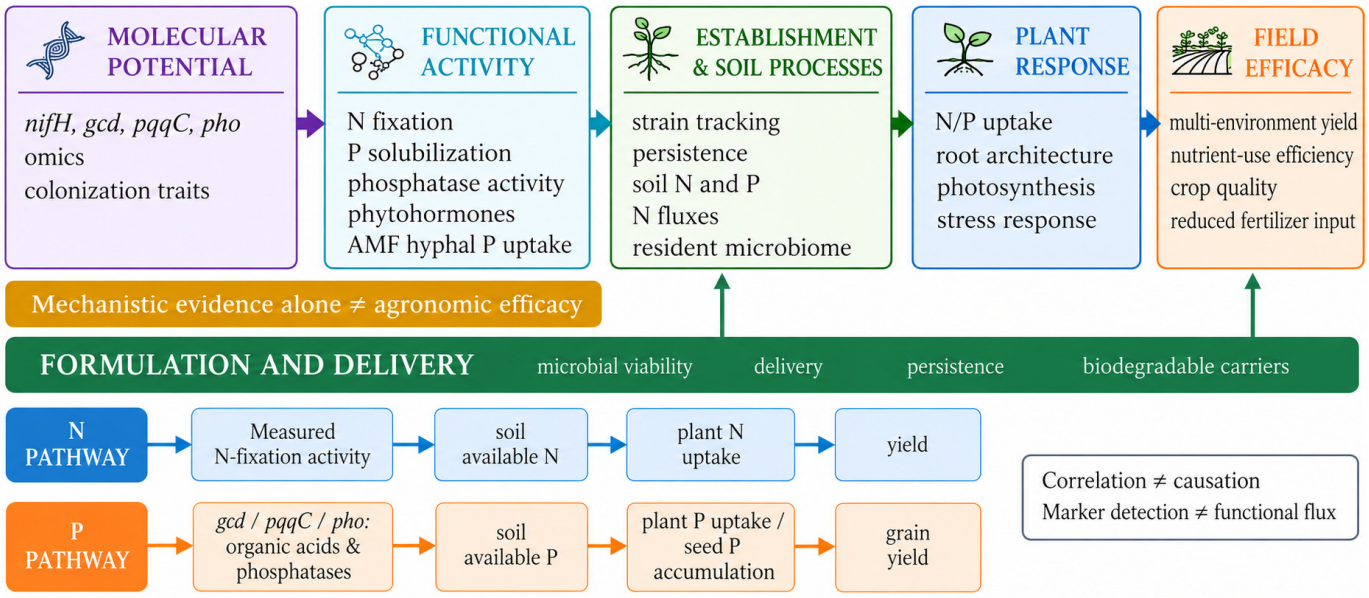


Figure 2. Functional basis of biofertilizer efficacy in plant–soil systems. The scheme summarizes how molecular potential and mechanistic evidence should be linked with functional activity, inoculant establishment, soil nutrient processes, plant responses, and field-level outcomes. It highlights that marker detection and omics-based signals do not by themselves demonstrate agronomic efficacy unless they are connected with nutrient fluxes, plant uptake, and reproducible field performance.

4. Resource-Efficient Production, Formulation, and Delivery of Microbial Biofertilizers

4.1. Production, Quality Control, and Resource Efficiency

The environmental performance of a microbial biofertilizer should be evaluated across its production and use rather than inferred from its biological origin. Although microbial cultivation may operate under milder conditions than conventional nitrogen-fertilizer synthesis, production requires medium preparation, sterilization, fermentation, aeration or agitation, control of pH and temperature, biomass recovery, stabilization, packaging, storage, and distribution [13,23,46–49]. Energy and water demand therefore depend on process efficiency and the physiological requirements of the strain. Evaluating production per unit mass of formulated product alone is insufficient: relevant operational indicators include viable-cell yield, batch-to-batch reproducibility, contamination rate, and the viable dose ultimately delivered to the field.

Renewable feedstocks and crop- or food-processing residues may replace refined ingredients in fermentation media, but their resource efficiency depends on composition, pretreatment requirements, sterilization, supplementation, and consistency between batches [48,50]. Water requirements include not only the culture medium but also cleaning and biomass separation. Accordingly, claims of lower production impacts should be supported by process-level inventories of electricity, water, raw materials, waste streams, product losses, and viable-cell recovery. Quality control must verify strain identity, purity, contamination limits, and functional competence; a nominally high fermentation yield has limited practical value if viability declines during subsequent formulation, transport, or storage [8,17,48,51].

Life-cycle assessment (LCA) and techno-economic assessment (TEA) should connect manufacturing inputs with the agronomic function actually delivered. For product-level comparisons, viable-cell recovery and stability provide more informative denominators than kilograms of formulation alone; for field-level comparisons, relevant functional units may include one hectare treated at a verified viable dose or a defined crop-yield outcome achieved

under comparable nutrient management. Claims of fertilizer substitution should additionally be assessed against a documented reduction in mineral input without an unacceptable yield penalty. Economic analysis should account for fermentation, contamination control, carrier preparation, packaging, shelf-life losses, transport, application, and field-response variability. Existing LCA syntheses of waste-derived fertilizers mainly address digestate, compost, biochar, or pellets rather than live microbial inoculants [52]. Consequently, environmental superiority cannot be inferred from renewable feedstocks or biodegradability without transparent, efficacy-adjusted comparisons [49,53].

4.2. Formulation, Carriers, and Delivery Systems

A production substrate, a microbial carrier, and a complementary nutrient amendment perform different functions and should not be treated as interchangeable. Fermentation substrates supply nutrients for microbial propagation, whereas carriers are intended to maintain viable cells during storage, transport, and application and to facilitate their establishment in the rhizosphere. Liquid formulations, peat- or compost-based materials, biochar, filter mud, and biodegradable polymers such as alginate, chitosan, starch, and cellulose derivatives offer different balances of protection, application compatibility, and material demand [13,17,54–60]. Encapsulation and biofilm-based delivery may improve cell retention or controlled release, but their benefits must be demonstrated for the particular strain, crop, and production system.

Formulation quality should be specified at the end of the declared shelf life, not only immediately after manufacture. Relevant measures include authenticated identity and viable count of each declared microorganism, product purity, contamination levels, moisture and temperature stability, and retention of the functional properties underlying the efficacy claim [8,17,48,51]. Field application should be expressed as a verifiable viable-cell dose per seed, plant, or unit area, where appropriate, together with the application route. Compatibility with seed-treatment chemicals, coating and drying procedures, sowing machinery, irrigation systems, and other fertilizers may determine whether the labeled dose actually reaches the intended soil–root environment. These operational requirements connect formulation performance with inoculant persistence and field validation.

Waste-derived matrices offer opportunities for circular resource use, but evidence of agronomic benefit is not automatically evidence of carrier quality. In wheat, a formulation combining filter mud with locally adapted phosphate-solubilizing bacteria was associated with improved P availability and crop performance under a 20% reduction in di-ammonium phosphate input [4]. By contrast, studies of spent mushroom substrate, vermicompost, and some composted residues primarily establish their value as amendments or growing-media components; they do not necessarily demonstrate microbial viability or shelf-life performance when these materials are used as inoculant carriers [59,61]. Direct comparisons should measure viable-cell retention, release and persistence after application, and agronomic responses at equivalent delivered doses. Field observations on biofilm-based delivery in rubber nursery plants are promising but remain specific to the tested formulation and production context [11].

Carrier selection also involves environmental and technological trade-offs. Porosity and moisture retention may favor survival, whereas variation in pH, salinity, nutrient content, contamination, or storage moisture may compromise consistency. Biochar properties depend on feedstock and pyrolysis conditions, while composted materials may require sanitization or stabilization. Biopolymer extraction, modification, cross-linking, and drying can consume resources even when the final carrier is biodegradable [13,17,54,55]. Comparative evaluation should therefore consider carrier production, transport distance, microbiological quality,

shelf life, compatibility with agricultural equipment, and field efficacy, rather than treating waste origin or biodegradability as sufficient evidence of sustainability.

Recovered mineral inputs such as biomass ash may complement circular nutrient management, but they should remain conceptually distinct from live microbial biofertilizers [62–64].

4.3. Nano-Enabled Microbial Formulations: Performance, Field Validation, and Environmental Trade-Offs

Nano-biofertilizers should be distinguished from conventional nanofertilizers. The former combine viable microorganisms with nanomaterials used as coatings, carriers, protective matrices, or nutrient-bearing components, whereas the latter deliver nanoscale nutrients without a microbial inoculant [65–67]. Nanomaterials may protect microbial cells against desiccation and environmental exposure, promote adhesion to carriers or roots, and enable gradual microbial or nutrient release. Their high specific surface area may additionally increase nutrient loading and local delivery efficiency [66,68]. Green synthesis using plant extracts, microbial metabolites, or agricultural residues may reduce reliance on some hazardous reducing and capping agents, but it does not by itself establish ecological safety or a lower life-cycle burden [69–71]. Experimental evidence remains promising but uneven. Nanoparticle-coated *Bacillus* strains combined with mesoporous silica, ZnO, or CuO improved wheat growth, nutrient uptake, and soil enzyme activity in a 45-day pot experiment, but responses differed among nanoparticle–strain combinations [72]. A recent systematic review found that only a minority of studies integrating nanoparticles with plant growth-promoting microorganisms formulated a true nano-biofertilizer; many evaluated nanoparticles and microorganisms as separate co-treatments, and responses depended strongly on nanoparticle type, concentration, microbial species, and crop [67].

Consequently, results obtained with conventional nanofertilizers should not be presented as direct field validation of microbial nano-biofertilizers. For example, Tarafdar et al. [73] evaluated a biosynthesised zinc nanofertilizer in pearl millet but did not test a formulation containing viable plant-beneficial microorganisms. The study is relevant to nanonutrient delivery, not to evidence for microbial nano-biofertilizer efficacy. The wider evidence base for novel fertilizers also remains constrained by limited multi-location field studies and inconsistent performance-testing protocols [53].

Safety assessment must address environmental transformation and exposure rather than only the composition of the material applied. Nanoparticles may dissolve, aggregate, adsorb to clay minerals and organic matter, or undergo chemical transformation, thereby changing their mobility, bioavailability, and toxicity. Their effects on native bacteria, fungi, nutrient-cycling processes, and plant–microbe associations may be beneficial, neutral, or inhibitory depending on concentration, material properties, soil type, and exposure duration [67,74–76]. Long-term accumulation in soil, movement into water, uptake by crops, and effects on non-target organisms remain insufficiently resolved. Regulatory evaluation is further complicated by inconsistent definitions, incomplete nanoparticle characterization, and differences between nominal and actual particle-size distributions in formulated products.

Nano-enabled microbial formulations should be assessed through staged validation: physicochemical characterization, strain-specific viability and release testing, soil-fate and non-target assays, controlled-environment comparisons, and multi-season field trials. Comparisons should include an equivalent non-nano microbial formulation delivering a comparable viable dose, so that any additional benefit of the nano-enabled carrier can be separated from inoculation itself. Life-cycle and techno-economic assessments should account for nanoparticle synthesis, purification, formulation, storage, application, transformation, and end-of-life behavior. Until benefits are confirmed under these conditions, nano-biofertilizers

should be regarded as emerging delivery systems rather than inherently greener or field-validated alternatives to conventional inoculants [53,67,75,76].

5. Field Performance, Nutrient-Use Efficiency and Management-Relevant Outcomes

5.1. Quantitative Evidence and Sources of Field Heterogeneity

Quantitative syntheses generally indicate positive average responses to microbial biofertilization, but the reported effect sizes are not directly interchangeable because the analyses differ in crop coverage, microbial categories, experimental scale, and outcome definitions. The global meta-analysis of Schütz et al. [16], based on 171 publications, reported an average yield increase of 16.2 ± 1.0% relative to non-inoculated controls. Biofertilization also increased apparent phosphorus-use efficiency by 7.5 ± 0.8 kg yield per kg P and nitrogen-use efficiency by 5.8 ± 0.6 kg yield per kg N. However, these indicators could not distinguish improved recovery of applied fertilizer from mobilization of native soil nutrient pools, and responses differed substantially among crop groups and environmental conditions. Root crops and the heterogeneous category of "other crops", which included cotton, oil crops, spices, and sugarcane, showed lower nutrient-use-efficiency responses than cereals and legumes.

Interpreted through a genotype × environment × management (G × E × M) perspective, these average effects conceal pronounced environmental gradients. Schütz et al. [16] reported yield gains of 20.0 ± 1.7% in dry climates, 14.9 ± 1.2% in tropical climates, 10.0 ± 3.7% in oceanic climates, and 8.5 ± 2.4% in continental climates. Importantly, 45% of dry-climate comparisons involved irrigation, so this contrast should not be interpreted solely as a drought response. The same analysis linked microbial-category responses to soil P availability: AMF responses were highest at 15–25 kg available P·ha^{-1}, P-solubilizing microorganisms at 25–35 kg·ha^{-1}, and N fixers at levels exceeding 45 kg·ha^{-1}. These are study-level associations, not universal application thresholds.

Complementary evidence links soil organic carbon (SOC) with microbial colonization resistance through plant immunity–microbiome interactions and reports a non-linear relationship between SOC and inoculant efficacy. These results support context-specific prediction but do not establish a universal SOC threshold for all crops, soils or formulations [77].

A crop-specific analysis provides an even clearer demonstration of field heterogeneity. Schmidt and Gaudin [6] evaluated bacterial biofertilizers in maize using 48 field publications comprising 442 observations and 21 controlled studies comprising 134 observations. Average productivity gains were 15.3% under field conditions and 18.4% in pots containing field soil, but controlled experiments poorly predicted the influence of climate, soil properties, and microbial taxonomy in the field. Importantly, strains confirmed in vitro to possess nitrogen-fixation, phosphorus-solubilization, or phytohormone-production traits did not consistently produce greater field yields. This result reinforces the conclusion that laboratory screening and mechanistic characterization cannot substitute for multi-environment field validation [6].

Larger mean responses have been reported in broader evidence syntheses, but their scope requires careful interpretation. Herrmann et al. [7] analyzed 1791 observations from 186 studies and found average increases of approximately 25% in plant growth and 30% in yield, together with improvements in crop nutrient and quality indicators. However, the analysis covered the broader category of bioeffectors, including non-microbial extracts and humic or amino-acid products, some of which produced larger effects than microbial treatments. These estimates should therefore not be cited as microbial-biofertilizer-specific effect sizes. A more recent field-based meta-analysis from China included 1818 comparisons

from 107 studies and reported positive yield responses in 21 of 23 crops, but effects in cotton and rapeseed were non-significant. The latter finding is particularly relevant to industrial crops and demonstrates that a positive overall mean can coexist with weak or absent responses in individual crop groups [15]. The main quantitative syntheses, their scope, principal findings, and interpretative limitations are summarized in Table 1.

Microbial identity and crop management further modify the interpretation of pooled estimates. In the maize field meta-analysis, *Azotobacter* inoculation was associated with a mean yield increase of 34.4%, while *Azospirillum* and *Pseudomonas* showed consistently positive responses; estimates for *Bacillus*, *Enterobacter*, and *Pantoea* included no effect within their 95% confidence intervals [6]. In the Chinese field synthesis, reported yield responses differed among AMF (+25.21%), *Rhizobium* (+20.65%), and *Bacillus* (+19.36%), while the average response under co-application with organic amendments reached +29.20% [15]. The latter finding does not by itself establish synergy. These subgroup comparisons illustrate microbial-group and management dependence, but crop genotype was rarely resolved sufficiently to quantify a complete G × E × M interaction.

Table 1. Comparative quantitative evidence on biofertilizer effects and major limitations of interpretation.

Study	Evidence Base and Scope	Principal Quantitative Findings	Critical Interpretation
Schütz et al. [16]	171 publications; microbial biofertilizers across multiple crops and climates	Yield: +16.2 ± 1.0%; PUE: +7.5 ± 0.8 kg·yield·kg^{-1}·P; NUE: +5.8 ± 0.6 kg·yield·kg^{-1}·N. Climate: dry +20.0%; tropical +14.9%; oceanic +10.0%; continental +8.5%.	Strong global synthesis, but responses differed by crop and climate; NUE and PUE were apparent agronomic indicators and could not distinguish fertilizer recovery from soil nutrient mobilization. AMF and N-fixer/P-solubilizer combinations showed strong responses; soil P moderated efficacy.
Schmidt & Gaudin [6]	Maize; 48 field publications and 442 observations; 21 pot-study publications and 134 observations	Field productivity: +15.3%; pot productivity: +18.4%; *Azotobacter* (field): +34.4%.	Greenhouse results poorly predicted field moderators; commonly screened in vitro traits did not reliably identify the most effective field strains. Genus-specific responses: *Azospirillum* and *Pseudomonas* showed consistently positive field responses; for *Bacillus*, the field 95% CI included zero, whereas pot studies showed a +23.0% yield response.
Herrmann et al. [7]	1791 observations from 186 studies; network meta-analysis of bioeffectors	Plant growth: approximately +25%; yield: approximately +30%; aboveground N: +28%; aboveground P: +40%.	Includes microbial and non-microbial products; the reported mean effects cannot be attributed specifically to microbial biofertilizers.
Pei et al. [15]	1818 field comparisons from 107 studies conducted in China; 23 crops	Positive yield responses in 21 of 23 crop categories; cotton and rapeseed responses were non-significant. AMF: +25.21%; *Rhizobium*: +20.65%; *Bacillus*: +19.36%; co-application with organic amendments: +29.20%.	Recent and field-focused, but geographically restricted and characterised by heterogeneous products and crop systems; especially useful for identifying crop-specific non-responses.

5.2. Nutrient-Use Efficiency and Partial Fertilizer Substitution

The most management-relevant question is not whether inoculation increases growth relative to an unfertilised control, but whether it maintains or improves crop performance under a defined reduction in mineral fertilizer input. Strong evidence therefore requires an experimental design in which fertilizer rates are explicit and nutrient availability, plant uptake, and yield are measured concurrently. In wheat, a filter-mud formulation containing autochthonous phosphate-solubilizing bacteria increased grain yield by 4–9%, seed P by 9%, soil available P by 8.5–11%, and phosphatase activity by 4–5% when di-ammonium phosphate application was reduced by 20% [4]. This study provides a relatively complete translational chain linking formulation performance and microbial persistence with nutrient acquisition and a field-level production outcome.

In legumes, crop-specific rhizobial symbioses, particularly in soybean, provide the most established evidence that microbial inoculation can make a substantial contribution to plant nitrogen nutrition [78]. Historical global accounting estimated that biological nitrogen fixation supplied approximately 50–70 Tg N per year to agricultural systems, including about 16.4 Tg N fixed annually by soybean; these are historical estimates, not current annual totals [79]. A field meta-analysis from Brazil reported a further 3.2% increase in soybean grain yield following co-inoculation with *Azospirillum brasilense* and *Bradyrhizobium* spp. compared with *Bradyrhizobium* inoculation alone, with responses modified by soil texture, climate, tillage, and cultivar [80]. However, yield responses to rhizobial inoculation may be limited under high soil mineral nitrogen availability or in soils containing abundant, well-established and effective indigenous rhizobial populations, where additional inoculation provides little incremental benefit [81]. This evidence should not, however, be extrapolated automatically to non-symbiotic diazotrophs or multifunctional consortia. Consortium-based field studies show that complementary microorganisms can modify nutrient cycling and plant performance, but their effects remain dependent on strain compatibility, soil fertility, and the resident microbiome [39].

Intensive tomato production provides evidence of partial mineral-fertilizer substitution under both greenhouse and open-field conditions. In a commercial greenhouse in Almería (Spain), repeated application of formulations enriched with plant growth-promoting bacteria (PGPB) increased cumulative fruit production by approximately 20–32% relative to the non-inoculated control, promoted larger fruit-size classes, and maintained productivity with a 20% reduction in mineral fertigation under the tested conditions [82]. A separate open-field tomato experiment showed that nitrogen-fixing bacterial synthetic communities could support marketable production under reduced fertilization and alter the native rhizosphere microbiota [83]. These results are relevant to management, but they represent particular formulations, sites, and application programs rather than a generally transferable substitution rate.

Contradictory evidence confirms the need for this caution. In 14 maize and wheat experiments conducted across five Mexican states, biofertilizer responses were observed mainly under moderately low soil P or N availability. Only 21% of the experiments produced a significant yield advantage from biofertilizer application, whereas responses were generally absent in higher-input systems [84]. Biofertilizers should therefore be positioned as targeted tools for improving nutrient acquisition under defined constraints, not as inputs that routinely allow a fixed proportion of mineral fertilizer to be replaced across crops and environments.

5.3. Crop-Specific Responses and Quality-Related Outcomes

Crop-quality endpoints do not necessarily follow yield responses. In the commercial greenhouse tomato experiment discussed in Section 5.2, higher fruit production did not

translate into appreciable improvements in most measured fruit-quality traits; differences were limited mainly to pH and electrical conductivity [82].

Evidence for industrial crops remains less consolidated than for cereals, grain legumes, and horticultural crops. This gap is not only taxonomic but also methodological: many studies report biomass or yield while omitting oil concentration, fiber properties, starch or sugar recovery, latex production, specialized metabolites, or other processing-relevant endpoints. The Chinese field meta-analysis by Pei et al. [15] illustrates this uncertainty. Although biofertilization improved average crop yield and several quality indicators, responses in rapeseed and cotton were not statistically significant, showing that benefits documented in vegetables or legumes cannot be assumed for industrial crops. Sugarcane provides a useful example of both potential and interpretative limitation. A formulation combining endophytic diazotrophic bacteria with humic substances was evaluated over three crop cycles and in large strip plots. Yield responses varied among years and crop cycles, while soluble solids and polarizable sugars in the juice remained unchanged. Moreover, because the product combined microorganisms with bioactive humic constituents, its effects could not be assigned exclusively to the microbial component. The absence of increased leaf N additionally suggested that improved nutrient-use efficiency or physiological stimulation, rather than quantitatively important biological N fixation, may have contributed to the response [14].

In rubber, a biofilmed formulation improved dry-matter production and nutrient uptake in *Hevea brasiliensis* nursery plants under field conditions [11]. This finding supports the relevance of microbial delivery systems to an industrial crop, but nursery growth should not be equated with evidence of improved mature-tree latex yield or technological latex quality. Cotton studies likewise indicate that formulation intensity does not necessarily translate into a stronger response. Escobar Diaz et al. [85] found that inoculum concentrations differing by six orders of magnitude produced comparable fiber and seed yields for several *Aspergillus* and *Bacillus* treatments. Greater microbial recovery from plant tissues at higher concentrations did not consistently result in greater plant productivity, demonstrating that viable-cell number alone is an inadequate predictor of field efficacy.

For crops in which composition and processing quality are important outcomes, field validation should therefore extend beyond yield and include crop-specific compositional and processing criteria. Relevant endpoints include oil and protein yield in oilseeds, fiber quantity and quality in cotton and other fiber crops, fermentable sugar and starch recovery, latex production, medicinal or aromatic metabolite profiles, and biomass suitability for biorefinery conversion. Multi-year comparisons against equivalent mineral-fertilizer programs are particularly important because an increase in vegetative biomass does not necessarily improve processing-relevant product yield or quality. At present, the evidence supports crop- and formulation-specific opportunities rather than a general conclusion that biofertilizers consistently improve crop performance across these production systems.

6. Integration of Biofertilizers with Sustainable Nutrient and Environmental Management

6.1. Integrated Nutrient and Organic Amendment Systems

Integrated nutrient management (INM) combines biological, organic, mineral, and recovered nutrient sources according to crop demand and site-specific soil constraints [86–88]. Biofertilizers can complement organic amendments by mobilizing nutrients and improving rhizosphere functioning, while the amendments provide carbon, nutrients, and protective microhabitats. Greenhouse evidence shows that phosphate-solubilizing bacteria, humic substances, and arbuscular mycorrhizal fungi can act complementarily, and combined biofertilizer and organic-fertilizer treatments have improved rice growth and photosyn-

thetic performance [28,36]. However, these results do not establish that all combinations are synergistic or that the microbial component alone caused the response.

The outcome depends on nutrient status, amendment properties, and ecological compatibility. Organic materials may support inoculant survival and nutrient mineralisation, but high nutrient availability can reduce plant dependence on microbial symbioses, while salinity, pH, contaminants, or decomposition products may inhibit establishment. Carrier and amendment effects may also mask the contribution of the inoculated strain. Factorial comparisons of the inoculant, amendment, and their combination are therefore needed. If the carrier itself supplies nutrients or organic carbon, a sterile-carrier or heat-killed inoculant control is additionally required to distinguish microbial effects from those of the formulation matrix. In organic and low-input systems, rotations, legume-derived nitrogen, composts, and microbial inoculants can reinforce nutrient cycling, but remain constrained by the timing of nutrient release and competition during residue decomposition [89,90].

6.2. Complementary Agroecosystem Practices and Targeted Delivery

The effectiveness of biofertilizers is shaped by the wider management system. Conservation tillage can preserve soil structure, moisture, and fungal hyphal networks, but long-term field evidence shows that tillage, nitrogen fertilization, and soil depth interact strongly in shaping bacterial and fungal communities [91]. Reduced disturbance should therefore not be assumed to improve inoculant establishment automatically; residue stratification, altered seedbed conditions, and nutrient redistribution may favor some microorganisms while limiting others. Direct inoculant × tillage field tests remain scarce.

Cover crops and rotations can extend periods of living roots, retain nutrients, and maintain hosts for arbuscular mycorrhizal fungi, but their effects are not necessarily additive with inoculation. In a two-year study across conventional, transitional, and organic maize systems, farming system and soil properties explained more variation than cover cropping or microbial seed inoculation. Inoculation increased yield in one conventional field, whereas cover crops improved yield in the organic and transitional systems, and no consistent synergistic effect on microbial diversity was detected [92]. Complementary practices may therefore operate under different constraints rather than producing a universal combined benefit.

Biochar may act as both an amendment and a microbial carrier by providing porous microsites, retaining moisture, and altering nutrient sorption [17,58]. A meta-analysis of 56 studies found that adding microbial inoculants to biochar increased crop productivity relative to biochar alone, although additional benefits varied by inoculum origin and experimental setting and were less consistent under field conditions [93]. Its interaction with inoculants nevertheless depends on feedstock, pyrolysis conditions, pH, dose, and soil properties. Biochar may protect cells, but can also immobilise nutrients, adsorb signalling compounds, or alter indigenous communities; it should therefore be treated as a formulation- and site-specific partner rather than an inherently synergistic amendment.

Seed coating, biopriming, and localized placement concentrate viable cells near the germination zone and can reduce the amount of inoculum required [94–96]. In a saline-soil field experiment, a microencapsulated microbial coating improved maize emergence, soil available P, and grain yield relative to uncoated seeds [97]. Nevertheless, delivery performance depends on cell survival during drying and storage, compatibility with chemical seed treatments, release kinetics, and sowing conditions. Precision agriculture could match inoculant type, dose, and placement to spatial variation in soil fertility and moisture, but validated prescription rules and predictive response maps for live microbial products remain largely undeveloped.

6.3. Climate Resilience and Environmental Outcomes

Biofertilizers may support adaptation to drought, salinity, and temperature stress through improved root development, osmotic adjustment, antioxidant responses, and mycorrhizal access to soil water [35,98,99]. However, responses observed under controlled stress do not necessarily translate into stable yield protection under variable field conditions. Stress timing and intensity, soil texture, native microbiota, and crop genotype can all modify the outcome. Claims of climate resilience should therefore be supported by multi-season field trials quantifying yield stability and water- or nutrient-use efficiency under defined stress exposure. Mitigation claims require equally careful attribution. Reducing synthetic fertilizer inputs can avoid emissions associated with fertilizer manufacture and may decrease nutrient losses, but the benefit depends on the quantity displaced without a yield penalty [1,47]. Direct field evidence for effects of microbial biofertilizers on N_2O emissions, nitrate leaching, and water quality remains limited [94]. Likewise, soil-carbon gains in integrated systems may arise mainly from cover crops, residue retention, compost, or biochar rather than inoculation. Studies should therefore separate component effects and report greenhouse-gas fluxes, nutrient balances, nitrate movement, soil-carbon stocks, and yield-scaled environmental indicators.

Overall, biofertilizers are best positioned as components of diversified nutrient and soil-management systems rather than stand-alone sustainability interventions. Their integration with organic amendments, cover crops, conservation tillage, biochar, and targeted delivery can create complementary functions, but can also introduce competition, confounding, and management-specific failure. The priority is therefore to identify which combinations work, under which soil–crop–climate conditions, and whether their agronomic and environmental benefits persist at field scale.

7. Challenges and Limitations

7.1. Determinants of Field Reliability

The reliability of biofertilizers under field conditions is constrained by interacting biological, environmental, and management factors rather than by a single failure mechanism. Meta-analyses consistently show substantial heterogeneity among crops, soils, climates, microbial groups, and fertilization regimes, while controlled screening does not reliably identify the strains that perform best in the field [6,16]. Because the limited standalone predictive value of molecular and in vitro markers has been evaluated in Section 3, the present section focuses on the operational determinants that govern whether microbial potential is retained during storage, establishment, and crop production [18]. These determinants and the corresponding validation requirements are summarized in Table 2.

Persistence remains the central biological constraint. Inoculated strains must survive outside production conditions, reach the rhizosphere, occupy a suitable niche, and remain active in competition with indigenous microorganisms. Molecular tracking studies show that population decline after application is common, although the rate and consequences depend on strain, soil, host, and inoculation method [45,101]. Community interactions can suppress the inoculant through competition and antagonism or support it through cross-feeding and complementary nutrient mobilization; therefore, successful establishment cannot be inferred from inoculum size alone [112–114].

Table 2. Major determinants limiting the reproducibility of biofertilizer performance under field and commercial conditions.

Determinant	Why Performance Becomes Unreliable	Consequence for Field or Commercial Use	Required Validation or Mitigation	Selected References
Inoculant persistence and root colonization	Populations that colonise roots in short controlled assays may decline rapidly after application because of dispersal, predation, competition, or insufficient adaptation to the target soil.	Initial functional activity is not maintained long enough to influence nutrient acquisition or yield consistently.	Strain-resolved tracking across crop development; measurement of persistence in contrasting soils; selection of locally compatible strains and delivery routes	[45,100,101]
Soil and climatic compatibility	Temperature, moisture, pH, texture, nutrient status, and resident microbiota regulate microbial survival and expression of beneficial traits.	A formulation effective at one site may be neutral or ineffective under another soil-climate combination.	Multi-location and multi-season testing; reporting of soil properties and weather; product recommendations linked to defined environmental domains	[18,102–104]
Host genotype and management interactions	Root architecture, exudation, immune regulation, fertilizer rate, crop protection products, and rotation history influence recruitment and activity of inoculated microorganisms.	Crop responsiveness may vary among cultivars and management systems even when the same product and dose are used.	Genotype × environment × management trials; compatibility tests with fertilizers and seed treatments; breeding for microbiome responsiveness	[105–108]
Formulation and storage stability	Viability and physiological competence change during processing, drying, packaging, transport, and storage and are strongly strain- and formulation-specific.	The dose delivered to the field may differ from the labeled or experimentally validated dose.	Viable-cell counts and functional checks at the end of shelf life; stability testing under realistic temperature and humidity profiles; clear storage and expiry information	[17,48,51,109]
Compatibility within microbial consortia	Members may differ in growth rate, nutrient requirements, stress tolerance, and antagonistic or cooperative interactions during production and after application.	The relative abundance and functional balance of strains may change during storage or rhizosphere establishment.	Pairwise and community-level compatibility testing; strain-specific enumeration; evidence that the consortium outperforms its components under field conditions	[39,48,110]
Product identity, purity, and viable-cell compliance	Taxonomic misidentification, contamination, batch variation, or declining viable counts can disconnect the commercial product from the formulation evaluated experimentally.	Uncertain efficacy, biosafety concerns, and loss of user confidence.	Authenticated strains, batch-level purity and contamination testing, viable counts throughout shelf life, traceability, claim-specific efficacy testing, and post-market surveillance	[8,10,23,111]

The host plant is an equally active source of variability. Genotype-dependent root traits, exudation chemistry, and immune responses influence microbial recruitment and the benefits obtained from inoculation [105–107]. Modern cultivars selected under high-input conditions may not retain the same responsiveness to microbiome-mediated nutrient acquisition, but this hypothesis requires crop-specific validation rather than generalization across breeding histories [108]. Consequently, reproducible recommendations require genotype × environment × management testing and clearly defined recommendation domains rather than universal product claims.

7.2. Formulation Stability and Product Quality

Formulation must preserve microbial identity, viability, and functional competence from production to field application. These attributes are affected by fermentation conditions, concentration, drying, carrier or liquid matrix, packaging, oxygen and moisture exposure, storage temperature, and transport [17,48,51]. Because responses are strain- and product-specific, broad assumptions that one formulation class has a uniformly longer shelf life than another are not sufficiently reliable. Product stability should instead be demonstrated under the storage conditions and time period stated on the label.

Microbial consortia add functional breadth but also create additional quality-control demands. Differences in growth kinetics and stress tolerance can alter strain proportions during fermentation and storage, whereas antagonism or competitive exclusion may emerge only after formulation or release into soil. Field evidence suggests that selected consortia can improve crop nutrition and productivity, but their additional benefit requires a single-strain comparator, as illustrated quantitatively for soybean in Section 5.2 [39,80,110]. Each member should therefore be authenticated and quantified throughout shelf life, and the final product should demonstrate both internal compatibility and agronomic benefit.

Quality assurance should extend beyond a viable-cell count measured immediately after manufacture. A defensible system should verify strain identity, purity, absence of relevant contaminants, concentration of each declared microorganism at the end of shelf life, stability of the formulation, compatibility with the intended application method, and efficacy for the specific claim and target crop [8,23,111]. Batch traceability and post-market sampling are particularly important because laboratory validation of a prototype does not ensure that every commercial batch retains the same composition and performance. Strain-level biosafety evaluation is also essential because certain plant-associated microorganisms within genera such as *Enterobacter*, *Klebsiella*, and *Ochrobactrum*, and within the *Burkholderia cepacia* complex, can have opportunistic pathogenic potential. These hazards cannot be inferred or excluded from plant growth-promoting traits alone, supporting identity verification, risk-based screening, and appropriate eligibility criteria [115].

7.3. Economic, Market, and Regulatory Trade-Offs

Commercial adoption depends on predictable agronomic value, product availability, storage and distribution capacity, technical advice, and confidence in label claims. Direct cost comparisons with mineral fertilizers are often misleading because microbial products differ in application rate, intended function, persistence, and the extent to which they replace or complement nutrient inputs. The more relevant economic question is whether the product produces a sufficiently consistent return under the defined soil, crop, and management conditions. Variable efficacy increases demonstration, advisory, and transaction costs and is particularly difficult for small and medium enterprises that must finance product development, multi-site validation, registration, and distribution simultaneously [10,51,116].

The European Union provides the most harmonized of the jurisdictions considered here. Regulation (EU) 2019/1009 establishes an EU route for microbial plant biostimulants,

requires substantiation of labeled effects, pathogen limits, identification and concentration of intentionally added strains, and production and expiry information. This approach supports comparable labeling and access to the common market. Its principal limitation is that component material category 7 (CMC 7: micro-organisms) currently permits only *Azotobacter* spp., mycorrhizal fungi, *Rhizobium* spp., and *Azospirillum* spp. for this harmonized route, which excludes many promising bacterial taxa and complex consortia. Compliance documentation and conformity assessment may also impose proportionally greater costs on smaller developers [8,9].

The United States model is more flexible but less uniform. Products without pesticidal or plant-regulator claims are generally marketed through state fertilizer or biostimulant pathways, whereas claims falling within plant-regulator or pesticidal functions may trigger federal regulation under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Imported products containing living microorganisms may also require authorization from the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA). This claims- and jurisdiction-dependent structure can facilitate entry for diverse technologies, but it creates variable definitions, labels, evidence requirements, and registration costs across states, thereby reducing comparability for users and predictability for manufacturers [10,117,118].

India uses a more prescriptive product-standard model under the Fertiliser (Control) Order. The framework specifies recognized biofertilizer categories, minimum viable counts that must be maintained throughout shelf life, contamination limits, physicochemical requirements, and formal lot-sampling procedures. These provisions offer operational clarity and a direct basis for inspection. However, some efficacy specifications remain based on laboratory proxy tests, such as solubilization zones, which do not establish consistent crop responses under diverse field conditions. The practical strength of this model therefore depends on analytical capacity, representative sampling, enforcement, and supplementation of product standards with claim-specific field validation [119].

Brazil provides a complementary model in which product-specific inoculant requirements coexist with a broader framework for agricultural bioinputs. Federal Law No. 15.070/2024 explicitly covers both inoculants and biofertilizers, while Normative Instruction SDA/MAPA No. 13/2011 specifies registration, authorized microorganisms, viable-cell guarantees, contamination controls, shelf-life requirements, and labeling for agricultural inoculants. These provisions make microbial product quality operationally assessable, although Brazilian regulatory terms such as biofertilizante and inoculante do not necessarily coincide with the narrower nutrient-related definition of microbial biofertilizer used in this review [120,121].

China regulates microbial fertilizers through a fertilizer registration framework complemented by product- and method-specific standards. The Measures for the Administration of Fertilizer Registration, revised in 2022, cover microbial materials, while GB 20287-2006 specifies requirements for agricultural microbial inoculants. The newer NY/T 1536-2025 establishes a technical framework for field experiments and efficacy evaluation of microbial fertilizers. These provisions support product standardization and structured performance assessment, but conformance with a product standard should not be interpreted as evidence of reproducible yield benefits across different crops, soils, and management systems [122–124].

Comparison of these five jurisdictions reveals distinct approaches to market access, microbial-group eligibility, viable-cell requirements, efficacy substantiation, and post-market oversight. The EU offers a harmonized route for specified microbial plant biostimulants; the US remains claims- and jurisdiction-dependent; India emphasizes defined product standards; Brazil combines bioinput legislation with inoculant-specific controls;

and China links fertilizer registration with technical standards for microbial products and field evaluation. No approach alone resolves the challenge of converting verified product quality into consistently demonstrated field performance. Greater international comparability should therefore emphasize strain identity, purity and viability at expiry, claim-specific multi-environment evidence, proportionate biosafety review, and transparent product labeling, without assuming that registration or laboratory compliance guarantees agronomic efficacy [8,10,120–127].

8. Critical Perspective: Research Priorities and Future Directions

The operational determinants of field reliability have been discussed in Section 7 and summarized in Table 2. Rather than repeating those requirements, this section identifies research questions that could change how microbial biofertilizers are selected, evaluated, and deployed. Three linked priorities emerge: establishing causal and transferable indicators of efficacy; defining ecological and management conditions under which inoculation adds value; and integrating agronomic, environmental, and economic evidence into practical decisions. These unresolved questions and proposed approaches are summarized in Table 3.

Table 3. Distinct research frontiers and decision-relevant questions for microbial biofertilizers.

Research Frontier	Decisive Research Question	Distinctive Approach and Intended Advance	Selected References
Causal, transferable efficacy indicators	Which microbial and plant signals precede a measurable nutrient-acquisition benefit across contexts?	Combine perturbation, strain tracking, nutrient-flux measurements, and external model validation; distinguish functional predictors from correlated markers.	[23,43,128]
Functional added value of microbial consortia	When does a designed consortium outperform its most effective component and the resident microbiome?	Use component-removal and host–soil cross-comparisons; quantify added function and persistence, not diversity alone.	[39,110,129–131]
Predictable response domains (G × E × M)	Which measurable combinations of genotype, soil and management predict benefit, non-response or failure?	Prospectively validate response boundaries in independent environments; report null outcomes and produce crop-specific decision rules.	[18,105,115,128,130]
Net benefits under climate and resource constraints	Do stress-tolerance and advanced-delivery gains exceed their resource and environmental costs?	Use multi-season, yield-scaled nutrient, water and emission endpoints, matched conventional comparators, and efficacy-adjusted LCA where appropriate.	[35,53,67,132–134]
Market-relevant and regulatory decision evidence	Which verified benefits justify product costs, adoption risks and evidence requirements?	Link field-response distributions with techno-economics, farm-level adoption research and proportionate claim substantiation.	[116,135–137]

8.1. From Molecular Associations to Transferable Predictions

A central research opportunity is to distinguish molecular markers that merely accompany inoculation from processes that causally improve nutrient acquisition. Longitudinal studies should combine strain-resolved tracking and microbial activity with measurable nutrient fluxes, plant nutrient uptake, and yield, preferably including experimental perturbations that test competing mechanisms [23,43]. Predictive models should then be

challenged with independent sites, seasons, cultivars, and management regimes rather than judged only by performance within their development dataset [128]. Their value will depend on identifying indicators that remain informative beyond the original experiment and on quantifying when predictions become unreliable.

8.2. Ecological Matching and Prospective Response Domains

A second frontier is to explain when introduced microorganisms contribute functions that the resident soil microbiome does not already provide. Host recruitment, root exudates, immune responses, and competition within native communities make establishment a relational property of the plant–soil–inoculant system [18,105,138,139]. For designed consortia, component-removal experiments and comparisons with the most effective single strain could establish whether added complexity produces a genuine functional advantage [39,110,129]. Prospective genotype × environment × management studies should test response boundaries across contrasting nutrient availability and soil microbiomes, including null or negative outcomes [128,130,131]. The intended result is a validated response domain for each product, not a universal ranking of strains.

8.3. Integrated Environmental and Economic Decision Evidence

A third priority is to establish whether biological gains produce net benefits under realistic farming conditions. Claims of stress protection, reduced nutrient losses, or emissions mitigation require repeated field measurements against comparable nutrient-management alternatives [35,53,132,134]. New delivery technologies, including nano-enabled formulations, should demonstrate added value over conventional inoculants after accounting for production, use, and end-of-life burdens [67,133]. Linking yield stability, environmental outcomes, and cost through joint agronomic, life-cycle, and techno-economic analyses would help identify conditions in which adoption is justified [116,135,137]. Regulatory research should translate such evidence into proportionate product claims without duplicating quality-control requirements discussed in Section 7.3 [136].

9. Conclusions

Biofertilizers offer substantial but context-dependent opportunities to improve nutrient acquisition, nutrient-use efficiency, and crop performance. Their principal value does not lie in universal replacement of mineral fertilizers, but in supporting more efficient nutrient management under conditions in which the microbial strain, crop genotype, soil environment, formulation, and management system are mutually compatible. Molecular and omics-based studies have clarified many potential modes of action, but the presence of functional traits or microbiome responses alone remains insufficient to predict persistent colonization, nutrient transfer, or reproducible field efficacy.

Quantitative evidence indicates positive average effects on crop yield and nutrient-use efficiency, although responses vary widely among crops, soils, climates, microbial products, and fertilization regimes. Benefits are generally more likely under identifiable nutrient or environmental constraints and when inoculants complement, rather than completely replace, mineral and organic nutrient sources. For crops in which composition and processing quality are important outcomes, efficacy should be assessed not only through biomass or yield, but also through crop-specific compositional and functional traits. Current evidence therefore supports crop- and formulation-specific opportunities rather than universal performance claims.

A broader resource-efficiency and environmental perspective shows that microbial origin, renewable feedstocks, or biodegradable carriers do not automatically establish environmental superiority. Production energy, water and material demand, storage stability,

application efficiency, and actual reductions in fertilizer use must be considered through comparative and efficacy-adjusted assessment. Similarly, claims concerning greenhouse-gas mitigation, soil carbon accumulation, or reduced nutrient losses require direct, long-term field measurements rather than inference from improved plant growth alone. Taken together, these findings highlight a key challenge in translating mechanistic potential into practical outcomes: microbial products with promising mechanistic properties and demonstrated quality compliance do not necessarily deliver reproducible agronomic or environmental benefits under field conditions. Reliable biofertilizer deployment will require field-adapted strains, stable formulations, traceable product identity, viable-cell compliance throughout shelf life, multi-environment validation, and proportionate regulatory standards. Biofertilizers should consequently be regarded as site-specific components of integrated nutrient and resource-management systems, rather than as self-sufficient solutions. Their long-term contribution to sustainable crop production will depend on whether mechanistic innovation can be translated into reproducible agronomic performance, measurable environmental benefits, and economically viable products under realistic production conditions.

Author Contributions: Conceptualization, M.C.; investigation, M.C. and E.S.-K.; resources, M.C. and E.S.-K.; writing—original draft preparation, M.C.; writing—review and editing, E.S.-K. and C.P.; visualization, M.C.; supervision, C.P.; funding acquisition, C.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article, as this is a review based on previously published literature.

Acknowledgments: During the preparation of this manuscript, the authors used a generative AI tool (OpenAI, GPT-5 series) to create conceptual graphical elements visualizing the described mechanisms. All content was critically reviewed and revised by the authors, who take full responsibility for the final content of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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