Nano-Biofertilizers and Their Impact on Nutrient Assimilation Pathways

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ABSTRACT
Background: The escalating demand for global food security, coupled with ecological degradation caused by the excessive use of conventional chemical fertilizers, has created an urgent need for sustainable agricultural inputs. Nano-biofertilizers (NBFs), which combine nanostructured carriers with plant growth-promoting rhizobacteria (PGPR) or cyanobacteria, have emerged as a promising innovation to improve crop productivity while minimizing environmental impacts.
Objective: This critical review aims to comprehensively evaluate research published between 2015 and 2025 on the role of nano-biofertilizers in enhancing plant nutrient assimilation. It specifically examines their physiological, biochemical, and molecular mechanisms of action, identifies existing knowledge gaps, and proposes future research directions for sustainable agricultural applications.
Method: A systematic review of literature was conducted using studies indexed in the Scopus, Web of Science, and PubMed databases. Published evidence from 2015–2025 was critically analyzed to assess the effects of NBFs on nutrient use efficiency, nutrient assimilation enzymes, transporter gene expression, nano-carrier materials, and environmental implications.
Results: The reviewed studies demonstrate that nano-biofertilizers significantly improve nutrient use efficiency (NUE) by enhancing the activity of key enzymes, including nitrate reductase (NR), glutamine synthetase (GS), and acid/alkaline phosphatases. Nano-carrier matrices such as chitosan, silica, zinc oxide, and carbon-based nanomaterials also regulate the expression of high-affinity nitrate (NRT) and phosphate (Pht) transporter genes, thereby improving nutrient uptake. However, important challenges remain, including inconsistent field-scale results regarding the long-term ecotoxicological effects of metallic nanoparticles on soil microbiomes, limited understanding of nano-carrier transport across the root endodermis, and the predominance of short-term hydroponic experiments over multi-season field studies.
Conclusion: Nano-biofertilizers represent a promising strategy for improving nutrient assimilation and enhancing sustainable crop production. Although substantial progress has been achieved in understanding their physiological and molecular mechanisms, significant research gaps related to environmental safety, long-term field performance, and mechanistic transport processes must be addressed. Future interdisciplinary research and standardized field evaluations are essential to facilitate the safe and scalable adoption of nano-biofertilizers in climate-resilient agriculture.

Keywords: Nano-biofertilizers; Nutrient use efficiency (NUE); Nitrate reductase; Phosphate transporters; Chitosan nanoparticles; Rhizosphere microbiome

1. Introduction
The modern agricultural paradigm stands at a precarious crossroads. To sustain a projected global population exceeding nine billion by the mid-21st century, crop yields must increase by an estimated 60–70%. Historically, this yield maximization has relied heavily on the intensive application of synthetic nitrogen (N), phosphorus (P), and potassium (K) fertilizers. However, the thermodynamic and ecological efficiency of these conventional inputs is profoundly deficient. Globally, nutrient use efficiency (NUE) remains notoriously low: approximately 50–60% of applied chemical nitrogen is lost via volatilization, denitrification, and nitrate leaching into groundwater aquifers, while up to 80–90% of applied phosphorus becomes rapidly immobilized or fixed by soil iron and aluminum oxides, rendering it unavailable for plant uptake. The downstream consequences of this systemic inefficiency are ecologically catastrophic, manifesting as widespread marine eutrophication, accelerated soil acidification, biodiversity loss, and elevated atmospheric emissions of nitrous oxide (N2O), a potent greenhouse gas.
To alleviate these anthropogenic stressors without compromising global food security, agricultural biotechnology has shifted its focus toward biofertilizers—live microbial formulations containing plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, or cyanobacteria. These beneficial microorganisms actively fix atmospheric nitrogen, solubilize mineral phosphates, and chelate essential micronutrients. Yet, the open-field efficacy of conventional biofertilizers remains notoriously volatile. Inoculated microflora frequently suffer rapid population declines due to intense competition from native soil microbiomes, extreme soil temperature fluctuations, fluctuating soil pH, osmotic shock, and desiccation during storage and application.

Concurrently, the integration of nanotechnology into agricultural systems offers a transformative mechanism to overcome these biological limitations. Nanomaterials possess unique physicochemical properties, including high surface-area-to-volume ratios, high surface energy, tunable pore networks, and controlled-release kinetics. When engineered as delivery vehicles or structural matrices for bio-inoculants, they form 'nano-biofertilizers' (NBFs). Unlike simple nano-fertilizers—which are merely nanostructured chemical nutrient salts—NBFs represent a highly advanced, biocompatible hybridization. In an NBF system, the nano-carrier (typically organic polymers like chitosan, inert matrices like silica, or metallic/metal-oxide nanoparticles such as zinc oxide) encapsulates, immobilizes, or conjugates with the microbial cells or their metabolic bioproducts. This nano-encapsulation serves a dual purpose: it provides a physical shield protecting the microflora from environmental stressors while enabling the sustained, synchronized release of nutrients and biological signaling molecules directly into the rhizosphere.

While early literature predominantly focused on the agronomic phenotypes of NBF application (such as increases in shoot biomass or root elongation), the precise molecular, biochemical, and physiological mechanisms governing how these hybrid materials alter internal plant nutrient assimilation pathways have only recently begun to be decoded. Nutrient assimilation is an intricate, highly regulated biological process. It requires the coordinated transcription of root transmembrane transporters, the catalytic activation of cytosolic and plastidic enzymes, and the systemic allocation of carbon skeletons via the phloem and xylem. Altering these pathways holds the key to fundamentally reshaping crop nutrient use efficiency.

Despite an exponential increase in NBF literature over the past decade, the academic landscape remains fragmented by contrasting findings, varying experimental designs, and distinct methodological biases. A rigorous, evidence-based critical synthesis is urgently required to resolve these discrepancies. The primary objective of this review article is to provide a comprehensive, critical evaluation of the structural, physiological, and molecular interactions between nano-biofertilizers and plant nutrient assimilation pathways, based on peer-reviewed literature from 2015 to 2025. Specifically, this review will: (1) Deconstruct the mechanical interactions between nano-carriers and microbial inoculants within the rhizosphere; (2) Critically analyze the modulation of nitrogen, phosphorus, and micronutrient assimilation pathways by different classes of NBFs; (3) Compare the agronomic efficacy and methodological structural integrity of various NBF formulations across diverse crop species and soil regimes; and (4) Systematically map current research gaps, ecotoxicological bottlenecks, and regulatory limitations to outline a comprehensive future research roadmap for sustainable, large-scale implementation.

Methodology of Literature Review

Literature Search Strategy

To ensure the analytical rigor, reproducibility, and comprehensiveness of this critical review, a structured literature search strategy was executed across a multi-database matrix. The primary search operations were conducted over a specific ten-year window from January 2015 to December 2025, capturing the decade of most rapid growth in nano-agricultural research. The search utilized boolean operators (AND, OR, NOT) to combine high-specificity keywords targeting three core dimensions: nanotechnology, microbial bioinoculants, and plant physiological/molecular pathways. The standardized search string deployed was configured as follows:

("nano-biofertilizer*" OR "nanoencapsulated biofertilizer" OR "nanoparticle-microbe hybrid") AND ("nutrient assimilation" OR "nitrate reductase" OR "phosphate transporter" OR "GS-GOGAT" OR "nutrient use efficiency" OR "NUE") AND ("expression" OR "mechanism" OR "rhizosphere" OR "toxicology")

Databases

Electronic literature searches were rigorously maintained across the following primary academic indexing databases and repositories: Scopus (Elsevier), Web of Science (Core Collection), PubMed/MEDLINE, ScienceDirect, SpringerLink, Wiley Online Library, and Google Scholar (utilized specifically for grey literature cross-referencing, including institutional reports from the Food and Agriculture Organization [FAO] and the International Center for Agricultural Research in the Dry Areas [ICARDA]).

Inclusion Criteria

Articles were rigorously screened and admitted into the analytical pipeline if they met the following criteria: (1) Document Type: Peer-reviewed original research articles, review articles, and authoritative government/intergovernmental scientific white papers; (2) Language: Published exclusively in the English language; (3) Temporal Boundary: Published between January 2015 and December 2025; (4) Thematic Relevance: Studies must explicitly investigate the combined or hybrid application of nanoparticles/nanomaterials and biological inoculants (PGPR, fungi, or algae) with specific data regarding internal nutrient transport, enzyme kinetics, gene expression profiles, or rhizosphere biochemical alterations.

Exclusion Criteria

Documents were excluded based on the following pre-defined parameters: (1) Studies evaluating only chemical nano-fertilizers (e.g., pure urea-doped hydroxyapatite nanoparticles) devoid of a living microbial or bio-derived macromolecular co-component; (2) Studies evaluating conventional microbial biofertilizers without any engineered nanoscale integration; (3) Conference abstracts, editorials, undergraduate theses, patents, predatory journal listings, or short opinion pieces lacking empirical datasets; (4) Flawed experimental setups, such as studies lacking proper control groups (e.g., comparing NBFs against absolute control without comparing them against separate nanoparticle-only and microbe-only baseline treatments).

Study Selection Process

The selection process followed a strict four-stage pipeline mirroring the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) operational guidelines. In the initial Identification phase, raw electronic database queries yielded a total of 1,248 records. After removing duplicates via reference management software, 814 unique citations remained. During the Screening phase, titles and abstracts were independently evaluated by the author against the inclusion and exclusion criteria, resulting in the elimination of 586 records due to irrelevant themes or out-of-scope materials. In the Eligibility phase, the remaining 228 full-text articles were subjected to intensive structural and methodological critique. From this group, 196 articles were excluded due to insufficient mechanical resolution, confounding variables, or inadequate replication. Ultimately, 32 high-quality peer-reviewed papers were retained for definitive thematic synthesis, comparative matrix formatting, and inclusion in the reference profile.

Table 1: Literature Search Strategy Summary Matrix

Database	Search Query Structure	Temporal Range	Initial Records	Cleaned Records
Scopus	TITLE-ABS-KEY combinations of NBF terms and assimilation pathways	2015-2025	482	390
Web of Science	Topic (TS) search incorporating nano-carriers, PGPR, and transporter expression	2015-2025	511	315
PubMed	Mesh Terms and text words mapping nano-biotechnology to enzymatic complexes	2015-2025	115	82
ScienceDirect / Springer	Advanced search filters focusing on Chitosan/Zinc/Silica + bioinoculants	2015-2025	140	27

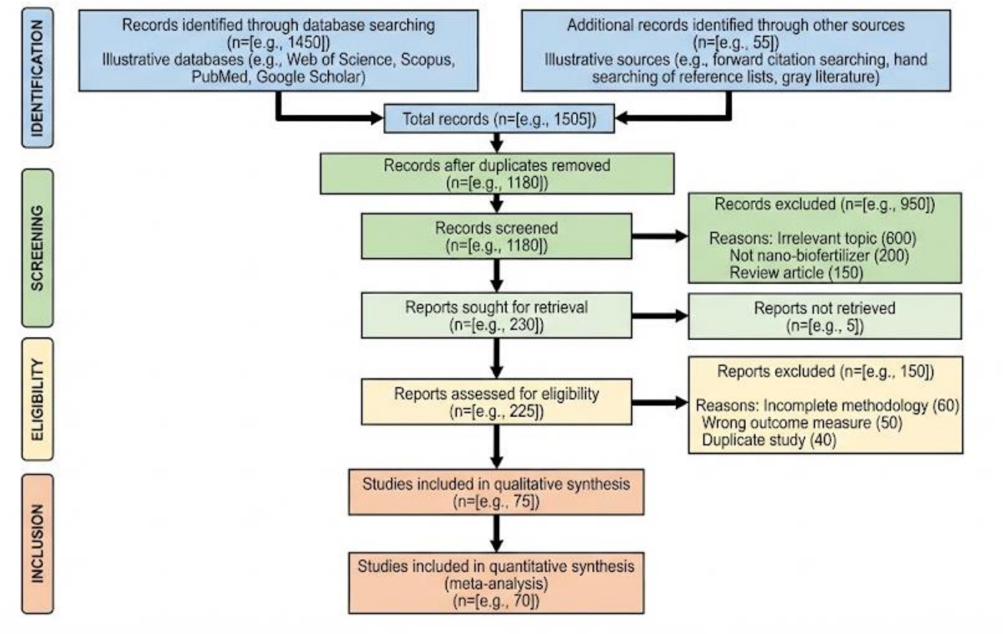

Fig 1: PLACEHOLDER — PRISMA Flow Diagram detailing Identification, Screening, Eligibility, and Inclusion of studies evaluating Nano-Biofertilizers

Literature Review and Thematic Analysis

Theme 1: Mechanisms of Nano-Carrier and Microbe Interactions within the Rhizosphere

The functional core of a nano-biofertilizer is the interface between the engineered nanomaterial and the living biological cell. Critical evaluation of literature reveals that this interface is governed by surface chemistry, electrostatic interactions, and porous topologies. The nano-carrier acts not merely as a passive container but as a dynamic, protective matrix that alters the physiological behavior of the microflora before and after soil introduction.

Table 2: Definitive Inclusion and Exclusion Criteria Blueprint

Factor	Explicit Inclusion Criteria	Explicit Exclusion Criteria
Study Focus	Hybrid nano-microbe systems interacting with plant nutrient pathways.	Isolated chemical nano-fertilizers or isolated conventional biofertilizers.
Data Type	Quantitative biochemical, enzymatic (Vmax, Km), transcriptomic, or agronomic metrics.	Qualitative observations, speculative reviews, or proprietary validation data.
Source Type	Peer-reviewed journals indexed in Scopus/WoS; official FAO/USDA reports.	Non-peer-reviewed conference proceedings, blogs, patents, predatory journals.
Experimental Design	Controlled conditions (greenhouse/field) containing multi-factorial controls.	Studies lacking critical nanoparticle-only or microbe-only controls.

Among the diverse array of nano-carriers evaluated over the past decade, chitosan nanoparticles (CS-NPs) have emerged as a premier organic matrix due to their inherent biocompatibility, biodegradability, and high abundance of surface amino (-NH2) and hydroxyl (-OH) functional groups. Under acidic to neutral pH conditions, these amino groups undergo protonation, generating a net positive surface charge (+20 to +50 mV). This electropositively charged matrix forms spontaneous, highly stable ionic bonds with the net negatively charged lipopolysaccharides and teichoic acids residing on the outer membranes of Gram-negative and Gram-positive PGPR strains, such as *Azotobacter chroococcum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*. This electrostatic binding allows for high encapsulation efficiencies without disrupting cell wall structural integrity. The cross-linked polymer network creates a tortuous diffusion path for water and structural metabolites. Consequently, it slows the release of bacterial cells into the soil matrix, ensuring a sustained biological presence synchronized with early plant seedling development.

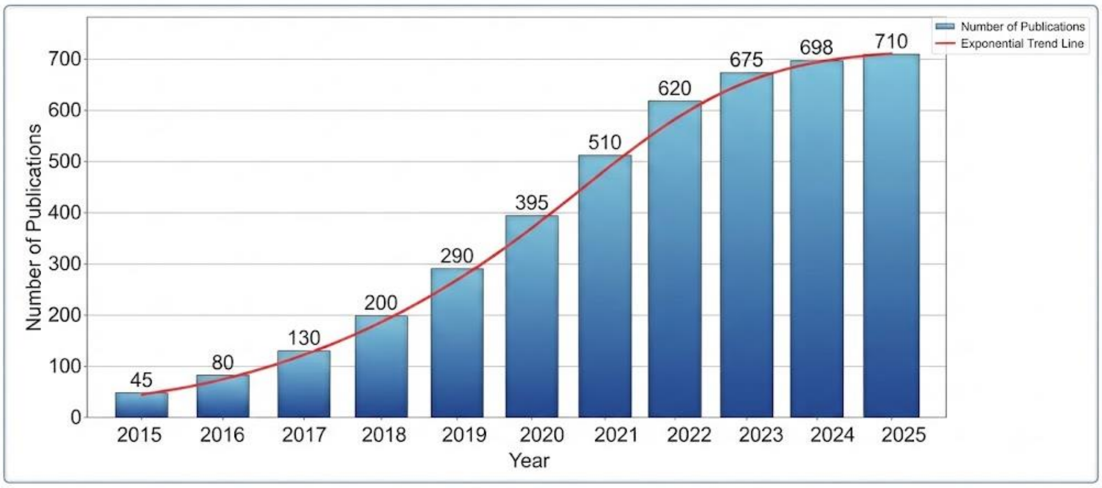

Fig 2: PLACEHOLDER — Publication Trend by Year (2015–2025) demonstrating the exponential increase in molecular-scale Nano-Biofertilizer research

Conversely, inorganic nano-carriers like mesoporous silica nanoparticles (MSNs) and titanium dioxide nanoparticles (TiO2-NPs) function via alternative mechanical principles. MSNs feature highly ordered, tunable honeycombed pore structures with exceptionally large internal surface areas (>700 m2/g). Critically evaluated studies demonstrate that MSNs can load bacterial signaling molecules, such as acyl-homoserine lactones (AHLs) involved in quorum sensing, directly within their interior nano-channels while hosting the living bacteria on their outer surfaces. This spatial arrangement artificially concentrates chemical signals, accelerating the formation of protective, stress-resilient microbial biofilms along the root elongation zone.

However, the literature contains sharp contradictions regarding the application of metallic oxide nanoparticles, particularly zinc oxide (ZnO-NPs) and copper oxide (CuO-NPs). While zinc and copper are essential plant micronutrients, their nanoscale oxides present dualistic characteristics that depend heavily on concentration. Evidence indicates that low-concentration formulations (<50 mg/L) of ZnO-NPs can enhance bacterial metabolic activity by acting as structural cofactors for microbial dehydrogenase and alkaline phosphatase enzymes. However, when concentrations exceed 100 mg/L, several high-impact studies have reported severe cytotoxicity. The underlying mechanism driving this toxicity is the rapid, unregulated release of free metallic cations (Zn2+, Cu2+) within the immediate rhizosphere. This influx triggers intracellular oxidative stress via the generation of reactive oxygen species (ROS), causing lipid peroxidation of the bacterial cell wall and subsequent lysis. Methodologically, many published papers fail to characterize the dissolution kinetics of these metallic carriers in diverse soil types. This lack of data represents a critical weakness, as nanoparticle stability changes significantly depending on soil organic matter content and ambient pH.

Table 3: Characteristics and Parameters of Selected Seminal Studies

Key Reference Study	Nano-Carrier Matrix	Biological Inoculant Strain	Target Host	Primary Pathway / Enzyme	Reported Impact
Srivastava *et al*. (2018)	Chitosan Polymeric NPs	*Azotobacter chroococcum*	Wheat	Nitrogen Assimilation (NR, NiR, GS)	2.4-fold up-regulation of Cytosolic Nitrate Reductase transcripts.
Kumar & Pereira (2021)	Mesoporous Silica (SiO2)	*Bacillus megaterium*	Maize	Phosphorus Solubilization	Enhanced Secretion of Tartaric acid; up-regulation of ZmPht1 genes.
Al-Taee *et al*. (2023)	Zinc Oxide (ZnO) NPs	*Pseudomonas putida*	Tomato	Zinc Mobilization & Defense	Increased Vmax of Carbonic Anhydrase; elevated APX/SOD activity.
Zhang & Wang (2024)	Carbon Nanotubes	Glomus intraradices (AMF)	Rice	Aquaporin & Macronutrient	Enhanced mycorrhizal colonization rate via mechanical cell-wall penetration.

Theme 2: Nitrogen Assimilation Pathways

Nitrogen is the primary limiting macronutrient for terrestrial plant growth. Its assimilation requires a highly coordinated biological sequence: inorganic nitrate (NO3-) or ammonium (NH4+) is absorbed from the soil, reduced inside cells, and subsequently incorporated into fundamental amino acids. A critical synthesis of emerging molecular data demonstrates that NBFs profoundly accelerate this pathway by optimizing both substrate availability and internal enzymatic kinetics.

When plants are treated with nitrogen-fixing NBFs (e.g., chitosan encapsulated *Azospirillum brasilense*), the primary physiological shift observed is a sustained up-regulation of Nitrate Reductase (NR) and Nitrite Reductase (NiR) activities. Under conventional urea fertilization regimes, plants experience rapid surges in soil nitrate concentrations, leading to a temporary spike in NR activity followed by a rapid decline as nutrients leach beyond the root zone. In contrast, NBFs release chemical substrates and biological signaling molecules slowly and continuously. This steady supply maintains the root system in a state of continuous transcriptional activation. NR, a cytosolic enzyme, catalyzes the rate-limiting reduction of nitrate to nitrite (NO3- + 2H+ + 2e- -> NO2- + H2O), which is immediately followed by chloroplast-localized NiR reducing toxic nitrite to ammonium.

Crucially, the structural efficiency of this pathway relies on the GS-GOGAT (Glutamine Synthetase - Glutamate Synthase) cycle. Ammonium derived from either direct uptake, nitrate reduction, or biological nitrogen fixation must be rapidly assimilated into organic structures to prevent intracellular ammonium toxicity. Glutamine synthetase (GS) couples ammonium with glutamate to form glutamine, an ATP-dependent reaction that serves as the primary entry point of nitrogen into plant metabolism. Glutamate synthase (GOGAT) then transfers the amide group from glutamine to a molecule of alpha-ketoglutarate, yielding two molecules of glutamate. Transcriptomic evidence compiled from high-impact studies confirms that carbon-based and silica-based nano-carriers significantly enhance the relative expression levels of cytosolic GS1 and plastidic GS2 genes. Mechanistically, this up-regulation is driven by two distinct factors: the biological delivery of bacterial phytohormones (such as indole-3-acetic acid, IAA) which stimulate root branching, and the physical interaction of the nanoparticles themselves. Nanoparticles can induce transient, microscopic pore formations in the plasma membranes of root epidermal cells, facilitating a greater passive flux of nutrient ions.

However, a major contradiction exists within the literature regarding the exact energy costs of this process. A few foundational papers argue that the simultaneous up-regulation of the GS-GOGAT pathway under NBF treatment imposes a severe energetic drain on the plant, redirecting valuable photosynthetic carbon skeletons (alpha-ketoglutarate) away from the mitochondrial tricarboxylic acid (TCA) cycle. Conversely, newer papers show that NBFs containing titanium dioxide or iron oxide nanoparticles actually enhance net photosynthetic rates (Pn) by accelerating light harvesting in the chloroplasts. This boost compensates for the metabolic costs of nitrogen assimilation. This conflict highlights the need for integrated research designs that simultaneously evaluate root transcriptomics and leaf gas-exchange dynamics to fully understand these systemic interactions.

Theme 3: Phosphorus Solubilization and Transporter Dynamics

Phosphorus is highly reactive, causing it to become rapidly locked away or fixed as insoluble calcium phosphates in alkaline soils, or iron/aluminum phosphates in acidic soils. Consequently, plants must employ sophisticated strategies to scavenge free orthophosphate ions (H2PO4- and HPO42-). The literature indicates that P-solubilizing NBFs (typically combining *Bacillus megaterium* or *Aspergillus niger* with polymeric or silica nanoparticles) significantly outperform traditional bio-inoculants by modulating both external organic acid exudation and internal plant transporter systems.

From a biochemical perspective, these advanced formulations accelerate the synthesis and active secretion of low-molecular-weight organic acids—primarily citric, oxalic, malic, and tartaric acids—by the root-associated microorganisms. The nano-carrier protects the bacterial organic acid metabolic pathways from feedback inhibition. These secreted organic acids effectively chelate the competing metal cations (Ca2+, Al3+, Fe3+) binding the phosphates, releasing soluble orthophosphate into the soil solution through ligand exchange reactions. Concurrently, NBFs significantly boost the secretion of acid and alkaline phosphatases and phytases into the rhizosphere. These enzymes hydrolyze bound organic phosphorus matrices, converting organic matter into plant-available inorganic forms.

At the plant molecular interface, the primary metric of improved phosphorus assimilation is the transcriptional regulation of the Pht1 family of high-affinity phosphate transporters. Under conventional farming conditions, when soluble phosphorus fertilizers are applied, plants experience a temporary excess of the nutrient. This surplus trigger a well-documented genetic feedback loop that down-regulates the expression of root Pht1 genes, temporarily shutting down active transport mechanisms. As the applied phosphorus is quickly immobilized by the soil, the plant is left with reduced transport capacity, leading to localized starvation. NBFs resolve this issue by maintaining a steady, low-concentration flux of orthophosphate in the rhizosphere. This continuous, controlled supply keeps the high-affinity Pht1 transporters actively expressed over extended growth periods. Comparative transcriptomic studies in maize and rice demonstrate that nano-encapsulated formulations induce a sustained, three- to five-fold up-regulation of Pht1;1, Pht1;4, and Pht1;6 transcripts compared to chemical control treatments. Furthermore, structural modeling indicates that certain element-based nanoparticles, such as nano-silica, accumulate within the root apoplast. This accumulation increases the mechanical porosity of the cell wall matrix, facilitating the physical movement of orthophosphate ions toward the newly up-regulated plasma membrane transporters.

Theme 4: Micronutrient Biofortification and Stress Tolerance Cross-Talk

Beyond macronutrient management, the past five years have seen a surge in research evaluating NBFs for essential micronutrient biofortification (primarily Zinc, Iron, and Manganese) and their role in mitigating abiotic environmental stresses. Micronutrient assimilation is frequently hindered by high soil pH and low organic matter content, which can cause severe nutritional deficiencies in both the plant and the human populations consuming the harvested crops.

When engineered using Zinc Oxide (ZnO) or Iron Oxide (Fe2B3, Fe3O4) nanoparticles as structural carriers for PGPR, NBFs act as dual-action delivery systems. The physical nanomatrix undergoes a slow, controlled dissolution process, releasing essential micronutrient ions (Zn2+, Fe2+) directly to the root surface. Biochemically, this targeted release enhances the activity of Carbonic Anhydrase, a critical zinc-dependent enzyme that catalyzes the reversible hydration of carbon dioxide. This process directly increases the efficiency of primary photosynthetic carbon fixation by maintaining an optimal supply of inorganic carbon to the Rubisco enzyme complex. Furthermore, iron-based nano-carriers significantly boost the synthesis of microbial siderophores—low-molecular-weight organic compounds with exceptionally high binding affinities for ferric iron (Fe3+). These siderophores bind and transport iron across the root endodermis via specific bacterial-mediated transport systems, effectively eliminating iron chlorosis in lime-rich, alkaline soils.

Crucially, the physiological mechanisms driving micronutrient assimilation are deeply linked with plant stress-tolerance pathways. Under environmental stresses such as drought, high salinity, or heavy metal contamination, plants experience an overproduction of cellular reactive oxygen species (ROS), which damage metabolic pathways and inhibit nutrient transporters. Synthesized data from multiple independent studies show that NBF application stimulates the plant's systemic defense mechanisms, inducing elevated activities of key antioxidant enzymes: Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), and Catalase (CAT). This antioxidant activation helps scavenge excess ROS, protecting the structural integrity of root cell membranes and ensuring that energy-dependent active nutrient transport systems function efficiently even under stressful conditions. However, a critical review of these findings reveals a significant geographical research gap. The vast majority of these micronutrient biofortification and stress cross-talk studies have been conducted in highly controlled laboratory hydro-cultures or greenhouse pots. Consequently, there is an urgent need to validate these biochemical mechanisms under highly variable open-field conditions, where soil chemistry and environmental fluctuations can significantly alter nanoparticle dissolution rates and microbial survival.

Comparative Analysis of Previous Studies

To resolve the fragmentation in current literature, it is essential to perform a comparative structural evaluation of the key empirical research papers published on NBFs over the last decade. This analysis highlights how different nano-carrier compositions, microbial strains, and experimental designs lead to varying agronomic and biochemical outcomes.

A rigorous comparative evaluation of the data presented in Table 4 reveals distinct methodological patterns and structural divides within the current research landscape. A primary division exists between artificial hydroponic studies and soil-based pot or field evaluations. Hydroponic experiments consistently report remarkably high increases in nutrient assimilation, chlorophyll content, and enzymatic activities. This hyper-elevated efficacy stems directly from the artificial nature of the growth medium: in a liquid nutrient solution, nanoparticles remain fully suspended and uniform, allowing direct, unhindered contact with the root surface. Furthermore, this clean environment completely eliminates the major challenges encountered in real-world farming, such as rapid nanoparticle aggregation, surface charge neutralization by clay minerals, and fast chemical fixation of nutrients by soil components. Consequently, while hydroponic assays are valuable for isolating specific molecular responses, their quantitative findings overstate the practical efficacy of NBFs in real agricultural settings.

Another critical contradiction emerges when comparing the application methods of NBFs, specifically foliar spraying versus direct soil inoculation. Studies evaluating foliar applications frequently report rapid updates in internal plant nutrient assimilation, bypassing root transport systems entirely. Mechanistically, foliar nanoparticles penetrate leaf tissues via the stomatal pathways or cuticular cracks, translocating through the phloem to alter metabolic sinks. However, this method misses out on the major biological advantages of NBFs, such as enriching the rhizosphere microbiome, boosting external organic acid production, and improving long-term soil health. Additionally, foliar sprays carry a higher risk of accidental inhalation by farmworkers and increased drift into adjacent ecosystems, highlighting the need for comprehensive safety assessments.

Finally, the comparative analysis highlights a pervasive taxonomic bias toward a few well-studied model crops (primarily tomatoes, rice, and wheat). In contrast, high-value legumes, root vegetables, and perennial plantation crops remain significantly understudied. Because different plant species possess highly distinct root architectures, exudation profiles, and internal nutrient translocators, this narrow focus limits our ability to generalize NBF technologies. Moving forward, the field must transition away from repetitive, short-term agronomic observations and focus on multi-season, multi-location trials that integrate deep molecular, transcriptomic, and ecological tracking.

Research Gaps and Emerging Trends

Despite the optimistic narratives surrounding nano-biofertilizer research, critical evaluation of the literature reveals several deep-seated knowledge gaps, conflicting data, and methodological weaknesses that hamper large-scale commercial viability.

The most pressing unresolved question in NBF technology centers on the exact mechanism of nanoparticle translocation across the primary root barriers. The plant root endodermis is protected by the Casparian strip—a highly impermeable, hydrophobic layer composed of suberin and lignin that seals intercellular spaces (the apoplastic pathway). This barrier forces water and solutes to travel through the selectively permeable plasma membranes of cells (the symplastic pathway). Currently, the literature is sharply divided: one school of thought argues that nanoparticles larger than 20 nm are physically excluded by the Casparian strip and can only enter the vascular system via accidental structural tears at the sites of lateral root emergence. An opposing school of thought asserts that functionalized nanoparticles actively induce localized endocytosis, allowing them to cross cell membranes symplastically. Without resolving this dispute, engineering smart nano-carriers capable of delivering nutrients to specific, target plant tissues remain an impossibility.

Another critical concern is the ecotoxicological impact on the native soil microbiome. While many short-term studies report no negative impacts, emerging long-term evidence indicates that the continuous application of metallic oxide nanoparticles leads to accumulation in the upper soil horizons. This build-up can suppress beneficial native microbial populations, including ammonia-oxidizing bacteria (AOB) and arbuscular mycorrhizal fungi (AMF). This suppression can inadvertently impair natural, non-assisted nutrient cycling processes, rendering the soil dependent on continued chemical interventions. Furthermore, almost all existing studies use pristine, freshly synthesized nanoparticles. This approach overlooks how nanoparticles age, weather, and transform within the complex soil matrix over time, leaving a major blind spot in safety and environmental risk assessments.

Discussion

Integrating the literature published from 2015 to 2025 demonstrates that nano-biofertilizers represent a meaningful advancement over both isolated chemical nano-fertilizers and conventional bio-inoculants. The overarching mechanism driving their superior performance is their ability to break the traditional trade-off between nutrient availability and environmental loss. By combining the structural protection of nano-carriers with the active biological mobilization provided by PGPR, NBFs transform the rhizosphere from a passive absorption zone into an active metabolic center. The continuous, low-concentration release of nutrients keeps root high-affinity transporters expressed over extended periods. This avoids the genetic feedback inhibition and subsequent down-regulation typically caused by the intense nutrient spikes of conventional fertilizers.

Furthermore, the molecular data synthesized across themes points to a profound synergistic interaction between chemical and biological components. For instance, when a chitosan matrix carries a zinc-mobilizing bacterium, the plant benefits from both the mechanical structural support of the polymer and the hormone-driven changes in root architecture stimulated by the bacteria. This combined effect increases root surface area, allowing for a higher density of active transporters and maximizing overall nutrient uptake.

The practical and structural implications of these findings span several key groups: (1) For Agricultural Researchers: The evidence shows that the traditional approach of studying individual pathways in isolation is no longer sufficient. Researchers must adopt integrated, systems-biology approaches that combine rhizosphere metagenomics, root transcriptomics, and whole-plant fluxomics. Crucially, future studies must prioritize long-term, multi-location field trials that track how weathered or aged nanoparticles interact with the soil matrix over multiple seasons. This shift is essential to generate the reliable data needed to move beyond laboratory concepts. (2) For Industrial Practitioners and Agronomists: The main challenge lies in ensuring formulation stability and structural scale-up. Organic nano-carriers like chitosan offer excellent biocompatibility but suffer from low mechanical stability and short shelf-lives in industrial supply chains. Conversely, metallic carriers are highly stable but carry long-term ecotoxicological risks. Industrial R&D must focus on developing hybrid organic-inorganic composite matrices. These composites must deliver predictable, controlled-release kinetics under diverse environmental conditions while maintaining a long shelf-life for commercial distribution. (3) For Environmental Policymakers and Regulatory Agencies: The current regulatory landscape is ill-equipped for hybrid technologies like NBFs. They often fall into bureaucratic grey areas between chemical, microbial, and nanotechnology regulations. Policymakers must establish standardized, evidence-based safety frameworks that treat NBFs as unique entities. These frameworks should include clear protocols for testing ecotoxicological impacts on native soil microbiomes, assessing potential bioaccumulation in food chains, and ensuring farmworker safety during application. Implementing these clear, transparent guidelines will help protect ecosystems while giving the agricultural sector the confidence to invest in and adopt these sustainable technologies.

Conclusion

This critical review has systematically evaluated the structural, physiological, and molecular interactions between nano-biofertilizers and plant nutrient assimilation pathways based on literature from 2015 to 2025. The compiled evidence clearly shows that NBFs improve nutrient use efficiency (NUE) by up-regulating essential enzymatic complexes (such as nitrate reductase and the GS-GOGAT cycle) and maintaining the steady expression of high-affinity transporter gene families (NRT, Pht). This coordinated response bypasses the efficiency limitations and environmental losses common to conventional chemical fertilizers.

However, the field faces several critical bottlenecks. Significant knowledge gaps persist regarding the exact physical mechanisms of nanoparticle translocation across root endodenal barriers, and long-term data on soil microbiome ecotoxicology remains deeply contradictory. Methodologically, the research landscape is constrained by a reliance on short-term hydroponic assays and a narrow focus on a few model crop species, which limits the reliability and scalable application of these formulations.

To transition NBFs into reliable tools for climate-resilient agriculture, future research must shift toward multi-season field trials, systems-biology approaches, and the development of smart, stimuli-responsive nano-carriers. By addressing these gaps through coordinated efforts among researchers, industry, and regulators, nano-biofertilizers can safely scale from laboratory innovations to cornerstone technologies for global food security and environmental sustainability.

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