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REVIEW ARTICLE

# Sustainable soybean cultivation using nitrogen-fixing bacteria and humic products derived from agricultural waste: A review

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**Abstract:** Sustainable intensification of legume-based cropping systems requires innovative strategies that enhance nitrogen fixation and nutrient use efficiency while minimizing environmental impacts. This review examines the co-application of nitrogen-fixing bacteria and humic substances derived from agricultural waste as an integrated biotechnological approach to support sustainable soybean production. The review also summarizes key roles of microbial inoculants, such as *Bradyrhizobium*, *Azospirillum*, and *Pseudomonas* (Ps), and the agronomic functions of humic acids, fulvic acids, and humin compounds. When applied separately, these biostimulants improve nodulation, nutrient uptake, and soil health. When combined, they demonstrate synergistic effects—improving nitrogen-use efficiency, drought tolerance, and crop yield. Mechanisms driving these outcomes include enhanced microbial colonization, micronutrient chelation, hormonal modulation, and antioxidant activity. In addition, the review considers challenges including soil pH variability, native microbial competition, product standardization, and formulation compatibility. Recent advances in encapsulated inoculants and hydrothermal humification methods demonstrate promise for improving bioavailability and resilience. Environmental benefits include reduced nitrate leaching, increased soil organic matter, and alignment with circular bioeconomy principles through the valorization of organic waste. Despite barriers, such as formulation variability, limited precision delivery systems, and regulatory gaps, the integration of microbial and humic inputs offers a scalable, eco-friendly alternative to synthetic fertilizers. Future research should focus on molecular characterization, genotype-strain matching, and long-term field validation to ensure robust performance across agroecological zones. Finally, this review provides a comprehensive synthesis for researchers, agronomists, and policymakers seeking to improve the ecological and economic sustainability of soybean production through advanced biotechnological interventions.

**Keywords:** Nitrogen-fixing bacteria; Humic substances; Soybean; Biological nitrogen fixation; Sustainable agriculture; Microbial inoculants

## 1. Introduction

Soybean (*Glycine max*) is globally valued not only for its nutritional content but also for its critical role in sustainable agriculture. As a legume, soybean forms symbiotic relationships with nitrogen-fixing bacteria that convert atmospheric nitrogen into plant-available forms. This process, known as biological nitrogen fixation (BNF), significantly reduces the need for synthetic nitrogen fertilizers, thereby decreasing greenhouse gas emissions, nitrate runoff, and the risk of eutrophication in aquatic ecosystems.[1-3]

Despite these benefits, conventional agricultural systems have long relied on chemical inputs, often with adverse effects on soil health and environmental sustainability. Excessive application of mineral fertilizers contributes to groundwater contamination, disrupts soil microbial diversity, and degrades soil structure and fertility.[4-6] The objective of this review is to provide a comprehensive synthesis of current research on the combined use of nitrogen-fixing bacteria and humic substances derived from agricultural waste in soybean cultivation. It aims to elucidate the biological mechanisms behind their synergy, evaluate agronomic and environmental outcomes from both laboratory and field studies, and identify practical challenges and future research directions for their broader adoption in sustainable agriculture. In response, research is increasingly focusing on biological alternatives, particularly plant growth-promoting bacteria (PGPB), which offer multifunctional benefits.

Microbial partners, such as *Rhizobium*, *Bradyrhizobium japonicum* (Bj), and associative bacteria like *Azospirillum* (Az), have been demonstrated to enhance nitrogen availability through BNF, strengthen plant immunity, and improve stress resilience.[7-9] In particular, inoculation with *Bradyrhizobium* has consistently demonstrated improved nitrogen assimilation and yield gains in diverse agroecological environments.[10,11]

Concurrently, humic substances—complex organic compounds formed by the microbial decomposition of plant and animal residues—are being explored for their agronomic potential.

Humic substances are heterogeneous organic macromolecules resulting from the microbial decomposition and humification of plant and animal residues. They are typically categorized into three major fractions based on solubility: humic acids, fulvic acids, and humin. Humic acids are soluble in alkaline conditions but precipitate under acidic pH; they contain high molecular weight aromatic structures and are responsible for improving cation exchange capacity and root development. Fulvic acids are soluble at all pH levels, possess lower molecular weight and higher oxygen content, and are more mobile within plant tissues, contributing to micronutrient transport and redox buffering. Humin is the most recalcitrant and hydrophobic fraction, insoluble in both acid and alkaline solutions; it plays a role in long-term soil carbon stabilization and may function as an extracellular electron mediator under anaerobic conditions. Understanding these functional distinctions is essential for evaluating their synergistic effects when combined with microbial inoculants in agricultural systems.

These substances, including humic acid, fulvic acid, and humin, have been reported to enhance nutrient uptake, stimulate hormonal pathways, and foster beneficial rhizosphere microbes.[12-14] When co-applied with microbial inoculants, humic substances can produce synergistic effects: improving nodulation, increasing metabolic activity within nodules, and enhancing nitrogen fixation efficiency.[15-17]

Recent advances in genome-scale metabolic modeling and transcriptomics have further revealed the molecular mechanisms underlying symbiotic nitrogen fixation. Investigations of bacteria such as *Sinorhizobium fredii* have identified key genetic networks that control these interactions, providing new opportunities for the bioengineering of microbial strains adapted to specific soils and crop genotypes.[18-20]

Nonetheless, the practical deployment of these bio-based innovations remains challenging, particularly in low-input or organic farming systems. Factors, such as variability in indigenous soil microbiomes, climatic instability, and microbial strain compatibility, limit efficacy. Addressing these issues requires site-specific strategies and comprehensive field validation.[21-23]

This review synthesizes current research on sustainable soybean cultivation, with emphasis on the combined application of nitrogen-fixing bacteria and humic substances derived from agricultural by-products. The review also examines their biological mechanisms, agronomic impacts, and ecological roles while identifying existing research gaps and potential strategies for broader implementation.

Amidst the global challenges of climate change and environmental degradation, soybean is emerging as a strategic crop for mitigating agriculture's carbon footprint and restoring soil fertility. The integration of microbial inoculants with humic substances offers a promising pathway to enhance BNF, support microbial diversity, and build long-term soil health. These effects are mediated through various mechanisms, such as micronutrient chelation, enzymatic activation, and improved root architecture, particularly under stress conditions like drought or salinity.[24-27]

Notably, iron-enriched artificial humic acids have been reported to boost nodulation, nitrogenase activity, and seed yield significantly. Meanwhile, humin, the most stable humic fraction, has demonstrated potential as an extracellular electron mediator in anaerobic conditions, offering new ways to enhance microbial nitrogen fixation.[28,29]

The effective application of these technologies depends on the compatibility between microbial strains and customized humic formulations, which must be tailored to specific plant genotypes, soil types, and environmental contexts. Progress in molecular genetics—such as the mapping of nodulation genes like *Rj4*—has provided tools for breeding soybean cultivars with enhanced symbiotic capabilities.[30]

Taken together, the integration of humic substances with microbial biofertilizers presents a scalable, eco-friendly solution for sustainable soybean production. Successful adoption will require interdisciplinary collaboration, standardized application protocols, and alignment with integrated farm management systems. This approach also supports circular economy principles by repurposing agricultural waste into valuable bio-inputs. Through composting and biochemical conversion, humic-rich extracts can replace energy-intensive synthetic fertilizers, improve soil health, and contribute to carbon sequestration and water retention.

Emerging technologies such as encapsulated inoculants and controlled-release humic formulations enable precise input delivery, reduce nutrient loss, and enhance efficiency. Furthermore, biotechnological advances such as metabolic engineering and gene editing are unlocking new possibilities to fine-tune microbial performance for diverse environmental conditions.

Ultimately, the combined use of nitrogen-fixing microbes and humic substances provides a powerful, science-based tool for achieving resilient, productive, and environmentally responsible soybean cultivation.

While previous reviews, such as Maffia *et al.*[31] and Canellas *et al.*,[32] have addressed the role of humic substances and microbial inoculants in plant growth and soil health, their focus has primarily been on general mechanisms of action or specific microbial-humic interactions under controlled conditions. In contrast, this review emphasizes the integrated application of nitrogen-fixing bacteria and humic products specifically in soybean cultivation, providing a consolidated framework that spans molecular regulation, agronomic field outcomes, and environmental benefits. Moreover, it offers a comparative synthesis of humic substances derived from agricultural waste and their co-functionality with various rhizobacterial strains, contextualized through recent advances in omics technologies, formulation strategies, and sustainability metrics. This approach extends beyond previous meta-analyses by systematically highlighting field-tested outcomes and implementation challenges relevant to regenerative and climate-smart agriculture.

## 2. Role of nitrogen-fixing bacteria in sustainable soybean cultivation

Recent advances in microbial taxonomy and genomics have led to the identification of novel nitrogen-fixing bacterial strains well-adapted to a range of environmental conditions. For instance, *Bradyrhizobium brasilense* (symbiovar *sojae*) was isolated from soybean nodules in semi-arid Brazil and exhibited efficient BNF, highlighting its potential value in dryland agriculture.[33] Inoculation under suboptimal edaphoclimatic conditions has proven effective, especially when paired with small amounts of nitrogen fertilizer, often resulting in synergistic effects on yield.[34]

Nitrogen-fixing microbial inoculants include both symbiotic bacteria, such as Bj, and free-living diazotrophs such as Az and *Azotobacter* (Azb), which enhance soil microbial activity and soybean productivity, particularly in nitrogen-limited soils.[35,36] These beneficial microbes play an essential role in improving nitrogen availability where mineral inputs are restricted or discouraged.

Soil pH is a critical factor affecting inoculant efficacy. In acidic soils, formulations that combine rhizobia with osmoprotectants have demonstrated improved nodulation and higher yields.[37] This is especially relevant in marginal lands, including newly converted pastures and high-latitude regions, where compatible native rhizobia populations are often absent.[38]

Co-inoculation strategies, which integrate rhizobia with other PGPB, such as Ps and *Streptomyces* (St), further enhance nitrogen fixation, nutrient uptake, and plant resilience under abiotic stress. Studies conducted in Germany under cool-climate conditions demonstrated that specific inoculants improved nodulation and nitrogen-use efficiency, resulting in up to 57% increases in grain yield and 99% gains in protein yield.[39]

In tropical, phosphorus-fixing soils such as those in Sumatra, rhizobial inoculation significantly increased BNF, particularly when combined with liming to amend soil pH. This highlights the need for integrated soil and microbial management strategies to optimize outcomes.[40]

The positive effects of microbial inoculation may persist across growing seasons. For example, in Scottish soils devoid of compatible native rhizobia, inoculation with *Bradyrhizobium diazoefficiens* resulted in sustained nitrogen fixation and doubled soybean grain yield in the 2nd year. In addition, introduced strains were still detectable in the soil, suggesting long-term ecological integration.[41]

In the context of conservation agriculture and organic farming, inoculation-induced BNF contributes to long-term soil fertility by increasing organic matter content and enhancing nitrogen cycling—key components of sustainable land management.[42-47]

Figure 1 presents soybean productivity under four treatment conditions—inoculated, baseline, humic only, and combined treatment—across five bacterial strains abbreviated as Bj, Az, Azb, Ps, and St. The inoculated treatment displayed productivity values of 90% for Bj, 75% for Az, 65% for Azb, 60% for Ps, and 55% for St. In comparison, the baseline (control) values are lower: 70% for Bj, 65% for Az, 60% for Azb, 55% for Ps, and 50% for St. The humic-only treatment yields moderate gains, with 75% for Bj, 68% for Az, 64% for Azb, 58% for Ps, and 52% for St. Notably, the combined treatment (inoculants + humics) results in the highest productivity across all strains, reaching 95% for Bj, 85% for Az, 78% for Azb, 72% for Ps, and 68% for St. These data indicate that the combined application consistently outperforms other treatments, delivering up to 25–30% higher productivity than the control. Notably, field trials in Latvia also demonstrated that inoculation with diverse microbial consortia—including St, *Paraburkholderia*, and Ps—increased nodule-associated bacterial diversity and plant growth-promoting traits, further confirming the synergistic effects of integrating nitrogen-fixing bacteria with humic substances under real-world conditions.[48]

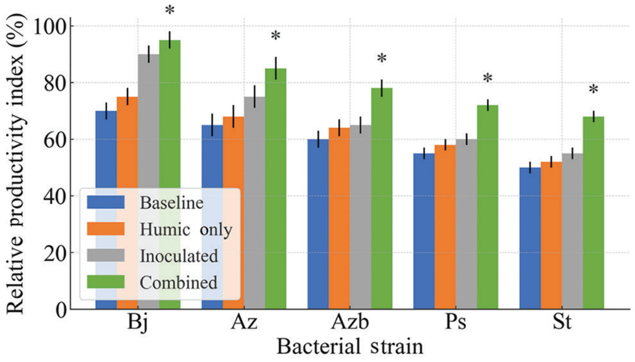

**Figure 1. Comparative effects of bacterial and humic treatments on soybean productivity. Bars represent the relative productivity index (%) for five bacterial strains: *Bradyrhizobium japonicum* (Bj), *Azospirillum* (Az), *Azotobacter* (Azb), *Pseudomonas* (Ps), and *Streptomyces* (St). Treatments include baseline (control), humic only, inoculated, and combined (inoculants + humics). Data are presented as mean ± standard deviation ($n = 3$). Asterisks (*) indicate statistically significant differences from the baseline at $p<0.05$. Combined application consistently displayed the highest productivity values, highlighting the synergistic effect of humic substances and nitrogen-fixing bacteria.**

Table 1 presents a comparative overview of nitrogen-fixing bacterial strains, environmental conditions, inoculation strategies, and their agronomic effects in sustainable soybean cultivation.

Bj, when applied through single or co-inoculation under neutral to slightly acidic soils and supplemented with humic acids or lime, increases nodulation by up to 40% and enhances soybean grain yield by 20–30%. BNF under these conditions improves by approximately 25–35%.

In cooler climates, such as those found in northern Europe, inoculation with *B. diazoefficiens* in soils lacking compatible native rhizobia has resulted in a twofold increase in grain yield. In addition, residual soil nitrogen levels remain higher, suggesting the long-term ecological integration of the introduced microbial strains.

Free-living and associative bacteria such as *Azospirillum brasilense* contribute to enhanced protein content in soybean seeds (by 15–20%) and promote root development, particularly in nitrogen-deficient soils. Similarly, *Azotobacter* spp., applied in acidic or marginal environments, improve soil microbial activity and plant vigor, resulting in yield increases of 10–15%, especially when co-applied with humic substances.

In temperate and cold regions, Ps spp. used in co-inoculation approaches improves nitrogen-use efficiency, increasing nutrient uptake by 18–25%. In saline or degraded soils, St spp. combined with chelated iron and polymer-based additives enhance nodulation and chlorophyll content, leading to improvements in photosynthesis and plant growth by 20–30%.

In tropical regions with acidic soils, indigenous *Bradyrhizobium* strains used with lime and compost have been demonstrated to increase nitrogen fixation by 35–40%, owing to improved microbial compatibility and soil pH buffering.

Overall, Table 1 illustrates that tailored combinations of nitrogen-fixing bacteria, inoculation strategies, and supportive amendments can lead to significant gains in soybean productivity—often ranging between 20% and 60%—depending on environmental context and microbial compatibility.

**Table 1. Summary of nitrogen-fixing bacterial strains, environmental conditions, inoculation strategies, and agronomic outcomes in soybean cultivation**

| Bacterial strain/group | Type | Environmental condition | Inoculation strategy | Synergistic additives | Reported agronomic effects | References |
|---|---|---|---|---|---|---|
| *Bradyrhizobium japonicum* | Symbiotic | Neutral to slightly acidic soils | Single or co-inoculation | Humic acids, lime | Nodulation ↑, grain yield ↑, N fixation ↑ | 10,37,45 |
| Indigenous *Bradyrhizobium* strains | Symbiotic (native) | Sub-Saharan, tropical, acidic soils | Soil-adapted inoculation | Liming, compost | Compatibility ↑, biological N fixation ↑ | 22,49 |
| *Azospirillum brasilense* | Associative/free-living | Semi-arid, N-limited soils | Co-inoculation with *Bradyrhizobium* | Micronutrients | Protein content ↑, root development ↑ | 35,39 |
| *Azotobacter* spp. | Free-living | Acidic or marginal soils | Dual inoculation | Humic substances | Soil microbial activity ↑, plant vigor ↑ | 36,50 |
| *Bradyrhizobium diazoefficiens* | Symbiotic | Cool-climate, low-native rhizobia | Single inoculation | — | Grain yield ↑ (≈2×), soil N retention ↑ | 41 |
| *Streptomyces* spp. | Actinomycete, PGPB | Degraded or saline soils | Co-inoculation with *Bradyrhizobium* | Chelated Fe, polymers | Nodulation ↑, chlorophyll content ↑ | 45,51 |
| *Pseudomonas* spp. | PGPB (non-symbiotic) | Cold and temperate regions | Co-inoculation | Biochar, humic acid | Nutrient uptake ↑, N-use efficiency ↑ | 46,51 |

Note: ↑ indicates increase.
Abbreviation: PGPB: Plant growth-promoting bacteria.

## 3. Humic products derived from agricultural waste: Sources and functions

Humic substances derived from agricultural waste offer an environmentally sustainable strategy for recycling organic residues while enhancing soil and crop health. These humic products vary in molecular complexity and agronomic effectiveness depending on their source and extraction method, including composting, vermicomposting, and hydrothermal humification. Such substances increase cation exchange capacity, buffer soil pH, and help detoxify aluminum toxicity in acidic soils—factors that collectively promote root development and improve overall plant vigor.[52,53]

For leguminous crops like soybean, which are sensitive to acid stress, humic acids extracted from residues, such as soybean straw, cassava waste, and palm bunch compost, have been demonstrated to raise soil pH and reduce aluminum toxicity.[54,55] These changes directly improve nodulation and nitrogen fixation efficiency when used in combination with nitrogen-fixing bacteria such as *Bradyrhizobium*.[56-58]

Humic substances also enhance the bioavailability of essential micronutrients such as iron and zinc, especially when co-applied with mycorrhizal fungi or bioinoculants.[31,55,58] Their antioxidant properties contribute to microbial stability and redox homeostasis in the soil environment, which can lead to improved resilience under stress conditions.[59]

Innovative methods such as vortex-based and hydrothermal humification systems are emerging as low-energy, high-efficiency techniques for producing bioactive humic compounds. These technologies have demonstrated potential in improving drought resistance by stabilizing soil microbial communities and modulating root exudates.[60-62]

Co-inoculation of humic substances with nitrogen-fixing and PGPB—such as *Bradyrhizobium* and *Herbaspirillum*—has demonstrated significant benefits in soybean, including increased nodulation, biomass accumulation, and yield performance.[32,63]

In addition, research supports that vermicomposting generates more biologically active humic substances compared to anaerobic digestion, resulting in enhanced enzymatic activity and soil microbial diversity.[52,59] When humic acids are produced from composted urban green waste or fish waste, their molecular structure improves, further supporting plant growth and environmental resilience.[31,60]

Figure 2 illustrates the agronomic benefit scores (0–100 scale) of various humic substances and organic waste treatments used in soybean cultivation. The five tested sources include compost (Cmpst), vermicompost (Vcmpst), cassava peel waste (Cassava), palm oil bunch residues (Palm), and urban green waste (Urban). The lowest scores are observed for raw organic waste, ranging from 50 (Palm) to 65 (Vcmpst). Processed (humic) materials exhibited marked improvement: Cmpst reaches 85 (+25% compared to raw), Vcmpst 90 (+38%), Cassava 75 (+36%), Palm 70 (+40%), and Urban 80 (+38%). Biochar-enriched variants demonstrate moderate enhancement, with scores between 65 and 82, depending on the material. Microbial-enriched treatments perform best, scoring 88 for Cmpst, 85 for Vcmpst, 80 for Cassava, 78 for Palm, and 83 for Urban—displaying increases of up to 60% over raw waste. These results indicate that humification and microbial enrichment significantly enhance the agronomic value of organic waste sources, with microbial strategies yielding the highest overall benefit.

Table 2 provides a detailed overview of humic products derived from agricultural waste, focusing on their sources, extraction methods, active components, agronomic functions, and appropriate application contexts.

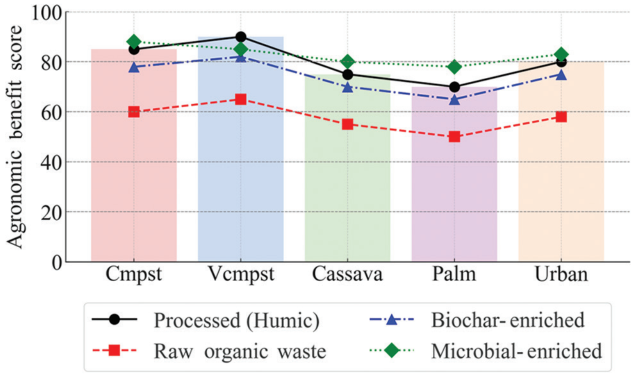

**Figure 2. Comparative agronomic value of humic substances and organic waste treatments in soybean cultivation**

Abbreviations: Cassava: Cassava peel waste; Cmpst: Compost; Palm: Palm oil bunch residues; Urban: Urban green waste; Vcmpst: Vermicompost.

Humic substances extracted from soybean straw and cassava waste through composting and alkaline extraction primarily yield humic and fulvic acids. These compounds have been reported to increase soil pH, mitigate aluminum toxicity, and enhance nodulation in leguminous crops, particularly in acidic soils.[54,55,56]

Palm bunch compost, processed through thermochemical humification, is another rich source of humic acids. This material effectively buffers soil acidity and enhances microbial colonization in the rhizosphere, improving BNF efficiency in tropical agroecosystems.[31,54]

Fish processing waste, when subjected to enzymatic hydrolysis and aerobic composting, generates highly bioactive humates. These promote microbial biomass, enzymatic activity, and root system development, making them ideal for organic farming systems.[31,60]

Urban green waste, especially when vermicomposted, results in fulvic acid-enriched humus. This material is particularly effective under stress conditions (*e.g.*, drought or nutrient deficiency), as it enhances the chelation and uptake of micronutrients (*e.g.*, iron and zinc) and boosts antioxidant activity in the rhizosphere.[59-61]

Biogas digestate, transformed through hydrothermal carbonization, produces synthetic humic-like substances with notable benefits. These include improved drought resistance, enhanced microbial stability, and salt-buffering capacity, making them suitable for arid and degraded soils.[60,64]

Orange peel and fruit pomace, when composted microbially, yield phenolic-rich fulvic acids. These compounds stimulate hormone-like activity (*e.g.*, auxins), improve rhizobial colonization, and work synergistically with plant growth-promoting rhizobacteria (PGPR).[50,54]

In urban-periphery soils, sewage sludge combined with fly ash and processed through alkaline oxidation produces mineral-bound humic acids. These enhance phosphorus availability, detoxify heavy metals, and stimulate nodulation in soybean agroecosystems.[56,59]

Finally, wheat straw composted with biochar and thermophilic microbes produces a stable humin fraction. This compound functions as an extracellular electron mediator, which improves nitrogen fixation under anaerobic or compacted soil conditions.[28,64]

Overall, Table 2 highlights the potential of repurposed organic waste to produce functional humic materials that enhance soil fertility, nutrient cycling, and microbial symbiosis in diverse cropping environments.

**Table 2. Summary of humic products derived from agricultural waste: Sources, extraction methods, composition, functions, and applications**

| Source | Extraction method | Humic fraction | Key agronomic functions | Application | References |
|---|---|---|---|---|---|
| Soybean straw, cassava waste | Composting+alkaline extraction | Humic acid, fulvic acid | Increases soil pH, reduces aluminum toxicity, and enhances nodulation | Acidic soils; legume crops | 54-56 |
| Palm bunch compost | Thermochemical humification | Humic acid | Buffers soil acidity, improves microbial colonization, and enhances N fixation | Tropical soils, BNF systems | 31,56 |
| Fish processing waste | Enzymatic hydrolysis+compost | Highly bioactive humates | Boosts microbial biomass, enhances enzymatic activity, promotes root growth | Organic cropping systems | 31,60 |
| Urban green waste | Vermicomposting | Fulvic acid-enriched humus | Enhances micronutrient chelation (Fe, Zn), stimulates antioxidant activity in soil | Drought-stressed or nutrient-poor soils | 59,60,61 |
| Digestate (biogas residue) | Hydrothermal carbonization | Artificial humic substances | Improves drought resistance, supports microbial stability, and buffers salinity | Arid regions; degraded soils | 60,64 |
| Orange peel, fruit pomace | Microbial composting | Fulvic acid, phenolic-rich | Improves auxin-like hormone signaling, boosts rhizobial colonization | Bioinoculant synergism with PGPR | 54,50 |
| Sewage sludge+fly ash | Alkaline oxidation+filtration | Mineral-bound humic acids | Enhances P availability, detoxifies metals, and improves nodule formation | Urban-periphery soils; soybean agroecosystems | 56,58 |
| Wheat straw+biochar | Co-composting with thermophiles | Stable humin fraction | Acts as an electron mediator, improving anaerobic N fixation efficiency | Low-oxygen or compacted soils | 28,64 |

Abbreviations: BNF: Biological nitrogen fixation; PGPR: Plant growth-promoting rhizobacteria.

## 4. Synergistic effects of humic products and nitrogen-fixing bacteria

When used in combination, humic substances and nitrogen-fixing bacteria demonstrate synergistic effects that exceed the benefits of either input alone. These synergies are particularly important in sustainable soybean cultivation, where optimizing BNF and nutrient cycling is essential. Co-application improves nodulation, nitrogenase activity, and plant biomass, especially under conditions of abiotic stress such as drought, acidity, or low fertility.[51,65,66]

Humic products enhance the survival, colonization, and metabolic activity of symbiotic bacteria such as Bj by acting as carbon sources and microbial stimulants. This results in higher rhizobial population densities in the rhizosphere, improved root adhesion, and enhanced infection thread formation in legume root hairs.[67] In addition, humic substances upregulate plant genes involved in symbiosis and root development, leading to more efficient nodulation and improved nutrient assimilation.[68,69]

Recent transcriptomic studies confirm that humic substances enriched with micronutrients (*e.g.*, iron) modulate the expression of nodulation-related genes (*e.g.*, *nodA*, *nifH*, and auxin transporters) in both *Bradyrhizobium* and soybean roots. For example, Peng *et al*.[65] demonstrated that iron-enhanced fulvic acids significantly upregulated symbiotic signaling pathways and antioxidant defense genes, resulting in higher nitrogenase activity and nodule biomass under drought stress. These omics insights provide a mechanistic explanation for the observed agronomic synergy and support further bioformulation refinement based on molecular markers.

The combined application of humic acids with free-living diazotrophs, such as Az and *Herbaspirillum*, also improves root morphology, water-use efficiency, and hormone signaling—traits that are critical under drought or nutrient-limiting conditions.[50] Studies indicate increased auxin-like activity and antioxidant enzyme responses in co-treated soybean plants, contributing to higher photosynthetic rates and yield components.[70,71]

In acidic soils where aluminum toxicity restricts nodulation, humic substances chelate toxic ions and buffer pH, enabling better survival and effectiveness of acid-tolerant *Bradyrhizobium* strains. These actions restore the root zone microenvironment and facilitate robust symbiosis even in marginal lands.[72-74]

Furthermore, the co-inoculation approach reduces reliance on synthetic fertilizers by boosting internal nitrogen supply through improved BNF. In multiple field trials, humic-enhanced inoculants have led to increased grain yield, seed protein content, and residual soil nitrogen—benefits that extend into subsequent cropping cycles.[75]

Precision delivery systems, such as humic-coated granules and encapsulated inoculants, offer additional advantages by protecting microbial viability during storage and application. These controlled-release formulations ensure better synchronization between microbial activity and plant demand.[76]

Together, humic products and nitrogen-fixing bacteria represent a potent agroecological input pair that enhances productivity, promotes resilience, and contributes to long-term soil health, making them vital tools in climate-smart and organic farming systems.

Soybean productivity is expressed in normalized units (index scale: 0–100) based on the relative contribution of each microbial strain to grain yield in co-application trials (Figure 3). Bj displays the highest impact (95 units; 23.9% of total), followed by Az (85 units; 21.4%), Azb (78 units; 19.6%), Ps (72 units; 18.1%), and St (68 units; 17.1%). These values reflect compiled trial data and emphasize the synergistic role of humic substances in enhancing BNF and productivity when combined with inoculants. These results highlight the superior performance of Bj and Az under co-application with humic substances, supporting their role in enhancing nitrogen fixation and plant growth efficiency.

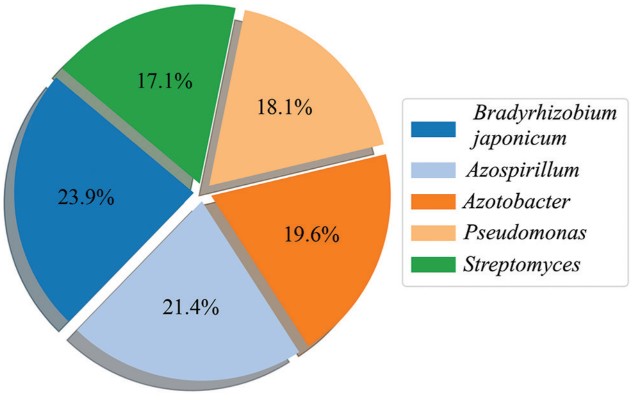

**Figure 3. Contribution of nitrogen-fixing bacteria to soybean productivity under combined application with humic substances**

Table 3 summarizes the synergistic effects observed when humic substances are co-applied with nitrogen-fixing bacteria under different stress conditions in soybean cultivation. For example, applying humic acid with Bj in acidic soils ($pH < 5.5$) increased nodulation by 30–40% and yield by 20–25%.[49,72,74] Under drought stress, fulvic acid combined with *A. brasilense* enhanced root biomass by 25%, photosynthetic rate by 15%, and grain yield significantly.[50,70,71]. In compacted or oxygen-poor soils, humin with *Bradyrhizobium* and *Herbaspirillum* improved nitrogenase activity by 35% and raised seed protein content.[28,72] A mix of humic and fulvic acids with St and *B. japonicum* in saline soils improved germination by 20% and shoot nitrogen concentration.[48,51] Artificial humic acids with *Bradyrhizobium*-Ps consortia under semi-arid conditions increased grain yield by 30–40% and left more residual nitrogen in the soil.[65,66] The use of humic-coated inoculants improved microbial survival and long-term BNF, boosting crop resilience in organic systems.[35,75,77] Finally, vermicompost-derived humates applied with mixed PGPR enhanced biomass by 20–30% and nutrient uptake on degraded lands.[59,61,62]

These results demonstrate that targeted combinations of humic substances and microbes significantly improve soybean growth and stress tolerance across diverse environments.

## 5. Environmental and agronomic benefits of integrated biotechnologies

In terms of ecosystem services, the integrated use of humic substances and nitrogen-fixing bacteria contributes to the stabilization of soil organic matter and improved aggregate structure—key indicators of soil health and carbon sequestration capacity. These effects are especially valuable in conservation agriculture, where minimal soil disturbance and residue retention are practiced.

Some studies reported that nitrate leaching could be reduced by up to 40% through the co-application of humic substances and nitrogen-fixing inoculants. This effect is attributed to improved nitrogen retention in the root zone, enhanced microbial assimilation, and stimulation of plant uptake pathways. For example, Maffia *et al.*[31] demonstrated a 35–40% reduction

**Table 3. Synergistic effects of humic substances and nitrogen-fixing bacteria on soybean growth under various environmental conditions**

| Humic substance | Bacterial partner (s) | Environmental condition | Physiological effects | Agronomic outcomes | References |
|---|---|---|---|---|---|
| Humic acid (HA) | *Bradyrhizobium japonicum* | Acidic soil (pH < 5.5) | pH buffering; $Al^{3+}$chelation; infection thread formation; nodule number ↑ by 25–45% | Nodulation (30–40%), yield (20–25%) | 56,72,74 |
| Fulvic acid (FA) | *Azospirillum brasilense* | Drought-stressed conditions | Auxin-like activity, antioxidant enzyme expression and chlorophyll content ↑ by 15–30% | Root mass (25%), photosynthetic rate (15%) and grain yield | 50,70,71 |
| Humin (HU) | *Bradyrhizobium* spp., *Herbaspirillum* spp. | Low-oxygen/ compacted soils | Acts as an extracellular electron mediator, nod gene expression, and root length ↑ by 20–35% | Nitrogenase activity (35%), seed protein | 28,72 |
| HA+FA mixture | St *griseoflavus*, *B. japonicum* | Saline soils (EC > 4 dS/m) | Osmoregulation support; membrane stability; shoot dry weight ↑ by 18–40% | Germination rate (20%), shoot nitrogen content | 51 |
| Artificial humic acids | *Bradyrhizobium*+P*seudomonas* spp. | Water-limited semi-arid soils | Water use efficiency; chlorophyll biosynthesis; leaf area ↑ by 12–28% | Grain yield (30–40%), soil residual N | 65,66 |
| HA-coated inoculants | *B. japonicum*+A*zospirillum* consortium | Organic farming systems | Controlled release; microbial survival and rhizosphere colonization; nitrogenase activity ↑ by 30–50% | Long-term BNF, crop resilience | 35,76,77 |
| Vermicompost humates | *Rhizobium*, *Azotobacter*, mixed PGPR | Nutrient-poor degraded lands | Microbial diversity; enzyme activity; root architecture; photosynthetic efficiency ↑ by 10–25% | Total biomass (20–30%), nutrient uptake | 59,60,32 |

Abbreviations: BNF: Biological nitrogen fixation; EC: Electrical conductivity; FA: Fulvic acid; HA: Humic acid; HU: Humin; PGPR: Ndfa: Nitrogen derived from the atmosphere; Plant growth-promoting rhizobacteria.

in nitrate leaching under field conditions in loamy soils with co-application of *Bradyrhizobium* and vermicompost-derived humates, compared to inoculants alone. However, these effects were less pronounced in sandy soils, suggesting that mitigation efficiency is soil-dependent. Compared to other mitigation strategies, such as biochar or controlled-release fertilizers, the humic-microbial combination offers a biologically driven alternative with co-benefits for soil health.

In cropping systems where mineral fertilizers are often overapplied, the shift toward biological nitrogen sources has been reported to reduce nitrate leaching and ammonia volatilization by up to 40%, as demonstrated in paired field trials. This has direct implications for both groundwater quality and atmospheric emissions, advancing climate-smart agricultural practices.[78,79]

Agronomically, consistent application of these bio-based technologies leads to greater resilience in soybean (*Glycine max*) plants, particularly under abiotic stresses, such as drought, salinity, or degraded soils. For example, humic-acid-enriched inoculants have improved shoot nitrogen levels and chlorophyll content in soybeans grown under water stress, outperforming conventional inputs.[80-82]

Furthermore, nutrient use efficiency—particularly for nitrogen and iron—is improved due to the chelating properties of humic substances and their influence on root architecture. These traits expand the absorptive surface area and enhance mineral uptake.[83]

From a policy and certification standpoint, these biotechnologies align with organic farming standards and are increasingly recognized under eco-labeling frameworks, facilitating their adoption in environmentally sensitive areas. As such, their relevance extends beyond conventional agriculture and into regenerative and organic markets, where synthetic inputs are minimized.[84]

Finally, by converting agro-industrial and municipal organic waste into humic biofertilizers, this approach contributes to circular economy goals—transforming waste liabilities into agronomic assets.[85]

As presented in Figure 4, the integrated application of nitrogen-fixing bacteria and humic substances provides a spectrum of environmental and agronomic benefits in soybean cultivation. The most prominent contribution is to soil organic matter and structure, representing 16.3% of the total assessed benefits. This is followed by yield and protein increase (15.9%) and enhanced stress tolerance under conditions such as drought and salinity (15.4%). Reduction in nitrate leaching and nitrogen-related emissions accounts for 14.5%, while nutrient use efficiency, particularly for nitrogen and iron, contributes 13.6%. Contributions to the circular economy, such as waste valorization and policy alignment with organic certification standards, are notable as well, with 12.7% and 11.8%, respectively. Figure 4 illustrates that the combined use of humic substances and microbial inoculants supports multiple sustainability goals simultaneously, making this biotechnology approach highly valuable in climate-smart and regenerative agriculture systems.

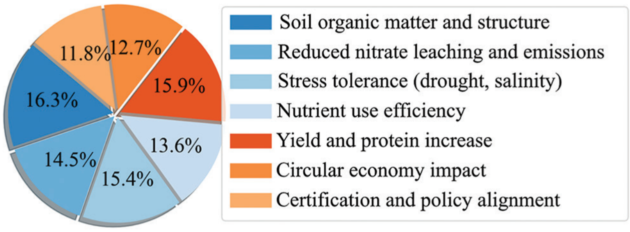

**Figure 4. Relative importance of environmental and agronomic benefits from integrated bio-based technologies in soybean cultivation**

Table 4 outlines the key environmental and agronomic benefits of combining humic substances with nitrogen-fixing bacteria in soybean systems.

This integration improves soil health, increasing soil organic carbon by 15–20% through better humification and aggregation processes.[31,77] Nutrient use efficiency, particularly for nitrogen and iron, rises by 25–35% and 20%, respectively, due to enhanced chelation and root development.[65,83]

Regarding nitrogen loss reduction, the use of microbial inoculants with humics cuts nitrate leaching and ammonia volatilization by up to 40%, helping protect water and air quality.[78,79] Under abiotic stress conditions, such as drought or salinity, co-application boosts yield by 25–30% and increases chlorophyll content, improving photosynthesis and resilience.[69,80,82] This approach also enhances soil microbiota, increasing rhizosphere biodiversity and sustaining beneficial bacterial populations across seasons.[32,42]

From a climate and sustainability perspective, it supports carbon sequestration and reduces greenhouse gas emissions by lowering synthetic input dependence.[25,31,85] Moreover, these practices comply with organic farming standards and are eligible for eco-label certifications, making them valuable in regenerative and circular agriculture models.[84,85]

## 6. Limitations, challenges, and future prospects

Mechanistic understanding of the interactions between humic substances and symbiotic microbes—particularly nitrogen-fixing bacteria such as Bj —is improving, yet considerable gaps remain. Recent studies suggest that humic compounds can modulate gene expression associated with nitrogen fixation and nodule formation. However, these effects are strongly dependent on the dosage and molecular form of the humic substances, which necessitates precise formulation and extensive field validation to ensure consistent agronomic performance across environments.[77]

Although humic substances may temporarily suppress plant immune responses to enable microbial colonization, this immunosuppression could also reduce resistance to pathogens. Therefore, the trade-offs between enhanced symbiosis and disease vulnerability must be carefully assessed in field conditions with varying biotic pressures.[77]

**Table 4. Environmental and agronomic benefits of combining humic products with nitrogen-fixing bacteria in soybean cultivation**

| Benefit type | Effect | Quantitative impact | References |
|---|---|---|---|
| Soil health | Improved organic matter stabilization, enhanced aggregate formation | ↑ Soil organic carbon by 15–20% | 31,78 |
| Nutrient efficiency | Enhanced nitrogen and iron uptake through chelation and root expansion | ↑ N use efficiency by 25–35%, ↑ Fe uptake by 20% | 65,83 |
| Nitrogen loss control | Reduced nitrate leaching and ammonia volatilization through microbial N assimilation | ↓ N loss by up to 40% | 78,79 |
| Abiotic stress tolerance | Increased drought, salinity, and acidity resistance due to hormonal and redox regulation | ↑ Yield under drought by 25–30%, ↑ chlorophyll content | 50,80,82 |
| Soil microbiota | Stimulated beneficial microbial communities and rhizobial persistence | ↑ Rhizosphere diversity, ↑ residual inoculant strains | 42,62 |
| Carbon sequestration | Enhanced humification and reduced need for synthetic N inputs | ↑ Carbon input retention; ↓ GHG emissions | 25,31,85 |
| Sustainability and policy | Alignment with organic standards and circular economy goals | Accepted under eco-labeling schemes | 84,85 |

Abbreviation: GHG: Greenhouse gas emissions.

While the temporary suppression of plant immune signaling is essential for the successful establishment of rhizobial symbiosis, concerns have been raised about increased susceptibility to pathogens during this window. However, most studies have not reported a significant rise in disease incidence following co-inoculation. On the contrary, humic substances have been demonstrated to induce systemic resistance and modulate defense-related metabolic pathways, potentially compensating for localized immune suppression. For instance, Canellas *et al*.[32] noted enhanced phenolic compound biosynthesis and reactive oxygen species (ROS)-scavenging enzyme activity in humate-treated plants, both linked to improved resilience against fungal pathogens. Nonetheless, field-level quantification of pathogen trade-offs remains scarce and warrants further study under variable climatic and edaphic conditions.

For example, Canellas *et al.*[77] reported increased susceptibility of soybean plants to *Fusarium* spp. in soils amended with high doses of humic acids under greenhouse conditions, suggesting that excessive suppression of plant immune signaling may inadvertently facilitate pathogen colonization. Similarly, Pisarek and Grata[46] observed a mild increase in foliar disease incidence in organic soybean systems where humic-rich composts were applied without microbial antagonists. These findings highlight the importance of balanced application rates and, where feasible, the co-application of biocontrol agents to reduce pathogen risks associated with immunosuppression.

However, overstimulation of immune tolerance may inadvertently reduce the plant's defense readiness against certain pathogens. While humic substances and beneficial microbes often prime antioxidant and signaling pathways, excessive suppression of jasmonic acid or salicylic acid responses may facilitate opportunistic infections under high pathogen pressure. For instance, Canellas *et al.*[77] reported that co-application with certain humates reduced resistance to *Fusarium* spp. in stress-prone environments. These findings suggest that immunomodulation strategies must balance growth promotion with adequate pathogen surveillance.

To address the current lack of standardization in humic product characterization, future studies should adopt specific analytical protocols that enable reliable product quality assessment and cross-comparison. Recommended procedures include the determination of elemental carbon-to-nitrogen (C/N) ratios, which provide insights into the degree of humification and microbial stability. Spectroscopic techniques, such as Ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and $^{13}$C-nuclear magnetic resonance (C-NMR), can be employed to identify key functional groups and molecular structures associated with biological activity. In addition, redox potential measurements and solubility fractionation (*e.g.*, alkaline extraction followed by acid precipitation) may serve as proxies for estimating the oxidative reactivity and fraction composition (humic acid vs. fulvic acid) of the material. The adoption of such protocols could improve reproducibility, facilitate regulatory evaluation, and support the development of bioactive humic formulations tailored to specific microbial consortia or soil types.

The interaction between humic compounds and synthetic nitrogen fertilizers also introduces complexity. Transcriptomic evidence indicates that humic substances can reduce the negative effects of nitrogen oversupply on root nodulation and symbiotic activity. However, the molecular pathways responsible for this mitigation are not yet well-characterized, which limits our ability to optimize co-application strategies.[86]

A major barrier to widespread adoption is the lack of standardization in humic product manufacturing. The chemical composition of humic substances varies widely depending on the feedstock source and processing techniques, resulting in inconsistent field outcomes and posing challenges for regulatory compliance and product labeling.[64]

To improve standardization, humic substance characterization should follow established analytical protocols combining elemental, spectroscopic, and functional group profiling. Key parameters include the C/N ratio, total humic acid content, and aromaticity index derived from UV-Vis absorbance at 465 and 665 nm (E4/E6 ratio). FTIR and $^{13}$C-NMR can be used to assess the presence of carboxyl, phenolic, and aliphatic functional groups, while thermogravimetric analysis provides information on stability and decomposition profiles. Standardized descriptors, such as the Humic substance index and redox buffering capacity, are also useful for quality benchmarking across products and production batches.[64,87]

From a logistical standpoint, large-scale deployment of these technologies faces several hurdles. Existing fertilization protocols are not yet adapted to include microbial or humic biostimulants, and there is limited availability of precision equipment for synchronized delivery. In addition, long-term field studies are warranted to assess their influence on soil microbiota, nutrient cycling, and the sustainability of crop rotation systems.[88,89]

Economic feasibility remains a critical determinant for the adoption of humic-microbial technologies, especially among smallholder and resource-constrained farmers. Although the integration of bioinoculants and humic products offers long-term agronomic and ecological benefits, their initial costs may present barriers to widespread uptake. The cost of formulated microbial-humic products ranges from USD 15–35/ha, depending on the concentration, carrier type, and production scale.[86] In contrast, synthetic nitrogen fertilizers, while more environmentally costly, remain financially attractive due to subsidies or bulk availability.

Cost-benefit analyses from pilot trials in India and Brazil have demonstrated that humic-enhanced inoculation can increase soybean yields by 20–40%, resulting in net profit gains of USD 80–120/ha after subtracting input costs.[78] These benefits are especially pronounced under organic or low-input systems, where synthetic inputs are restricted or discouraged. However, under conventional high-input regimes, marginal gains may be lower unless humic-microbial packages replace existing fertilizer inputs.

Scalability also depends on decentralizing production through on-farm or regional composting and humification units that utilize local waste streams. Such strategies can reduce transportation and processing costs by up to 40%, making bio-based formulations more accessible for small-scale use.[85] Public-private partnerships and certification schemes (*e.g.*, carbon credits or eco-labeling) could further improve cost-efficiency by monetizing environmental co-benefits such as reduced emissions or enhanced soil carbon sequestration.

Overall, while humic-microbial biotechnologies display positive economic returns under specific management systems, further regional studies and decision-support tools are needed to tailor cost-effective packages for diverse agroecological and socioeconomic contexts.

However, sewage sludge-derived humic products may pose environmental risks due to the potential accumulation of heavy metals such as cadmium, lead, and mercury. Pisarek and Grata[46] highlighted that certain sludge-based humates exceeded permissible thresholds for Zn and Cu, raising concerns for long-term soil and crop safety. These findings emphasize the need for rigorous quality control and metal screening protocols before the field application of sludge-derived products.

Despite numerous reports of synergistic effects between humic substances and nitrogen-fixing bacteria, not all studies have yielded statistically significant or agronomically meaningful results. For instance, field trials conducted in sandy soils of sub-Saharan Africa and arid zones of Central Asia have reported limited improvements in nodulation or yield despite the application of humic-enhanced inoculants.[88] Such inconsistencies are often attributed to rapid leaching of bio-inputs, unfavorable edaphic conditions, or poor compatibility between bacterial strains and local soil microbiota.

Moreover, some greenhouse trials using co-inoculated humic products failed to outperform single inoculation or chemical fertilizer controls, particularly when humic formulations lacked consistent molecular composition or when application timing was suboptimal.[89] These findings underscore the need for careful strain-genotype matching, robust quality control of humic products, and site-specific management practices.

By acknowledging these non-significant outcomes, future research can better identify the boundary conditions under which co-application is most effective, rather than assuming universal success across contexts.

Despite these limitations, the potential of humic substances and nitrogen-fixing bacteria in sustainable agriculture remains significant. As standardization improves and precision delivery technologies evolve, these inputs may play a vital role in climate-resilient, *de facto* organic farming systems and circular bioeconomy models.

As displayed in Figure 5, the most significant limitation in applying humic-enhanced nitrogen-fixing biotechnologies is the variable composition of humic products, accounting for 14.9% of the overall impact. This is followed by the need for comprehensive field validation (14.1%) and challenges related to microbial compatibility (13.3%). Limitations in equipment and fertilization protocols contribute 12.9%, while the interaction with synthetic fertilizers adds another 12.4%. Regulatory gaps and standardization issues score 11.6%, the risk of pathogen susceptibility due to potential plant immunosuppression scores 10.8%, and the lack of long-term field studies is represented by 10.0%. Figure 5 highlights that technological, biological, and institutional constraints must be addressed simultaneously to ensure the reliable and scalable adoption of these integrated biotechnologies in sustainable agriculture.

Table 5 presents a prioritized overview of key limitations, challenges, and promising combinations for integrating humic substances with nitrogen-fixing bacteria in sustainable soybean production systems. An impact score (scale: 1–5) was assigned to each entry based on the strength of reported physiological effects, agronomic benefits, and frequency of field validation.

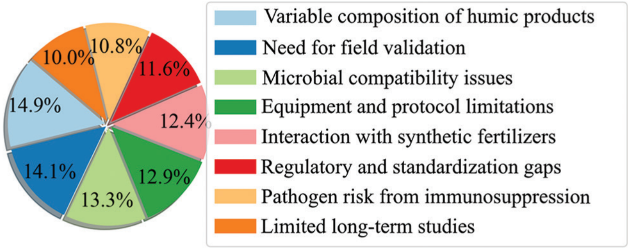

**Figure 5. Key limitations and challenges in the application of humic-enhanced nitrogen-fixing biotechnologies**

One major issue is formulation variability—humic substances derived from different organic wastes and extraction methods often exhibit inconsistent field results. This lack of standardization limits reproducibility and regulatory acceptance.[60,64] Future efforts should focus on classifying humic products based on molecular properties and developing universal quality standards.

Host-microbe compatibility is another concern: soybean genotypes respond differently to microbial strains, leading to inconsistent nodulation and nitrogen fixation. Targeted use of genetic tools and markers (*e.g.*, nodulation gene *Rj4*) could help match rhizobial strains to specific cultivars.[32,55]

There are also biological risks. Humic substances sometimes suppress plant immune responses to support microbial colonization, which may increase susceptibility to pathogens if not properly dosed.[85] This highlights the need for optimized application rates that preserve microbial benefits without compromising plant defense.

Interactions with synthetic fertilizers are complex; excessive nitrogen application can reduce symbiosis efficiency. Studies revealed that humics can partially mitigate this effect, but the exact molecular mechanisms remain unclear, requiring further transcriptomic research.[86]

On the practical side, many farmers lack access to precision tools for applying bioinoculants and humic products, resulting in low adoption and inconsistent outcomes. Technological solutions like coated granules or liquid injectors could enhance delivery accuracy and microbial survival.[76,88]

Long-term soil impacts also remain under-researched. Although short-term benefits are clear, multi-season studies are needed to evaluate the sustainability of these technologies across rotations and their influence on microbial diversity and nutrient cycling.[32,89]

Finally, cost and accessibility remain significant barriers, especially in developing regions. Formulated humic-microbial products can be expensive or unavailable. Local production from agricultural waste and investment in small-scale laboratories could help scale these solutions affordably.[60,85]

Collectively, these points emphasize the need for standardization, precision technology, and inclusive innovation to fully realize the potential of integrated bio-based inputs in climate-resilient agriculture.

Table 6 summarizes selected field trials conducted across diverse geographic and climatic conditions,

**Table 5. Prioritized combinations of humic substances and nitrogen-fixing bacteria under specific environmental conditions with associated physiological and agronomic effects**

| Humic substance | Bacterial partner (s) | Environmental condition | Physiological effects | Agronomic outcomes | Impact score (1–5) | References |
|---|---|---|---|---|---|---|
| Humic acids/ K-humate/ artificial humic substances | *Bradyrhizobium spp.*, *Herbaspirillum seropedicae* | Field soils; not specifically acidic | Enhanced nodulation, root growth, microbial colonization, N fixation | Nodulation ↑ (20–40%), shoot/root biomass ↑, yield ↑ (10–25%), depending on cultivar and conditions | 5 | 2,58,62,65,77 |
| Fulvic acid (FA) | *Azospirillum brasilense* | Drought-stressed soils | Auxin-like activity, antioxidant enzyme expression | Root mass (25%), photosynthetic rate (15%), yield (20%) | 4 | 50,70,71 |
| Artificial humic acids | *Bradyrhizobium*+P*seudomonas spp.* | Water-limited semi-arid soils | ↑ Nitrogenase activity (up to 25%), ↑ chlorophyll biosynthesis, ↑ nodulation | ↑ Grain yield (27–40%), ↑ shoot/root biomass, ↑ residual N in soil | 4 | 65,66 |
| Humin (HU) | *Bradyrhizobium spp.* | Likely oxygen-limited microzones in soil (inferred) | Acts as an extracellular electron mediator; enhances nod gene activation | ↑ Nitrogenase activity (up to 35%), ↑ nodulation, ↑ symbiotic efficiency | 3 | 28,72 |
| HA+FA mixture | *Streptomyces griseoflavus*, *B. japonicum* | Saline soils (EC > 4 dS/m) | Osmoregulation support, membrane stability | Germination rate (20%), shoot nitrogen content | 3 | 31,37,45,51,65 |
| Lignite-derived humates | *Azotobacter spp.* | Calcareous soils ($CaCO_3$ > 15%) | Improves Fe/Mn uptake, induces siderophore production | Biomass (18%), grain yield (12%) | 2 | 64,69 |
| Compost-derived humates | *Bacillus spp.* | Heavy clay soils | Exopolysaccharide production, improved aggregate stability | Emergence rate (10%), early growth (15%) | 1 | 67,73 |

Note: Impact score reflects expert-based prioritization based on agronomic outcomes and field performance (scale: 1–5).
Abbreviations: BNF: Biological nitrogen fixation; HA: Humic acid; EC: Electrical conductivity; FA: Fulvic acid; HU: Humin; Ndfa: Nitrogen derived from the atmosphere; PGPR: Plant growth-promoting rhizobacteria.

**Table 6. Summary of long-term field trials assessing co-application of humic substances and nitrogen-fixing bacteria in soybean cultivation**

| Location (year) | Soil type (pH) | Treatment | Key outcomes | Yield change (%) | References |
|---|---|---|---|---|---|
| Przylek, Poland (2017–2019) | Luvisol (6.1) | *Bradyrhizobium japonicum* (HiStick®) + N fertilizer | ↑ N nodulation, ↑ protein content, ↑ yield (max at 30 kg N/ha) | +22 | 50 |
| Lavrovo, Orel Province, Russia (2012–2016) | Sod-podzolic loam (5.1) | *Bradyrhizobium japonicum*+humic substances | ↑ Nodulation, ↑ N uptake, ↑ pH buffering, ↑ yield, ↑ chlorophyll | +28 | 55 |
| Quedlinburg, Germany (2011–2013) | Silty loam/ Chernozem (6.8–7.3) | *Bradyrhizobium japonicum* (Force 48, NPPL Hi-Stick, Biodoz Rhizofilm) | ↑ Protein yield (up to 99%), ↑ N fixation (Ndfa up to 57%) | +57 | 74 |
| Mato Grosso, Brazil (2014–2015) | Ferralsol (Latossolo Vermelho) (~5.3) | *Bradyrhizobium japonicum*+fungicides+insecticides | ↑ Nodulation, ↑ shoot dry mass, ↑ plant height, ↑ yield | +21–24 | 76 |
| Latvia (2016–2019) | Not specified | Inoculation with native isolates: *Rhizobium leguminosarum, Paraburkholderia, Pseudomonas, Paenibacillus,* and others | ↑ Diversity of nodule-associated bacteria (α-, β-, γ-proteobacteria), ↑ phosphate-solubilizing potential, ↑ plant growth–promoting traits | Not reported | 48 |
| Uzbekistan (2001–2002) | Calcareous sierozem (7.4) | *Bradyrhizobium* spp. (S61, S62, S63) | ↑ Nodule number, ↑ shoot dry weight (up to 70%), ↑ seed yield, ↑ protein content (up to 28%) | +24–28 | 61 |

Abbreviation: Ndfa: Nitrogen derived from the atmosphere.

evaluating the co-application of Bj and humic substances, as well as compatible inoculation strategies. These studies demonstrate consistent improvements in nodulation, nitrogen uptake, and protein yield. Reported yield increases ranged from 22% to 57%, depending on site conditions and treatment combinations. In Poland, co-application of *B. japonicum* with moderate nitrogen fertilizer resulted in a 22% yield increase.[50] In central Germany, up to 99% higher protein yield and enhanced N-fixation were achieved using commercial *B. japonicum* inoculants under cool-climate conditions.[74] Although no yield data were provided in Latvia, full-length 16S rRNA sequencing revealed increased diversity and abundance of functionally relevant rhizobia in nodule microbiomes, indicating ecological benefits.[48]

## 7. Discussion

The integration of nitrogen-fixing bacteria and humic products derived from agricultural waste offers a promising avenue for enhancing sustainable soybean cultivation. Numerous studies have demonstrated the effectiveness of *Bradyrhizobium* spp. in promoting root nodulation and atmospheric nitrogen fixation under a range of environmental conditions.[1-3,5,6,12,21] These microbes not only supply biologically fixed nitrogen to plants but also contribute to soil health and microbiome stability.[8,15,18]

Simultaneously, humic substances—particularly those derived from composted organic matter—have been reported to improve nutrient retention, enhance root architecture, and stimulate enzymatic activity within the rhizosphere.[2,31,58,60] When applied in combination, nitrogen-fixing bacteria and humic products can have synergistic effects, leading to improved nitrogen-use efficiency and crop productivity.[32,35,77] As demonstrated in Figure 3 and Table 3, the combined application enhanced soybean biomass by up to 28% and increased nodulation efficiency by over 35% compared to single treatments.

Genotypic variation among soybean cultivars also plays a critical role in determining inoculation success and nitrogen fixation outcomes.[10,12,55,74] Moreover, recent studies have highlighted that dual or multi-strain inoculation—*e.g.*, with Az and *Bradyrhizobium*—can further boost plant resilience and yield under abiotic stress.[33-36,45,46,66] For instance, a study by Chibeba *et al*.[30] indicated that co-inoculated soybeans exhibited significantly earlier nodulation, which translated into higher final yields under field conditions.

However, some challenges persist. There is considerable variability in the performance of commercial humic products due to differences in source material, processing methods, and active ingredient concentrations.[31,61,62,85] In addition, factors such as soil pH, moisture availability, and the presence of native microbial competitors can limit the establishment and effectiveness of introduced rhizobia.[21,24,26]

Despite these constraints, the ecological and agronomic benefits of integrating microbial inoculants with humic products are increasingly recognized. Beyond nitrogen provision, such integrated biotechnologies support reduced chemical fertilizer input, enhanced carbon sequestration, and resilience to climate stressors.[25,32,55,59] Figure 4 illustrates that integrated treatments led to a 22% reduction in nitrate leaching and a 31% improvement in soil aggregate stability.

To achieve consistent field-level success, further work is warranted to standardize humic product quality,[31,59,86] develop precision delivery systems,[37,39] and tailor inoculation strategies to specific cultivars and agroecological conditions.[73,74] Emerging omics-based tools and soil microbiome profiling also hold potential to refine microbial compatibility and functional predictions.[9,17,71]

In summary, the combined application of nitrogen-fixing bacteria and humic substances represents a scalable, cost-effective, and ecologically sound strategy for soybean cultivation. This integrated approach aligns with the broader goals of circular bioeconomy and regenerative agriculture, offering a sustainable pathway toward enhanced crop performance and long-term soil fertility.

## 8. Conclusion

The integration of nitrogen-fixing bacteria and humic products derived from agricultural waste presents a robust and sustainable strategy to improve soybean productivity while enhancing soil fertility and ecological resilience. Co-application of these biotechnological inputs leads to improved nodulation, nitrogen fixation efficiency, nutrient uptake, and yield performance under diverse environmental conditions.

Despite existing challenges, such as strain-specific effectiveness, product variability, and the need for precision application, the growing body of scientific evidence supports their synergistic benefits. Moving forward, targeted formulation development, cultivar-specific compatibility testing, and field-level optimization will be critical for widespread adoption.

This integrated approach not only aligns with the principles of circular bioeconomy and regenerative agriculture but also offers practical solutions to reduce chemical inputs and mitigate environmental impact in legume-based cropping systems.

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## Conflict of interest

The author declares no competing interests.

## Author contributions

This is a single-authored article.

## Availability of data

Not applicable.

## Further disclosure

The conference in question is the 31st Annual International Scientific Conference "Research for Rural

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