Associative Biological Nitrogen Fixation in Maize: From Diazotroph Recruitment to Sustainable Crop Nutrition

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ABSTRACT

Biological nitrogen fixation (BNF) represents a promising approach for decreasing maize's reliance on synthetic nitrogen fertilisers while at the same time improving the sustainability of low-input agricultural production systems. Unlike the highly efficient symbiotic nitrogen fixation seen in the association between legumes and rhizobium bacteria, maize mainly depends on associative and endophytic diazotrophic microorganisms which colonise the rhizosphere, the root surfaces and the internal tissues of the plant. Recent discoveries, especially the identification of mucilage-associated nitrogen-fixing microbiomes in Sierra Mixe maize, have once again stimulated interest in the possible role that BNF can play in the nitrogen nutrition of maize. This review brings together current knowledge of the biological, biochemical, genetic, and ecological mechanisms responsible for associative BNF in maize. The main areas covered are the diversity and functional roles of diazotrophic bacteria such as *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter*, *Azoarcus* and *Klebsiella*; the regulation of nitrogenase activity and the nitrogen assimilation pathways; and the impact of carbon supply, oxygen availability and host-microbe interactions on the efficiency of fixation. The review also covers recent developments in maize genetics, including genome-wide association studies, quantitative trait locus mapping, and the identification of candidate genes involved in aerial root development and mucilage secretion. Moreover, it assesses the effects of soil fertility management, environmental conditions, and agronomic practices on microbial recruitment and nitrogen fixation. Lastly, the review examines opportunities and challenges in breeding, biotechnology, and microbiome engineering, highlighting their potential to improve associative BNF and support climate-smart, resource-efficient maize production. Combining knowledge of host genetics, rhizosphere ecology, and microbial function may reduce the need for fertilizers and improve nitrogen use efficiency in future maize production systems.

Keywords: Associative biological nitrogen fixation; diazotrophs; nitrogenase; aerial root mucilage; Sierra Mixe maize; rhizosphere microbiome; epiphytic bacteria; plant-microbe interactions.

INTRODUCTION

Plant growth and productivity depend substantially on beneficial interactions between their roots and associated microorganisms. These partnerships contribute to growth promotion, suppression of soil-borne diseases, and improved nutrient availability. Among them, the contribution of free-living and root-associated diazotrophic bacteria to biological nitrogen supply has received comparatively little attention, despite its potential to enhance nitrogen availability and support more efficient, sustainable crop production.

Maize is a crop with a high demand for nitrogen and generally needs large amounts of synthetic fertilizers if it is to achieve its optimal yield. Although the nitrogen fertilizers produced by the Haber–Bosch process account for almost half of the quantity taken up by the world's main cereal crops, this input is used in an inefficient way since a great deal of it is lost through leaching and gaseous emissions rather than being absorbed by the plant (Martinez-Feria et al., 2025). In sub-Saharan Africa, nitrogen deficiency is a leading constraint on yield because high cost, poor access, and environmental risk limit fertilizer use among smallholder farmers (Van Deynze et al., 2018). In higher-input regions, the opposite problem prevails: excessive fertilizer application drives nitrate

pollution, eutrophication, and nitrous oxide emissions (Bloch et al., 2020). Nitrogen management is therefore a global sustainability challenge with strikingly different faces in different agricultural systems.

Unlike legumes, maize does not form root nodules. However, it can still benefit from associative diazotrophs such as *Azospirillum, Herbaspirillum, Klebsiella,* and *Kosakonia* that colonize the rhizosphere, root tissue, and mucilage exudates and fix atmospheric nitrogen without an intracellular symbiosis (Higdon et al., 2020). Ground-breaking work on maize landraces in Sierra Mixe, Mexico found that mucilage-associated diazotrophic communities can supply 29–82% of the plant's nitrogen needs, demonstrating that associative fixation can be agronomically meaningful in cereals (Van Deynze et al., 2018). More recent work on engineered diazotrophs, including *Kosakonia sacchari* and *Klebsiella variicola* strains genetically modified for nitrogenase activity that persists under nitrogen-rich conditions, shows that associative biological nitrogen fixation (BNF) can be enhanced to partially displace synthetic fertilizer even in conventionally managed systems (Martinez-Feria et al., 2025). Taken together, this evidence positions associative BNF as a sustainable strategy for intensification of maize nutrition: it can lower input costs, reduce negative environmental externalities, and improve resilience to N-depleted soils.

This review examines associative BNF in maize from four complementary angles: the host genetic and molecular basis of microbial recruitment; the biochemical mechanisms that make nitrogen fixation possible; the root physiological and signaling context in which the association occurs; and the breeding, biotechnological, agronomic, and policy pathways through which the trait might be deployed at scale. The aim is to synthesize current understanding, highlight open questions, and identify practical directions for improving nitrogen self-sufficiency in maize, with particular relevance to low-input food systems in the global south.

2. Associative versus Symbiotic Nitrogen Fixation

Symbiotic fixation occurs inside root nodules formed between legumes and *Rhizobium* species. Within these specialized structures, nitrogenase activity is tightly controlled by the host plant, ensuring efficient fixation of atmospheric nitrogen and its assimilation into plant metabolism. Associative N-fixation, by contrast, involves no root nodulation: diazotrophic bacteria colonize the rhizosphere, root surface, and internal root tissue of cereals such as maize and sorghum, fixing atmospheric nitrogen and releasing ammonium or amino acids that the host can take up (Bloch et al., 2020). Metabolically, associative BNF is generally less efficient than symbiotic fixation, but its significance lies in its availability to the non-leguminous cereals that dominate global low-input food systems.

Several bacterial genera recur across the maize-associative BNF literature (Table 1). *Azospirillum* species are well-studied plant growth-promoting rhizobacteria (PGPR) that combine nitrogen fixation with auxin-mediated root stimulation (Giri et al., 2025). *Herbaspirillum* species behave as root and stem endophytes, contributing to both nitrogen assimilation and stress tolerance (Irineu et al., 2023). *Klebsiella* and *Kosakonia* species are free-living diazotrophs capable of colonizing maize mucilage and rhizosphere soil with measurable nitrogenase activity under field conditions (Martinez-Feria et al., 2025). Meanwhile, some *Burkholderia* strains combine nitrogen fixation with suppression of soil-borne pathogens, making them valuable both as diazotrophs and as biocontrol agents in maize and other crops (Heo et al., 2022).

Ecologically, associative BNF is most consequential where soils are nitrogen-poor and synthetic inputs are unaffordable. Heavy rainfall and associated nutrient leaching make tropical soils particularly prone to nitrogen deficiency (Russo et al. 2017). In these conditions, associative diazotrophs contribute most to plant nitrogen budgets, as shown by the mucilage-associated communities of Sierra Mixe maize mentioned above. Beyond the direct nitrogen contribution, sustained diazotroph activity supports broader soil health, thus reducing dependence on external inputs.

Genetic regulation of BNF in Diazotrophs

Diazotrophs possess specialized genetic systems that enable them to fix atmospheric nitrogen into ammonia, a process central to global nitrogen cycling. The core machinery is encoded by the *nif* gene cluster, particularly *nifHDK*, which encodes the nitrogenase enzyme complex (Dixon & Kahn, 2004; Dos Santos et al., 2012). *nifH*

produces the Fe protein (dinitrogenase reductase), which transfers electrons from ferredoxin/flavodoxin to the MoFe protein (Hoffman et al., 2014). *nifD* and *nifK* encode the MoFe protein (dinitrogenase) (Einsle & Rees, 2020), which together

Table 1. Main genera of associative (non-symbiotic, plant-associated) nitrogen-fixing diazotrophs, their e**cological diversity** and how environmental stressors (temperature, moisture, pH, nutrient status) modulate their efficiency

Genus	Colonization site(s)	Main benefits/mechanisms	Key limitations	References
Azospirillum	Rhizosphere and root surface of cereals	Nitrogen fixation; produces IAA; improves root growth and nutrient uptake	Requires low oxygen; inhibited by high nitrogen; sensitive to drought/salinity	Steenhoudt & Vanderleyden, 2000; Bashan et al., 2004; Dixon & Kahn, 2004; Bashan & de-Bashan, 2010; Cassán et al., 2020
Gluconacetobacter	Endophyte in sugarcane and related crops	Nitrogen fixation; hormone production; nutrient solubilization	Reduced by nitrogen fertilizer; depends on host genotype	Cavalcante & Döbereiner, 1988; Reinhold-Hurek & Hurek, 1998; Boddey et al., 2003; Santi et al., 2013;
Herbaspirillum	Root tissues and xylem of cereals	Nitrogen fixation; IAA, GA and ACC deaminase; promotes root branching	Poor survival outside plant; sensitive to drought and oxygen	(Baldani et al., 1986; Reinhold-Hurek & Hurek, 1998; James et al., 2002; Dixon & Kahn, 2004; Santi et al., 2013; Pankievicz et al., 2015
Azoarcus	Internal root tissues of grasses and rice	Nitrogen fixation with direct nitrogen transfer to host	Limited persistence in soil; host dependent	Reinhold-Hurek & Hurek, 1998; Hurek & Reinhold-Hurek, 2003; Dixon & Kahn, 2004; Krause et al., 2006;
Klebsiella	Roots and internal tissues of cereals	Nitrogen fixation; useful model for nif regulation	Some strains are human pathogens; fixation suppressed by nitrogen	Dixon & Kahn, 2004; Iniguez et al., 2004; Rosenblueth et al., 2018)(Bloch et al., 2020)
Burkholderia /Paraburkholderia	Rhizosphere and endosphere	Nitrogen fixation; phosphate solubilization; disease suppression	Regulatory concerns due to related pathogenic species	Estrada-De Los Santos et al., 2001; Caballero-Mellado et al., 2004; Santi et al., 2013; Rosenblueth et al., 2018)
Azotobacter	Free-living in rhizosphere	Aerobic nitrogen fixation; growth-promoting compounds	Performs poorly in acidic, saline, or nutrient-poor soils	Sabra et al., 2000; Santi et al., 2013; Bageshwar et al., 2017; Rosenblueth et al., 2018;

Pseudomonas	Root surface and rhizosphere	Some strains fix nitrogen; strong root colonization ability	Only a few strains fix nitrogen; performance varies	Yan et al., 2008; Dixon & Kahn, 2004; Fox et al., 2016
Enterobacter/ Kosakonia	Rhizosphere, endosphere and maize mucilage	Nitrogen fixation in mucilage-rich environments	Requires suitable mucilage and high humidity	Dixon & Kahn, 2004; Van Deynze et al., 2018; Rosenblueth et al., 2018;
Pantoea	Rhizosphere, endosphere and phyllosphere	Nitrogen fixation; IAA and phosphate solubilization	Some species are plant pathogens	Dixon & Kahn, 2004; Santi et al., 2013; Rosenblueth et al., 2018; Van Deynze et al., 2018;
Bacillus/ Paenibacillus	Rhizosphere and some root tissues	Nitrogen fixation; spores improve shelf life; disease suppression	Field performance can be inconsistent	Santi et al., 2013; Rosenblueth et al., 2018)
Rhizobium/ Bradyrhizobium	Rhizosphere and cereal roots	Mainly growth promotion through hormones and nutrient mobilization	Nitrogen fixation usually low in cereals	Dixon & Kahn, 2004; Santi et al., 2013; Santi et al., 2013; Rosenblueth et al., 2018)

catalyze the reduction of N_2 to NH_3 at the expense of at least 16 ATP per molecule of nitrogen fixed. Regulation of nitrogen fixation is tightly controlled by environmental cues (Dixon & Kahn, 2004). The *nifA–nifL* regulatory system ensures that nitrogenase is expressed only under low-oxygen and nitrogen-deficient conditions. *nifA* acts as a transcriptional activator, while *nifL* inhibits *nifA* activity when ammonium or oxygen levels are high, preventing wasteful energy expenditure . Oxygen is sensed through NifL's FAD cofactor (Hill et al., 1996), and nitrogen status is sensed through 2-oxoglutarate, adenine nucleotides and PII signalling proteins (Little et al., 2002). This prevents wasteful energy expenditure, since nitrogen fixation requires at least 16 ATP per N_2 reduced, and it also protects oxygen-sensitive nitrogenase (Burgess & Lowe, 1996).

Comparative genomics reveals that diazotrophy is widely distributed across bacteria and archaea, including heterotrophic genera such as *Klebsiella* and *Burkholderia*, as well as cyanobacteria. While the *nif* cluster is evolutionarily conserved, modular variations exist that reflect adaptation to diverse ecological niches (Nichio et al., 2020). These adaptations include alternative nitrogenases (e.g., V- and Fe-only nitrogenases) that function under molybdenum-limited conditions.

Molecular adaptations also address the oxygen sensitivity of nitrogenase (Gallon, 1992). Diazotrophs employ strategies such as high respiratory rates (Oelze, 2000), protective proteins (Schlesier et al., 2016), and biofilm formation to create favorable microaerophilic niches (Wang et al., 2017). In associative systems, diazotrophs rely on host-derived carbon exudates, while free-living cyanobacteria couple nitrogen fixation directly to photosynthesis (Ustick et al., 2021). Together, these genetic and molecular features highlight the versatility of diazotrophs in sustaining nitrogen fixation across ecosystems, underscoring their ecological importance in both free-living and plant-associated contexts.

Genetic control of BNF in Maize

Maize genotypes differ considerably in their capacity to recruit and sustain diazotrophic communities. Much of this variation traces back to root exudation. Under genetic control, root-released sugars, amino acids, and organic acids serve as chemo-attractants for nitrogen-fixing bacteria; landraces such as Sierra Mixe maize produce

unusually abundant, polysaccharide-rich mucilage that creates a favorable colonization niche (Van Deynze et al., 2018).

Several quantitative trait locus (QTL) mapping studies have identified maize chromosomal regions associated with aerial root mucilage (ARM) exudation and microbial recruitment. In a genome-wide association study (GWAS) of a recombinant inbred line population, Gao et al. (2023) identified 17 candidate genes associated with aerial root mucilage secretion. One of the QTLs identified, on chromosome 7, coincides with the candidate gene ZmSBT3 (GRMZM2G363552); knockouts of this subtilase-family gene increased both mucilage secretion and nitrogen content following inoculation with diazotrophic bacteria, indicating that ZmSBT3 normally suppresses the trait. In a larger multi-year, multi-environment study built on a teosinte-derived recombinant inbred population grown under low-nitrogen conditions, Zhao et al. (2025) mapped fifteen QTLs and sixty-eight quantitative trait nucleotides for aerial-root mucilage secretion, including a significant nucleotide on chromosome 9 (QTN 9_154921657) near the candidate gene ZmAco3, whose disruption reduced mucilage secretion. Complementing these mucilage-focused studies, a heritability and QTL mapping analysis of aerial root and yield-related traits across a *Zea* species panel spanning teosinte, landraces, and improved lines localized three QTLs for aerial root node count to chromosomes 1 and 9, which influence the capacity for diazotroph recruitment (O'Donnell et al., 2025). Together, these studies point to chromosomes 1, 7, and 9 as recurrent locations for BNF-relevant aerial root and mucilage traits. However, QTL detection varies across environments and populations, and these loci often overlap with genes governing root architecture more broadly, suggesting that root morphology and exudation jointly shape BNF efficiency (Gao et al., 2023; Zhao et al., 2025).

Transcriptomic and proteomic studies of colonized roots show upregulation of genes involved in sugar transport, defense signaling, and nitrogen assimilation, alongside increased abundance of enzymes linked to carbon metabolism (Prinsi & Espen, 2018; Brusamarello-Santos et al., 2017). These findings point to a dynamic molecular cross-talk in which host genotype sets the boundary conditions for how efficiently associative BNF can proceed, making QTLs for exudation and colonization attractive targets for marker-assisted selection.

Biochemical Basis of BNF

The biochemical mechanism of biological nitrogen fixation is centered on the nitrogenase enzyme complex, which serves as the catalytic machinery responsible for converting atmospheric dinitrogen (N_2) into ammonia (Hoffman et al., 2014). The nitrogenase complex comprises two metalloproteins, dinitrogenase reductase, commonly referred to as the Fe protein (Georgiadis et al., 1992), and dinitrogenase, or the MoFe protein (Einsle & Rees, 2020). The Fe protein receives electrons from cellular electron donors such as reduced ferredoxin or flavodoxin and transfers them, in an ATP-dependent process, to the MoFe protein. Electrons are subsequently delivered through the P-cluster to the FeMo-cofactor, where N_2 undergoes successive reduction and protonation to form ammonia. The process is highly energy intensive, with at least 16 molecules of ATP hydrolysed for every molecule of N_2 reduced as H_2 is produced as an obligatory by-product. The high energetic cost of fixation makes an adequate supply of carbon and reducing power essential for diazotrophic activity (Seefeldt et al., 2009; Bloch et al., 2020).

Associative diazotrophs obtain much of the carbon required to support this energy-demanding process from plant-derived compounds in the rhizosphere. Root exudates, including sucrose and other soluble sugars, as well as organic acids such as malate and citrate, provide substrates for microbial respiration and generate the ATP and reducing equivalents required for nitrogenase activity (Dakora & Phillips, 2002; Bais et al., 2006; Badri & Vivanco, 2009). Consequently, carbon availability can influence both diazotrophic population growth and their capacity to fix atmospheric nitrogen. In maize, root-derived carbon compounds and mucilage can create nutrient-rich microsites that support microbial colonization and activity. Therefore, maize genotypes that release greater quantities of root exudates may provide a more favorable carbon environment for associative diazotrophs and sustained nitrogen fixation (Van Deynze et al., 2018).

Nitrogenase activity presents an additional biochemical challenge because the enzyme is extremely sensitive to molecular oxygen (Gallon, 1992). Exposure to oxygen can irreversibly damage the enzyme's metal-containing centers required for catalysis (Schlesier et al., 2016). Consequently, diazotrophs employ several mechanisms to

protect nitrogenase from oxygen (Gallon, 1992; Dixon & Kahn, 2004). These include high respiratory activity, which rapidly consumes oxygen (Oelze, 2000), occupation of microaerophilic niches within the rhizosphere and root-associated mucilage (Van Deynze et al., 2018), and physiological regulation that restricts nitrogenase activity under unfavorable oxygen conditions. In plant-associated systems, the physical and chemical characteristics of the root environment can further influence oxygen availability. Root architecture, mucilage production, microbial respiration, and local diffusion gradients can collectively generate microsites in which oxygen concentrations remain sufficiently low to support nitrogenase activity (Dixon & Kahn, 2004; Bloch et al., 2020).

Once ammonia is produced, the fixed nitrogen enters microbial nitrogen metabolism, principally through assimilation into glutamine and glutamate. The glutamine synthetase-glutamate synthase pathway provides a major route for incorporating ammonium into organic nitrogen compounds (Suárez et al., 2002; Hodges, 2002). In associative interactions, subsequent transfer of nitrogen from the diazotroph to the plant depends on the particular microbial species, plant genotype, colonization site, and physiological conditions (Carvalho et al., 2014; Sheoran et al., 2021). Thus, BNF represents an integrated biochemical process in which electron supply, ATP generation, carbon metabolism, oxygen regulation, nitrogenase catalysis, and ammonium assimilation operate together. In maize, the effectiveness of this process ultimately depends on the ability of the plant-microbe association to maintain an adequate carbon supply while providing a suitable microenvironment for diazotrophic activity (Van Deynze et al., 2018).

The Role of Root Physiology and Rhizosphere Niche

Root exudates simultaneously function as chemical signals and metabolic substrates for diazotrophs. Sugars, amino acids, organic acids, and secondary metabolites attract colonizing bacteria while also fueling their metabolism and activating nitrogen-fixation pathways. Given that exudate composition varies genetically among maize lines, it is a key determinant of rhizosphere microbiome composition and, by extension, of BNF efficiency (Bloch et al., 2020; Van Deynze et al., 2018).

Mucilage is a particularly important physical and chemical niche. In landraces such as the Sierra Mixe maize, polysaccharide-rich mucilage secreted at aerial roots creates a carbon-dense, low-oxygen microenvironment that is well suited to diazotroph colonization and nitrogenase activity. This is the physiological basis for the high nitrogen contributions reported in that system. More broadly, rhizosphere pH and other microenvironmental factors modulate colonization and enzyme activity: mildly acidic conditions tend to favor diazotroph growth, while extreme acidity is inhibitory, and root architecture and surface area jointly determine how much colonizable surface is available (Martinez-Feria et al., 2025).

Molecular Cross-Talk between Maize and Diazotrophs

The molecular cross-talk and carbon-for-nitrogen exchange at the root-mucilage interface is very elaborate (Figure 1). .Root colonization by the diazotrophs begins with bidirectional chemical signaling: root-derived flavonoids, sugars, and organic acids act as chemo-attractants, while bacterial surface molecules such as lipopolysaccharides and flagellin are recognized by the host. This signaling exchange allows selective recruitment of beneficial diazotrophs while limiting opportunities for pathogens (Bloch et al., 2020). Plant hormones shape the outcome of this exchange. Diazotroph-derived auxins stimulate root elongation and branching, expanding the surface area available for colonization, whereas elevated ethylene acts as a negative regulator of microbial entry; genotypes that modulate ethylene signaling tend to be more permissive to diazotroph association. Abscisic acid, meanwhile, links root exudation and stress physiology, so that drought or nutrient stress can indirectly reshape microbial recruitment (Martinez-Feria et al., 2025). Nitrogen deficiency itself upregulates genes for sugar transport and mucilage secretion, thus enhancing colonization, while antioxidant systems moderate reactive-oxygen signaling so that immune activation does not overwhelm beneficial colonization (Van Deynze et al., 2018). The net effect is a finely balanced cross-talk between plant defense and mutualism.

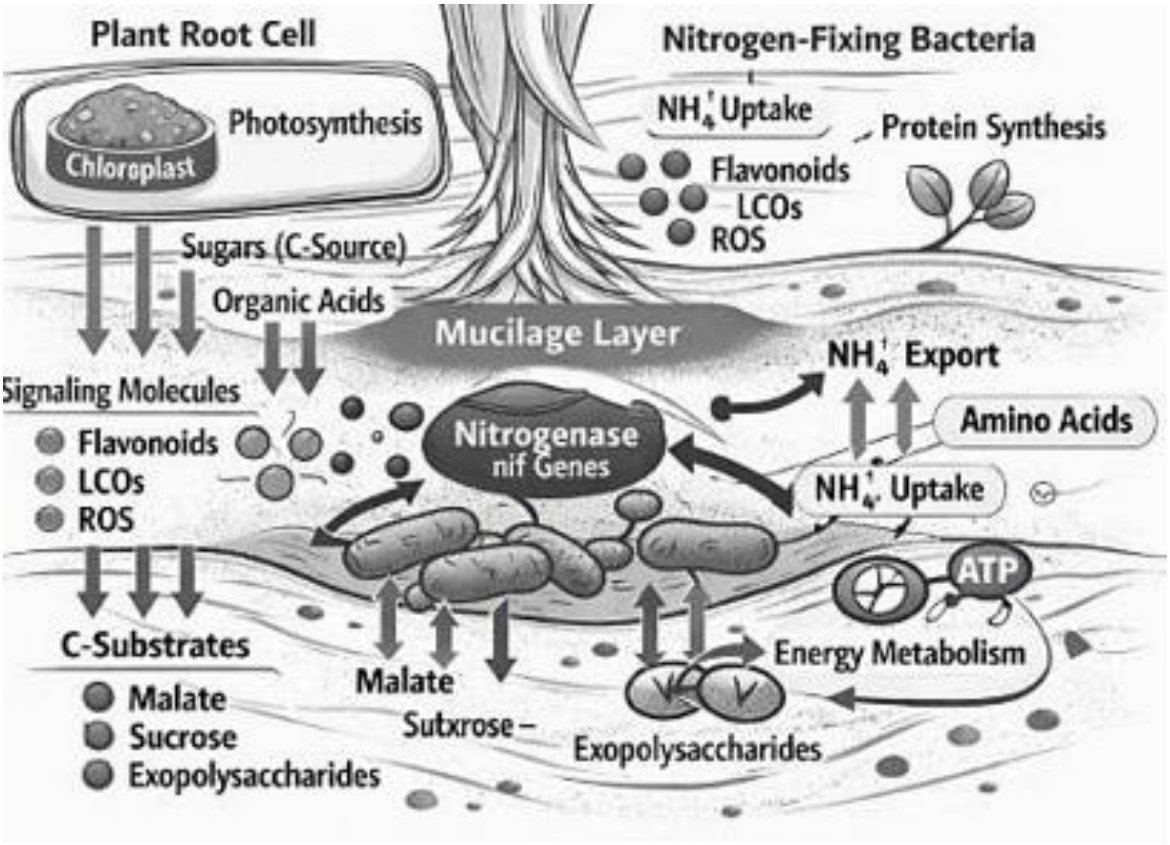


Figure 1. Root Mucilage Carbon–Nitrogen Exchange

Breeding and Biotechnological Approaches

Marker-assisted selection allows breeders to track QTLs and candidate genes linked to root exudation, ARM production, and microbial recruitment, accelerating selection for favorable alleles without relying solely on costly field screening (Martinez-Feria et al., 2025). Transgenic approaches have focused on overexpressing transporter genes that increase carbon flux to root-associated diazotrophs, or on engineering maize to secrete specific sugars or organic acids that favor colonization by genera such as *Azospirillum* and *Herbaspirillum*; direct transfer of nitrogenase genes into the maize genome remains far more difficult, given the enzyme's sensitivity to oxygen and relatively high energy demands (Bloch et al., 2020).

CRISPR/Cas editing offers a more precise route, allowing targeted modification of regulatory genes controlling root exudation, mucilage secretion, and immune response. For instance, fine-tuning transcription factors in the auxin and ethylene pathways can favor diazotroph recruitment without compromising pathogen defense. In principle, the same tools can be applied to the microbial partner, improving nitrogenase efficiency or environmental resilience directly. Increasingly, breeding programs are combining genomic selection with marker-assisted selection to integrate BNF-associated traits into elite germplasm, with participatory breeding helping ensure that resulting cultivars are adapted to the nitrogen-deficient soils of sub-Saharan Africa and Latin America (Van Deynze et al., 2018).

Agronomic and Environmental Considerations

Soil fertility management interacts directly with associative BNF: high synthetic nitrogen inputs suppress nitrogenase expression in root-associated diazotrophs, whereas the low-input conditions typical of much of sub-Saharan Africa are precisely where associative fixation becomes a critical nitrogen source (Van Deynze et al., 2018). Balanced fertilization with phosphorus and micronutrients, rather than nitrogen alone, tends to support root exudation and microbial recruitment without suppressing fixation (Brisson et al., 2022)

Conservation agriculture practices, including minimum tillage, residue retention, and crop rotation, improve soil organic matter and microbial diversity, producing more stable rhizosphere conditions for diazotroph

colonization. Biochar amendments complement this by improving soil structure, raising cation exchange capacity, reducing nutrient leaching, and providing physical microhabitats that support diazotroph survival. A meta-analyses of African agroecosystems found that conservation agriculture combined with biochar improves nitrogen cycling and grain yield, consistent with a supportive role for associative BNF (Namatsheve et al., 2024). As climate change intensifies rainfall variability, nutrient leaching, and soil acidification, associative BNF stands out as a comparatively low-cost, climate-resilient nitrogen strategy for smallholder systems, and one that pairs naturally with conservation agriculture and biochar to build long-term soil health and carbon sequestration (Bloch et al., 2020).

Future Directions and Challenges

Systems biology approaches that integrate genomics, transcriptomics, proteomics, and metabolomics offer a more holistic view of maize–diazotroph interactions than any single technique. Computational modeling built on these datasets could help predict how specific genotypes will respond to microbial inoculation across diverse soils (Bloch et al., 2020).Because machine learning applied to multi-omics data (such as GWAS, QTL mapping, RNA-seq and metabolomics) is able to rank candidate loci more reliably than single-marker tests, particularly in the case of a noisy phenotype, genomic selection models can be used to predict which breeding lines will harbor more diazotrophs or secrete more mucilage without having to measure each plant in the greenhouse. Moreover, computer vision applied to images taken in the field or in the greenhouse can assess aerial roots and mucilage on a large scale, something which at present is a major bottleneck in phenotyping. A parallel line of research is moving from single-strain inoculants toward synthetic microbial consortia that combine complementary traits such as nitrogen fixation, phosphate solubilization, and stress tolerance. Early field trials suggest that these systems sustain fixation more reliably across variable climates than single strains alone (Martinez-Feria et al., 2025). On the biotechnology side, CRISPR editing of microbial genomes and synthetic-biology efforts to design lower-ATP-cost nitrogenase variants could further improve field reliability, alongside continued engineering of host exudation pathways.

Realizing these gains at scale will require more than technical progress. Adoption depends on investment in inoculant production and distribution, extension services that train farmers in the use of BNF-enhanced cultivars, and policy incentives for low-input, climate-resilient practices, with international collaboration especially important for scaling these technologies in nitrogen-deficient regions of sub-Saharan Africa and Latin America (Van Deynze et al., 2018). Substantial challenges remain: BNF efficiency varies widely across soil types, climates, and genotypes; transgenic and microbial inoculant technologies face significant regulatory hurdles; farmer adoption depends as much on affordability and accessibility as on efficacy; and the long-term ecological consequences of introducing engineered diazotrophs into open-field systems are not yet well understood.

The development of gene-edited crops and genetically engineered biological nitrogen-fixing diazotrophs faces a complex set of biosafety and regulatory hurdles. A central issue is classification ambiguity: under frameworks such as the *Cartagena Protocol on Biosafety*, many countries treat gene-edited microbes as genetically modified organisms (GMOs), requiring full GMO-level regulation even when no foreign DNA is present. This precautionary stance, particularly in the European Union, has slowed approvals and created uncertainty for researchers and companies (Vengadesena, Abdul Aziz, & Jones, 2025). Regulatory misalignment across regions further complicates commercialization, with some jurisdictions adopting more flexible, product-based approaches while others enforce strict process-based rules (Heaton et al., 2026).

Challenges associated with field release are equally significant. Regulators demand extensive ecological risk assessments, including studies on horizontal gene transfer, persistence in soil, and unintended effects on native microbiomes. These requirements are costly and time-consuming, often exceeding the resources of small research groups or startups (Fernández Ríos et al., 2025). Moreover, containment and monitoring protocols for field trials add logistical burdens, while traceability requirements,, though scientifically difficult to achieve for microbes in open environments—are often mandated by regulators (Heaton et al., 2026).

Even with regulatory approval, farmer adoption barriers remain (Areal et al., 2011; O'Callaghan et al., 2022). Concerns often center on biosafety, liability, and consumer acceptance, particularly in export-oriented markets

where GMO skepticism persists (Baldani et al., 2014). High regulatory costs translate into expensive products, limiting accessibility for smallholder farmers in the Global South. In addition, weak extension services and limited training hinder effective use of engineered diazotrophs, while consumer resistance to GMOs discourages adoption due to fears of market rejection (Fernández Ríos et al., 2025

CONCLUSION

Associative biological nitrogen fixation in maize sits at the intersection of genetics, biochemistry, and agronomy. Host genetic factors (e.g. root exudation, mucilage secretion, and immune regulation) interact with the biochemical demands of a highly energy-intensive, oxygen-sensitive nitrogenase system to determine how much nitrogen a given maize genotype can obtain from diazotrophic partners such as *Azospirillum*, *Herbaspirillum*, *Klebsiella*, and *Kosakonia*. Landraces such as Sierra Mixe maize demonstrate that this contribution can be agronomically significant, and emerging genetic and breeding tools (e.g. artificial intelligence, marker-assisted selection, genomic selection, and CRISPR/Cas editing) offer plausible routes to introducing comparable traits into elite germplasm.

Realizing that potential as a practical alternative to synthetic fertilizer will depend on integrating genetic innovation with supportive agronomic practices (e.g. conservation agriculture, biochar amendment, and balanced fertilization) and with policy frameworks that make BNF-enhanced cultivars accessible to the smallholder farmers who stand to benefit most, particularly across the nitrogen-deficient soils of sub-Saharan Africa. Advances in systems biology and synthetic microbial consortia are likely to refine this picture further, but the core opportunity is already clear: associative BNF offers a sustainable route toward reduced fertilizer dependence, improved food security, and more climate-resilient maize production systems.

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