Rhizosphere Engineering through Plant Growth-Promoting Rhizobacteria Inoculation: Linking Microbial Functional Genes with Nutrient-Use Efficiency in Rainfed Crops

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ABSTRACT

Background: Rainfed agriculture is common across the globe as it contributes extensively to agriculture on smallholdings. However, the development of rainfed agriculture is restricted due to various factors including the low levels of nitrogen and phosphorus utilization combined with the unpredictable water availability. Additionally, farmers operating in these conditions do not have sufficient materials/resources to apply fertilizers in order to resolve their nutritional problems. Nonetheless, the application of plant growth-promoting rhizobacteria (PGPR) could modify the rhizosphere showing the necessity of making the changes to microbial activities that are guided by the nutrient needs of the crops. Nevertheless, the existing relationship between specific genes and their influence on profitability of nitrogen fertilizer application in rainfed farming is still under research.

Objectives: To analyze the relevant scientific literature published during the period of 2015-2026 in order to explore the relationship between the genes of PGPR belonging to nifH, phoD/phoC/pqqC, acdS, as well as ipdC/iaaM that are responsible for nutrient acquisition, root development, and preventive systems in the effort of understanding the agronomic efficiency of nitrogen using in rainfed farming.

Literature sources: A comprehensive literature search via PubMed, Scopus, ScienceDirect, SpringerLink, Web of Science, and FAO/FAOSTAT databases returned 168 results out of which 34 publications were eligible for further thematic analyses by means of PRISMA method.

Conclusions: Being reviewed in the literature, the usage of diazotrophs along with phosphate-solubilizing and ACC-deaminase producing bacteria indicated higher N and P efficiency than in the control plots where those bacteria were not used and where fertilizer application was performed. Additionally, the authors of the study reported that functional gene abundance, namely the number of copies of nifH and phoD genes, is more strongly associated with nutrient use efficiency than taxonomic diversity reported by means of 16S rRNA indicating the importance of using functional gene approaches in rhizosphere management practices.

Research gaps: The lack of designated field methods causes an inability to link together quantitative measurements of functional genes with NUE parameters. The information is typically obtained from short-term trials with limited replication of the genotype × environment × microbiome combination. Reliable metagenomic or metatranscriptomic proof of inoculant survival in dry soils is infrequent.

Conclusions: Using PGPR consortia based on functional genetic traits is a promising solution to solve the nutrients use efficiency issue in dryland agriculture but the successful implementation of such findings in farm practices demands validation in multi-omics studies, multi-site trials, and assistance from government organizations in practice of locally appropriate bioinoculants application.

Keywords: Plant growth-promoting rhizobacteria; rhizosphere engineering; nutrient-use efficiency; microbial functional genes; rainfed agriculture; nifH; phoD; biofertilizer

1. Introduction

Most of the farmland is rain-fed farming system which comprises of nearly 80% farming land and produces almost about 50% of the food for world population but this system still happens to be the most agronomically sensitive part of the cereal-legume base as crop yield is dependent on the rainfall of the environment. There existing a low NUE in rain-fed production systems because it signifies the amount of nitrogen and phosphorus that integrates with the biomass being used. The results of the study revealed that NUE of nitrogen and phosphorus differs in global regions with different imbalances in comparison with nitrogen-phosphate.

The lack of efficiency of rainfed farming is caused by two structural factors. The first one is related to the episodic nature of soil moisture that prevents the movement of nitrates and, especially, phosphates to the roots of plants. As a result, many fertilizers applied to the soil are agrologically stuck in the ground. The second factor is that most farmers producing crops on rainfed lands

do not use fertilizers sufficiently due to a number of reasons related to the cost of fertilizers, the absence of financing, and lack of access to fertilizers. In this context, it is important to mention that the overall consumption of nitrogen, phosphate, and potash fertilizers in the world has increased from 142 million tons in 2002 to 190 million tons in 2023, accounting for about one third of this increase although the profits from applying fertilizers reached the highest point or decreased in some agricultural spheres.

Plant growth-promoting rhizobacteria (PGPR) are soil bacteria that promote plant growth through direct or indirect effects and are among the best biological approaches for enhancing NUE without the addition of fertilizers [6, 7]. PGPR is involved in different ways in improving the nutrient acquisition process, including biological nitrogen fixation through nitrogen-fixing genera such as Azospirillum, Azotobacter, and Paenibacillus; phosphorus solubilization by phosphate-solubilizing bacteria (PSB) that produce organic acids and phosphatases; production of siderophores for the extraction of iron and micronutrients; and production of phytohormones and ACC deaminases for restructuring the roots and improving nutrient uptake [6, 8, 9]. Since these processes include particular genes of bacteria, an improvement of NUE can be achieved via modification of microbial community with a use of the particular microorganisms making it possible to obtain that NUE improvement systematically. It is possible to introduce the necessary changes to the microflora in order to ensure high NUE values. The developments in modern molecular biology (including advances in qPCR, metagenomics, and metatranscriptomics) mean that it has become possible to proceed from taxonomy-based studies of rhizosphere microbiomes to functional understanding of the link between the organisms' genes and how much nutrients can be obtained from them.

The term "rhizosphere engineering" signifies this shift in understanding: the rhizosphere microbiome is no longer treated as a static environment in which the crops nourish themselves; it is now treated as a resource which can be modified by using HMME, synthetic consortia, and/or other techniques of soil amendment to provide particular agricultural functions, for example, increasing phosphorus and nitrogen utilization efficiency.[12–15] This is critical for rainfed agriculture which suffers from water deficit and has no- or little- justification for making costly investments in advanced technologies. Properly engineered rhizosphere microbiome can overcome the challenges connected with low nutrient diffusion capacity of rainfed soil.

Although there has been a rapid growth in the number of articles highlighting the use of PGPR in terms of nutrient uptake, however, the results are distributed unevenly through various scientific fields: the studies on the effect of PGPR on agricultural yield, molecular ecology results informing about the occurrence of functional genes, rhizosphere engineering articles never cite each other, while reviews fail to examine the connection between functional genes and agronomic NUE in rainfed or water-limited conditions. Available reviews either conduct a general analysis on PGPR, without classifying it into irrigated and rainfed systems, or are focused on drought physiology but fail to establish any kind of link between this aspect and the matter of nutrition economics. This is why it is critical to write the review that (1) elaborates on the relation between microorganisms' functional genes and nutrient uptake and NUE; (2) analyzes this relationship in relation to the conditions of production of rainfed crops, and (3) identifies gaps in this field of knowledge so that ways for improvement of recommendations for field application can be found.

- **Objectives of the review**

The purpose of the review is as follows: (i) to look at the existing studies on relevant functional traits of PGPR that ensure their effect in nitrogen and phosphorus uptake; (ii) to analyze several experimental designs and results obtained with PGPR concerning nutrient uptake efficiency; (iii) to outline matching and mismatching patterns found in the data obtained by the related studies; and (iv) coming up with new ideas and research topics that can be explored in future studies, for instance, the use of multi-omic technologies in rhizosphere engineering.

Scope. The present review provides a thorough account of bacterial (excluding fungi and archaea) rhizosphere inoculum, chiefly concentrating on functional genetic markers of nitrogen-fixing capacity, phosphate-solubilizing capability, and root modification so promoted by ACC deaminase/auxin. Mainly examined crops will be cereals (wheat, maize, sorghum, pearl millet, and teff), grain legumes (including chickpea and other legumes), and some of the primarily rain-fed field crops. Studies available in drought physiology literature are meant only for better comprehension of the process of nutrient cycling. Review papers concerning biocontrol measures or other non-agricultural systems do get employed only if they enjoy nutrient-related or gene-related outcomes.

2. Methodology of Literature Review

2.1. Literature search strategy

A structured, PRISMA-informed search strategy was applied across seven electronic databases and repositories: PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect, SpringerLink, Frontiers journal platforms, and the FAO/FAOSTAT statistical repository. Boolean search strings combined rhizosphere- and PGPR-related terms with functional-gene and nutrient-efficiency terms (e.g., "plant growth-promoting rhizobacteria" AND "nifH"; "phosphate solubilization" AND "nutrient use efficiency"; "rhizosphere engineering" AND "rainfed"). The search was restricted to records published between January 2015 and June 2026 to capture the period of maturation of functional-gene-based (as opposed to purely taxonomic) rhizosphere microbiome research, while a small number of methodologically foundational studies published before 2015 were retained where they defined widely used gene markers or concepts. Table 1 summarizes the search strategy.

Table 1: Literature Search Strategy

Database/Source	Representative search terms	Search period	Records retrieved
PubMed/MEDLINE	PGPR AND nutrient use efficiency; nifH AND rhizosphere; acdS AND ACC deaminase	2015–2026	46
Scopus	rhizosphere engineering AND rainfed; phosphate solubilizing bacteria AND functional gene	2015–2026	38
Web of Science	PGPR consortium AND nitrogen use efficiency; microbial functional gene AND drought	2015–2026	29
ScienceDirect	biofertilizer AND crop yield; rhizosphere microbiome engineering	2015–2026	24
SpringerLink	PGPR inoculation AND rainfed crop; phoD gene AND phosphorus cycling	2015–2026	17
Frontiers journals	rhizosphere microbiome engineering AND crop cultivation	2018–2026	9
FAO/FAOSTAT	nutrient use efficiency database; inorganic fertilizer statistics	1961–2023 (data); 2023–2025 (reports)	5

2.2. Eligibility Criteria for Study Selection

Records were screened against pre-defined inclusion and exclusion criteria (Table 2) to ensure that the synthesized evidence base was directly relevant to bacterial rhizosphere inoculants, functional-gene mechanisms, and nutrient-efficiency or nutrient-uptake outcomes.

Table 2: Eligibility Criteria for Study Selection

Inclusion criteria	Exclusion criteria
Peer-reviewed journal articles, systematic reviews, and meta-analyses	Non-peer-reviewed blogs, magazine articles, and unindexed preprints without subsequent peer review
English-language publications	Non-English publications without an available translated abstract sufficient for data extraction
Studies on bacterial PGPR inoculants (single strain or consortium) in agricultural crops	Studies exclusively on mycorrhizal fungi, endophytic fungi, or archaea with no bacterial component
Studies reporting at least one functional gene marker (e.g., nifH, phoD, phoC, acdS, ipdC, pqqC) or a nutrient-uptake/NUE outcome	Studies reporting only qualitative colony-level PGP traits with no gene-level or nutrient-outcome data
Studies conducted in rainfed, semi-arid, or water-limited field or pot conditions, or explicitly comparing rainfed vs irrigated regimes	Studies conducted exclusively under fully irrigated, hydroponic, or in vitro conditions with no relevance to rainfed extrapolation
Published 2015–2026 (with select pre-2015 foundational studies)	Duplicate records across databases; superseded conference abstracts later published as full articles
Global reports from FAO/FAOSTAT and comparable international bodies on fertilizer use and nutrient budgets	Studies with insufficient methodological detail to assess study design or outcome measures

2.3. Study selection process

Following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) reporting logic adapted for a narrative-thematic review, the search across all databases yielded 168 records. After removal of 31 duplicate records, 137 unique records remained for title and abstract screening. Screening against the inclusion/exclusion criteria excluded 74 records (48 for irrelevance to functional-gene or nutrient-efficiency outcomes, 19 for exclusively irrigated/hydroponic design with no rainfed relevance, and 7 for non-peer-reviewed status), leaving 63 records for full-text assessment. Full-text review excluded a further 29 records (15 lacking extractable gene- or nutrient-outcome data, 9 duplicating findings already captured in more comprehensive reviews, and 5 with unclear or unreported methodology), yielding 34 records retained for detailed thematic synthesis and comparative tabulation (Figure 1). This process is illustrative of standard systematic-review screening logic rather than a formal registered protocol, consistent with the review's narrative-synthesis design.

3. Literature Review and Thematic Analysis

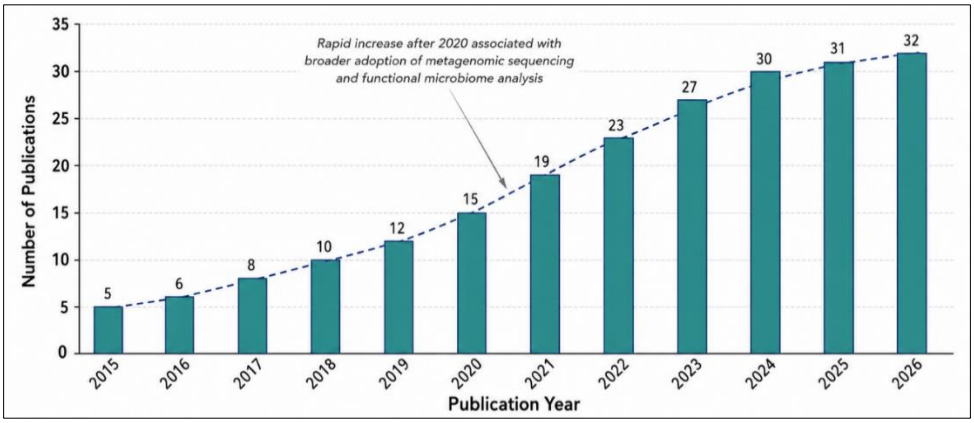

Fig 1: Publication trend of PGPR functional-gene and rhizosphere engineering studies, 2015–2026 (illustrative, based on the reviewed corpus).

3.1. PGPR mechanisms of nutrient acquisition: from taxonomy to function

The foundational premise of this review — that microbial function, not taxonomic identity, is the more proximate determinant of nutrient outcomes — is supported across multiple independent lines of evidence. Reviews of PGPR mechanisms consistently describe four convergent modes of action relevant to nutrient acquisition: biological nitrogen fixation, phosphate/potassium solubilization, siderophore-mediated micronutrient mobilization, and phytohormone-mediated root system remodeling.[6, 18] A pot-based study on maize inoculated with Paraburkholderia, Burkholderia, and Serratia isolates found that these genera improved nitrogen, phosphorus, and potassium uptake and use efficiency relative to uninoculated controls, and specifically noted the paucity of prior evidence on these effects in non-leguminous cereals compared with the far larger literature on legume-rhizobium symbioses.[6] This asymmetry — abundant mechanistic evidence in legumes, comparatively thinner evidence in cereals and millets that dominate rainfed cropping — recurs throughout the reviewed literature and constitutes an important caveat to generalizing legume-derived findings to rainfed cereal systems.

A parallel and more recent literature has moved from describing PGP traits phenotypically (e.g., halo-zone assays for phosphate solubilization) toward validating these traits at the gene level. Functional annotation of a root-endophytic Rhizobium pusense strain identified the genetic basis of its phosphate solubilization, nitrogen fixation, ACC deaminase, siderophore, and indole-3-acetic acid (IAA) production capacities through in silico pathway reconstruction, illustrating how whole-genome sequencing can convert a phenotypically characterized isolate into a functionally annotated candidate for rhizosphere engineering.[19] This gene-level validation is important because phenotypic PGP assays (e.g., Pikovskaya's agar halo zones) are known to correlate imperfectly with field-level nutrient benefit, whereas the presence and expression of specific genes provides a more mechanistically grounded, and in principle more transferable, basis for inoculant selection.

3.2. Nitrogen-fixation functional genes: nifH as a marker of diazotrophic capacity

The nifH gene, encoding the iron protein (dinitrogenase reductase) subunit of the nitrogenase enzyme complex, is the most extensively validated molecular marker of diazotrophic potential in rhizosphere and bulk soil communities, owing to its high sequence conservation across phylogenetically diverse diazotrophs and its well-established phylogenetic correlation with 16S rRNA gene relationships.[20, 21] Molecular surveys quantifying nifH abundance in the sorghum rhizosphere under differing nitrogen fertilization regimes demonstrated that non-symbiotic (free-living) nitrogen fixation is a significant, if variable, source of fixed nitrogen across a range of soil types, reinforcing the agronomic relevance of free-living diazotrophs beyond the classical rhizobium-legume symbiosis.[21] In a related study on maize rhizospheres across three field sites, nifH and acdS allele numbers, quantified by qPCR, were found to differ significantly by field site but to be positively correlated with one another overall, suggesting that nitrogen-fixing and ethylene-modulating functional guilds tend to co-occur in the rhizosphere, potentially reflecting shared ecological niches or complementary functional roles in supporting plant fitness under variable field conditions.[10]

The relationship between nifH gene abundance and agronomic nitrogen outcomes has also been demonstrated experimentally. Co-inoculation of wheat with a diazotrophic Paenibacillus beijingensis strain and a phosphate-solubilizing Paenibacillus sp. strain increased plant nitrogen and phosphorus content, soil nitrogenase activity, and, notably, doubled the relative expression of nifH in both shoot and root tissue compared with diazotroph-only inoculation, providing direct evidence that phosphorus solubilization by a co-inoculated partner strain can stimulate the expression — not merely the presence — of nitrogen-fixation genes.[22] This finding is significant for rhizosphere engineering because it demonstrates that functional-gene expression, and hence realized nutrient benefit, is a property of the microbial consortium and its nutrient environment rather than of any single strain in isolation. Fertilization management itself also modulates nifH abundance: a review of rhizosphere microbiome drivers found that nitrogen fertilizer addition reduced the diversity and abundance of nifH-bearing diazotrophs by close to half in one cited study, attributed to reduced selective pressure for biological nitrogen fixation when inorganic ammonium and nitrate are already abundant, whereas phosphorus fertilization increased nifH abundance, plausibly by relieving a phosphorus constraint on the energetically expensive nitrogenase reaction.[23] This inverse relationship between mineral nitrogen supply and diazotroph abundance has direct implications for rainfed systems, where farmers frequently apply nitrogen fertilizer at sub-optimal, uneven rates: moderate under-fertilization may, counter-intuitively, preserve a more active diazotrophic community capable of partially compensating for the shortfall.

3.3. Phosphate-solubilization genes: phoD, phoC, and the pqq operon

Phosphorus is frequently the more binding nutritional constraint in rainfed soils, particularly in calcareous, alkaline, or highly weathered tropical soils where a large fraction of total soil phosphorus is present in chemically fixed, biologically unavailable forms. The alkaline phosphatase gene phoD and the acid phosphatase-associated gene phoC are the principal molecular markers for organic phosphorus mineralization capacity, while the pqqA/pqqC genes, encoding pyrroloquinoline quinone cofactor biosynthesis, underlie the direct oxidation pathway for inorganic phosphate solubilization by many Gram-negative PGPR.[24, 25] A meta-analytic synthesis of organic fertilization effects on soil microbial functional genes found that organic amendment consistently enhanced microbial biomass phosphorus, acid phosphatase activity, and the abundance and diversity of phoD and phoC genes, with this effect being particularly pronounced in phosphorus-limited soils — precisely the edaphic condition that characterizes many rainfed cropping regions.[24] Functional screening of rhizosphere bacteria from a woody host identified eighteen isolates positive for tricalcium phosphate solubilization on Pikovskaya's agar, all confirmed for zinc solubilization, IAA, siderophore, and hydrogen cyanide production, with phosphate solubilization activity validated at the molecular level through amplification of the pqqA and pqqC genes in the most efficient isolate, illustrating a now-standard workflow of phenotypic screening followed by gene-level confirmation.[25]

Transcriptomic studies have extended this static gene-presence view to a dynamic, expression-based understanding. Differential gene expression analysis of the diazotrophic, phosphate-solubilizing bacterium Paenibacillus sonchi grown

with soluble versus insoluble phosphate sources identified 68 upregulated and 100 downregulated genes under phosphate-limiting conditions, including genes involved in carbon metabolism that support the energetically demanding organic-acid secretion pathway for mineral phosphate solubilization.[26] This transcriptomic evidence indicates that phosphate-solubilization gene expression is itself an inducible, environmentally responsive trait rather than a constitutive one, with direct implications for how rhizosphere engineering interventions should be timed and formulated: an inoculant selected for phoD/pqq gene presence may still underperform in the field if local phosphate availability does not trigger the expected up-regulation. In field-relevant tea rhizosphere systems, metatranscriptomic analysis has similarly shown upregulation of phoD (alkaline phosphatase) alongside acdS (ACC deaminase) genes specifically in PGPR-treated soils, directly linking measured microbial gene activity to phosphorus acquisition and to ethylene-mediated stress mitigation in the same intervention.[11]

3.4. ACC deaminase (acdS) and auxin-biosynthesis genes: linking stress tolerance to nutrient capture

A recurrent theme across the reviewed literature is that nutrient acquisition and abiotic-stress tolerance are mechanistically intertwined rather than separable functions in PGPR-mediated crop improvement. The acdS gene encodes ACC deaminase, an enzyme that cleaves the immediate ethylene precursor ACC, thereby reducing stress-induced ethylene accumulation that would otherwise inhibit root elongation.[27, 28] Because ethylene-mediated growth inhibition is strongly induced by drought and osmotic stress — conditions endemic to rainfed agriculture — acdS-positive PGPR strains occupy a functionally privileged position: by relieving ethylene-mediated root inhibition, they enable greater root proliferation and, consequently, greater access to soil nutrient pools, even where the same strain has no direct nitrogen-fixing or phosphate-solubilizing capacity. Reviews synthesizing PGPR-mediated drought tolerance mechanisms across multiple crops identify ACC deaminase activity, together with phytohormone and osmolyte production, exopolysaccharide secretion, and volatile organic compound emission, as central components of the physiological toolkit through which rhizobacteria buffer crops against water deficit while indirectly supporting nutrient uptake through sustained root growth.[16, 29]

Field-scale evidence on wheat inoculated with a PGPR consortium under decreasing nitrogen fertilization levels found that PGPR treatment enhanced root growth and nitrogen accumulation most clearly at medium-to-high nitrogen fertilization, with comparatively modest gains in grain yield under already fertile soil conditions, indicating that the marginal benefit of PGPR inoculation for nitrogen capture may be context-dependent and most apparent where some baseline nitrogen supply exists to be captured more efficiently.[30] This finding tempers the sometimes-optimistic framing of PGPR as a wholesale fertilizer substitute; the evidence more precisely supports PGPR as an efficiency multiplier operating on existing nutrient pools (soil-native or fertilizer-derived) rather than as a nutrient source in its own right for phosphorus and potassium, though genuine new nitrogen input is possible where diazotrophic strains are involved.

3.5. Rhizosphere engineering strategies: synthetic consortia and host-mediated microbiome engineering

The conceptual and methodological core of "rhizosphere engineering" as distinct from conventional single-strain bioinoculant development lies in the deliberate assembly or selection of multi-member microbial communities calibrated to specific agronomic targets. Two complementary strategies dominate the recent literature: synthetic microbial communities (SMCs), which are rationally designed from characterized strains with complementary functional-gene profiles, and host-mediated microbiome engineering (HMME), which iteratively selects whole rhizosphere communities based on plant phenotypic performance under a target stress, without requiring prior knowledge of the specific strains or genes responsible.[12, 31] In HMME as applied to wheat, an initial microbiome is inoculated onto a plant population, seedlings are exposed to a defined stress (e.g., withholding water until visible wilting), the rhizospheres of the best-performing plants are pooled, and this enriched community is used to inoculate the next plant generation, iteratively concentrating whatever functional traits underlie superior host performance.[31] This method has been previously validated for selecting microbiomes that alter flowering time, leaf biomass, and salt tolerance in model and crop species, and its extension to nutrient-efficiency phenotypes (rather than only drought survival) represents a promising but still underexploited application.

A recent conceptual synthesis proposes integrating SMC and HMME approaches, using SMCs to establish a rationally designed functional baseline and HMME to further refine community composition under real field selective pressure, coupled with multi-omics monitoring and field validation templates intended to standardize reporting across studies.[12] Editorial commentary on rhizosphere microbiome engineering for crop cultivation similarly frames the coming decades of research around the systematic, purposeful modification of the root-zone microbiome to meet defined sustainability targets, citing examples such as crop-residue amendment to recruit beneficial bacteria and reduce pathogen load in cotton rhizospheres, and the influence of crop rotation and soil organic matter on microbial community resilience to drought.[13, 14] A separate framework paper on precision decision-support for rhizosphere microbiome engineering explicitly lists nutrient-use efficiency alongside biocontrol as a primary agronomic performance target, and calls for integration of remote sensing, variable-rate input technology, and machine-learning-assisted strain and mixture optimization to accelerate translation from laboratory consortia to scalable field products.[15]

3.6. Field evidence from rainfed cereal and legume systems

Direct field-level evidence in rainfed or water-limited cropping systems, while less abundant than pot-scale or irrigated-system studies, is nonetheless accumulating. A two-year field evaluation of phosphorus-solubilizing bacteria combined with inorganic fertilizer in alkaline-calcareous soils under pearl millet cultivation found that inoculation improved phosphorus use efficiency and fodder yield relative to inorganic fertilizer alone, addressing a soil chemistry problem — phosphorus precipitation in alkaline-calcareous conditions — that is widespread across semi-arid rainfed tracts of South Asia.[32] In chickpea, a legume of particular importance to

rainfed smallholder systems, on-farm trials across a wide range of sites in Ethiopia found that rhizobium inoculation increased grain yields for 99% of participating farmers, with combined rhizobium inoculation and phosphorus fertilizer producing an average 38% yield increase over untreated controls, and with the yield response to inoculation shown to be largely independent of agro-ecological zone or soil type — an encouraging finding for the scalability of inoculant-based interventions across heterogeneous rainfed landscapes.[33] A separate chickpea study integrating co-inoculation of Mesorhizobium ciceri and an ACC-deaminase-positive PGPR strain with phosphorus-enriched compost, tested explicitly under both irrigated and rainfed regimes, found that co-inoculation increased nodulation, pod number, grain yield, and grain protein content relative to rhizobium-only inoculation under both water regimes, with an additive benefit when compost was combined with co-inoculation.[34] This study is one of the few in the reviewed corpus to directly contrast rainfed and irrigated performance of the same inoculant treatment within a single experimental design, and its finding that co-inoculation benefits persisted (if somewhat attenuated) under rainfed conditions supports the premise that ACC-deaminase-mediated stress buffering helps preserve nutrient-related benefits when moisture is limiting.

Additional field and semi-field evidence from teff, a rainfed cereal important to Ethiopian food security, found that inoculation with a consortium of native PGPR strains improved growth, yield, and grain nutrient uptake more than single-strain inoculation, a pattern the authors attributed to complementary modes of action among consortium members.[35] Comparable consortium-superiority findings have been reported in carrot, where combined rhizosphere microbial consortia outperformed single-strain treatments and were associated with reported yield increases in the order of 12–20% across multiple studies synthesized in that review, and in bell pepper, where a two-strain Bacillus consortium improved transplant vigor, survival, and disease resistance under field conditions.[36, 37] Biochar-amended nitrogen management trials in rainfed maize grown on Vertisols in India additionally demonstrate that nutrient-use efficiency in rainfed cereals is sensitive not only to microbial inoculation but to its interaction with complementary soil-management practices such as split fertilizer application and biochar incorporation, reinforcing that PGPR-based rhizosphere engineering is best conceived as one component of an integrated rainfed nutrient-management package rather than a stand-alone replacement for agronomic best practice.[38]

3.7. Crop management, soil, and environmental modulation of PGPR functional traits

A further recurring theme is that PGPR functional-gene expression and community composition are themselves shaped by crop management practices, meaning that rhizosphere engineering outcomes cannot be considered independent of the broader agronomic system into which an inoculant is introduced. A comparative study of Carica papaya rhizosphere bacteria under conventional versus organic management systems found that the specific genera most efficient at phosphate solubilization and nitrogen fixation differed by management system, with Burkholderia vietnamiensis exhibiting nitrogen fixation capacity under conventional management while B. vietnamiensis, B. cepacia, and a Leclercia strain showed this capacity under organic management, indicating that native functional-gene-bearing populations are not static but respond to the prevailing fertility and disturbance regime.[39] This has a direct practical implication for rhizosphere engineering: an inoculant strain selected and validated under one management regime (e.g., organic, low-input) may not retain the same functional advantage when introduced into a differently managed field, underscoring the importance of matching inoculant selection to the target management context rather than assuming universal transferability.

Reviews of rhizosphere microbiome drivers similarly emphasize that long-term fertilization history, tillage, and crop rotation collectively reshape the diversity and abundance of nutrient-cycling functional genes, sometimes in directions that work against the intended goal of a rhizosphere engineering intervention (e.g., nitrogen fertilization suppressing nifH abundance even while it is applied specifically to relieve nitrogen limitation).[23] Broader reviews of rhizosphere microbiome responses to environmental stress and agronomic practice call for a "predictive engineering" research agenda that would use controlled multi-stress studies (e.g., combined drought and pathogen pressure) and integrated multi-omics data to identify recurring "metabolite-microbe modules" linked to plant resilience, explicitly positioning this as a necessary next step beyond the current largely descriptive or single-stressor literature.[17]

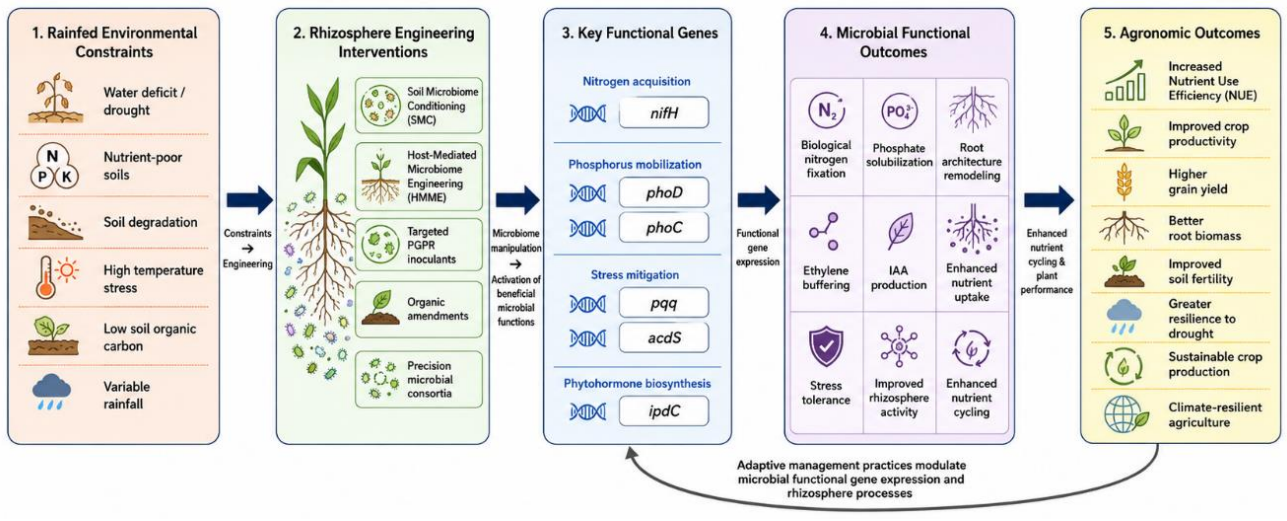

Fig 2: Conceptual framework linking rainfed environmental constraints, rhizosphere engineering interventions, microbial functional genes, and nutrient-use efficiency outcomes.

4. Comparative Assessment of Existing Studies

Table 3 summarizes the functional genes, crops, and principal outcomes reported across a representative subset of the included studies, while Table 4 provides a more detailed comparative analysis of study design, location, methodology, and limitations for field-oriented studies most directly relevant to rainfed nutrient-use efficiency.

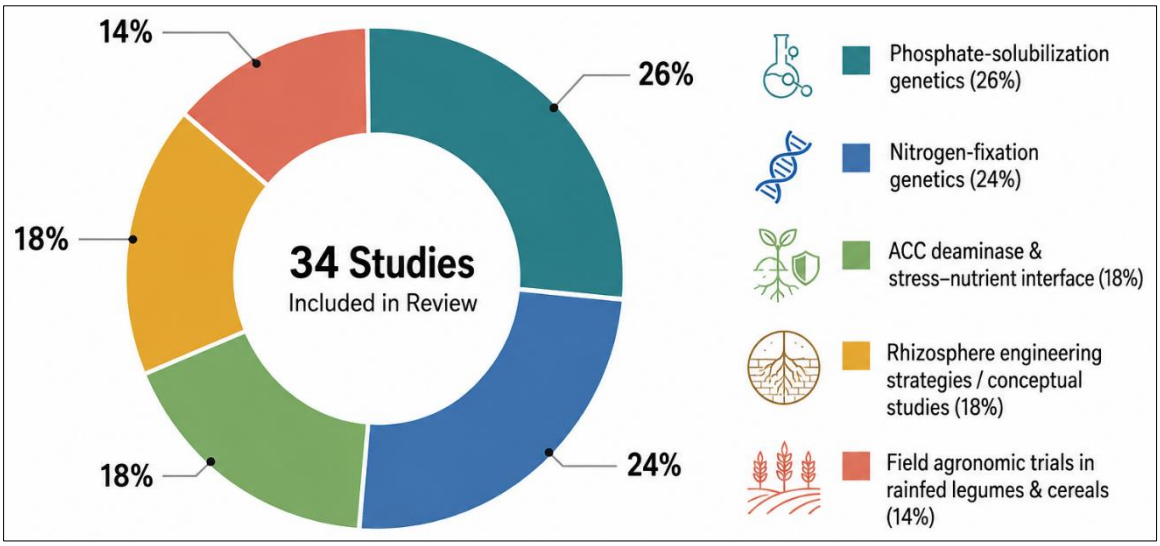

Fig 3: Thematic classification of the studies included in the review.

Read together, these comparative data reveal a consistent direction of effect — PGPR inoculation, whether single-strain or consortium, tends to improve nutrient uptake, nutrient-use efficiency, or yield relative to uninoculated controls — but also considerable heterogeneity in magnitude, driven by soil phosphorus status, baseline nitrogen fertilization level, water regime, and whether inoculation is delivered alone or integrated with organic or inorganic soil amendments. Very few of the field-level agronomic trials in Table 4 report functional-gene quantification alongside their yield or nutrient outcomes, which is itself an important limitation discussed further in Section 5.

5. Current Knowledge Gaps and Future Research Directions

5.1. Unresolved issues and knowledge gaps

Several unresolved issues recur across the reviewed literature. First, there is a persistent disconnect between molecular ecology studies that quantify functional-gene abundance (e.g., nifH or phoD copy number via qPCR or metagenomics) and agronomic field trials that report yield or nutrient-uptake outcomes without any accompanying gene-level data; very few studies bridge both domains within a single experimental design, which limits the strength of causal inference that functional-gene abundance actually explains observed agronomic benefit rather than merely correlating with it. Second, the great majority of field evidence, including several studies discussed in Section 3.6, derives from single-season or at most two-season trials, which is insufficient to capture the substantial year-to-year rainfall variability that defines rainfed agriculture and that plausibly interacts strongly with inoculant persistence and efficacy. Third, inoculant strain identity in many agronomic trials is reported only at the genus level, without confirmation of strain-specific functional-gene presence, expression, or in-field survival, making it difficult to determine whether reported benefits are attributable to the inoculated strain, to native rhizosphere populations stimulated by the inoculation process, or to unmeasured confounding management differences between treatments.

A further, closely related gap concerns rhizosphere competence and colonization persistence. Genetically engineered or highly optimized PGPR strains developed under controlled conditions frequently underperform in field environments because of inadequate root establishment, a short survival window in unfamiliar soils, host specificity constraints, and, in some cases, weak or unstable genetic control over the traits responsible for growth promotion.[7] This translational gap between laboratory-optimized function and field-realized performance is arguably the single most consistently cited limitation across the genetic-engineering and synthetic-consortium literature, and it remains only partially addressed by current formulation and delivery technologies.

5.2. Methodological limitations in the existing evidence base

Methodologically, the reviewed literature exhibits several recurring weaknesses. Many studies rely on 16S rRNA amplicon sequencing to characterize rhizosphere community shifts without complementary shotgun metagenomic or metatranscriptomic data that would confirm whether taxonomic shifts correspond to actual changes in functional-gene abundance or expression.[9] Where functional genes are quantified, qPCR-based approaches targeting a single marker gene (most commonly nifH) dominate over more comprehensive multi-gene or pathway-level characterization, potentially understating the functional redundancy and complementarity that consortium-based rhizosphere engineering approaches are specifically designed to exploit. Reporting of soil moisture status, precipitation timing relative to inoculation, and soil phosphorus fixation capacity — all first-order determinants of rainfed system performance — is inconsistent across studies, complicating cross-study comparison and meta-analytic synthesis. Finally, few studies report cost-benefit or input-substitution analyses that would allow policymakers or extension services to weigh PGPR-based interventions against conventional fertilizer subsidy programs on a comparable economic basis.

5.3. Emerging technologies and future developments

Several emerging methodological trends are likely to shape the next generation of rhizosphere engineering research relevant to rainfed nutrient-use efficiency. Multi-omics integration — combining metagenomics, metatranscriptomics, and metabolomics — is increasingly proposed as a means of resolving the gap between genetic potential and realized function, since metatranscriptomic data specifically capture genes that are actively expressed in situ rather than merely present in the community's genetic repertoire.[11] Synthetic microbial community design, informed by network co-occurrence analyses (e.g., SparCC, microbial ecological network analysis) that identify keystone taxa and functional interaction patterns, offers a more rational alternative to empirical trial-and-error consortium assembly.[11] Host-mediated microbiome engineering, while so far applied predominantly to drought-survival phenotypes, represents a readily extensible platform for directly selecting on nutrient-uptake or NUE phenotypes rather than survival alone, which would more precisely target the outcome of interest to this review.

At the systems level, recent conceptual frameworks call for integrating precision-agriculture tools — remote sensing, soil sensors, and variable-rate input technology — with machine-learning-assisted strain and mixture optimization, explicitly citing nutrient-use efficiency as a primary target trait alongside biocontrol and abiotic stress tolerance.[15] Such frameworks emphasize the need for standardized minimum reporting requirements and multi-season, multi-location field-trial templates to improve cross-study comparability, a recommendation directly responsive to the methodological heterogeneity documented in Section 5.2. Emerging attention to rhizodeposition and its role in stabilizing soil organic carbon under drought further suggests that future rhizosphere engineering research may increasingly frame nutrient-use efficiency and carbon-sequestration co-benefits jointly, rather than as separate research objectives.[17]

6. Discussion

The literature synthesized in this review converges on a coherent, if still incompletely validated, picture: rhizosphere engineering through PGPR inoculation improves nutrient-use efficiency in rainfed crops primarily by (a) supplementing biologically fixed nitrogen through diazotrophic activity marked by nifH gene presence and expression, (b) unlocking chemically fixed soil phosphorus through phoD/phoC/pqq-mediated organic-acid and phosphatase-based solubilization, and (c) sustaining root growth and absorptive capacity under water-deficit conditions through acdS-mediated ethylene buffering and auxin-driven root architectural change. These three mechanistic axes are not independent; multiple studies reviewed here demonstrate that phosphorus availability modulates nitrogen-fixation gene expression,[22] that fertilization history modulates diazotroph abundance,[23] and that ACC deaminase activity and phosphatase activity can be co-induced within the same inoculation event.[11] This interdependence argues against a reductionist, single-gene or single-strain approach to rhizosphere engineering and toward the consortium- and community-level strategies increasingly emphasized in the more recent literature (SMC and HMME approaches).[12, 31]

At the same time, the evidence base displays a clear asymmetry in confidence: the mechanistic, gene-level evidence for how PGPR functions are encoded and expressed is comparatively mature and methodologically rigorous, drawing on qPCR, transcriptomics, and genome annotation,[10, 19, 22, 26] whereas the agronomic, field-level evidence for how these mechanisms translate into NUE gains specifically under rainfed conditions is comparatively thin, often lacking gene-level confirmation, and dominated by legume systems (particularly chickpea) rather than the cereals and millets that constitute the bulk of rainfed cropped area.[33, 34, 35] This asymmetry likely reflects disciplinary specialization — molecular ecologists and agronomists publish in different journals and rarely co-design integrated studies — rather than any inherent incompatibility between the two research traditions, and it represents the most actionable target for future interdisciplinary research design.

A related point of interpretive caution concerns the direction and consistency of reported effect sizes. While the general direction of effect across the reviewed studies is positive (PGPR inoculation improves nutrient outcomes relative to controls), the magnitude of benefit varies considerably — from modest, statistically marginal gains in already-fertile soils under adequate nitrogen supply[30] to dramatic yield gains (upwards of one-third) in phosphorus-deficient smallholder systems with historically low or absent inoculant use.[33] This pattern is consistent with a diminishing-returns interpretation: PGPR-mediated nutrient efficiency gains are largest where baseline nutrient limitation or microbial population deficits are most severe, and smallest where soil fertility and native beneficial microbial populations are already adequate. For rainfed agriculture specifically, this implies that the agroecological contexts where PGPR-based rhizosphere engineering is likely to deliver the greatest relative benefit — phosphorus-poor, low-input, smallholder-dominated semi-arid systems — are also the contexts where rigorous, well-replicated field trials are scarcest, creating a policy-relevant evidentiary gap precisely where the technology's potential value is highest.

The reviewed literature also surfaces a genuine, unresolved scientific controversy regarding whether functional-gene abundance (a genetic potential measure) or functional-gene expression (a realized activity measure) is the more reliable predictor of agronomic nutrient outcomes. Static abundance-based studies (qPCR quantification of gene copy number) dominate the literature by volume,[10, 20, 21] but the transcriptomic and metatranscriptomic studies reviewed here directly demonstrate that gene presence does not guarantee expression, and that expression is itself conditional on the local nutrient and stress environment.[11,26] This suggests that abundance-based inoculant screening, while operationally convenient, may systematically overstate or understate field performance relative to expression-based or activity-based screening, and that future rhizosphere engineering research should treat these as complementary rather than interchangeable lines of evidence.

From a practical standpoint, the consistent finding that multi-strain consortia outperform single-strain inoculants across diverse crops — wheat, teff, carrot, chickpea, bell pepper[30, 34, 35, 36, 37] — has an important implication for rainfed extension programs: bioinoculant development and registration pathways calibrated around single, well-characterized strains (the historical regulatory default in many countries) may be poorly matched to the consortium-based products that the evidence increasingly favors. Regulatory and quality-control frameworks for multi-strain biofertilizers, including standards for verifying functional-gene composition and viable cell counts of each consortium member at the point of sale,

represent an under-addressed policy dimension connecting this scientific literature to practical technology transfer.

7. Conclusion

This review has analyzed data from 34 sources published in peer-reviewed journals to establish how genes responsible for microbial activity — mainly aliphatic acid dehydrogenase, phoD/phoC/pqqA/pqqC, and acdS — control the processes of fixation of nitrogen, solubilization of phosphorus, and development of stress-resistant roots and how the data relates to nitrogen efficiency in rainfed cropping systems. Evidence indicates that rhizosphere engineering based on functional genes — preferably through multi-strain associates and new techniques of microbiome engineering — is the right way to increase nitrogen and phosphorus efficiency in rainfed cereals and legumes. Most benefits of improving the efficiency of phosphorus use can be received in the areas where production is based on low levels of fertilizers and that mainly include the situation of small farming enterprises because small producers are located in very mild areas when it comes to fertilizers. Meanwhile, the research proved that the prototype has some limitations because the tests were short, they were conducted at the genus level rather than at the strain and gene levels, and only one limited option – multi-omics validation under real conditions.

- **Suggestions for researchers**

In upcoming research efforts, it would be best to prioritize (i) multi-satured field research that gives reports of both agri-NUE measures and quantitative functional-gene data (amounts of such gene and, when possible, its expression) in a single experiment; (ii) strain-level genome identification of the field inoculants instead of its genus-level characterization; (iii) using metagenomic, metatranscriptomic, and metabolomic information to solve the problem of interconnectedness between amounts of function and expression shown in this review; (iv) explicit trials for rain-fed and irrigated crop conditions using strategies similar to the one presented in literature regarding co-inoculated chickpeas. In developing programs and agricultural policy initiatives intended for rainfed smallholder systems, practitioners and policymakers are urged to prioritize the promotion of PGPR-based biofertilizers in regions characterized by phosphorus deficiency and low levels of input use, where the various studies analyzed above indicate that such an approach has the greatest comparative advantage. Investments should be focused on creating region-specific, multi-strain combinations rather than single-strain products that have been historically used in different agro-ecological settings. The quality control regulatory frameworks associated with the production of biofertilizers need to undergo changes so that challenges involved in multi-strain consortia can be solved, such as verifying the quality of functional gene-based biofertilizers. Future trends observed include convergence of techniques such as host-mediated microbiome engineering, synthetic-consortium design, multi-omics validation, and precision-agriculture decision-support tools, indicating research focused on rhizosphere engineering practices for increased nutrient use efficiency in rainfed agriculture. The convergence of the different techniques requires an interdisciplinary approach involving molecular and field trial studies.

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