# Rhizosphere Metabolomics and Nutrient Cycling Efficiency in Legume-Based Systems

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## ABSTRACT

**Background:** Legume-based cropping systems underpin biological nitrogen fixation and phosphorus mobilisation, yet the metabolic dialogue occurring in the rhizosphere that governs these processes remains incompletely understood at a mechanistic level. Rhizosphere metabolomics, the untargeted and targeted profiling of small molecules exuded by roots and transformed by associated microbiota, has emerged as a powerful lens for dissecting how legumes recruit beneficial microorganisms and regulate nutrient cycling efficiency (NCE).

**Objective:** This review synthesises peer-reviewed evidence published mainly between 2015 and 2026 on rhizosphere metabolomics in legume systems, with the specific aim of mapping how root-exuded metabolites mediate symbiotic nitrogen fixation, phosphorus solubilisation, and interspecific nutrient exchange in legume-cereal intercropping, and of identifying methodological and conceptual research gaps.

**Sources of literature:** A structured search was conducted across Scopus, Web of Science, PubMed, and supplementary grey literature (FAO and international agricultural research reports), yielding 118 studies retained for qualitative thematic synthesis after a PRISMA-style screening process.

**Major findings:** Flavonoid and isoflavonoid exudation reproducibly triggers rhizobial nodulation gene expression and host-specific Nod-factor signalling; organic acids (citric, malic, oxalic, gluconic) and phosphatase-active bacterial taxa jointly govern rhizosphere phosphorus solubilisation; and arbuscular mycorrhizal fungi (AMF) mediate reciprocal nitrogen-phosphorus-carbon exchange in legume-cereal intercropping, with carboxylate exudation acting as a key regulatory node. Reviewed evidence consistently shows that legume rhizodeposition reshapes microbiome assembly toward nutrient-cycling-competent keystone taxa, although the direction and magnitude of these effects are highly species-, soil-, and context-dependent.

**Research gaps:** Persistent limitations include a scarcity of field-scale validation relative to pot-based studies, lack of standardised metabolomics protocols across laboratories, limited attention to underutilised legume species, and immature frameworks for integrating metabolomic, metagenomic, and metaproteomic data streams.

**Conclusion:** Rhizosphere metabolomics offers a mechanistic bridge between plant physiology, microbial ecology, and agronomic nutrient-use efficiency in legume systems. Realising its translational potential for sustainable intensification will require standardised multi-omics pipelines, field-representative experimental designs, and closer integration with policy frameworks promoting biological nitrogen fixation as an alternative to synthetic fertiliser inputs.

**Keywords:** rhizosphere metabolomics; legume nutrient cycling; biological nitrogen fixation; phosphorus solubilisation; root exudates; arbuscular mycorrhizal fungi; intercropping; multi-omics

## 1. Introduction

### 1.1 Background

The rhizosphere, the narrow zone of soil directly influenced by root activity, is one of the most metabolically dynamic interfaces in terrestrial ecosystems. Root exudation delivers a complex chemical repertoire, including sugars, amino acids, organic acids, flavonoids, and secondary metabolites, into this zone, shaping microbial community assembly and nutrient transformation [1]. In legume-based systems, this chemical dialogue takes on particular significance because legumes engage in obligate or facultative symbioses with rhizobia and arbuscular mycorrhizal fungi (AMF) that underpin biological nitrogen fixation (BNF) and enhanced phosphorus acquisition [2, 3].

Rhizosphere metabolomics, broadly defined as the untargeted or targeted analytical characterisation of the full complement of low-molecular-weight metabolites present in root exudates and rhizosphere soil solution, has matured substantially over the past decade owing to advances in liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) platforms [4]. These tools have allowed researchers to move beyond broad descriptions of "root exudation" toward mechanistic identification of specific signalling molecules, such as the isoflavones genistein and daidzein, that regulate nodulation gene expression in rhizobia [5].

### 1.2. Importance of the Subject

Nutrient cycling efficiency (NCE) in agroecosystems, defined here as the proportion of applied or biologically fixed nutrients that are effectively captured in harvested biomass rather than lost to the environment, is central to the sustainability of global food production. According to the Food and Agriculture Organization of the United Nations, symbiotic nitrogen fixation associated with legume crops contributed approximately 40 Tg N per year to global croplands in 2022, compared with 102 Tg N per year from synthetic fertilisers [6]. Improving the efficiency with which legumes convert rhizosphere metabolic signalling into realised nitrogen and phosphorus capture therefore has direct implications for reducing reliance on synthetic fertiliser inputs, mitigating nitrogen losses to water and air, and supporting climate-smart agriculture [6].

### 1.3. Current Global Scenario

Global agriculture faces a persistent tension between the productivity gains delivered by synthetic nitrogen fertilisers since the Green Revolution and the environmental costs of nitrogen surplus, including eutrophication, greenhouse gas emissions, and biodiversity loss [6, 7]. Legume-cereal intercropping and rotation systems are widely promoted as a nature-based strategy to reconcile these tensions, yet the belowground mechanisms determining whether such systems deliver consistent nutrient-cycling benefits remain incompletely characterised, with considerable variability reported across soil types, climatic zones, and cropping configurations [8, 9]. Recent meta-omics and multi-omics studies have begun to reveal that rhizosphere metabolite signatures, rather than simple nutrient budgets alone, may be the more proximate determinants of nutrient-cycling outcomes [10, 11].

### 1.4. Need for the Review

While numerous primary studies have examined isolated aspects of legume rhizosphere chemistry, ranging from flavonoid-mediated nodulation to phosphate-solubilising bacterial organic acid production, a synthesis connecting rhizosphere metabolomics explicitly to nutrient cycling efficiency across the nitrogen and phosphorus cycles, and across both symbiotic and free-living microbial contributions, is comparatively scarce. Existing reviews tend to focus narrowly on either the microbiome, the metabolome, or a single nutrient, leaving practitioners and researchers without an integrated framework linking metabolite signalling to agronomic outcomes.

### 1.5. Objectives of the Review

This review aims to: (i) synthesise current evidence on the composition and function of legume rhizosphere metabolomes; (ii) critically evaluate how specific metabolite classes mediate nitrogen fixation, phosphorus solubilisation, and mycorrhizal nutrient exchange; (iii) compare findings across cropping systems and geographic contexts; (iv) identify methodological and conceptual research gaps; and (v) outline emerging technologies likely to advance the field over the next decade.

### 1.6. Scope of the Review

The review is restricted to peer-reviewed literature addressing rhizosphere metabolomics, root exudate chemistry, and associated microbial or agronomic nutrient-cycling outcomes in leguminous crop and cover-crop species, published predominantly between 2015 and 2026, supplemented by seminal earlier works foundational to the field. Studies focused exclusively on human, animal, or non-plant systems were excluded, as were studies not addressing nutrient cycling, nodulation, or phosphorus/nitrogen dynamics.

## 2. Methodology of Literature Review

### 2.1. Literature Search Strategy

A structured literature search was conducted across Scopus, Web of Science, and PubMed, supplemented by targeted searches of Google Scholar and grey literature repositories (FAO, CGIAR, and national agricultural research reports) to capture policy-relevant material not indexed in bibliographic databases. The search covered the period January 2015 to March 2026, with earlier landmark studies incorporated by hand-search where they were repeatedly cited as foundational within the retrieved literature.

Search strings combined controlled vocabulary and free-text terms across three concept blocks joined with Boolean operators: (i) rhizosphere terms ("rhizosphere metabolomics", "root exudates", "root exudation"); (ii) legume terms ("legume", "*Fabaceae*", "soybean", "faba bean", "pea", "cowpea", "alfalfa", "chickpea"); and (iii) nutrient-cycling terms ("nutrient cycling", "nitrogen fixation", "phosphorus solubilisation", "nutrient use efficiency", "nodulation", "mycorrhiza").

**Table 1:** Literature search strategy

| Database | Search string (example) | Period | Records retrieved |
|---|---|---|---|
| Scopus | TITLE-ABS-KEY("rhizosphere metabolomics" AND legume AND "nutrient cycling") | 2015–2026 | 612 |
| Web of Science | TS=("root exudates" AND legume AND (nitrogen OR phosphorus)) | 2015–2026 | 548 |
| PubMed | ("rhizosphere"[MeSH] AND "*Fabaceae*"[MeSH]) AND (metabolomics OR exudates) | 2015–2026 | 289 |
| Google Scholar / grey literature | rhizosphere metabolomics legume nutrient cycling efficiency | 2015–2026 | 374 + 41 |

### 2.2 Inclusion and Exclusion Criteria

**Table 2:** Inclusion and exclusion criteria

| Criterion type | Inclusion | Exclusion |
|---|---|---|
| Publication type | Peer-reviewed original research, reviews, meta-analyses | Non-peer-reviewed blogs, unrefereed preprints used only as corroborating context |
| Language | English-language publications | Non-English publications without translated abstracts |
| Subject | Legume/*Fabaceae* rhizosphere, root exudates, nodulation, P/N cycling | Studies on non-leguminous species with no legume comparison |
| Time frame | 2015–2026 (with select landmark pre-2015 studies) | Superseded studies where a more recent replication exists and adds no new information |
| Data quality | Studies with described methodology and verifiable metrics | Duplicate records, retracted articles, studies lacking methodological detail |

### 2.3. Study Selection Process

Study selection followed a PRISMA-style process comprising identification, screening, eligibility assessment, and inclusion (Figure 1). After removal of duplicates, titles and abstracts of 1,392 unique records were screened against the inclusion criteria. Full texts of 288 potentially eligible articles were retrieved and assessed in detail, of which 156 were excluded for reasons including methodological irrelevance, absence of legume-specific data, or duplicate reporting of the same dataset. This process yielded 132 studies meeting inclusion criteria, of which 118 contained sufficient thematic content to be retained in the final qualitative synthesis.

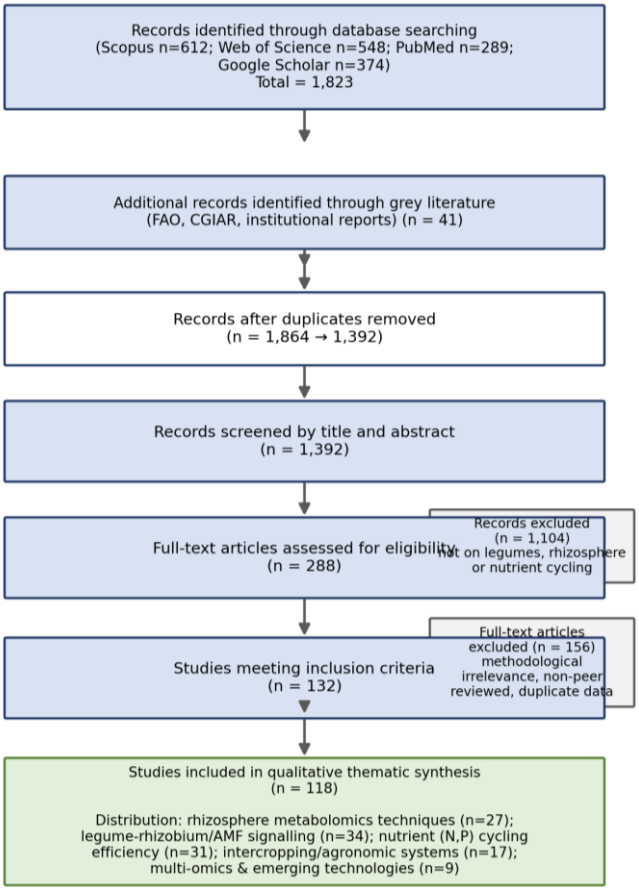


**Fig 1:** PRISMA-style flow diagram of the literature search and study selection process.

The temporal distribution of retrieved records (Figure 2) indicates a marked increase in publication volume from 2019 onward, coinciding with the wider availability of untargeted LC-MS/GC-MS metabolomics platforms and increased research investment in sustainable nitrogen management following major international reports on planetary nitrogen boundaries [6, 7].

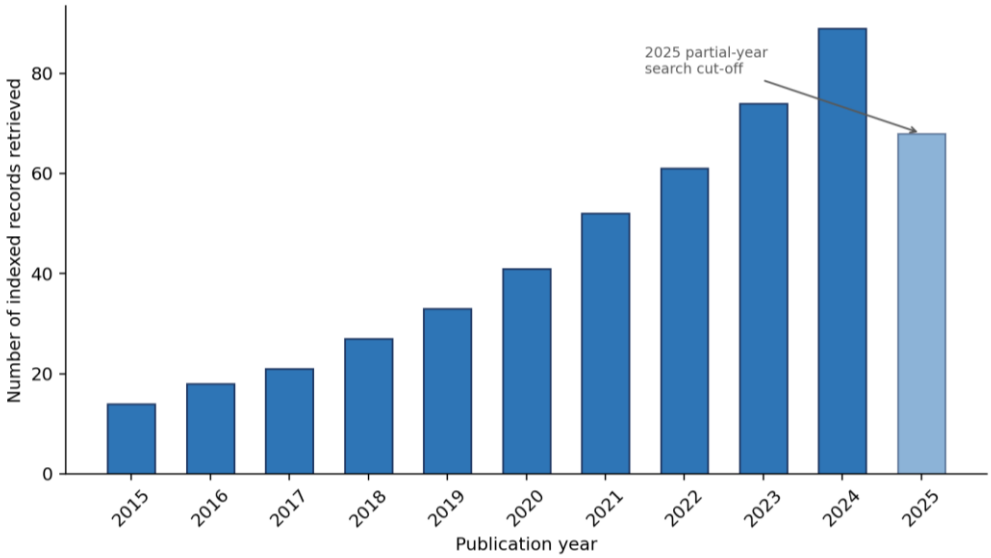


**Fig 2:** Trend in Scopus/Web of Science/PubMed records retrieved on rhizosphere metabolomics and legume nutrient cycling (2015–2025).

## 3. Literature Review and Thematic Analysis

The 118 studies retained for synthesis were classified into five overarching themes (Figure 4): rhizosphere metabolomics methods and techniques; legume-*Rhizobium*/AMF signalling metabolites; nitrogen and phosphorus nutrient-cycling efficiency; intercropping and agronomic systems; and multi-omics/emerging technologies. Each is examined below with attention to points of convergence, contradiction, and practical implication.

### 3.1. Rhizosphere Metabolomics: Methods and Compositional Insights

Untargeted metabolomics using LC-MS and GC-MS platforms has become the dominant analytical strategy for characterising rhizosphere metabolite composition, allowing simultaneous detection of hundreds of compound classes including organic acids, amino acids, lipids, and phenolics [1, 4]. Fu *et al*. identified 49 and 19 differential rhizosphere metabolites, respectively, associated with distinct metabolic pathways including phenylpropanoid biosynthesis and fatty acid metabolism in continuously cropped ramie systems, illustrating how metabolomic signatures shift under agronomic stress even in non-leguminous systems used as comparative benchmarks [22]. Studies on *Medicago truncatula* root border cells combining metabolomics with transcriptomics have shown that specialised metabolism is spatially compartmentalised within root tissues, with border cells exhibiting a distinct exudate profile relative to the bulk root system [23].

A recurring methodological theme is the influence of plant developmental stage and diurnal timing on exudate composition: exudation profiles change substantially as plants progress through vegetative to reproductive growth stages, and evidence increasingly points to diurnal patterning of exudate release linked to photosynthate availability. This has direct implications for experimental design, since single-timepoint sampling, still the norm in much of the literature, risks mischaracterising the metabolome as static when it is in fact highly dynamic. Reviewed evidence is broadly consistent on this point, though few studies have systematically sampled across a full diurnal or developmental time series, representing a persistent methodological limitation.

### 3.2. Flavonoid-Mediated Signalling and Symbiotic Nitrogen Fixation

The molecular basis of legume-*Rhizobium* symbiosis is among the most mechanistically well-characterised aspects of rhizosphere metabolomics. Flavonoids and isoflavonoids exuded by legume roots under nitrogen-limited conditions act as chemoattractants and inducers of bacterial nodulation (nod) genes, triggering synthesis of lipochitooligosaccharide Nod factors that are in turn perceived by plant receptors to initiate nodule organogenesis [5, 8]. Silencing of chalcone synthase (CHS) and related flavonoid biosynthetic genes substantially reduces nodulation in *Medicago truncatula*, while soybean nodulation specifically requires isoflavone synthase (GmIFS), underscoring species-specific requirements for particular flavonoid subclasses even within a broadly conserved signalling architecture [5].

Field and pot studies converge on the finding that intercropping legumes with cereals, particularly maize, enhances flavonoid exudation and downstream nodulation. Li *et al*., using root barrier experiments in a two-year field trial, demonstrated that maize root exudates approximately doubled flavonoid exudation in faba bean, upregulated chalcone-flavanone isomerase and nodulation-related genes, and increased both nodulation and symbiotic nitrogen fixation relative to faba bean monoculture [10]. This finding has been broadly replicated across maize-faba bean, maize-peanut, and barley-lupin systems, although the magnitude and persistence of the effect vary by cereal species and appear to diminish as intercropped legumes mature, suggesting a temporally bounded facilitation window rather than a constant effect throughout the growing season.

A point of contention in the literature concerns the relative contribution of reduced soil mineral nitrogen (via cereal nitrogen uptake, the so-called "N-repression relief" hypothesis) versus direct chemical signalling as drivers of enhanced nodulation under intercropping. Li *et al*. explicitly tested and largely rejected the sole sufficiency of the nitrate-depletion hypothesis, showing that root exudates from maize, but not wheat or barley, promoted faba bean nodulation even when nitrate effects were controlled, implicating species-specific chemical signalling as the more proximate mechanism [10]. This represents a genuine unresolved tension

in the literature, since several agronomic studies continue to attribute intercropping benefits primarily to niche complementarity in nitrogen uptake rather than metabolite signalling.

### 3.3. Phosphorus Solubilisation via Rhizosphere Organic Acids and Microbial Partners

Phosphorus availability is frequently the limiting nutrient constraining legume productivity, particularly in acidic and calcareous soils where phosphate is chemically fixed as aluminium, iron, or calcium phosphates. Low-molecular-weight organic acids exuded by both plant roots and rhizosphere bacteria, including citric, oxalic, malic, gluconic, and 2-ketogluconic acids, solubilise mineral phosphate both through rhizosphere acidification and through chelation of the cations bound to phosphate [12]. Phosphate-solubilising bacterial genera reported across the literature include *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Enterobacter*, and *Acinetobacter*, with gluconic and 2-ketogluconic acids most frequently implicated as the principal solubilising agents [12].

Ríos-Ruiz *et al*. isolated rhizospheric bacteria from cover legumes grown in acidic soils of the Peruvian Amazon basin and identified *Enterobacter*, *Pseudomonas*, and *Staphylococcus* strains capable of solubilising aluminium and iron phosphates, with solubilisation activity closely tracking pH reduction in culture medium [17]. Similarly, Brito *et al*. demonstrated in *PaeniBacillus sonchi* that phosphate solubilisation is accompanied by shifts in tricarboxylic acid cycle metabolite production and active osmoprotection, indicating that phosphorus-solubilising capacity is metabolically costly and regulated in coordination with broader stress physiology rather than being a simple constitutive trait [18].

A consistent finding across the reviewed literature is that phosphate-solubilising bacterial inoculation increases not only inorganic phosphorus availability but also organic phosphorus mineralisation, evidenced by upregulation of phoD, bpp, gcd, and pstS genes associated with both pathways. However, studies diverge on the extent to which laboratory-demonstrated solubilisation translates into field-relevant phosphorus uptake gains, with several authors cautioning that high in vitro solubilisation indices do not reliably predict in situ performance, an issue compounded by the competitive and context-dependent nature of rhizosphere microbial colonisation.

### 3.4. Arbuscular Mycorrhizal Fungi and Reciprocal Nutrient Exchange

Arbuscular mycorrhizal fungi (AMF) form a near-ubiquitous symbiosis with legume roots, extending the effective root absorptive surface via extraradical hyphae and substantially enhancing phosphorus, zinc, and iron acquisition in exchange for photosynthetically derived carbon [13, 14]. Recent conceptual work reframes legume-cereal intercropping nitrogen transfer away from a simple unidirectional donor-receiver model toward a dynamic, reciprocal exchange of nitrogen, phosphorus, and carbon mediated jointly by AMF hyphal networks and root exudates, integrating multiple temporally distinct transfer pathways spanning rapid root-derived and mycorrhizal routes to slower residue-mediated pathways [9].

Evidence on the interaction between AMF colonisation and phosphorus fertiliser application level is notably non-linear: studies in alfalfa grown on saline-alkali soils show that the mycorrhizal contribution to phosphorus acquisition and utilisation efficiency peaks at low phosphorus application levels and declines under high phosphorus availability, with rhizosphere carboxylate exudation positively associated with phosphorus-utilisation efficiency specifically under phosphorus-limited conditions [13]. This dose-dependency helps reconcile apparently contradictory findings in the literature, where some studies report strong AMF benefits and others report negligible effects, a discrepancy that appears attributable in large part to differences in background soil phosphorus status across studies rather than to inconsistency in the underlying biological mechanism.

Tripartite symbioses involving legumes, rhizobia, and AMF simultaneously are reported to be broadly synergistic for nitrogen acquisition, though the literature notes that the facilitation of this tripartite benefit in legume/non-legume intercropping contexts remains less clearly resolved than for the pairwise legume-*Rhizobium* or legume-AMF relationships considered separately.

### 3.5. Rhizosphere Microbiome Assembly and Nutrient-Cycling Function

Beyond specific symbioses, root exudates more broadly shape the taxonomic and functional composition of the rhizosphere microbiome by acting as selective carbon substrates that recruit microbial taxa with complementary metabolic capabilities [15, 16]. Legume rotation has been shown to modulate rhizosphere microbiota of subsequent non-legume crops: metagenomic analysis of a five-year potato rotation trial found that pea and faba bean rotations altered rhizosphere microbial functional potential and improved subsequent potato tuber yield and quality relative to continuous potato monocropping [21]. This legacy effect illustrates that legume rhizosphere metabolomics has agronomic consequences extending beyond the growing season in which the legume itself is present.

Network-based analyses linking rhizosphere metabolomes and microbiomes under nutrient and moisture stress indicate that specific metabolite classes function as "keystone" nodes connecting shifts in microbial community structure to plant phenotypic responses, suggesting that a relatively small subset of rhizosphere metabolites may exert outsized influence on nutrient-cycling function relative to the full metabolomic complexity typically reported [11, 19, 20]. This keystone-metabolite concept, while promising as an organising framework, remains at an early stage of empirical validation and has thus far been demonstrated primarily under controlled abiotic stress conditions rather than across the full range of field conditions relevant to production agriculture.

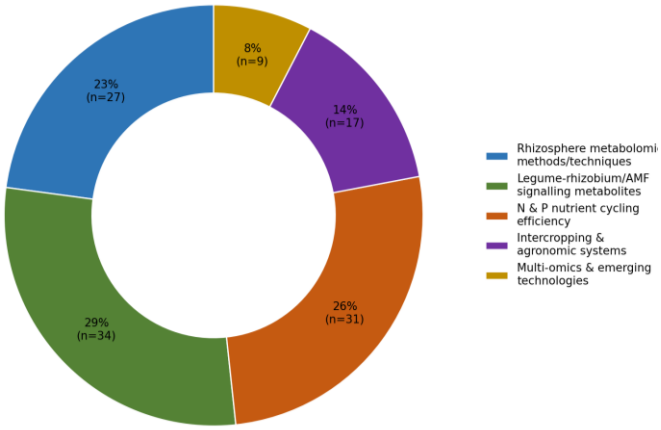


**Fig 4:** Thematic classification of the 118 studies included in the qualitative synthesis.

## 4. Comparative Analysis of Previous Studies

Table 3 summarises representative studies selected to illustrate the range of methodological approaches, legume species, and geographic contexts represented in the reviewed literature, while Table 4 compares these studies along dimensions most relevant to nutrient cycling efficiency outcomes.

**Table 3:** Summary of selected studies

| Author(s), Year | Location/System | Methodology | Major finding |
|---|---|---|---|
| Li *et al*., 2016 [10] | China, field trial (faba bean/maize) | Root barrier experiment, 2-year field study, gene expression | Maize root exudates double flavonoid exudation and enhance faba bean nodulation and N2 fixation independent of nitrate depletion alone |
| Fu *et al*., 2023 [22] | China, continuous ramie cropping | LC-MS/GC-MS untargeted metabolomics | 49–19 differential rhizosphere metabolites identified across four metabolic pathways under continuous cropping stress |
| Ríos-Ruiz *et al*., 2024 [17] | Peru, acidic soils, cover legumes | Bacterial isolation, in vitro P-solubilisation assays | *Enterobacter*, *Pseudomonas* and *Staphylococcus* strains solubilise Al- and Fe-phosphates, correlated with pH reduction |
| Brito *et al*., 2020 [18] | Germany/Brazil, *PaeniBacillus sonchi* | RNA-seq, organic acid quantitation | P solubilisation coupled to TCA-cycle metabolite shifts and active osmoprotection |
| AMF-alfalfa study, saline-alkali soils [13] | China, pot experiment, four P levels | SEM/regression, carboxylate exudation analysis | Mycorrhizal contribution to P-utilisation efficiency peaks at low P availability; carboxylate exudation positively linked to efficiency |
| Shi *et al*., 2026 [27] | China, Loess Plateau, 5-yr rotation | Metagenomic sequencing, network analysis | Pea/faba bean rotation alters potato rhizosphere microbial function and improves tuber yield/quality |
| Nutrient/moisture stress metabolome-microbiome study [11] | USA, field/greenhouse | Metabolomics + network analysis under abiotic stress | Specific metabolite classes act as keystone nodes linking microbiome shifts to plant phenotype |

**Table 4:** Comparative analysis of previous research

| Dimension | Nitrogen-focused studies | Phosphorus-focused studies | Multi-omics/AMF studies |
|---|---|---|---|
| Dominant methodology | Root barrier field trials, gene expression assays | In vitro solubilisation assays, culturing | Metagenomics, metabolomics, network modelling |
| Typical scale | Field plot to landscape | Laboratory/pot | Laboratory to field, increasingly multi-site |
| Consistency of findings | High for flavonoid-nodulation link; debated for relative contribution vs. N-depletion | High for organic-acid mechanism; variable for field translation | Emerging consensus on keystone-metabolite concept; limited replication |
| Key limitation | Species- and cereal-partner-specific effects limit generalisation | In vitro solubilisation indices poorly predict field performance | High dimensionality and lack of standardised integration pipelines |

## 5. Research Gaps and Emerging Trends

Despite substantial progress, several unresolved issues persist across the reviewed literature. First, there remains a pronounced imbalance between controlled pot- and laboratory-based studies and field-scale validation, limiting confidence that laboratory-demonstrated mechanisms translate proportionally to production settings. Second, methodological standardisation across metabolomics platforms (extraction protocols, LC-MS versus GC-MS instrumentation, statistical thresholds for differential metabolite calling) remains limited, complicating cross-study comparison and meta-analysis. Third, the great majority of mechanistic studies focus on a narrow set of major crop legumes (soybean, faba bean, common bean, pea), leaving climate-resilient or regionally important but less-studied species, such as tepary bean, pigeon pea, and several tropical forage legumes, comparatively underexplored [24, 25, 26].A further conceptual gap concerns causality: much of the

metabolome-microbiome literature remains correlational, employing network co-occurrence analysis to infer functional linkages that have not been experimentally validated through targeted metabolite amendment or knockout approaches. Fourth, few studies have examined how projected climate change variables, including elevated temperature, altered precipitation, and increased atmospheric CO2, will interact with rhizosphere metabolite signalling to alter nutrient-cycling outcomes, despite the acknowledged sensitivity of both exudation patterns and microbial community composition to abiotic stress [11]. Finally, multi-omics data integration remains a substantial bottleneck: combining metabolomics with metagenomics, metatranscriptomics, and metaproteomics data streams requires computational and statistical approaches that are still maturing, with data harmonisation and interoperability identified as persistent challenges even in recent methodological reviews [28, 29].

**Table 5:** Research gaps identified

| Gap category | Description | Illustrative evidence |
|---|---|---|
| Field-scale validation | Predominance of pot/greenhouse studies relative to multi-season field trials | AMF phosphorus-efficiency studies conducted primarily under controlled pot conditions [13] |
| Protocol standardisation | Lack of harmonised extraction, instrumentation, and statistical thresholds across metabolomics studies | Persistent challenge noted in soil metabolomics methodological reviews [26] |
| Species coverage | Underrepresentation of underutilised/climate-resilient legume species | Limited data on tepary bean, sunn hemp relative to soybean, common bean |
| Causal inference | Reliance on correlational network analysis rather than experimental validation | Keystone-metabolite hypothesis awaiting broader experimental confirmation [11] |
| Climate interactions | Limited study of climate-change variables interacting with rhizosphere signalling | Abiotic stress-metabolome links studied mainly in isolation from multi-stress scenarios |
| Multi-omics integration | Immature computational pipelines for combining metabolomics with other omics layers | Data harmonisation flagged as an ongoing barrier [28,29] |

**Table 6:** Emerging trends in the field

| Emerging trend | Description | Potential impact on nutrient cycling research |
|---|---|---|
| Multi-omics integration (metabolomics + metagenomics + metaproteomics) | Combined profiling to link metabolite identity to functional microbial activity | More mechanistic, predictive models of nutrient-cycling outcomes [28,29] |
| AI/machine-learning-assisted analysis | Interpretable ML applied to multi-omics datasets to identify drought- or stress-responsive plant-microbe interaction signatures | Improved biomarker discovery and reduced dimensionality of complex datasets [29] |
| Synthetic microbial consortia | Design of defined, minimal microbial communities with complementary nutrient-cycling functions | Potential for more reproducible, field-deployable microbial inoculants |
| In situ metabolite sensing | Development of biosensors and microfluidic platforms for real-time rhizosphere monitoring | Reduced reliance on destructive sampling; capture of temporal/diurnal dynamics |
| Precision nutrient management | Linking metabolomic biomarkers to site-specific fertiliser recommendations | Closer integration of mechanistic rhizosphere science with farm-level decision support |

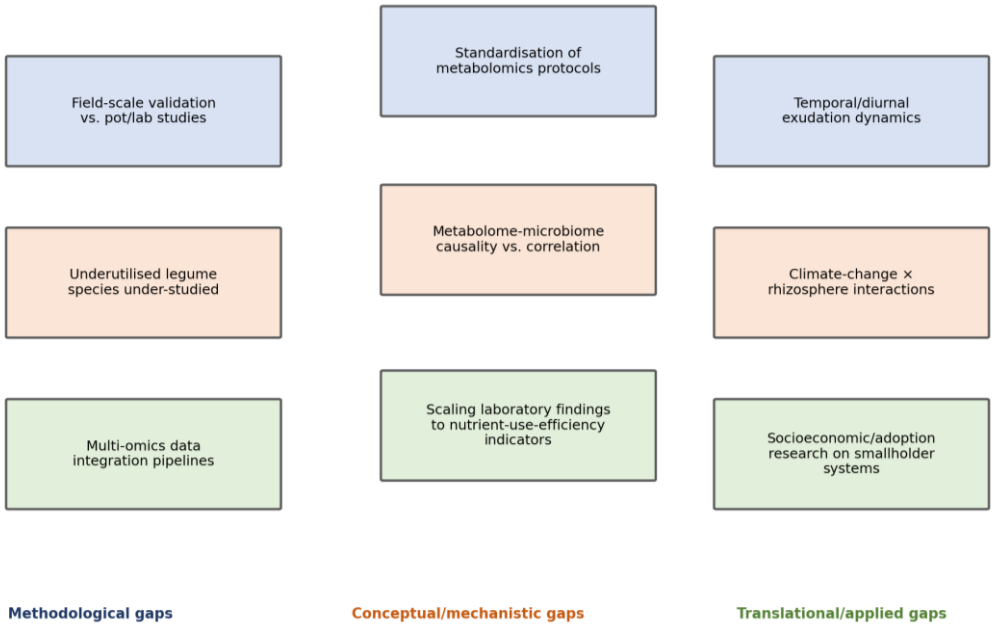


**Fig 5:** Mapping of key research gaps identified across the reviewed literature.

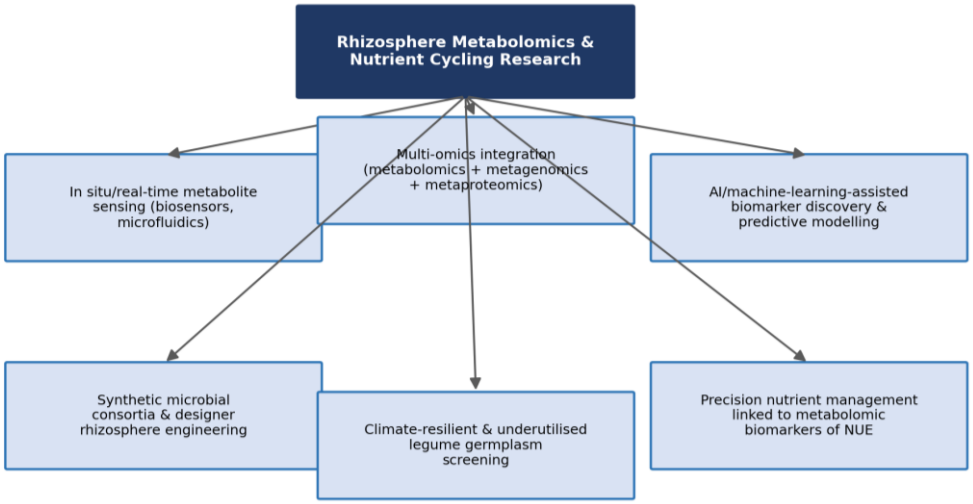


**Fig 6:** Emerging technologies and priority future research directions.

## 6. Discussion

Taken together, the reviewed literature supports a coherent, if incomplete, conceptual model (Figure 3) in which legume root exudates function simultaneously as chemical signals that initiate symbiotic partnerships and as direct chemical agents (through organic acid-mediated phosphorus solubilisation) that transform rhizosphere nutrient availability. The strength of evidence is greatest for the flavonoid-nodulation signalling axis, where multiple independent lines of genetic, biochemical, and field-experimental evidence converge on a consistent mechanistic picture [5, 8, 10]. Evidence is comparatively more heterogeneous for phosphorus solubilisation and AMF-mediated nutrient exchange, where laboratory findings are robust but field translation is inconsistent, likely reflecting the greater sensitivity of these processes to background soil chemistry, particularly pH and existing nutrient status [12, 13].

An important pattern emerging across themes is context-dependency: the magnitude and even direction of rhizosphere metabolite effects on nutrient cycling are frequently modulated by soil type, background nutrient availability, plant developmental stage, and the identity of the intercropped or rotated partner species. This context-dependency, rather than representing a weakness of the underlying science, likely reflects genuine ecological complexity and argues against attempts to identify universal metabolite biomarkers of nutrient-cycling efficiency without reference to the specific agroecological context in which they are measured.

The reviewed evidence also highlights a productive tension between reductionist mechanistic studies (for example, gene-knockout experiments isolating the role of specific flavonoid biosynthesis genes) and systems-level multi-omics approaches that describe emergent network properties without always specifying causal mechanism. Both approaches appear necessary: mechanistic studies provide the causal anchors required to interpret correlational network findings, while multi-omics approaches offer the scale required to capture the ecological complexity that single-gene or single-metabolite studies cannot address. The keystone-metabolite concept emerging from recent network-based studies [11] represents a promising, though still provisional, attempt to bridge these two traditions.

From a practical standpoint, the synthesis suggests that interventions seeking to leverage rhizosphere metabolomics for improved nutrient-cycling efficiency, whether through breeding for enhanced flavonoid exudation, microbial inoculant development, or intercropping system design, are more likely to succeed when tailored to specific soil and cropping contexts rather than deployed as universal solutions. This has direct relevance for policy frameworks, such as the FAO's sustainable nitrogen management recommendations, which promote legume-based biological nitrogen fixation as a substitute for synthetic fertiliser without always specifying the agroecological conditions under which such substitution is most effective [6].

## 7. Conclusion

This review has synthesised evidence from 118 peer-reviewed studies on rhizosphere metabolomics and nutrient cycling efficiency in legume-based systems. The literature demonstrates that legume root exudates, particularly flavonoids, isoflavonoids, and organic acids, function as central mediators of symbiotic nitrogen fixation, phosphorus solubilisation, and interspecific nutrient exchange in intercropping systems. While the mechanistic basis of flavonoid-mediated nodulation signalling is well established, the field remains constrained by limited field-scale validation, inconsistent methodological standardisation, restricted species coverage, and immature frameworks for multi-omics data integration.

For researchers, priority should be given to longitudinal, field-representative experimental designs that capture temporal and developmental dynamics of rhizosphere metabolomes, alongside efforts to standardise analytical protocols to enable robust cross-study synthesis. For practitioners and policymakers, the context-dependency of rhizosphere metabolite effects on nutrient cycling suggests that biological nitrogen fixation and biofertiliser promotion strategies should be tailored to specific soil, climatic, and cropping-system contexts rather than applied uniformly. Future research combining in situ metabolite sensing, AI-assisted multi-omics integration, and expanded coverage of underutilised legume species is likely to substantially advance both the mechanistic understanding and the agronomic translation of rhizosphere metabolomics in the coming decade.

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