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Rhizobial diversity, symbiotic genomics, and non-rhizobial nodule endophytes in Moroccan Fabaceae: a systematic review

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Abstract

Biological nitrogen fixation (BNF) by rhizobia–legume (Fabaceae) symbioses underpins sustainable agriculture globally. Morocco, situated at the convergence of Mediterranean, arid and semi-arid biomes and harbouring more than 100 strictly endemic Fabaceae taxa, represents an exceptional biodiversity hotspot for legume-nodulating bacteria. Despite three decades of primary research, no integrative analysis of Moroccan legume-nodulating bacteria and their non-rhizobial nodule endophyte (NRE) co-inhabitants exists. We conducted a systematic search of Scopus, Web of Science, ScienceDirect, PubMed, Springer Nature and Google Scholar (updated April 2026), retaining 293 peer-reviewed articles (1982–April 2026). A narrative systematic review design was adopted owing to heterogeneous methodological frameworks. At least seven rhizobial genera and twelve symbiovars are documented across Moroccan ecosystems, dominated by *Rhizobium*, *Ensifer*, *Mesorhizobium* and *Bradyrhizobium*. NREs including *Pseudomonas*, *Pantoea*, *Stenotrophomonas*, *Serratia*, *Burkholderia* and *Bacillus* constitute up to 44% of culturable nodule isolates and display plant growth-promoting traits. Indigenous Moroccan rhizobia exhibit remarkable abiotic-stress tolerance exceeding that of commercial inoculant strains, positioning Morocco as a globally significant bioprospecting reservoir. We show that horizontal gene transfer (HGT) of symbiosis islands, rather than strict co-evolutionary divergence, best explains the observed host-range patterns. Metagenomic profiling of nodule communities, dedicated endemic Fabaceae surveys and multi-year field validation trials are identified as priority investments for climate-smart agriculture across the Maghreb and Mediterranean Basin.

Introduction

Nitrogen (N), an indispensable macronutrient for plant biosynthesis, is paradoxically abundant in the atmosphere as biologically inert dinitrogen (N_2). Biological nitrogen fixation (BNF), the enzymatic reduction of N_2 to bioavailable ammonia (NH_3) via the nitrogenase complex, constitutes the principal natural pathway through which fixed nitrogen enters terrestrial ecosystems. Among diazotrophic microorganisms, those that engage in endosymbiosis with leguminous plants (Fabaceae) contribute an estimated 35–47 Tg N yr^{-1} to global agricultural systems, combining 21.5 Tg N yr^{-1} from grain legumes with 12–25 Tg N yr^{-1} from forage, fodder and green-manure legumes (Herridge et al., 2008), underpinning agricultural productivity and soil fertility in both managed and natural agrosystems.

The Fabaceae (Leguminosae) encompasses more than 750 genera and approximately 20,000 species. Morocco, situated at the crossroads of the Mediterranean Basin, Atlantic coastline, and Saharan transition zone, hosts over 700 Fabaceae species, including more than 100 strictly endemic taxa (Fennane and Ibn Tattou, 1998; Rankou et al., 2013). Economically, Moroccan agriculture depends substantially on legume crops: faba bean (*Vicia faba* L.) is the most important, followed by chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.), common bean (*Phaseolus vulgaris* L.), and fenugreek (*Trigonella foenum-graecum* L.).

Morocco's soils impose extreme selective pressures on rhizobial populations: alkalinity (pH 7.5–10), salinity (up to 513 mM NaCl), high temperatures (> 40°C), severe drought (> 60% of territory classified as semi-arid to arid), and heavy-metal contamination in abandoned mining districts notably the Touissit, Sidi Boubker, and Zaida mine tailings in eastern and Middle Atlas Morocco (Alami et al., 2021, 2025b; Lamin et al., 2019; Missbah El Idrissi et al., 2020a; Zerhari et al., 2000). These conditions have generated indigenous rhizobial strains with tolerance traits far exceeding those of commercial inoculants, positioning Morocco as a globally important bioprospecting hotspot for climate-resilient microsymbionts. In alfalfa-growing regions alone, Elboutahiri et al. (2010) demonstrated that 55% of isolates tolerated NaCl concentrations exceeding 513 mM.

Root nodules were historically regarded as the exclusive domain of nitrogen-fixing rhizobia. However, mounting evidence demonstrates that they routinely harbour a diverse second community of non-rhizobial nodule endophytes (NREs): organisms residing within surface-sterilised nodule tissue that neither induce nodule formation autonomously nor fix nitrogen symbiotically, yet possess plant growth-promoting properties and interact synergistically with rhizobia (Fig. 1). The global systematic review by Hnini and Aurag (2024) confirmed that *Bacillus* and *Pseudomonas* are the most prevalent NRE genera worldwide. A 2025 *Frontiers in Microbiology* editorial (Menéndez and Brígido, 2025) highlighted this synthesis as a key contribution to understanding NRE–rhizobial tripartite interactions, now corroborated by independent systematic reviews (Hassen et al., 2025).

Despite three decades of productive research, no study has yet synthesised the full complement of Moroccan legume-nodulating bacteria and their NRE co-inhabitants into an integrative analysis a gap this review directly addresses. We pursue four explicit aims: (i) to map the full taxonomic and functional breadth of rhizobia and NREs associated with Moroccan Fabaceae; (ii) to evaluate how abiotic stressors, soil properties, and host-plant identity shape microbial communities and symbiotic efficiency; (iii) to assess the roles of horizontal gene transfer (HGT), host-range expansion and ecological sorting in structuring Moroccan rhizobial diversity; and (iv) to identify priority research directions and biotechnological applications emerging from this body of work.

Methodology

1. Search strategy, inclusion, and exclusion criteria

This review was conducted as a comprehensive narrative systematic review. Because the synthesised studies employ heterogeneous methodological frameworks, quantitative units, and outcome measures that preclude pooling by meta-analysis, we did not register a protocol with PROSPERO; a PRISMA 2020 screening flow diagram is provided as Supplementary Figure S1. Individual study risk-of-bias assessment was not performed, consistent with standard practice for narrative systematic reviews in microbial biodiversity, where studies are predominantly observational or descriptive and lack randomised controlled designs; to mitigate this limitation, studies employing multilocus sequence analysis (MLSA)

and whole-genome sequencing were prioritised over those relying on 16S rRNA alone in all comparative statements, and readers are referred to the original studies for full experimental and statistical details.

A comprehensive systematic search was conducted in January–February 2025, with an update sweep performed in April 2026 to capture publications from September 2025 onwards. Databases searched included Scopus, Web of Science, ScienceDirect, PubMed, Springer Nature and Google Scholar. Search strings combined the geographic keyword “Morocco” with rhizobial genus names (*Rhizobium*, *Ensifer*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Microvirga*, *Burkholderia*) and biological terms including “Fabaceae”, “legume”, “nitrogen fixation”, “symbiosis”, “nodule”, “endophyte”, and “biofertilizer”.

Inclusion criteria were: (1) primary research articles or systematic reviews; (2) explicit Moroccan geographic context; (3) rhizobia or NREs as the focal organism or a principal study outcome; and (4) publication between 1982 and April 2026. Exclusion criteria were: (1) conference abstracts and grey literature lacking peer review; (2) studies without accessible full text; and (3) reviews that did not report primary data from Moroccan isolates. A total of 293 peer-reviewed primary research papers and systematic review articles were retained after duplicate removal and application of these criteria. Of these, 135 contained sufficient geographic locality data for geocoding (Fig. 2); the remainder contributed to the taxonomic and functional synthesis but could not be precisely mapped.

2. Data extraction and quality assessment

For each retained study the following variables were extracted: rhizobial or NRE genus and species designation; host legume species; geographic origin within Morocco; molecular characterisation methods (16S rRNA, MLSA, symbiotic gene analysis, whole-genome sequencing); phenotypic traits; and quantitative symbiotic efficiency metrics. Studies employing MLSA of multiple housekeeping genes and symbiotic gene (*nodC*, *nifH*, *nodA*) phylogenies were prioritised over those relying on 16S rRNA alone. Quantitative outcomes (e.g., biomass gains, yield increases) are reported as described by the primary authors; readers are referred to original studies for full statistical details including sample sizes, replication, and significance tests. Reference strains and type species designations were cross-checked against the List of Prokaryotic Names with Standing in Nomenclature (LPSN; https://lpsn.dsmz.de), including the recent reclassification of *Ensifer kummerowiae* as a later heterotypic synonym of *E. meliloti* and of *Rhizobium indigoferae* as *R. leguminosarum* (Menéndez et al., 2024).

Rhizobial diversity nodulating Moroccan Fabaceae

1. Taxonomic overview

Rhizobia are phylogenetically diverse Gram-negative bacteria distributed across the Alphaproteobacteria and Betaproteobacteria. In Morocco, four major genera dominate: *Rhizobium*, *Ensifer*, *Mesorhizobium*, and *Bradyrhizobium*. Table 1 provides a comprehensive and updated inventory of genera, species, host associations, and key supporting literature, incorporating new species and genomic data from 2025–

2026. The phylogenetic relationships among these genera and the host–microsymbiont specificity patterns are summarised in Fig. 3 and Fig. 4, respectively.

Table 1
Rhizobial genera, species, and their principal host legumes documented in Moroccan ecosystems (updated April 2026).

Genus	Key species / strains	Principal Moroccan hosts	Key references
Rhizobium	*R. leguminosarum*, *R. laguerreae*, *R. acidisoli*, *R. sullae*, *R. phaseoli*	*Vicia faba*, *Pisum sativum*, *Lens culinaris*, *Hedysarum* spp., *Sulla flexuosa*, *Phaseolus vulgaris*, *Colutea arborescens*	Benidire et al., 2018; Missbah El Idrissi et al., 2020a; M'saouar et al., 2024; Sijilmassi et al., 2021; Taha et al., 2022; Ourarhi et al., 2011
Ensifer	*E. meliloti* (syn. *E. kummerowiae*), *E. medicae*, *E. aridi*, *E. fredii*	*Medicago sativa*, *Trigonella foenum-graecum*, *Retama monosperma*, *Vachellia* spp., *Lotus creticus*, *Robinia pseudoacacia*, *Colutea arborescens*	Alami et al., 2024; Belkadi et al., 2022; Bouhnik et al., 2019, 2024; Elboutahiri et al., 2010; Hnini et al., 2023a; Lamin et al., 2019; Le Quéré et al., 2017; Ourarhi et al., 2011
Mesorhizobium	*M. ciceri*, *M. opportunistum*, *M. camelthorni*, *M. qingshengii*, *M. plurifarium*, *M. maamorense* sp. nov.	*Cicer arietinum*, *Astragalus* spp., *Ononis repens*, *Sulla spinosissima*, *Colutea arborescens*	Alami et al., 2025a, 2025b, 2026; Benjelloun et al., 2019, 2021; Guerrouj et al., 2013b; Laadraoui et al., 2023; Lamin et al., 2021a
Bradyrhizobium	*B. canariense*, *B. rifense*, *B. retamae*, *B. valentinum*, *B. lupini*, *B. algeriense*, *B. cytisi*; *Bradyrhizobium* sp. nov. (×4)	*Lupinus* spp., *Retama* spp., *Cytisus villosus*, *Chamaecytisus albidus*, *Astragalus algarbiensis*	Bouhnik et al., 2021a, 2021b; Chaddad et al., 2023; Chahboune et al., 2011, 2012; Kaddouri et al., 2024, 2025; Lamrabet et al., 2022, 2023; Lamrabet and Missbah El Idrissi, 2025
Microvirga	*M. tunisiensis*, *Microvirga* sp. sv. mediterranense	*Retama sphaerocarpa*, *Lupinus cosentinii*, *L. angustifolius*, *L. albus*	Missbah El Idrissi et al., 2020b, 2021; Mouad et al., 2020
Burkholderia	*B. phymatum* (nodulating); *Burkholderia* spp. (non-nodulating endophytes)	*Phaseolus vulgaris*	Talbi et al., 2010, 2013; Benjelloun et al., 2019
Agrobacterium-lineage	Cluster C (*Agrobacterium*-like)	*Acacia gummifera*, *A. raddiana*, *A. cyanophylla*	Khbaya et al., 1998

2. Cultivated Fabaceae

2.1. Faba bean (*Vicia faba*): *R. laguerreae* and *R. leguminosarum* dominate; trehalose underpins osmotolerance

Vicia faba is the most extensively cultivated legume in Morocco and has been the subject of detailed diversity studies spanning more than three decades (Benidire et al., 2018; Hajjam et al., 2016; Robert et al., 1982). Benidire et al. (2018) isolated 106 rhizobial strains from nodules across five major cultivation regions (Taounat, Taza, Fez, Meknes, Marrakech), identifying 102 as *Rhizobium leguminosarum* or *R. laguerreae*, with four affiliating unexpectedly with *Ensifer meliloti*. Trehalose content was measured in cultured cells of the rhizobial isolates themselves (not in planta or in nodules): isolates recovered from nodules of faba bean plants grown under saline conditions accumulated higher intracellular trehalose than isolates from non-saline conditions, and trehalose content was positively correlated with osmotolerance in vitro, providing a biochemical mechanism for salinity tolerance (Benidire et al., 2018). A complementary MLSA study on 76 isolates placed all strains in *Rhizobium*, related to *R. leguminosarum* (including isolates formerly assigned to *R. indigoferae*, now reclassified as *R. leguminosarum*; Menéndez et al., 2024), *R. laguerreae*, *R. anhuiense*, and *R. acidisoli*. Missbah El Idrissi et al. (2020a) subsequently formalised symbiovar viciae within *R. acidisoli* for strains co-nodulating *Pisum sativum* and *V. faba* in Morocco. Hajjam et al. (2016) identified strains PSR19, PSR26, and PSR29, isolated in the Marrakech–Haouz region and recovered from faba bean root nodules, representing Rhizobium spp., as the most stress-tolerant faba bean inoculants, with PSR29 achieving 81.35% symbiotic efficiency ($n = 3$ independent replicates; $p < 0.05$ vs. uninoculated control). Maghraoui et al. (2016) demonstrated that strain RHOF147 improved phosphorus uptake in the Aguadulce variety relative to triple superphosphate controls. Under the same experimental programme, strain RHOF174 improved P uptake in the Defes variety, with inoculation performing comparably to P fertilisation (Maghraoui et al., 2016). Wahbi et al. (2016) further demonstrated that mycorrhizal inoculation increased N transfer from faba bean to companion wheat by up to 50% of biologically fixed N.

2.2. Chickpea (*Cicer arietinum*): endemic *Mesorhizobium* diversity; NREs constitute 44% of nodule isolates

The seminal study by Benjelloun et al. (2019), covering 135 bacterial isolates from chickpea nodules across two Moroccan growing regions, revealed that 56% (~ 75 strains) nodulated chickpea and clustered by MLSA into six clades affiliated with *M. ciceri*, *M. opportunistum*, *M. qingshengii*, *M. plurifarium*, and two unnamed genospecies, with the majority distinct from all known reference species. The remaining 44% of nodule isolates were non-nodulating NREs. Earlier work by Maâtallah et al. (2002) demonstrated wide physiological diversity some isolates tolerated pH 5–8, 1–1.5% NaCl, and 30–35°C with a remarkable first report of chickpea nodulation by *Sinorhizobium* (now *Ensifer*). Benjelloun et al. (2021) showed that combining *M. ciceri* with *Bacillus* sp. and *Enterobacter aerogenes* in phosphate-deficient soils increased nodulation by > 270%, shoot dry weight by 192%, and grain yield by 242% relative to uninoculated controls (the experimental design included an uninoculated unfertilised control

and a fully fertilised control receiving 120 kg N ha^{-1} plus 80 kg P_2O_5 ha^{-1}; the dual inoculation matched the performance of the fertilised control).

2.3. Alfalfa (*Medicago sativa*): *Ensifer* dominance; near-complete strain-level genotypic uniqueness

The microsymbionts of *M. sativa* in Morocco are dominated by *Ensifer meliloti* and *E. medicae*. The largest study (Elboutahiri et al., 2010) characterised 157 isolates from arid and semi-arid regions, assigning 136 to *E. meliloti* and 21 to *E. medicae* by 16S RFLP; rep-PCR genotyping yielded 148 distinct genotypes from 157 isolates, demonstrating near-complete strain-level uniqueness (i.e., 148 of the 157 isolates each represented a unique rep-PCR fingerprint, indicating extremely high strain diversity within a single host species). A defining ecological finding was that 55% of isolates tolerated NaCl exceeding 513 mM (approximately 3% w/v, an exceptionally high salinity threshold for rhizobia). Guerrouj et al. (2013a) showed that all 61 isolates from *Medicago arborea* across four arid eastern soils were *E. meliloti*, demonstrating restricted host specificity. Raklami et al. (2024) demonstrated that a consortium of *Ensifer meliloti* strains (RhOL6, RhOL8) combined with *Pseudomonas* sp. and *Proteus* sp. improved alfalfa root dry weight by 138% under salt stress and 195% under water stress relative to uninoculated controls (uninoculated, unfertilised control plants were used in this study; the inoculated strains originated from metal-contaminated sites, adding phytostabilisation relevance).

2.4. Lentil (*Lens culinaris*): *R. laguerreae* dominance with evidence of geographic local adaptation

Taha et al. (2018) identified *Rhizobium laguerreae* as the dominant lentil microsymbiont across 40 cultivated fields in Morocco, with high genetic differentiation for *nodC* among geographic regions suggesting local adaptation. Sijilmassi et al. (2021) characterised 14 rhizobial strains from lentil accessions at the ICARDA Marchouch station: all except one clustered with *R. laguerreae* and *R. leguminosarum*; the outlier affiliated with *Mesorhizobium huakuii* at 100% 16S rRNA identity a novel cross-genus association. Co-inoculation with *R. laguerreae* LMR655 + *Enterobacter aerogenes* LMR696 + *Bacillus* sp. LMR698 increased shoot biomass by 38% under well-watered conditions and maintained productivity comparable to N-fertilised controls under drought (30% water retention; Taha et al., 2022).

2.5. Fenugreek (*Trigonella foenum-graecum*): *Ensifer medicae* as exclusive microsymbiont

Belkadi et al. (2022) provided the first detailed characterisation of fenugreek rhizobia in Morocco. Forty-seven nodule isolates from six northwestern locations grouped by rep-PCR into 13 clusters; all representative strains affiliated with *Ensifer*, sharing 100% 16S rRNA similarity with *E. medicae* WSM419^T; *nodC* and *nifH* placed all within symbiovar meliloti.

2.6. Common bean (*Phaseolus vulgaris*) and related hosts: beta-rhizobial nodulation documented

Common bean rhizobia in Morocco exhibit exceptional taxonomic breadth. Mouhsine et al. (2007) identified five species (*R. etli*, *R. tropici*, *R. gallicum*, *R. leguminosarum*, *Ensifer meliloti*) across five salt-affected soils, with species distribution correlated to salinity (in the most saline site, Skhirat (EC 418 mS m^{-1}), *R. tropici* represented ~ 90% of isolates, whereas *R. gallicum* dominated at Benslimane and *R. etli* at Ain Atiq, indicating that salinity, rather than geography alone, structures community composition; overall, 61%, 24%, 10% and 4% of isolates belonged to *R. tropici*, *R. leguminosarum*, *R. gallicum* and *R. etli*, respectively, with E. meliloti at ~ 1%). *Burkholderia phymatum GR01N* the first documented beta-proteobacterial nodulant of common bean in Morocco accumulated trehalose, mannitol, and alanine under saline stress and displayed higher nodule occupancy and N fixation than *R. tropici* $CIAT899^R$ in salt-stressed plants (Talbi et al., 2010, 2013). El Attar et al. (2019) documented coexistence of endophytic bacteria *Rhizobium miluonense* and *R. yanglingense* (two non-nodulating, endophytic *Rhizobium* species that do not fix nitrogen symbiotically) together with nodulating *Agrobacterium* spp. and other symbiotic strains inside bean nodules; of the 113 isolates examined, the collection comprised a mixture of nodulating rhizobia and non-nodulating endophytes, and 45 isolates showed plant growth-promoting (PGP) traits.

2.7. *Sulla* species: dual inoculation with AMF delivers highest biomass gains documented for Moroccan cultivated legumes

M'saouar et al. (2024) demonstrated that dual inoculation of *Sulla flexuosa* with arbuscular mycorrhizal fungi (AMF) and *Rhizobium sullae a* strain isolated locally from root nodules of *Sulla spinosissima* growing near Bni Boufrah (Al Hoceima province, northern Morocco), not a commercial inoculant increased stem height by 38%, root length by 46%, and shoot and root dry biomass by 153% and 144%, respectively, relative to uninoculated controls (uninoculated plants received no inoculation and no fertiliser amendment). Hamane et al. (2023) isolated *Enterobacter hormaechei*, *Pseudomonas moraviensis*, and *Serratia inhibens* from nodules of *Sulla aculeolata* and *S. flexuosa* in northeastern Morocco alongside nodulating *Ensifer* and *Rhizobium* symbionts; that is, both nodulating rhizobia and non-nodulating endophytes were recovered from the same nodules, with documented antifungal activity against *Botrytis cinerea*, *Aspergillus ochraceus*, and Fusarium oxysporum.

3. Wild and woody Fabaceae

3.1. *Retama* species: multi-genus microsymbiont profiles driven by soil pH and metal contamination

The description of *Bradyrhizobium retamae* sp. nov. and symbiovar retamae by Guerrouj et al. (2013c) established the taxonomic framework for *Retama* microsymbiont biology. Three *Retama* species show distinct microsymbiont profiles in Morocco, and unusually among legumes *Retama* is nodulated by three rhizobial genera: *Bradyrhizobium* (sv. retamae), *Microvirga* (sv. mediterranense), and *Ensifer* (sv.

vachelliae). *Retama monosperma*: in the Touissit mine tailings, Lamin et al. (2019) showed effective symbiosis with *Ensifer aridi* a desert species tolerant to 48°C, pH ≥ 9, and lead up to 1000 µg ml^{-1} (Lamin et al., 2019) genomically validated by Le Quéré et al. (2017); Lamin et al. (2021b) additionally documented *Bradyrhizobium* sp. sv. retamae nodulating *R. monosperma* at the same site. *Retama dasycarpa* endemic to Morocco's High Atlas now has the most genomically characterised microsymbiont community of any Moroccan wild legume. Lamrabet and Missbah El Idrissi (2025) published comparative genomics of five native strains: four likely represent novel *Bradyrhizobium* species (average nucleotide identity [ANI] / digital DNA–DNA hybridisation [dDDH] below species delineation thresholds), with a small core genome (4.36%), large accessory genome (39.41%), and substantial strain-specific gene fraction (56.23%). The draft genome of *Bradyrhizobium* sp. strain RDM4 (Kaddouri et al., 2025) spans 8.4 Mb (GC content 63%; 8,141 predicted genes).

3.2. *Lupinus* species: *Bradyrhizobium* and *Microvirga* co-nodulate in Maamora forest

Lupinus species are nodulated primarily by *Bradyrhizobium* and additionally by *Microvirga* in the Maamora forest. Lamrabet et al. (2022) identified *B. lupini*, *B. cytisi*, *B. canariense*, and a novel genospecies in *L. albus* and *L. angustifolius*, all within symbiovar *genistearum*. Missbah El Idrissi et al. (2020b) reported *Microvirga* sp. sv. *mediterranense* nodulating L. *cosentinii* the first *Microvirga* isolation from an African *Lupinus* host.

3.3. Ononis repens and Mesorhizobium maamorense sp. nov.: first genome-supported novel species from a Moroccan wild legume

We emphasise that *Ononis repens* is a wild legume native to Morocco but not endemic to the country (it also occurs in southern Europe and Macaronesia). Laadraoui et al. (2023) characterised bacteria nodulating *O. repens* in the Maamora cork oak forest: 20 nodC-positive isolates clustered as *Mesorhizobium*, with *nodC* phylogeny diverging from all described type strains, forming a separate clade that prompted the formal proposal of symbiovar maamori. Cross-inoculation assays showed that strains ORM6, ORM8.1, ORM13.1 and ORM16 nodulate *O. repens*, *Astragalus gombiformis* and *A. armatus*, whereas only ORM16 additionally nodulated *Vachellia gummifera*, indicating a broader host range than initially inferred from the single original host. Alami et al. (2026) subsequently proposed *Mesorhizobium maamorense* sp. nov. (type strain ORM16^T = DSM 120599^T = CCMM B1359^T), confirming a distinct taxonomic position: ANI = 87.63% and dDDH = 31.2% vs. M. opportunistum WSM2075T, both below the species delineation thresholds of ANI ≥ 96% and dDDH ≥ 70% (Chun et al., 2018). Canonical *nod* genes reside on chromosomal DNA, and the type strain shows moderate salinity, osmotic, and thermal stress tolerance. This represents the first novel rhizobial species formally described from a Moroccan wild legume with whole-genome support (earlier Moroccan species descriptions, e.g., *Bradyrhizobium retamae* in Guerrouj et al., 2013c, preceded the genome-based era). We further note that *M. maamorense* does not appear to be restricted to Morocco: the genome of *Mesorhizobium* sp. B2-1-1, isolated from soil

in Australia, shares an ANI value of ~ 98% with ORM16, indicating that this species has a much wider geographic distribution than the Moroccan sampling alone would suggest.

3.4. *Vachellia* and *Acacia* species and other wild hosts

Vachellia species. For *Vachellia tortilis* subsp. *raddiana* in the Anti-Atlas, Hnini et al. (2023a) showed *E. aridi* sv. vachelliae dominance, with strains LMR688, LMR692, and LMR687 achieving up to 231% symbiotic efficiency relative to uninoculated controls the highest documented value for any wild Moroccan legume system. For *Vachellia gummifera*, Bouhnik et al. (2019) documented nodulation by *Ensifer fredii* sv. vachelliae in semi-arid Moroccan areas, and Bouhnik et al. (2024) showed that inoculation with these native *Ensifer* strains combined with phosphorus fertilisation increased *V. gummifera* seedling biomass by 74.87–140.3% across measured growth parameters relative to uninoculated controls.

Acacia species. The earliest systematic Moroccan rhizobial diversity study was conducted by Khbaya et al. (1998) on four *Acacia* species, identifying three 16S rDNA clusters: two in the *Ensifer* (then *Sinorhizobium*) lineage and one near the Agrobacterium–Rhizobium galegae group. Benata et al. (2008) characterised *Prosopis juliflora* microsymbionts in arid soils of eastern Morocco, discovering Achromobacter xylosoxidans within nodules a non-nodulating endophyte, reported for the first time for any legume.

Other wild hosts. For *Sulla spinosissima* in Sidi Boubker mine tailings, Lamin et al. (2021a) identified Mesorhizobium camelthorni sv. aridi. Mohamed et al. (2021) showed that field-dose glyphosate and paraquat directly impaired rhizobia, reducing nodulation, nitrogen content, and symbiotic efficiency in *Bituminaria bituminosa* the only study addressing pesticide–BNF interactions across the full range of compounds used in Moroccan agriculture.

3.5. *Chamaecytisus albidus*: an endemic host nodulated by multiple *Bradyrhizobium* species

Chamaecytisus albidus (also known as *Cytisus albidus* or *Chamaecytisus mollis*, and locally known as “Ghandoul”) belongs to the tribe Genisteae within the subfamily Papilionoideae. It is a perennial shrub endemic to Morocco. It thrives in semi-arid bioclimatic conditions and demonstrates resilience in arid climatic zones (Naggar, 1993). This plant provides protection against soil erosion and serves as a source of fodder for livestock (Bouhnik et al., 2021b).

Bouhnik et al. (2021a, b) analysed the symbiotic performance and diversity of rhizobial strains isolated from *C. albidus* plants growing in the soils of four distinct agroforestry ecosystems representing arid, semi-arid, and sub-humid forest zones in Morocco (Amhach, Bounaga, Sahel Doukkala, and Maâmora). All isolated strains were found to belong to the genus *Bradyrhizobium*. MLSA using the housekeeping genes *rrs*, *gyrB*, *recA*, *glnII*, *atpD*, and *rpoB* revealed that these strains were distributed across several species, including *B. canariense*, *B. lupini*, *B. cytisi*, *B. valentinum*, and *B. retamae*. Analysis of symbiotic genes indicated that these strains belonged to three symbiovars: lupini, genistearum, and retamae.

Strains isolated from the arid regions of Bounaga and Amhach exhibited greater tolerance to salinity and drought stress than those isolated from the Doukkala (semi-arid) and Maâmora forest (sub-humid) sites. The majority of these strains significantly improved plant yield ($p < 0.05$) compared with uninoculated, non-nitrogen-fertilised control plants; some strains showed symbiotic efficiencies two to three times higher than control plants treated with potassium nitrate (KNO_3).

Symbiovar diversity and horizontal gene transfer

The symbiovar concept defining biologically meaningful clusters of rhizobial strains by symbiotic gene (*nodC*, *nifH*, *nodA*) phylogeny and host-range profile (Rogel et al., 2011; Martínez-Romero et al., 2024) is particularly powerful in the Moroccan context. Horizontal gene transfer (HGT) of symbiotic islands or plasmids (pSym) generates new host-range capacities in chromosomally unrelated backgrounds, creating discordance between core-genome and symbiotic-gene phylogenies. Table 2 provides an updated comprehensive inventory of the twelve symbiovars documented or formally proposed from Moroccan rhizobial material.

Table 2

Symbiovars identified or proposed from Moroccan rhizobial isolates (updated April 2026). Symbiovar assignments follow the guidelines of Martínez-Romero et al. (2024). □ = newly described or first-recorded since 2025. The "Genetic signature" column uniformly reports the location of the symbiosis loci (chromosomal vs. plasmid-borne) together with the placement of the *nodC* lineage.

Symbiovar	Genus / species	Key Moroccan hosts	Genetic signature	Reference
sv. viciae	*Rhizobium leguminosarum*, *R. acidisoli*	*Vicia faba*, *Pisum sativum*, *Lens culinaris*	*nod* genes plasmid-borne; *nodC* distinct from sv. trifolii; formally defined in Morocco	Missbah El Idrissi et al., 2020a
sv. meliloti	*Ensifer meliloti* (syn. *E. kummerowiae*)	*Medicago sativa*, *Trigonella foenum-graecum*, *Robinia pseudoacacia*	*nod*/*nif* plasmid-borne; *nodC* 94–100% identity to sv. meliloti type lineages	Alami et al., 2024; Belkadi et al., 2022
sv. medicae	*Ensifer medicae*	*Medicago* spp., *Trigonella*	*nod*/*nif* plasmid-borne; *nodC* in the meliloti–medicae clade	Belkadi et al., 2022
sv. lancerottense	*Ensifer meliloti*	*Lotus creticus* (saline soils, N. Morocco)	*nod* plasmid-borne; *nodC* closely related to sv. meliloti; first mainland North Africa record	Belechheb et al., 2021
sv. retamae	*B. retamae*, *B. valentinum*, *B. algeriense*	*Retama monosperma*, *R. sphaerocarpa*, *R. dasycarpa*	*nod* island chromosomal; *nodC* forms a distinct *Retama*-specific clade (HGT across species)	Guerrouj et al., 2013c; Lamin et al., 2021b; Lamrabet et al., 2023
sv. genistearum	*Bradyrhizobium* spp.	*Lupinus* spp., *Retama* spp., *Chamaecytisus albidus*, *Lupinus luteus*	*nod* island chromosomal; *nodC* in the genistearum clade; broad Genisteae host range	Bouhnik et al., 2021a, 2021b; Chaddad et al., 2023; Lamrabet et al., 2022
sv. aridi	*Mesorhizobium camelthorni*	*Sulla spinosissima*, *Astragalus gombiformis*, *A. armatus*	Symbiosis island chromosomal; *nodC* divergent from other *Mesorhizobium* symbiovars; narrow host range	Lamin et al., 2021a; Guerrouj et al., 2013b
sv. maamori □ (2026)	*Mesorhizobium maamorense* sp. nov.	*Ononis repens* (Maamora cork forest)	*nod* genes chromosomal; *nodC* forms a separate clade; species delineation ANI 87.63%, dDDH 31.2%	Alami et al., 2026; Laadraoui et al., 2023
sv. vachelliae	*Ensifer aridi*, *E. fredii*	*Vachellia gummifera*, *V. tortilis* subsp.	*nod* genes plasmid-borne (pSym); *nodC* shared across chromosomally	Bouhnik et al., 2019,

Symbiovar	Genus / species	Key Moroccan hosts	Genetic signature	Reference
		raddiana; *Acacia* spp.	distinct *Ensifer* species (HGT)	2024; Hnini et al., 2023a
sv. mediterranense	*Microvirga tunisiensis*	*Retama sphaerocarpa*, *Lupinus cosentinii*, *L. albus*	Symbiosis island chromosomal; *nodC* distinct from all *Ensifer*/*Bradyrhizobium* symbiovars; alkaline-soil specialist	Missbah El Idrissi et al., 2020b; Mouad et al., 2020
sv. hedysari (2025)	*Mesorhizobium* sp.	*Astragalus armatus* (Zaida mine tailings)	Symbiosis island chromosomal; *nodC* affiliated with sv. hedysari; Pb/Zn-tolerant	Alami et al., 2025a, 2025b
sv. rigiduloides	*Ensifer meliloti*	*Robinia pseudoacacia* (Pb-contaminated soils)	*nod*/*nif* plasmid-borne; *nodC* within sv. meliloti; Pb-tolerant	Alami et al., 2024

Three lines of evidence from the Moroccan literature illustrate this process. First, HGT of symbiosis islands between genera is directly observable: sv. retamae, originally defined in *Bradyrhizobium retamae* (Guerrouj et al., 2013c), is also carried by *B. valentinum* and *B. algeriense* on *Retama* hosts (Lamin et al., 2021b; Lamrabet et al., 2023), whereas sv. vachelliae occurs in chromosomally distinct *Ensifer aridi* and *E. fredii* backgrounds on *Vachellia* hosts (Bouhnik et al., 2019; Hnini et al., 2023a). Second, host-range expansion within a genus occurs through acquisition of novel symbiotic gene clusters, as in *Ensifer* strains on *Vachellia gummifera* (Bouhnik et al., 2024). Third, ecological sorting then acts on this HGT-generated diversity: the distinct genera nodulating *Retama* carry non-sister *nodC* lineages (sv. retamae in *Bradyrhizobium*, sv. mediterranense in *Microvirga*, sv. vachelliae in *Ensifer*), indicating that soil pH and contamination status, rather than shared symbiotic ancestry, determine which lineage occupies a given site (Mouad et al., 2020; Lamin et al., 2019, 2021b).

The formal description of *Mesorhizobium maamorense* sp. nov. (Alami et al., 2026) anchors sv. maamori in a formally deposited type strain with full genomic characterisation. The pangenome architecture of *Bradyrhizobium* strains from *R. dasycarpa* (Lamrabet and Missbah El Idrissi, 2025) provides direct genomic evidence of HGT-driven plasticity: the very small core genome (4.36% of gene families) together with the large accessory (39.41%) and strain-specific (56.23%) fractions indicates that most genes are frequently gained and lost across strains (see also Supplementary Figure S4). Because the symbiosis island itself is a mobile genetic element, a pangenome dominated by accessory and strain-specific genes is the expected genomic signature of a population in which horizontal transfer of symbiotic and accessory loci is ongoing and extensive.

Non-rhizobial nodule endophytes (NREs)

1. Conceptual framework and global context

Root nodules were long presumed to be exclusive sanctuaries of nitrogen-fixing rhizobia. Mounting evidence from culture-dependent isolation and amplicon-sequencing studies demonstrates that legume nodules routinely harbour a diverse second community of NREs. A global systematic review by Hnini and Aurag (2024) synthesised published data on NRE occurrence and functionality, confirming that *Bacillus* and *Pseudomonas* are the most prevalent NRE genera worldwide, succeeded by *Paenibacillus*, *Enterobacter*, *Pantoea*, *Agrobacterium*, and Microbacterium. Hassen et al. (2025) published an independent systematic review confirming synergistic roles of NREs in tripartite plant–microbe interactions, with particular emphasis on ACC deaminase activity as a key PGP mechanism under stress.

2. NREs documented in Moroccan legumes

The most comprehensive characterisation of NREs from Moroccan legumes is provided by Benjelloun et al. (2019): of 135 bacterial isolates from surface-sterilised chickpea nodules, 44% (59 isolates) were non-nodulating NREs, spanning three *Burkholderia* spp. (*Betaproteobacteria*) non-nodulating endophytes rather than rhizobia, as these isolates lack nod/nif symbiosis genes and dominant Gammaproteobacteria comprising *Pseudomonas* (31 isolates; 97% 16S identity), *Pantoea* (10; 95%), *Stenotrophomonas* (8; 97%), *Serratia* (5; 99%), one *Bacillus* sp. (99%) and one *Brevibacterium* sp. (99.8%) (Benjelloun et al., 2019). Additional NRE diversity has been documented across other Moroccan host legumes. Hamane et al. (2023) isolated *Enterobacter hormaechei*, *Pseudomonas moraviensis*, and *Serratia inhibens* from *Sulla* nodules as non-nodulating endophytes co-existing with the nodulating *Ensifer* and *Rhizobium* symbionts, with strong antifungal activity and PGP traits. Hnini et al. (2023b) characterised *Pseudomonas* and *Bacillus* endophytes from *Vachellia tortilis* subsp. *raddiana* nodules in southern Morocco, documenting phosphate, K, and Zn solubilisation, N_2 fixation, auxin, siderophore, HCN, and ACC deaminase production (Table 3).

Table 3
Non-rhizobial endophyte (NRE) genera documented from Moroccan legume nodules, with PGP roles (updated April 2026). All listed taxa are non-nodulating endophytes except *Burkholderia phymatum*, which is a nodulating beta-rhizobium.

Genus	Class	Isolates (host)	Documented PGP / ecological role	Reference
Pseudomonas	γ-Proteobacteria	31 (chickpea); *Vachellia*, *Sulla* nodules	IAA, siderophores, phosphate solubilisation, biocontrol, ACC deaminase	Benjelloun et al., 2019; Hamane et al., 2023; Hnini et al., 2023b
Pantoea	γ-Proteobacteria	10 (chickpea nodules)	IAA production, ethylene modulation	Benjelloun et al., 2019
Stenotrophomonas	γ-Proteobacteria	8 (chickpea nodules)	ACC deaminase, siderophore production	Benjelloun et al., 2019
Serratia	γ-Proteobacteria	5 (chickpea); *Sulla* nodules	Phosphate solubilisation; growth promotion; antifungal activity	Benjelloun et al., 2019; Hamane et al., 2023
Bacillus	Firmicutes	1 (chickpea); *Vachellia* nodules	IAA, ACC deaminase, drought-tolerance co-inoculant; antifungal	Hnini et al., 2023b; Taha et al., 2022
Enterobacter	γ-Proteobacteria	Lentil rhizosphere; *Sulla* nodules	IAA, ACC deaminase; synergy with rhizobia under drought; biocontrol	Hamane et al., 2023; Taha et al., 2022
Burkholderia	β-Proteobacteria	3 (chickpea nodules)	Osmotolerance; *B. phymatum* strains nodulate *P. vulgaris*	Benjelloun et al., 2019; Talbi et al., 2010

3. Functional roles and co-inoculation synergism

NREs modulate the legume–rhizobium symbiosis through at least four mechanisms: (i) phytostimulation production of IAA, cytokinins, and gibberellins promoting root architecture and nodule initiation (Patten and Glick, 2002); (ii) biofertilization phosphate solubilisation (Vurukonda et al., 2016) and siderophore synthesis enhancing Fe and P bioavailability in alkaline Moroccan soils (Compant et al., 2005); (iii) biocontrol competition with soil pathogens and antimicrobial metabolite production (antifungal activity against *Fusarium oxysporum*, *Botrytis cinerea*, and *Aspergillus ochraceus* documented in Moroccan strains; Hamane et al., 2023); and (iv) stress-tolerance facilitation exopolysaccharide production, ACC deaminase activity reducing ethylene-mediated stress responses (Glick, 2014), and synthesis of compatible solutes protecting rhizobia under osmotic stress.

Unless otherwise stated, the synergistic effects described below were quantified relative to a baseline of rhizobial inoculation alone (the standard control in all cited co-inoculation studies); fully uninoculated and unfertilised controls were additionally included in most studies and are noted where relevant. Co-inoculation experiments consistently demonstrate synergistic outcomes. Taha et al. (2022) showed that combining *R. laguerreae* LMR655 with *E. aerogenes* LMR696 and *Bacillus* sp. LMR698 increased lentil shoot biomass by 38% versus single inoculation under well-watered conditions, and maintained dry weights comparable to N-fertilised controls under drought (30% water retention). Benjelloun et al. (2021) demonstrated > 270% nodulation, 192% shoot dry weight, and 242% grain yield increases in chickpea under phosphate deficiency. Raklami et al. (2024) showed 138% and 195% root dry weight gains under salt and water stress, respectively, through multi-species Ensifer–PGPB consortia. These data position NRE-inclusive multipartite inoculants as the next frontier in Moroccan biofertilizer technology. Comprehensive effect-size data for all co-inoculation experiments are provided in Supplementary Table S1.

Environmental drivers of rhizobial and NRE diversity

1. Soil pH and salinity

Moroccan soils span an exceptionally wide pH range, from acidic Maamora forest soils (pH < 6) to highly alkaline calcareous substrates (pH 8.5–10), and salinities from negligible to > 513 mM NaCl. Elboutahiri et al. (2010) documented 55% of Moroccan alfalfa rhizobia tolerating salinity above this threshold. The Maamora forest itself is pedologically heterogeneous: the western part of the forest, on sandstone parent material under cork oak (*Quercus suber*) cover, has acidic soils (pH < 6), whereas the eastern part, on calcareous material under eucalyptus plantations, has alkaline soils (pH > 7.5). The alkaline soils of the Maamora forest drive ecological dominance of *Microvirga* alongside *Bradyrhizobium* at *Retama* and *Lupinus cosentinii* roots consistent with the preference of *Microvirga* for alkaline, calcareous substrates demonstrated by Mouad et al. (2020) one of the clearest documented examples of pH-mediated microsymbiont community selection in the Moroccan literature. Bouhmouch et al. (2001) identified ten osmotolerant common bean rhizobial strains from Moroccan saline soils and showed that exogenous addition of glutamate and aspartate to the culture medium could relieve growth inhibition under high salinity.

2. Heavy metal contamination

Mining districts in eastern and Middle Atlas Morocco have created unique selection environments for metal-tolerant rhizobia. *Ensifer aridi* strains from the Touissit mine tolerated lead up to 1000 µg ml^{-1} Pb-acetate (Lamin et al., 2019). *Mesorhizobium camelthorni* sv. aridi exhibited multi-metal tolerance (Pb, Zn, Cd) (Lamin et al., 2021a). Alami et al. (2024) demonstrated that *Ensifer meliloti* sv. rigiduloides significantly improved *Robinia pseudoacacia* shoot biomass, chlorophyll content, and leaf nitrogen in soils contaminated with up to 1200 µg ml-1 Pb-acetate. Alami et al. (2025b) extended phytostabilisation

evidence to the Zaida Middle Atlas mine tailings: *Mesorhizobium* sp. strains (sv. hedysari) from *Astragalus armatus* nodules tolerated high Pb/Zn concentrations and produced ACC deaminase and siderophores, with greenhouse inoculation enhancing growth and chlorophyll content in metal-rich substrates.

3. Drought and forest soil chemistry

Drought affects approximately 60% of Morocco's territory. *Bradyrhizobium* strains from *Retama dasycarpa* (Lamrabet and Missbah El Idrissi, 2025) tolerated PEG-6000 at severe water potential deficits, produced exopolysaccharides, siderophores, IAA, and phosphate-solubilising enzymes under osmotic stress, and promoted host biomass under three water-availability regimes. The Maamora cork oak forest represents a unique low-pH Atlantic-influenced soil system, where the divergent microsymbiont communities of *Ononis repens* (*Mesorhizobium maamorense* sp. nov. sv. maamori) suggest that microheterogeneity in soil chemistry mediated by *Quercus suber* canopy cover, organic matter accumulation, and water retention drives fine-scale niche differentiation among rhizobial lineages. Legeay et al. (2024) demonstrated that, along a Moroccan precipitation gradient, the endosphere of non-legume *Malva sylvestris* was dominated by *Rhizobium* underscoring the broad ecological competence of Moroccan *Rhizobium* populations beyond conventional nodulation niches.

Discussion

1. Principal findings and global context

The principal finding of this review is that Morocco harbours one of the most taxonomically diverse and ecologically adapted legume-nodulating bacterial communities documented for any single country, encompassing at least seven rhizobial genera, twelve symbiovars including two newly described since 2025 and a rich NRE community with substantial biotechnological potential. The formal description of *Mesorhizobium maamorense* sp. nov. (Alami et al., 2026) from the wild legume *Ononis repens* represents a milestone: it anchors Moroccan rhizobial novelty in a whole-genome-supported, formally deposited type strain, validating the broader pattern of high novelty that prior studies had suggested but not formally demonstrated.

Contextualised against the global rhizobial literature, Morocco's microsymbiont diversity is consistent with and in several respects exceeds comparable surveys from other biodiversity hotspots. The 44% NRE fraction in Moroccan chickpea nodules (Benjelloun et al., 2019) is among the highest reported globally (Hassen et al., 2025; Hnini and Aurag, 2024). The salinity tolerance of Moroccan *Ensifer meliloti* strains (55% exceeding 513 mM NaCl; Elboutahiri et al., 2010) exceeds thresholds reported for European and Australian strains of the same species. The four likely novel *Bradyrhizobium* species from *Retama dasycarpa* (Lamrabet and Missbah El Idrissi, 2025) add to a growing recognition that arid-zone *Bradyrhizobium* lineages are systematically undersampled relative to their true species richness a view

reinforced by continuing taxonomic revision within the genus (e.g., the reclassification of *B. japonicum* group Ia strains as *B. diazoefficiens*; Delamuta et al., 2013).

2. HGT, host-range expansion and ecological sorting

The mechanistic interpretation of Morocco's rhizobial diversity centres on HGT-driven host-range expansion followed by ecological sorting, rather than strict co-evolutionary divergence between plant and bacterial lineages. First, the symbiovar framework reveals that symbiotic gene clusters have been laterally transferred across chromosomally unrelated backgrounds on multiple independent occasions for example, sv. retamae occurs in *B. retamae*, *B. valentinum* and *B. algeriense* (Guerrouj et al., 2013c; Lamin et al., 2021b; Lamrabet et al., 2023), and sv. vachelliae in *E. aridi* and *E. fredii* (Bouhnik et al., 2019; Hnini et al., 2023a) demonstrating that a given host can be colonised by bacteria that acquired their symbiotic competence horizontally rather than by descent from a common symbiotic ancestor. Second, the pangenome architecture of *Bradyrhizobium* strains from *R. dasycarpa* large accessory genome (39.41%), large strain-specific fraction (56.23%), small core genome (4.36%) is the signature of a population in which HGT is ongoing and extensive, and in which symbiosis islands behave as mobile genetic elements. Third, the Moroccan data themselves argue against a strict co-evolutionary model: the plant *Ononis repens* is not endemic to Morocco, and the bacterium *M. maamorense* is not restricted to Morocco (the Australian isolate *Mesorhizobium* sp. B2-1-1 shares ~ 98% ANI with the type strain ORM16), so a scenario of paired localised co-evolutionary divergence is untenable for this flagship example. We therefore propose that HGT generates the raw symbiotic diversity, and that soil pH, salinity, metal contamination and drought then sort which of these horizontally acquired partnerships persist at a given site.

This interpretation also accommodates the global context. The same symbiovars recur on the same host genera across the Mediterranean Basin and beyond (sv. retamae on *Retama* in Spain and Morocco; sv. mediterranense on *Retama* and *Lupinus*; sv. genistearum across Genisteae hosts in the Mediterranean and South America; Rogel et al., 2011; Martínez-Romero et al., 2024), consistent with frequent dispersal and HGT of symbiosis loci followed by local ecological filtering. Whether the particularly high symbiovar richness observed in Morocco reflects genuinely elevated HGT rates under strong abiotic selection, or simply denser sampling, cannot be resolved from the current data; both possibilities are compatible with the evidence reviewed here, and we advance them as hypotheses rather than conclusions.

3. Functional ecology of NREs

The functional ecology of Moroccan NREs requires dedicated investigation. The current evidence base, while compelling, rests almost exclusively on culture-dependent isolation and single-season greenhouse trials. The NRE community structure documented by Benjelloun et al. (2019) in chickpea provides a baseline, but no amplicon-sequencing or metagenomic characterisation of Moroccan nodule NRE

communities has been published. Transcriptomic analyses of tripartite plant–rhizobia–NRE interactions, which have begun to illuminate the molecular dialogue between these partners in model systems (Hassen et al., 2025; Menéndez and Brígido, 2025), are entirely absent for Moroccan legume systems. This represents the single largest functional knowledge gap identified by this review.

4. Limitations

This review has several limitations that should be acknowledged. First, the synthesis is necessarily constrained by the primary literature's heavy reliance on culture-dependent methods: the "culturability bias" means that fastidious or slow-growing nodule inhabitants are systematically underrepresented in the documented diversity. Second, research effort is geographically uneven: Atlantic Morocco (Rabat–Casablanca corridor, Maamora forest, Meknes–Fez) accounts for the majority of published studies, while southern Morocco, the Anti-Atlas, and the Draa–Tafilalet regions are severely understudied. Third, the heterogeneous molecular methods across studies ranging from 16S rRNA-only characterisation to full MLSA and whole-genome sequencing limit cross-study comparability. Fourth, quantitative outcomes (biomass gains, yield increases) are reported as described by primary authors; direct comparison across studies is confounded by differences in experimental design, soil type, plant variety, and inoculant concentrations.

5. Agricultural and environmental implications

The agricultural and environmental implications of this body of work are substantial. Indigenous Moroccan rhizobia displaying remarkable abiotic-stress tolerance (substantially exceeding that of current commercial inoculants) are available candidates for next-generation biofertilizers tailored to the increasingly harsh conditions projected under climate change for the Maghreb. The convergence of metal-tolerant legumes (*Retama* spp., *Sulla spinosissima*, *Astragalus armatus*) with heavy-metal-resistant rhizobia provides a practically available, ecologically sound strategy for phytostabilisation of Morocco's numerous abandoned mining sites without costly soil remediation. Multi-year, multi-site field validation of the most promising strain–host combinations identified in greenhouse trials is the most urgent translational research priority.

The pangenome architecture revealed by Lamrabet and Missbah El Idrissi (2025) small core genome (4.36%), large accessory (39.41%) and strain-specific (56.23%) fractions provides a genomic mechanism for adaptability rather than co-evolutionary specialisation: HGT of symbiotic gene clusters allows rapid host-range acquisition without disrupting the chromosomal background, while strain-specifgenes encode fine-tuned adaptations to local soil environments. In this framework, the promiscuity of *Retama* which is nodulated by *Bradyrhizobium*, *Microvirga* and *Ensifer* carrying non-sister *nodC* lineages (sv. retamae, sv. mediterranense and sv. vachelliae, respectively; Mouad et al., 2020; Lamin et al., 2019, 2021b) reflects ecological sorting by soil pH and contamination status rather than a shared co-evolutionary history. Non-canonical partnerships further illustrate the flexibility of host-range constraints.

The simultaneous nodulation of *Colutea arborescens* by three rhizobial genera (Ourarhi et al., 2011), the extension of *Microvirga tunisiensis* host range from Australasian to North African Genisteae legumes (Mouad et al., 2020), and the reciprocal incompatibility between *Hedysarum* rhizobia documented by Glatzle et al. (1986) collectively suggest that the boundaries of host-range specificity are actively being tested and expanded in Morocco's heterogeneous environments.

Research gaps and priority directions

1. The endemic Fabaceae gap

Despite three decades of productive research, only about 25–30 of Morocco's more than 100 endemic Fabaceae taxa fewer than a third of the endemic flora (Fennane and Ibn Tattou, 1998; Rankou et al., 2013) have been investigated for rhizobial associations. Genera of immediate research priority include *Argyrocytisus battandieri*, *Hesperolaburnum platycarpum*, *Ononis mogadorensis*, *Lotus maroccanus*, *Genista ifniensis*, *Astragalus maroccanus*, *Adenocarpus*, and *Argyrolobium* species many restricted to montane or Saharan habitats yet entirely uninvestigated for rhizobial associations.

2. Seven structural research priorities

Seven critical gaps define the priority research agenda for Moroccan rhizobial science (Fig. 5):

1. **Culture-independent metagenomics.** All published Moroccan nodule diversity data derive from culturability-biased isolation. 16S rRNA V3–V4 amplicon sequencing and shotgun metagenomics of nodule communities are essentially absent and represent the single highest-impact methodological investment.
2. **NRE functional characterisation.** Taxonomic identity of Moroccan NREs is documented for chickpea, *Sulla*, common bean, and *Vachellia*, but tripartite co-inoculation experiments and transcriptomic analyses of plant–rhizobia–NRE interactions remain absent.
3. **Whole-genome sequencing pipeline.** The successful description of *M. maamorense* sp. nov. (Alami et al., 2026) and comparative genomics of Bradyrhizobium–R. dasycarpa (Lamrabet and Missbah El Idrissi, 2025) provide a template for systematic genome-based species characterisation of other novel Moroccan lineages.
4. **Geographic coverage.** Research is concentrated in Atlantic Morocco, the Meknes–Fez corridor, and eastern Morocco. Southern Morocco, the Anti-Atlas, and the Draa–Tafilalet regions remain severely understudied.
5. **Longitudinal field dynamics.** No studies have examined how rhizobial community composition shifts seasonally or in response to changing land-use practices in Moroccan agroecosystems. Multi-year field trials are essential for inoculant validation, regulatory approval, and farmer adoption.
6. **Pesticide–BNF interactions.** Beyond Mohamed et al. (2021) on glyphosate and paraquat, pesticide impacts on indigenous rhizobial populations remain largely uninvestigated. Future biofertilizer

development programmes must integrate pesticide-compatibility screening as a standard criterion for strain selection.

7. **Moroccan rhizobial culture collection.** Establishment of a curated, cryopreserved Moroccan rhizobial culture collection linked to georeferenced soil metadata is an urgent conservation priority; the formal deposition of *M. maamorense* sp. nov. (ORM16^T = DSM 120599^T = CCMM B1359^T) is a first step.

Agricultural and environmental applications

1. Biofertilizer development

The exceptional abiotic-stress tolerance of Moroccan indigenous rhizobia positions them as high-priority candidates for next-generation biofertilizer development. Field-validated outcomes include: lentil biomass gains of 38% under well-watered conditions and maintained productivity under drought through *R. laguerreae* + PGPB co-inoculation (Taha et al., 2022); chickpea grain yield increases of 242% in phosphate-deficient soils through *M. ciceri* + phosphate-solubilising *Bacillus* sp. and *Enterobacter aerogenes* (Benjelloun et al., 2021); *V. faba* P uptake equivalent to triple superphosphate through rock phosphate-solubilising *Rhizobium* strains (Maghraoui et al., 2016); and alfalfa root dry weight increases of 195% under water stress through multi-species PGPB consortia (Raklami et al., 2024). The integration of AMF further amplifies benefits: M'saouar et al. (2024) achieved 153%/144% increases in *Sulla flexuosa* shoot and root biomass through AMF + *R. sullae* dual inoculation, while Wahbi et al. (2016) demonstrated that mycorrhizal networks can mediate transfer of ~ 50% of biologically fixed N from faba bean to companion wheat.

2. Phytostabilisation of contaminated soils

The convergence of metal-tolerant legumes (*Retama* spp., *Sulla spinosissima*, *Anthyllis vulneraria*, *Astragalus armatus*) with heavy-metal-resistant rhizobia represents a practically available, ecologically sound strategy for phytostabilisation of Morocco's numerous abandoned mining sites. Three model legume–microsymbiont phytostabilisation systems are now documented: (i) *Retama monosperma* + *E. aridi* (Lamin et al., 2019); (ii) *Robinia pseudoacacia* + *E. meliloti* sv. rigiduloides (Alami et al., 2024); and (iii) *Astragalus armatus* + *Mesorhizobium* sp. sv. hedysari (Alami et al., 2025b).

3. Climate adaptation

Under climate change projections for Morocco and the Maghreb increased drought frequency, rising temperatures, expanding aridity sustaining BNF requires microsymbionts capable of functioning under substantially more severe conditions than current commercial inoculants tolerate. The comparative genomics of *Bradyrhizobium* strains from *R. dasycarpa* (Lamrabet and Missbah El Idrissi, 2025) provides a genome-level foundation for identifying stress-responsive gene repertoires enabling survival at high

temperature and low water potential a blueprint for targeted strain selection for climate-smart biofertilizers.

Conclusions

Morocco constitutes a globally significant hotspot for the diversity, ecology, and applied potential of legume-nodulating bacteria. This comprehensive systematic review directly addresses the four aims stated in the Introduction. (i) At least seven rhizobial genera and twelve symbiovars are documented across 293 retained studies, including the newly formally described *Mesorhizobium maamorense* sp. nov. anchoring sv. maamori and a new sv. hedysari record for *Astragalus armatus*; numerous candidate novel species have been identified across *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, and *Ensifer*. (ii) Soil pH, salinity, heavy-metal contamination, and drought are identified as the primary selective forces shaping rhizobial and NRE community composition, with indigenous strains displaying remarkable abiotic-stress tolerance that exceeds commercial inoculant benchmarks. (iii) The Moroccan evidence is best explained by HGT-driven host-range expansion followed by ecological sorting, not by strict co-evolutionary divergence: neither the flagship host (*Ononis repens*) nor its microsymbiont (*M. maamorense*) is restricted to Morocco, and symbiovars recur across chromosomally unrelated genera. (iv) Seven priority research directions are identified, and the agricultural, phytostabilisation, and climate-adaptation applications of Moroccan rhizobial diversity are synthesised.

Two findings demand immediate research action. First, the endemic *Fabaceae* gap fewer than a third of Morocco's endemic legume flora investigated must be systematically addressed through targeted field surveys leveraging next-generation sequencing. Second, the functional ecology of NREs in Moroccan legumes requires dedicated tripartite co-inoculation experiments and transcriptomic analyses. Addressing these priorities will unlock the full biotechnological potential of Morocco's rhizobial heritage for sustainable agriculture, land restoration, and climate-smart food systems across the Maghreb and the broader Mediterranean Basin.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Mohamed Hnini: Conceptualisation, literature search, data extraction, writing—original draft, writing—review & editing.

Data availability

All data supporting this review are available within the article and its supplementary materials. Raw data underlying Supplementary Figures S1–S5 and Supplementary Table S1 are available from the corresponding author upon reasonable request.

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Figures

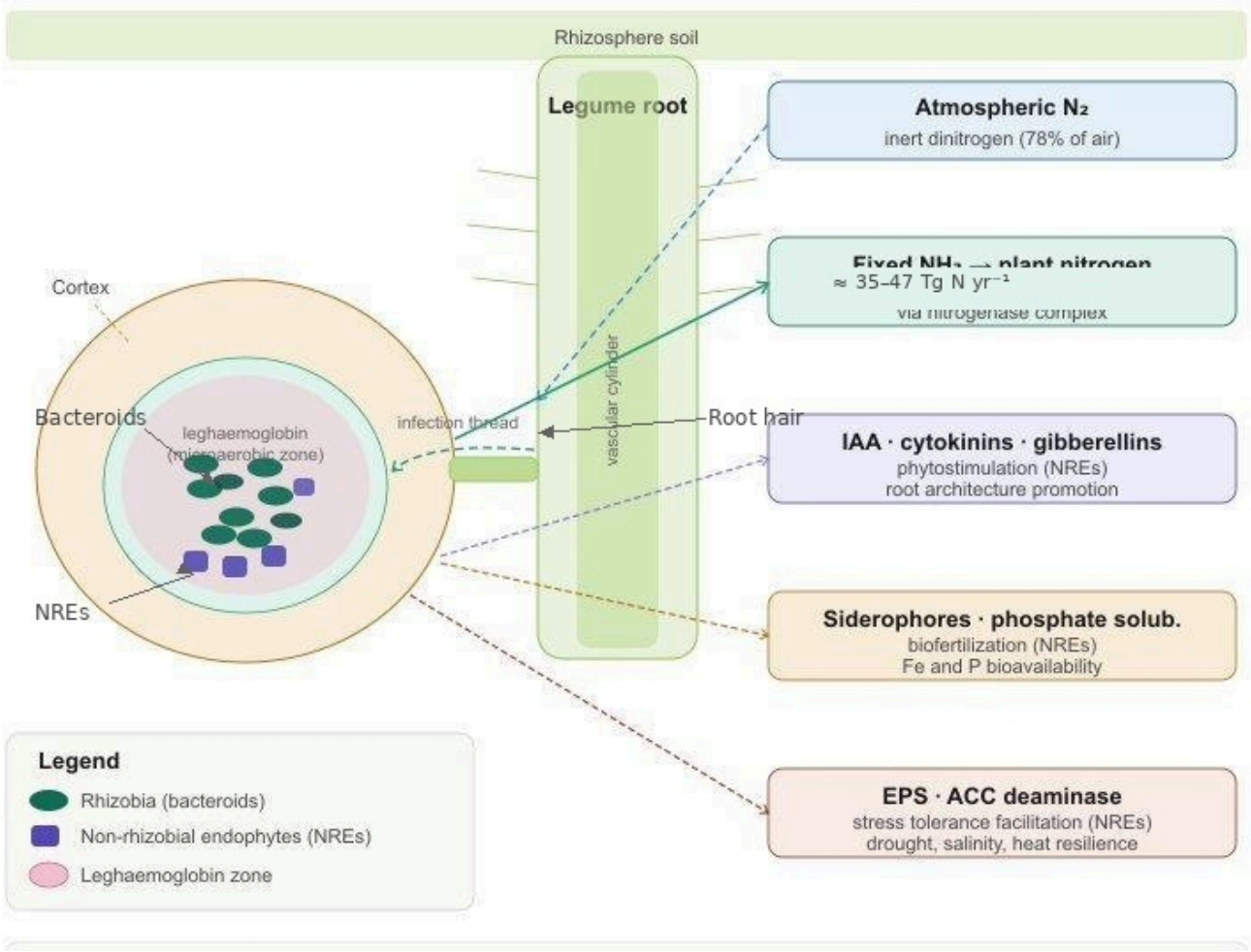

Figure 1

The legume nodule ecosystem: rhizobia and non-rhizobial endophytes. The legume root forms root nodules through molecular signalling with soil rhizobia entering via infection threads. Inside the nodule, rhizobia differentiate into bacteroids (green ellipses; position labelled) within a leghaemoglobin-maintained microaerobic zone, fixing atmospheric N_2 into NH_3 (global agricultural inputs ≈ 35–47 Tg N yr^{-1}; Herridge et al., 2008). Non-rhizobial endophytes (NREs; purple squares; position labelled) co-inhabit the nodule, contributing phytostimulation, biofertilization, and stress-tolerance functions through plant growth-promoting (PGP) traits. The root hair and infection-thread entry point are also indicated.

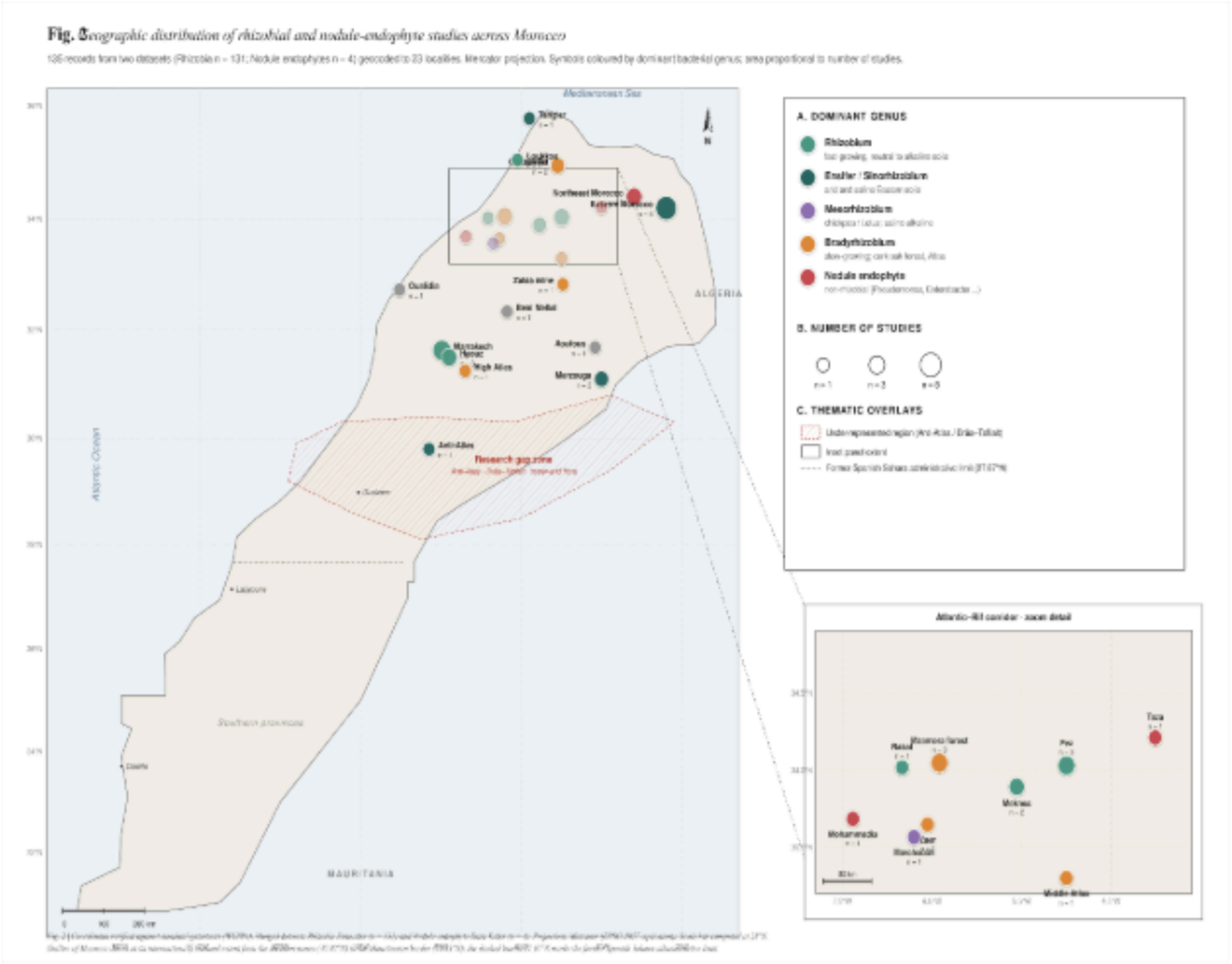

Figure 2

Geographic distribution of rhizobial diversity studies across Moroccan Fabaceae. Circles mark documented research localities at their real geographic coordinates (Mercator projection), colour-coded by dominant rhizobial genus (Rhizobium: mint green; Ensifer: teal; Mesorhizobium: purple; Bradyrhizobium: orange). The hatched red zone marks the under-represented southern Morocco region (Anti-Atlas, Draa–Tafilalet). Zoom inset (bottom-left) expands the dense Atlantic–Rif corridor (Rabat, Maamora, Meknes, Fez, Taza, Casablanca).



Figure 3

Simplified consensus phylogenetic cladogram of rhizobial species documented in Moroccan Fabaceae, based on MLSA of housekeeping genes (recA, atpD, glnII, gyrB, rpoB) and symbiotic gene (nodC, nifH) phylogenies. One box is shown per species (or genospecies), so that no taxa are conflated as synonyms. Five Alphaproteobacterial genera (Rhizobium, Ensifer, Mesorhizobium, Bradyrhizobium, Microvirga) and one Betaproteobacterial genus (Burkholderia, dashed) are represented. Ensifer melilotiincludes the later heterotypic synonym E. kummerowiae(Menéndez et al., 2024). Novel symbiovars proposed from Moroccan material (sv. aridi, sv. maamori) and novel species (Bradyrhizobium sp. nov. ×4) are highlighted. Symbiovar assignments follow the guidelines of Martínez-Romero et al. (2024). Branch lengths are schematic only.

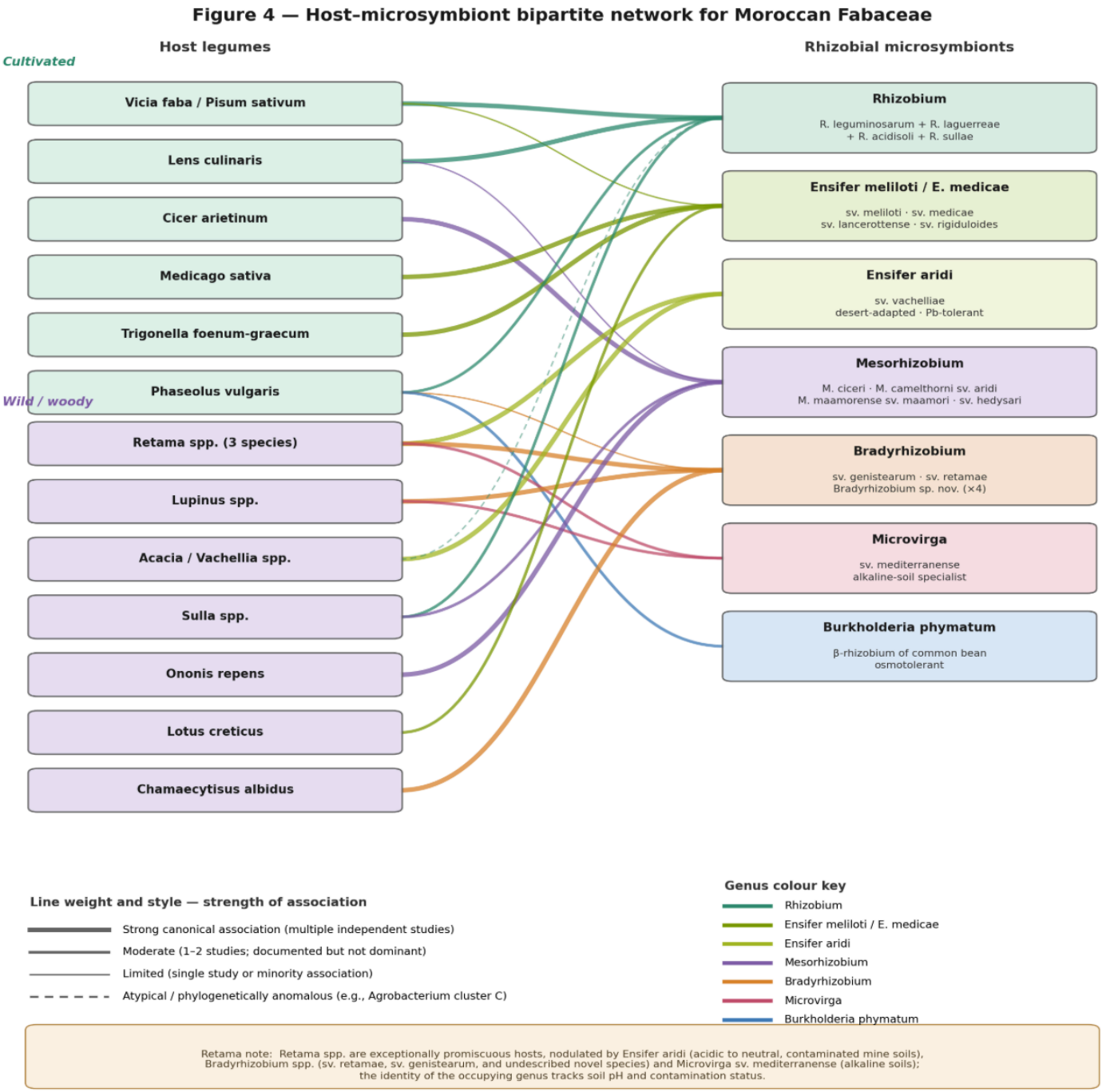

Figure 4

Host–microsymbiont bipartite network for Moroccan Fabaceae. Left column: host legumes (cultivated in teal; wild/woody in purple). Right column: rhizobial genera/groupings (one box per genus, with two-line subtitles). Connecting lines are colour-coded by rhizobial genus and weighted by association strength. The promiscuity of Retama spp. nodulated by three genera (Bradyrhizobium, Microvirgaand Ensifer) carrying divergent nodC lineages (sv. retamae, sv. mediterranense and sv. vachelliae, respectively), with the realised partnership sorted by soil pH and contamination status is annotated in the full-width band at the bottom.

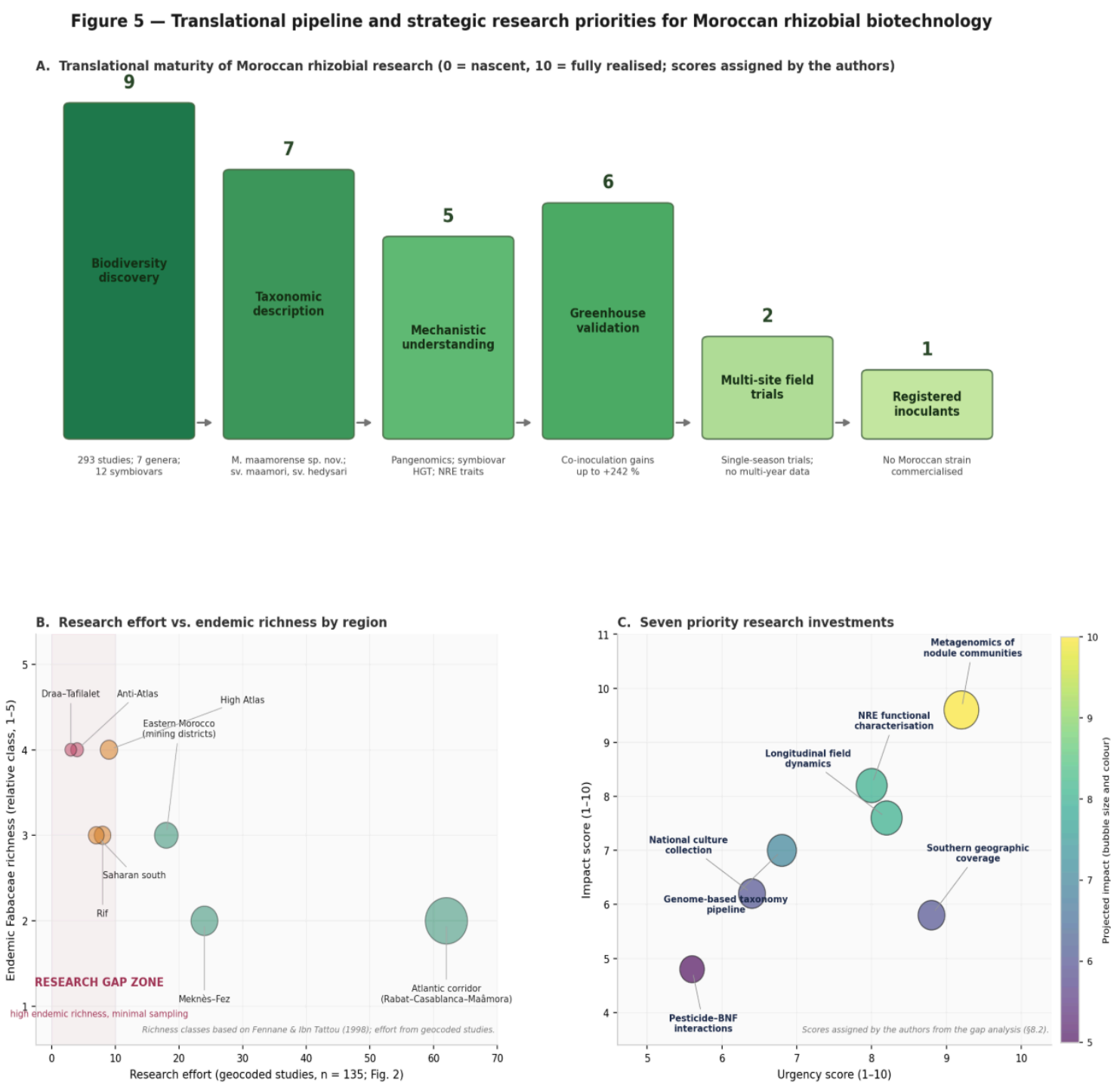

Figure 5

Translational pipeline and strategic research priorities for Moroccan rhizobial biotechnology. (A) Translational maturity score (0–10, author-assigned) across the discovery-to-application pipeline. (B) Research effort (number of geocoded studies, of 135 with locality data) vs. endemic legume richness by region (relative classes 1–5 based on Fennane and Ibn Tattou, 1998), showing critical gaps in Anti-Atlas and Draa–Tafilalet; the red zone marks regions with high endemism but low research effort. (C) Seven priority research investments plotted by urgency and impact scores (author-assigned); bubble size = projected impact.