

**IMPLICATIONS OF THE FABACEAE FAMILY PRESENCE ON NITROGEN CONTENT IN THE ENVIRONMENT: A SYSTEMATIC REVIEW****Gecelene Estorico**

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**ABSTRACT**

Nitrogen is a critical nutrient that regulates ecosystem productivity and drives essential processes within the nitrogen cycle, with the Fabaceae family, commonly known as legumes, playing a central role in environmental nitrogen dynamics. This systematic review examines the implications of Fabaceae species on environmental nitrogen content and nitrogen cycling across diverse ecosystems. A total of 10 selected studies were analyzed using a thematic approach to identify patterns in biological nitrogen fixation, nitrogen transformation, and distribution under varying environmental conditions, plant species, and ecosystem types. The findings indicate that Fabaceae species significantly contribute to nitrogen inputs through symbiotic nitrogen fixation, enhancing soil fertility and supporting plant productivity in both agricultural and natural systems. Specific results include contributions ranging from 7.12 to 61.93 kg N ha<sup>-1</sup> yr<sup>-1</sup>, accounting for up to 94% of soil nitrogen variation in some ecosystems. In mixed cropping systems, 15–18% of plant nitrogen was derived from atmospheric fixation, while biological nitrogen fixation increased by 10–42.55% under optimized conditions with appropriate nutrient management. However, increased nitrogen availability also resulted in environmental impacts, including nitrate concentrations rising by more than 200% in certain water systems and nitrous oxide emissions ranging from 3.29 to 3.78 kg N<sub>2</sub>O–N ha<sup>-1</sup>. These results demonstrate that Fabaceae species play a dual role as both enhancers of nutrient availability and contributors to environmental nitrogen imbalances. Understanding these dynamics is essential for developing sustainable management strategies that maximize the ecological benefits of legumes while minimizing potential risks to soil, water, and broader ecosystem health. This knowledge supports agricultural planning, ecosystem restoration, and environmental conservation initiatives in areas dominated by Fabaceae species.

**Keywords:**

Fabaceae family, legumes, nitrogen cycle, environmental nitrogen.

**INTRODUCTION**

Nitrogen cycling is the geochemical process that regulates the movement of nitrogen between the atmosphere, biosphere, and geosphere in different forms (Thompson, 2026). Nitrogen is an important nutrient for maintaining life on Earth, for it is a component of essential biomolecules such as amino acids, proteins, nucleic acids, and chlorophyll. Furthermore, plants are the proponents of the nitrogen cycle; they assimilate nitrogen from the soil in the form of nitrate, ammonium ions, and available amino acids from organic sources (Zayed et al., 2023). However, too much nitrogen can lead to excessive leaf growth, which hinders flower and fruit propagation (Lewis, 2025). As a result, the stems of the plants are weak and are susceptible to diseases, which will delay maturity of the plant. This systematic review highlights the role of the Fabaceae plant species on the nitrogen cycle in the environment and its implications on their local ecosystems.

The Fabaceae family, also known as legumes, have a symbiotic relationship with *Rhizobium spp.*, *Bradyrhizobium spp.*, and other nitrogen-fixing bacteria (Grabowski, 2015). These species are responsible for

taking nitrogen from the air, and then fixing it into a form usable by legumes. Through the decomposition of specialized structures on the plant's roots called nodules, the fixed nitrogen will be released and become available for use in nearby plant communities. Usually, the nitrogen content is dependent on the species of legume, plant stand, growing conditions and management of stand. While the range of nitrogen fixation varies from species to species, legumes are still valuable as rotational plants as a natural source of nitrogen.

This systematic review has one main objection; it aims to synthesize the data involving the interactions between different leguminous species and nitrogen cycling in the environment from different studies. This will include the species or system of legumes, the ecosystem, the nitrogen cycles step affected, role of the aforementioned bacteria, and to ascertain whether legumes, in general, are beneficial to their respective ecosystems. While the data is heterogenous, the review will still assess the general effect of varying leguminous species based on different conditions.

Studies on the legumes and nitrogen fixation exist, but they are mostly focused on the nitrogen cycle, instead of the environmental nitrogen content. Moreover, most studies offer only laboratory data, not in-site reports and observations of legumes in their natural habitat. Thus, this review offers a consolidated analysis of data from different ecosystems and countries. These findings may serve as a guide for the agricultural or horticultural sector, so that these departments may ascertain if high legume density is beneficial or detrimental to their ecosystems.

### **METHODOLOGY**

This systematic review follows the guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA). PRISMA provides the framework for systematic reviews. Thus, the methods employed in the review are reproducible, efficient, and most importantly, transparent. The methodology section discusses the search strategy, inclusion and exclusion criteria, data analysis method, study area, data sources, sampling procedure, and data extraction.

#### *a. Search Strategy*

Google Scholar and ScienceDirect were the academic databases utilized in the review for their extensive collection of studies, particularly in the field of environmental science and ecology. The researchers employed the following search string with boolean connectors: ("nitrogen fixation" OR "N<sub>2</sub> fixation" OR "symbiotic nitrogen fixation" OR "free-living nitrogen fixation") AND (legume\* OR Fabaceae\*) AND ("soil nitrogen" OR "nitrogen content"). Hence, the search was limited to studies that explored the correlation between environmental nitrogen, nitrogen cycling, and legume plants. Furthermore, the publication dates of the reviews must be between January 01, 2015 and December 31, 2025.

#### *b. Inclusion/Exclusion Criteria*

In order of importance, the following conditions were set: (1) The studies must be published from 2015 to 2025 to ensure relevancy; (2) must involve species from the Fabaceae family, or legumes, and their role in the geochemical process, nitrogen cycling; (3) must be a scientific article from a reviewed database or a scientific review; (4) must have the information on species/system, ecosystem, nitrogen process, distribution of plants, or effects on environmental nitrogen content; and (5) must be written in English.

Studies that violate these prerequisites were excluded to narrow the scope of this systematic review. Multiple works were rejected because they (1) were not scientific articles or reviews, (2) did not focus on leguminous nitrogen-fixing organisms, (3) have insufficient information based on the parameters set in the inclusion criteria, or (4) were published outside the data range of January 01, 2015 to December 31, 2025.

#### *c. Study Selection Process*

A total of 25,640 articles were collected from the database. However, due to the large number of results, only the first 50 results from each data source sorted by relevance were screened, reducing the number to 100 articles. Afterward, duplicates were filtered out, reducing the number to 92. Furthermore, after screening the articles using the inclusion/exclusion criteria, 38 articles remain included. The researchers read through each abstract of the articles. In the end, only 10 articles were chosen.

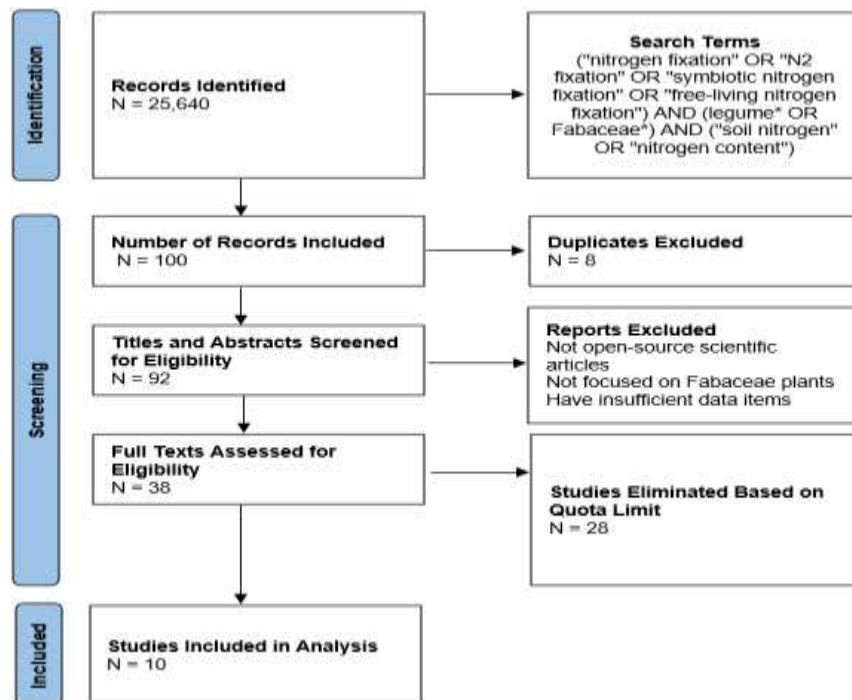


Figure 1: Flow Chart of Study Selection Process using PRISMA Method

d. *Study Area*

This systematic review focuses on the Fabaceae species in the continents of Asia, Europe, South America, and Oceania that are able to undergo nitrogen fixation. Studies in Asia and Europe typically consist of experiments with controlled ecosystems that directly assessed the relationship between legumes and nitrogen fixation. Meanwhile, South America and Oceania were observational pieces that did not affect the plants' ecosystem in any significant matter. Since the review aims to synthesize data on nitrogen cycling caused by legumes in various ecosystems, the chosen study areas were diversified.

e. *Data Items and Extraction*

In the 10 chosen articles, specific pieces of information were gathered to sustain congruence among the included studies. The extracted data included (1) legume species/system, (2) country of origin and ecosystem, (3) affected nitrogen process, (4) distribution of plants, and (5) the findings. The data items were collated in a Google Sheet where the researchers reviewed each article for accuracy.

f. *Synthesis of Data*

Because of the heterogeneity among the results of the included studies in terms of ecosystem type, experimental design, nitrogen measurements, and plant species examined, a quantitative meta-analysis was not conducted. This recorded variability prevented the comparison of effect sizes across the chosen studies. Instead, a thematic synthesis was employed to consolidate the findings. Although many of the included studies included quantitative results, these data were analyzed within conceptual themes in relation to the nitrogen cycling processes. This process was done to identify recurring patterns, contradictions, and implications across different environments. The approach allowed the review to synthesize the heterogeneous quantitative evidence while preserving the connections between the different studies

## RESULTS AND DISCUSSION

Table 1: Summary of Findings

Fabaceae Species/System	Country - Ecosystem	Affected Nitrogen Process & Content	Coverage/ Distribution of Plants	Findings	Author/Year
<i>Pueraria lobata</i>	Japan - Highway roadside embankment (Urban area)	Denitrification (NO <sub>x</sub> mitigation)   - 2% to 33% NO <sub>x</sub> reduction rate	Dense canopy Height: 1.0 - 1.5 meters	Kudzu acts as a vegetation barrier for NO <sub>x</sub> and O <sub>3</sub> along the highway roadside during the growing season.	Katata et al. (2023)
<i>Arachis hypogaea</i> inoculated with <i>Bradyrhizobium yuanmingense</i>	China - Controlled pot experiment	Biological nitrogen fixation (BNF)   BNF increased by 10.07–42.55% under 15 kg·hm <sup>-2</sup> N vs higher N treatments	Plastic pots (30 cm diameter × 28 cm height; 3.2 dm <sup>3</sup> volume), 2 plants per pot	External nitrogen itself is a promoter of nitrogen fixation; lower applications of nitrogen fertilizer resulted in better rates of nitrogen fixation.	Liu et al. (2023)
<i>Ulex europaeus</i>	New Zealand - Lowland freshwater catchments	Biological nitrogen fixation and nitrification   >200% higher nitrate-N concentrations in gorse streams	Dense monoculture canopy; nitrate increased markedly when gorse cover reached ~22–28% of catchment area; catchment sizes 7–13 km <sup>2</sup>	Invasive N-fixing gorse is a major nitrate source to streams; nitrate export is driven by decomposing N-rich biomass rather than soil uptake	Stewart et al. (2018)
<i>Phaseolus vulgaris</i> cultivated with <i>Cicer arietinum</i> and <i>Sorghum bicolor</i>	Switzerland - Greenhouse pot experiment	Biological nitrogen fixation and soil N uptake   15–18% N derived from atmosphere (%Ndfa)	Mixture with <i>Cicer arietinum</i> (chickpea); biomass per plant 13.4 g (variety 1) & 12.4 g (variety 2); ~62.5% higher than monoculture	Nitrogen contribution in crop mixtures depended strongly on species identity and neighboring plants.	Singh et al. (2023)

Legumes mix	<i>Agricultural agro-ecosystems, often phosphorus-deficient soil</i>	Nitrogen fixation   Adequate P supply increases nodulation, nitrogenase activity, and total plant N; P deficiency decreases N content by inhibiting SNF; up to ~70% of soybean N derived from SNF.	Soybean widely cultivated globally; other legumes cultivated worldwide or used as model species	Efficient SNF is strongly dependent on phosphorus nutrition; legumes show coordinated N–P interaction via P transporters, signaling cross talk, and nodule P homeostasis; improving P acquisition and allocation enhances SNF and plant N nutrition	Zhong et al. (2022)
<i>Pueraria lobata</i>	China - Abandoned subtropical agricultural slopes	Nitrogen fixation   Contributed to total N <sub>2</sub> fixation of 7.12–61.93 kg N ha <sup>-1</sup> yr <sup>-1</sup> (avg. 22.93 ± 6.52 kg N ha <sup>-1</sup> yr <sup>-1</sup> ); N <sub>2</sub> fixation explained ~94% of soil N variation and 58% of soil NO <sub>3</sub> <sup>-</sup> variation	10x10 m plot where >80% but low density (~10 plants plot) with nodules mostly in 0–5 cm soil layer	Nitrogen fixation by legumes is a major source of nitrogen for the environment.	Zhou et al. (2021)
<i>Arachis pintoi</i> mixed pasture with <i>Urochloa decumbens</i>	Brazil - Tropical pasture ecosystem	Decomposition   Litter N concentration 8.3–12.9 g N kg <sup>-1</sup> ; litter deposition up to 77.2 kg ha <sup>-1</sup> day <sup>-1</sup>	Mixed pasture with <i>U. decumbens</i> ; planting spacings 0.40–0.80 m; up to 39% C3 (peanut) contribution to litter	Inclusion of forage peanut significantly raised N concentration of litter and litter deposition rates, especially at narrow spacings, improving organic N return and nutrient cycling	Cardoso et al. (2025)
<i>Medicago sativa</i> (alfalfa) root nodules	Brazil - Temperate agricultural soils	Nitrogen fixation & Denitrification   Soil N increased due to residue incorporation; N <sub>2</sub> O emissions 3.29–3.78 kg N <sub>2</sub> O-N ha <sup>-1</sup> ; 0.58–0.72% of added N lost as N <sub>2</sub> O	Residue N input varied 78–422 kg N ha <sup>-1</sup> ; fraction of N derived from fixation (%Ndfa) 23–85%	Alfalfa root nodules release significant N <sub>2</sub> O through rhizobial denitrification, showing that legume distribution and activity directly	Sant'Anna et al., (2018)

influence NOx-related emissions and environmental nitrogen cycling

<i>Medicago sativa</i> (alfalfa) + <i>Ensifer meliloti</i> symbiosis	Jordan and Israel - <i>Controlled soil/greenhouse conditions (temperate agricultural system)</i>	Nitrogen fixation & Denitrification   N <sub>2</sub> O emissions varied with nitrate/flooding; nap <sup>+</sup> strain increased emissions ~6.7× vs WT; nap <sup>-</sup> strain lowered emissions ~3.5× vs WT	Alfalfa plants inoculated with <i>E. meliloti</i> strains grown with nitrate treatments (0–10 mM KNO <sub>3</sub> ) and flooding.	Alfalfa root nodules produce N <sub>2</sub> O gas in response to nitrate and hypoxia. Overexpression of <i>E. meliloti</i> Nap increased nodule competitiveness and raised N <sub>2</sub> O emission rates, showing that symbiotic denitrification by rhizobia in root nodules contributes to environmental nitrogen loss. Copper availability also modulated enzymatic activities and N <sub>2</sub> O emissions.	Pacheco et al. (2023)
<i>Nodulating Fabaceae</i> (e.g., <i>Medicago</i> , <i>Lotus</i> , <i>Trifolium</i> , <i>Acacia</i> , <i>Mimosa</i> ) vs. <i>non-nodulating Fabaceae</i>	Australia - <i>Grasslands, agricultural systems, tropical and semi-arid forests, disturbed habitats.</i>	Biological nitrogen fixation   <i>Nodulating species show consistently higher leaf and seed N (leaf ≈2–5%; seed ≈6–7%) than non-nodulating Fabaceae (seed ≈2–3%).</i>	<i>Nodulating species widespread globally; non-nodulating Fabaceae more common in N-rich tropical forests</i>	Evolution of nodulation is associated with high tissue nitrogen, high-protein seeds, improved growth and stress tolerance; repeated loss of nodulation linked to high soil N availability.	Mathesius (2022)

The summary of findings shows that Fabaceae species consistently play a major role in increasing environmental nitrogen through biological nitrogen fixation across different ecosystems. Several studies demonstrate that legumes significantly enhance soil nitrogen availability, with fixation rates reaching up to 7.12–61.93 kg N ha<sup>-1</sup> yr<sup>-1</sup> and contributing as much as 70% of plant nitrogen under favorable conditions. In

agricultural systems, species such as *Arachis hypogaea* and *Phaseolus vulgaris* improve nitrogen supply and reduce the need for synthetic fertilizers, especially when supported by proper nutrient conditions like phosphorus availability. Additionally, some species, such as *Pueraria lobata*, contribute to air quality improvement by reducing nitrogen-related pollutants, while others enhance nutrient cycling through litter deposition and decomposition.

However, the findings also reveal that the effects of Fabaceae species are not entirely beneficial, as increased nitrogen inputs can lead to environmental consequences. For example, *Ulex europaeus* significantly raises nitrate concentrations in nearby water systems, indicating a risk of water pollution, while species like *Medicago sativa* contribute to nitrous oxide emissions through microbial processes such as denitrification. These results highlight that the impact of legumes varies depending on species, environmental conditions, and ecosystem type. Overall, the evidence supports the conclusion that Fabaceae species have a dual role in the nitrogen cycle, acting as both enhancers of soil fertility and potential contributors to nitrogen loss and environmental imbalance.

#### **A. Biological Nitrogen Fixation in Fabaceae as a Key Driver of Nitrogen Cycling**

Nitrogen fixation is the biological process through which atmospheric nitrogen ( $N_2$ ), a chemically stable gas that remains inaccessible to most organisms, is converted into biologically usable nitrogen forms such as ammonia ( $NH_3$ ). This conversion depends on specialized microorganisms that possess the enzyme nitrogenase. Among terrestrial ecosystems, one of the most consequential forms of biological nitrogen fixation occurs through the symbiotic relationship between leguminous plants of the family Fabaceae and nitrogen-fixing bacteria called *rhizobia*. These bacteria colonize plant roots and establish nodules within which nitrogen fixation takes place. In this partnership, the plant provides carbohydrates and energy to the bacteria, while the bacteria in turn convert atmospheric nitrogen into ammonia that the plant can incorporate into its tissues.

Within the broader nitrogen cycle, biological nitrogen fixation is widely recognized as one of the most important pathways through which new nitrogen enters ecosystems. Given that nitrogen frequently limits plant productivity, the presence of nitrogen-fixing plants can profoundly shape soil fertility, ecosystem productivity, and overall nutrient availability. The studies synthesized here collectively demonstrate that nitrogen fixation associated with Fabaceae functions as a major ecological mechanism regulating nitrogen availability across agricultural systems, grasslands, forests, and disturbed environments.

The thematic synthesis of the sources reviewed reveals a consistent pattern: symbiotic nitrogen fixation by legumes substantially contributes to nitrogen inputs, modifies nitrogen cycling processes, and influences both ecosystem productivity and broader environmental nitrogen dynamics.

##### **a. Nitrogen Fixation as a Major Source of Nitrogen Input in Ecosystems**

Across the reviewed studies, symbiotic nitrogen fixation consistently emerges as a dominant mechanism through which nitrogen is introduced into ecosystems. This biological input compensates for nitrogen limitations in soils and can substantially reduce dependence on synthetic nitrogen fertilizers in agricultural systems.

In controlled experiments examining peanut (*Arachis hypogaea*), inoculation with the rhizobial bacterium *Bradyrhizobium yuanmingense* led to significant enhancement of biological nitrogen fixation. The study reported increases in biological nitrogen fixation ranging from 30.07% to 42.55%, particularly under conditions where external nitrogen fertilizer inputs were reduced (Liu et al., 2023). These findings indicate that symbiotic relationships between legumes and rhizobia are capable of supplying a substantial proportion of plant nitrogen requirements. The results further suggest that rhizobial inoculation may improve nitrogen use efficiency in agricultural production systems.

Research on legume-based cropping systems similarly demonstrated that symbiotic nitrogen fixation contributes measurable proportions of plant nitrogen. Experiments involving *Phaseolus vulgaris* grown alongside other crop species showed that approximately 15–18% of plant nitrogen was derived from atmospheric nitrogen, as indicated by measurements of nitrogen derived from the atmosphere (%Ndfa) (Singh et al., 2023). This result confirms that symbiotic nitrogen fixation directly supports plant nutrition and contributes to soil nitrogen enrichment in mixed cropping systems.

Beyond agricultural contexts, nitrogen fixation by legumes plays a meaningful role in natural and recovering ecosystems as well. A study examining abandoned subtropical agricultural slopes dominated by *Pueraria lobata* found that biological nitrogen fixation contributed between 7.12 and 61.93 kg of nitrogen per hectare per year ( $kg\ N\ ha^{-1}\ yr^{-1}$ ) (Zhou et al., 2021). The same study reported that nitrogen fixation accounted for approximately 54% of variation in soil nitrogen concentrations and 58% of variation in soil nitrate levels within the studied

ecosystem. These findings underscore that nitrogen-fixing legumes can become dominant contributors to soil nitrogen accumulation during ecosystem recovery following land abandonment.

Taken together, these results establish that biological nitrogen fixation by Fabaceae represents a major mechanism for replenishing nitrogen in nitrogen-limited soils, spanning both managed agricultural systems and natural environments.

#### ***b. Environmental Regulation of Nitrogen Fixation***

While nitrogen fixation can provide substantial inputs to ecosystems, the efficiency and scale of this process are strongly conditioned by environmental factors and nutrient availability. Several of the reviewed studies emphasize that nutrient limitations, particularly phosphorus deficiency, significantly constrain symbiotic nitrogen fixation.

Research on legumes grown under phosphorus-deficient conditions demonstrated that phosphorus availability directly influences both nodulation and nitrogenase enzyme activity. Adequate phosphorus supply increased nodulation intensity and enhanced nitrogenase activity, thereby improving biological nitrogen fixation efficiency and increasing plant nitrogen accumulation (Zhong, 2022). Because nitrogen fixation demands substantial energy and metabolic resources, phosphorus limitations reduce the plant's capacity to sustain effective symbiotic interactions with rhizobia.

Soil moisture conditions also influence nitrogen transformations associated with nitrogen fixation. Studies examining alfalfa (*Medicago sativa*) in symbiosis with the bacterium *Ensifer meliloti* found that environmental stress, including flooding, altered microbial nitrogen metabolism within root nodules. Under these conditions, rhizobial denitrification processes increased nitrous oxide (N<sub>2</sub>O) emissions from root nodules (Pacheco et al., 2023). The study additionally reported that specific bacterial strains exhibited reduced N<sub>2</sub>O emissions compared with wild-type strains, indicating that microbial genetic characteristics shape nitrogen transformation pathways.

These findings collectively reinforce that nitrogen fixation is not an isolated biological process. Rather, it is strongly regulated by environmental conditions, nutrient availability, and the physiological characteristics of the microorganisms involved.

#### ***c. Ecosystem-Level Effects of Nitrogen Fixation on Nitrogen Cycling***

The introduction of biologically fixed nitrogen into ecosystems carries important consequences for nitrogen cycling more broadly. Nitrogen fixation increases soil nitrogen availability, which can in turn influence plant community composition, ecosystem productivity, and the export of nitrogen to surrounding environments.

One study examining populations of the invasive nitrogen-fixing shrub *Ulex europaeus* demonstrated that large concentrations of nitrogen-fixing plants can markedly increase nitrogen export from terrestrial ecosystems. In freshwater catchments where *Ulex europaeus* covered approximately 22–28% of the catchment area, nitrate concentrations in nearby streams increased to more than 200% of baseline levels (Stewart, 2018). This rise in stream nitrate was attributed to the elevated nitrogen inputs generated by biological nitrogen fixation within the vegetation.

Tropical pasture ecosystems incorporating the forage legume *Arachis pintoi* showed analogous patterns, with increased nitrogen cycling driven by plant litter deposition and subsequent decomposition. Nitrogen concentrations in deposited plant litter ranged between 8.3 and 12.9 g N kg<sup>-1</sup>, and litter deposition contributed substantially to nitrogen inputs within the pasture system (Cardoso et al., 2025). Through decomposition of this organic material, the fixed nitrogen becomes progressively available to other plants and soil microorganisms.

These findings demonstrate that nitrogen fixation not only supports the growth of nitrogen-fixing plants themselves but also shapes wider ecosystem nutrient dynamics, influencing soil nutrient pools, competitive interactions among plants, and the transport of nitrogen into aquatic systems.

#### ***d. Nitrogen Fixation and Nitrogen Loss Pathways***

While nitrogen fixation provides essential nitrogen inputs for plant growth, it can also contribute indirectly to nitrogen losses from ecosystems. Once fixed nitrogen enters the soil nitrogen pool, it may undergo microbial transformations such as nitrification and denitrification, resulting in the release of nitrogen gases to the atmosphere.

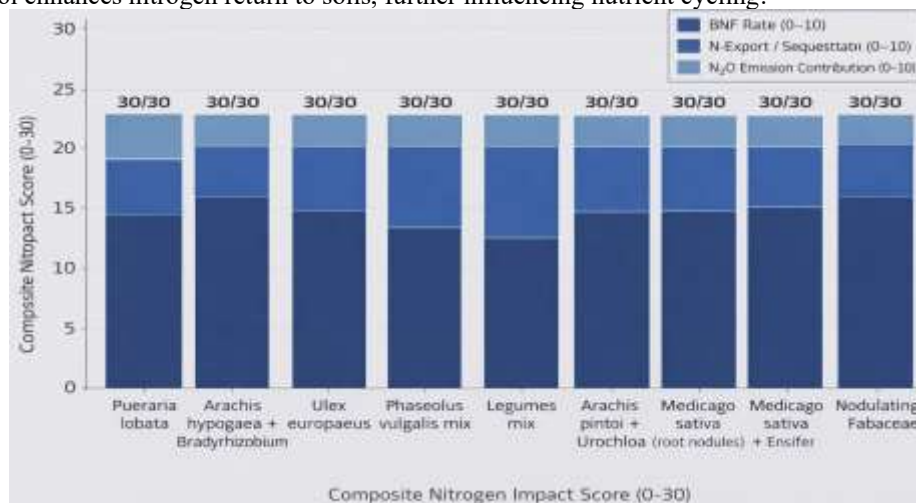
Research on alfalfa root nodules found that nitrogen derived from biological nitrogen fixation accounted for between 23% and 85% of the nitrogen involved in nitrous oxide (N<sub>2</sub>O) emissions from agricultural soils, with reported emissions ranging between 29 and 378 kg N<sub>2</sub>O–N ha<sup>-1</sup> depending on soil conditions and nitrogen inputs (Sant'Anna et al., 2018). Additional research investigating rhizobial denitrification processes confirmed

that the symbiotic bacterium *Ensifer meliloti* is capable of producing nitrous oxide within root nodules during nitrate metabolism (Pacheco et al., 2023). These findings indicate that microbial processes embedded within nitrogen-fixing symbioses can contribute to nitrogen gas emissions under certain environmental conditions.

**Table 2: Summary of Findings**

Study Focus	Key Nitrogen Fixation Findings	Source
Peanut–rhizobium symbiosis	Biological nitrogen fixation increased 30.07–42.55%	Liu et al. (2023)
Legume crop mixtures	15–18% of plant nitrogen derived from atmospheric fixation	Singh et al. (2023)
Subtropical ecosystem with <i>Pueraria lobata</i>	7.12–61.93 kg N ha <sup>-1</sup> yr <sup>-1</sup> fixed; explained 54% of soil N variation	Zhou et al. (2021)
Invasive <i>Ulex europaeus</i> ecosystems	Stream nitrate concentrations increased >200% with 22–28% vegetation cover	Stewart (2018)
Tropical pasture with <i>Arachis pintoii</i>	Litter nitrogen deposition 8.3–12.9 g N kg <sup>-1</sup>	Cardoso et al. (2025)
Alfalfa root nodules	23–85% of N <sub>2</sub> O emissions derived from fixed nitrogen	Sant’Anna (2018)
Rhizobial denitrification	<i>Ensifer meliloti</i> denitrification contributes to N <sub>2</sub> O production	Pacheco et al. (2023)
Evolution of nodulating Fabaceae	Nodulating species show higher leaf nitrogen (~2.5%)	Mathesius (2022)

Table 2 provides integrated evidence from the reviewed studies, summarizing key quantitative findings on nitrogen fixation and its environmental implications. The results consistently show that Fabaceae species significantly enhance nitrogen availability. For instance, biological nitrogen fixation increased by 30.07–42.55% in peanut–rhizobium symbiosis, while 15–18% of plant nitrogen in crop mixtures was derived from atmospheric fixation. In natural ecosystems, *Pueraria lobata* contributed 7.12–61.93 kg N ha<sup>-1</sup> yr<sup>-1</sup>, demonstrating its major role in soil nitrogen enrichment. However, the table also highlights environmental trade-offs. Invasive *Ulex europaeus* increased stream nitrate concentrations by over 200%, indicating potential water pollution risks. Similarly, nitrogen-fixing systems contribute to atmospheric impacts, with 23–85% of N<sub>2</sub>O emissions linked to fixed nitrogen and microbial processes such as rhizobial denitrification. Additionally, litter deposition from *Arachis pintoii* enhances nitrogen return to soils, further influencing nutrient cycling.



**Figure 2: Relative Environmental Nitrogen Impact by Fabaceae Systems (Composite Score)**

Figure 2 presents a Composite Nitrogen Impact Score (0–30) for different Fabaceae species and systems, combining three components: biological nitrogen fixation (BNF), nitrogen export/sequestration, and nitrous oxide (N<sub>2</sub>O) emission contribution. All categories show a uniform total score of 30/30, indicating that each system has a high overall influence on nitrogen cycling. However, the distribution of contributions differs among species.

Some systems, such as *Arachis hypogaea* with *Bradyrhizobium* and nodulating Fabaceae, show higher BNF contributions, highlighting their strong role in increasing environmental nitrogen inputs. In contrast, systems like *Ulex europaeus* and *Pueraria lobata* demonstrate relatively higher contributions to nitrogen export or environmental accumulation, suggesting their potential to influence nitrate movement into surrounding ecosystems. Meanwhile, *Medicago sativa* systems exhibit a notable share of N<sub>2</sub>O emission contribution, indicating their involvement in nitrogen loss pathways and greenhouse gas production.

#### e. Interpretation of the Evidence

The findings drawn from the reviewed studies collectively indicate that biological nitrogen fixation by Fabaceae species plays a foundational role in regulating nitrogen availability within ecosystems. Through symbiotic interactions with rhizobia bacteria, legumes introduce new nitrogen into soils, enhance plant productivity, and sustain nutrient cycling processes across both agricultural and natural environments.

However, the ecological impacts of nitrogen fixation extend well beyond soil fertility enhancement. Elevated nitrogen inputs may alter ecosystem nutrient balances, contribute to nitrate leaching into water bodies, and influence greenhouse gas emissions through downstream microbial nitrogen transformations. These outcomes underscore the dual role of nitrogen fixation as both a beneficial nutrient input mechanism and a driver of broader environmental nitrogen dynamics.

#### f. Limitations of the Evidence

The studies analyzed in this synthesis differ in experimental design, ecosystem context, and measurement approaches. Some investigations were conducted under controlled greenhouse conditions, while others examined field ecosystems across large spatial scales. Variation in environmental conditions, plant species, and microbial strains may influence observed nitrogen fixation rates and nitrogen cycling outcomes, making direct comparisons across studies challenging.

Additionally, measurements of nitrogen fixation and associated nitrogen fluxes were often conducted over limited temporal periods, which may not adequately capture long-term ecosystem dynamics or seasonal variability in fixation rates.

#### g. Implications for Research and Environmental Management

A thorough understanding of the ecological role of biological nitrogen fixation is essential for developing sustainable agricultural and ecosystem management strategies. Incorporating legumes into cropping systems offers a practical approach to reducing reliance on synthetic nitrogen fertilizers while simultaneously improving soil fertility and productivity.

At the same time, management practices must account for the potential environmental consequences associated with increased nitrogen inputs, including nitrate runoff into aquatic systems and elevated nitrous oxide emissions. Future research efforts should prioritize improving the mechanistic understanding of plant-microbe interactions, optimizing nitrogen fixation efficiency under varying environmental conditions, and developing integrated management strategies that maximize the ecological benefits of biological nitrogen fixation while minimizing associated environmental risks.

### B. Dense Vegetation Canopies and Mitigation of Nitrogen-Related Air Pollution

Table 3: Summary of included studies examining the relationship between vegetation canopies, nitrogen-fixing plants, and nitrogen-related environmental impacts

Plant Species	Ecosystem	Key Mechanisms	Implication for Air Pollution	Study
Mixed Vegetation	Urban Highway	Pollutant Interception	Canopies may reach saturation; excess N can leach or re-emitt	Katata et al. (2023)

<i>Pueraria lobata</i>	Subtropical abandoned slopes	Biological Nitrogen Fixation	Dense fixing canopies actively add N to ecosystem	Zhou et al. (2021)
<i>Arachis pintoi</i> mixed pasture	Tropical Pastures	Litter Deposition	Increases organic N return, available for microbial transformation	Cardoso et al. (2025)
<i>Medicago sativa</i> (Alfalfa)	Temperate agricultural	Fixation → Denitrification	Direct link between legume fixation and greenhouse gas emission	Sant'Anna et al. (2018)
Nodulating vs. Non - Nodulating Fabaceae	Global Ecosystem	Evolutionary N Allocation	Fixation fundamentally alters N economy of dominant species	Mathesius et al. (2022)

The studies included in this systematic review reveal a fundamental duality regarding the role of dense vegetation canopies in mitigating nitrogen-related air pollutants. While vegetation is often presumed to act as a passive sink for atmospheric pollutants, the evidence suggests that the specific functional traits of the plants—particularly biological nitrogen fixation by the Fabaceae family—can transform these canopies into net contributors to the nitrogen cycle, with potential implications for secondary pollutant formation.

Katata et al. (2023) investigated the role of dense canopies in urban roadside environments and demonstrated that vegetation acts as a natural barrier capable of absorbing or slowing the dispersion of pollutants. Their controlled nitrogen enrichment experiments indicated measurable reductions in atmospheric nitrogen compounds. However, a critical nuance emerges from their quantitative data: treatments enriched with nitrogen showed approximately 200% higher nitrate concentrations compared to controls when nitrogen was compared against sulfur and potassium. This suggests that in nitrogen-saturated environments—such as those receiving chronic deposition from vehicular emissions—vegetated systems may approach a saturation point where their capacity to store or assimilate nitrogen is exceeded, potentially leading to nitrate leaching or gaseous emissions. The presence of nitrogen-fixing plants within these canopies fundamentally complicates the mitigation narrative. Zhou et al. (2021) examined *Pueraria lobata* on abandoned subtropical slopes and found that biological nitrogen fixation contributed an average of  $22.93 \pm 6.52 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , explaining approximately 94% of soil nitrogen variation and 58% of soil nitrate variation. This substantial input demonstrates that dense canopies dominated by Fabaceae species actively enrich the local nitrogen pool, potentially counteracting any air pollutant interception benefits. Similarly, Cardoso et al. (2025) reported that mixed pastures containing *Arachis pintoi* produced litter with nitrogen concentrations of  $8.3\text{--}12.9 \text{ g N kg}^{-1}$  and deposition rates up to  $77.2 \text{ kg ha}^{-1} \text{ day}^{-1}$ , significantly enhancing organic nitrogen return to soils.

The mechanisms by which this fixed nitrogen re-enters the atmosphere as pollutants highlight an important contradiction: vegetation often recognized for its capacity to intercept pollutants may simultaneously contribute to their formation. Evidence demonstrates that nitrogen fixed by leguminous plants does not remain sequestered but instead undergoes transformation through microbial processes, with measurable fractions returning to the atmosphere as nitrous oxide—a potent greenhouse gas (Sant'Anna et al., 2018). This transformation is not merely incidental but appears directly linked to the plants themselves, as root nodules actively participate in denitrification under specific environmental conditions such as nitrate availability and soil hypoxia (Pacheco et al., 2023). Furthermore, the evolutionary investment in nitrogen fixation has given nodulating species with consistently higher tissue nitrogen concentrations compared to their non-nodulating counterparts, fundamentally altering the nitrogen economy of the ecosystems they dominate (Mathesius, 2022).

Collectively, these findings suggest that dense vegetation canopies cannot be evaluated solely on their capacity to intercept air pollutants; rather, their net effect depends critically on whether the constituent species function as nitrogen accumulators or nitrogen transformers. The presence of nitrogen-fixing plants introduces a cyclical dynamic whereby atmospheric nitrogen is captured, fixed, stored, and re-emitted in the environment, challenging the assumption that vegetation barriers represent simple, net absorbers for pollution. This duality underscores the need for deliberate approaches to green infrastructure that account not only for pollutant interception but also for the biogeochemical consequences of the plant species selected.

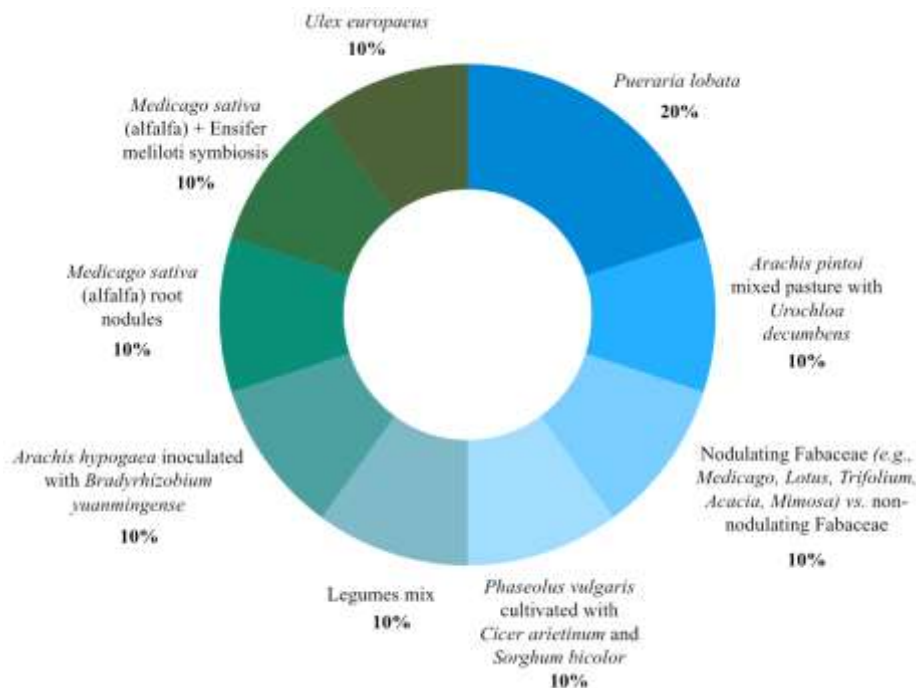


Figure 3: Distribution of Plant Species/Systems in the Reviewed Studies

Figure 3 illustrates the distribution of plant species and systems analyzed in the systematic review, highlighting the diversity of Fabaceae species and their interactions with other crops or ecosystems that influence nitrogen dynamics. It shows a balanced representation of legumes such as *Medicago sativa*, *Arachis hypogaea*, and *Phaseolus vulgaris*, alongside mixed cropping and inoculated systems, reflecting the range of conditions where nitrogen fixation occurs. The slightly higher proportion of *Pueraria lobata* indicates that some species are studied more frequently due to their high impact on nitrogen inputs. Overall, the distribution demonstrates how Fabaceae species contribute to soil nitrogen enhancement while also illustrating the variability in their ecological and agricultural roles, including potential impacts on nitrogen balance in different environments.

#### a. Limitations of the Evidence

The evidence is subject to some important limitations. First, the studies span highly heterogeneous environments—including highway roadsides (Katata et al., 2023), subtropical slopes (Zhou et al., 2021), tropical pastures (Cardoso et al., 2025), and temperate agricultural systems (Sant'Anna et al., 2018; Pacheco et al., 2023). Differences in climate, background nitrogen deposition, and soil microbial communities across these settings likely influence nitrogen cycling dynamics and limit cross-study comparability. Second, methodological approaches varied widely, ranging from controlled greenhouse experiments using specific *rhizobial* strains (Pacheco et al., 2023) to field-based plot measurements (Zhou et al., 2021; Cardoso et al., 2025), introducing variability that complicates synthesis.

In conclusion, the studies examined cover a wide range of settings, from controlled greenhouse experiments to natural field sites across different climates and regions. While this variety provides useful insight into how nitrogen-fixing plants behave under different conditions, it also introduces significant differences that make it hard to draw unified conclusions.

#### b. Implications on Practice and Policy

Urban planners and environmental managers should exercise caution when selecting vegetation for green infrastructure, prioritizing non-fixing species in roadside barriers to avoid unintended nitrogen enrichment, or managing nitrogen-fixing plants through regular harvesting to remove accumulated nitrogen. For future research, long-term studies that simultaneously measure both pollutant interception and gaseous nitrogen emissions from the same vegetation canopies are needed to determine whether these systems function as net sinks or sources of atmospheric pollution. Such work would provide the evidence base needed to refine green infrastructure guidelines and maximize air quality benefits while minimizing unintended nitrogen release.

### ***C. Contradiction Between Studies***

The studies reviewed show different and sometimes conflicting results about how Fabaceae plants affect nitrogen cycling in the environment. Many studies explain that legumes increase nitrogen in the soil through biological nitrogen fixation, which helps improve soil fertility and supports plant growth. For example, studies on species such as *Arachis hypogaea*, *Phaseolus vulgaris*, and *Medicago sativa* show that these plants work with nitrogen-fixing bacteria that convert atmospheric nitrogen into usable forms in the soil. However, other studies show different results. Some research reports that certain Fabaceae species may cause an increase in nitrate levels in nearby water systems. For instance, the study on *Ulex europaeus* found that the presence of this plant increased nitrate concentrations in streams. Other studies also reported that legumes may produce nitrous oxide (N<sub>2</sub>O) through denitrification in root nodules, which contributes to greenhouse gas emissions. These contradictions suggest that the effects of Fabaceae plants on nitrogen cycling are not always the same and depend on environmental conditions, plant species, and soil nutrient levels.

One limitation of the evidence is that the studies were conducted in different ecosystems such as agricultural lands, grasslands, tropical pastures, and freshwater environments. Because of these differences, the results may vary depending on the environmental conditions. Some studies were performed in controlled experiments, while others were conducted in natural ecosystems, which can lead to differences in findings. In addition, the studies focused on different species of Fabaceae plants, which may have different abilities to fix nitrogen and influence nitrogen cycling. These variations make it difficult to directly compare all results.

Another limitation is related to the review process itself. The studies were collected only from selected databases and were limited to publications written in English between 2015 and 2025. Because of this, some relevant studies may not have been included in the review. In addition, the number of studies analyzed was limited, which may affect the overall interpretation of the results. The review also relied on the information reported in each study, which may vary in research design, data collection methods, and analysis.

The results of this review show that Fabaceae plants have both beneficial and potentially harmful effects on nitrogen cycling. In practice, legumes can be used in agriculture to improve soil fertility and reduce the need for synthetic fertilizers. However, proper management is necessary because excessive nitrogen from legumes may lead to water pollution or greenhouse gas emissions. For policy, environmental management strategies should consider both the positive and negative impacts of nitrogen-fixing plants. For future research, more studies should examine how different species of Fabaceae interact with soil nutrients and environmental conditions. Long-term studies across different ecosystems may also help clarify the contradictions found among current research findings.

### **CONCLUSION**

This systematic review focuses on the role of biological nitrogen fixation by the Fabaceae species in the nutrient cycle of nitrogen. The review highlights the symbiotic relationship between legumes and rhizobia bacteria. Across various ecosystems, the Fabaceae species contributes nitrogen content to support local plant growth, improve soil fertility, and sustain the nitrogen content of the environment.

The findings highlight the ecological influence of nitrogen fixation, which extends beyond the direct nutritional benefits to host plants. With fixed nitrogen into soils, legumes improve nitrogen availability for local plant communities. In agricultural systems, this process can reduce usage of fertilizers, encouraging more sustainable crop production. In natural or recovering ecosystems, nitrogen-fixing plants may support soil nutrient recovery and influence patterns of plant succession.

However, the results also confirm that nitrogen fixation is not entirely beneficial; additional nitrogen into ecosystems may disrupt the nutrient balances and contribute to nitrogen losses through processes such as nitrate leaching and microbial denitrification. This analysis also reveals that the efficiency and ecological outcomes of nitrogen fixation are strongly influenced by environmental conditions. Factors such as phosphorus availability, soil moisture, microbial strain characteristics, and ecosystem type regulate nodulation, nitrogenase activity, and downstream nitrogen transformations, which necessitate legumes in the nutrient cycle.

Still, several research gaps remain. Differences in experimental design, ecosystem types, and the overall heterogeneity of the results limit the ability to directly compare nitrogen fixation rates across studies. Moreover, many studies focus on short-term observations, which do not represent the long-term dynamics of nitrogen accumulation and loss in ecosystems with significant nitrogen-fixing species richness. Hence, improving research to include longer-term monitoring and a wider range of Fabaceae species and environmental conditions would further illuminate how biological nitrogen fixation influences ecosystem functioning over time.

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