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ENGINEERING NITROGENASE FOR PLANETARY SUSTAINABILITY: RENEWABLE-POWERED BIOHYBRID AMMONIA AND THE ROAD BEYOND HABER–BOSCH

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HIGHLIGHTS

- Nitrogen fertiliser enabled modern yields but drives primary energy use, emissions, and nitrogen pollution
- Engineered biofertilisers and plant-centred symbiosis or organellar strategies can reduce inputs but are constrained by oxygen sensitivity and metabolic cost
- Renewably powered, purified-nitrogenase systems (electro- and photo-biohybrids) offer a modular route to decentralised ammonia yet hinge on wiring, stability and robust $^{15}N_2$ validation
- Protein engineering (stability, interface tolerance, electron delivery) is a shared enabling tool across all three deployment routes

GRAPHICAL ABSTRACT

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ABSTRACT

The Haber–Bosch process made synthetic ammonia abundant and helped feed a growing population, but it also created a sustainability debt: Substantial fossil-energy demand, significant CO_2 emissions, and pervasive nitrogen losses that drive eutrophication and N_2O emissions. Biological nitrogen fixation (BNF) is attractive because nitrogenase reduces N_2 to NH_3 at ambient conditions, yet the enzyme's oxygen lability, complex metallocluster biosynthesis, and high energy demand complicate implementation beyond its native microbial contexts. This review synthesises three routes to reduce fertiliser dependence: Engineered nitrogen-fixing biofertilisers that excrete ammonium; plant-centred strategies that extend symbiosis or express nitrogenase components in organelles; and, purified nitrogenase or biohybrid systems powered by renewable electricity or light. We emphasise biohybrid devices because they decompose the challenge into

modular interfaces (enzyme, power, microreactor environment) but require credible solutions for wiring, stability, continuous operation, and product capture. Across routes, protein engineering for stability, interface tolerance, and electron delivery is a shared enabling lever for system-level impact.

Introduction

Few technologies have reshaped the planet's nitrogen cycle as industrial ammonia synthesis. The expansion of synthetic nitrogen fertiliser is strongly linked to yield gains and global food security. However, there is a significant increase in reactive nitrogen losses and negative downstream impacts on air and water quality (Erisman *et al.*, 2008; Galloway *et al.*, 2008; Fowler *et al.*, 2013). At the same time, ammonia production is deeply embedded in energy and industrial infrastructure, and the emissions profile depends on hydrogen source, plant efficiency, and policy context (Smith *et al.*, 2020; Rouwenhorst *et al.*, 2022).

Biological nitrogen fixation (BNF) is nature's route for converting N_2 into plant-available nitrogen. Diazotrophic bacteria and archaea use nitrogenase to reduce N_2 to NH_3 under ambient conditions, but the enzyme is exceptionally oxygen-sensitive and energetically expensive (Seefeldt *et al.*, 2009; Hoffman *et al.*, 2014). The canonical Mo-nitrogenase reaction requires at least 8 electrons and $\geq$ 16 ATP per N_2, and obligatorily evolves H_2, an intrinsic efficiency penalty unless captured or mitigated (Seefeldt *et al.*, 2009; Seefeldt *et al.*, 2018). These constraints mean that "replacing Haber–Bosch with biology" is not a single invention, but a portfolio of engineering strategies that adapt nitrogenase to new contexts.

This review examines how to translate nitrogenase chemistry into interventions that reduce fertiliser dependence. We organise progress around three routes: (i) Engineered biofertilisers that deliver nitrogen to crops, (ii) plant-centred strategies using symbiosis or organellar expression, and (iii) purified-enzyme biohybrids powered by renewable electricity or light. Routes (i) and (ii) face ecological and regulatory complexity (Rogers & Oldroyd, 2014; Wen *et al.*, 2021). Route (iii) is engineering-tractable because it uses modular interfaces: Enzyme, power source, and reactor (Brown *et al.*, 2016; Milton *et al.*, 2016; Lee *et al.*, 2020). Protein engineering improves stability, oxygen resilience, and electron delivery across all routes (Bennett *et al.*, 2023). The three routes differ in the primary system they must control: Ecology and evolution [Route (i)], plant development and metabolism [Route (ii)], or reactor or device physics [Route (iii)], which are reflected in Table 1.

Table 1: Analytical comparison of nitrogenase deployment routes

Route	What is Engineered	Primary Upside	Dominant Technical Risks	Dominant Non-Technical Risks	Realistic Timeline to Broad Impact
Engineered biofertilisers	Diazotroph regulation or excretion, colonisation traits	Works with existing crops, avoids transgenic plant burden	Field variability, carbon limitation, trait loss, O_2 exposure	Environmental release, HGT, regulation, trust	Near- to mid-term if reliability improves
Plant-centred strategies	Host recognition or symbiosis, organelle expression of components	Potentially significant impact at crop scale	High fitness penalty, gene stack complexity, O_2 control, cofactor trafficking	Trait stewardship, regulatory, and public acceptance	Long-term, high risk, or high reward
Purified nitrogenase biohybrids	Catalyst immobilisation + renewable electron delivery	Modular, controlled conditions, "device" scaling logic	Enzyme lifetime, continuous operation, capture or separation, energy cost	Capex or opex, durability, safety, maintenance	Mid-term for niches, long-term for displacement

Biological Utilisation Routes

Biology-first strategies introduce fixed nitrogen into the plant-soil system through the use of living catalysts. The two primary approaches are engineered microbial biofertilisers and plant-focused symbiotic or organelle-based methods. Both approaches may benefit from recent advances in nitrogenase genetics and protein engineering; however, they continue to face fundamental limitations such as oxygen sensitivity and high energetic requirements (Rogers & Oldroyd, 2014; Burén & Rubio, 2018; Bennett *et al.*, 2023).

Engineered Nitrogen-Fixing Biofertilisers

Microbial biofertilisers can fit existing practice by partially replacing synthetic N, but most diazotrophs retain fixed nitrogen and repress nif expression when ammonium is available (Rogers & Oldroyd, 2014; Burén *et al.*, 2020). The engineering goal is therefore ammonia delivery: Strains that fix N_2 and release plant-available nitrogen while maintaining fitness and genetic stability in the rhizosphere (Ambrosio *et al.*, 2017; Batista *et al.*, 2021).

Early work on engineering ammonium excretion focused on simple proof-of-concept systems, but recent designs incorporate condition-responsive regulation for better control. One strategy is to downregulation of ammonium assimilation or uptake so that fixed nitrogen is more likely to leak to the environment. This is possible by partial inhibition of glutamine synthetase activity, modulation of PII signaling, or alteration of ammonium transport (Ambrosio *et al.*, 2017; Plunkett *et al.*, 2020). Another strategy focuses on transcriptional control. In many Proteobacteria, when fixed nitrogen is abundant or when carbon or energy is limiting, the NifL–NifA system integrates nitrogen and carbon signals to gate nif transcription and prevent nitrogenase expression. Batista *et al.* (20221) demonstrated that disrupting hierarchical nitrogen control can enable carbon-dependent regulation of ammonia excretion: The engineered diazotrophs excrete ammonia primarily when supplied with carbon, aligning nitrogen fixation with an external "payment" that could plausibly come from plant root exudates (Plunkett *et al.*, 2020; Batista *et al.*, 2021).

Using these biofertilisers adds ecological and evolutionary filters because engineered strains must compete with native microbiomes. The competition is to maintain colonisation and avoid losing costly engineered traits under selection (Wen *et al.*, 2021; Martinez-Feria *et al.*, 2024). Many diazotrophs usually shut down nif genes in the presence of fertiliser nitrogen. However, some studies have shown that regulatory remodelling can enable biological nitrogen fixation to contribute to fertilised fields (Wen *et al.*, 2021). More recently, genetic remodelling of soil diazotrophs was reported to partially replace synthetic nitrogen fertiliser with biological nitrogen fixation in maize under field-relevant conditions (Martinez-Feria *et al.*, 2024).

Protein engineering improves nitrogenase stability under fluctuating oxygen levels, expanding the range of environments where engineered diazotrophs can operate (Burén *et al*., 2020; Bennett *et al*., 2023). Tuning electron delivery pathways raises productivity per unit carbon, decreasing fitness penalties and stabilising engineered traits (Seefeldt *et al.*, 2018; Bennett *et al.*, 2023). Engineered biofertilisers are advancing toward partial replacement, but progress is constrained by ecology, regulation, and enzyme sensitivity.

Plant-Centred Strategies: Synthetic Symbioses and Organelles

A second route attempts to move fixed nitrogen closer to the crop by engineering plant-microbe partnerships or by expressing nitrogenase components within plant cells. Because legumes achieve high BNF rates through organ-level microaerobiosis (root nodules) and coordinated signalling, a long-standing ambition has been to extend comparable benefits to cereals (Charpentier & Oldroyd, 2010; Rogers & Oldroyd, 2014). Conceptually, this requires re-creating multiple layers: Recognition and accommodation of bacteria, allocation of plant carbon, local oxygen buffering, and feedback regulation that prevents parasitism (Rogers & Oldroyd, 2014; Bennett *et al.*, 2023). Progress is steady but incremental and most results remain in model systems or controlled environments.

Organelle targeting is often viewed as the most plausible biochemical entry point for direct nitrogenase expression, as mitochondria and chloroplasts can supply ATP and reducing power and provide compartmental control. Work in yeast mitochondria showed that an oxygen-labile nitrogenase component can be expressed and retain activity in aerobically grown cells, providing proof of principle for eukaryotic expression of nitrogenase components (López-Torrejón *et al.*, 2016). Subsequent studies demonstrated the formation of nitrogenase subassemblies, such as NifDK tetramers, in yeast mitochondria, clarifying which proteins can fold, assemble, and avoid degradation in organellar environments (Burén *et al.*, 2017; Burén & Rubio, 2018). In plants, multi-gene targeting to mitochondria has revealed additional constraints, including proteolytic processing and unwanted cleavage sites that can inactivate nitrogenase proteins; engineering cleavage-resistant variants of key components, such as NifD, therefore an explicit protein-engineering requirement (Allen *et al.*, 2017; Allen *et al.*, 2020).

Even if complete nitrogen fixation inside a cereal cell is a longer-term goal, plant-centred strategies remain valuable because they can improve the "host context" for diazotrophs, thereby amplifying biofertiliser performance. Engineering root exudation profiles, local oxygen buffering, or nutrient exchange could create controllable niches that support carbon-responsive ammonium excretion by engineered microbes (Van Deynze *et al.*, 2018; Batista *et al.*, 2021). Thus, rather than treating plant engineering and microbial inoculants as competing approaches, a practical view is that

they may converge: Modest plant edits that improve microbial accommodation paired with engineered diazotrophs optimised for regulated nitrogen delivery (Rogers & Oldroyd, 2014; Wen *et al.*, 2021).

In legumes, BNF is costly and tightly regulated, nodules draw a meaningful fraction of photosynthate, and symbiosis can impose yield penalties when conditions do not favour fixation (Minchin & Witty, 2005; Kaschuk *et al.*, 2010). For non-legumes, imposing nitrogenase expression and support could create a direct energetic burden (ATP and reductant), plus metal and sulfur demands, and proteostasis stress from the expression of cofactor-rich proteins (Rogers & Oldroyd, 2014; Burén & Rubio, 2018). Even if nitrogenase were functional, the plant would need regulatory logic to turn the system on only when beneficial, otherwise yield penalties could overwhelm benefits.

The feasibility of plant strategies is not precluded. However, progress should be evaluated against stringent criteria such as net yield and resilience, rather than solely enzyme activity. A practical milestone involves achieving partial nitrogen contribution in specific tissues or developmental stages, as demonstrated by nitrogen mass balances and field-relevant performance metrics.

Purified Nitrogenase and Renewable-Powered Biohybrids

Route (iii) is conceptually different: Instead of relying on living systems to supply energy, protection, and regulation, biohybrid systems aim to modularise nitrogenase as a catalyst in a controlled, anaerobic reactor powered by renewable electricity or light (Milton *et al.*, 2016). This modularity is the main novelty: It decouples nitrogenase catalysis from growth and ecology, but forces an explicit engineering reckoning about durability, continuous operation, and economics.

Canonical Mo-nitrogenase operates via the Fe protein (NifH), delivering electrons to the MoFe protein (NifDK), which is coupled to ATP hydrolysis and conformational gating (Seefeldt *et al.*, 2009; Hoffman *et al.*, 2014). The minimum stoichiometry is commonly written as:

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i \quad (1)$$

H_2 evolution is not a minor side effect, it is integral to the catalytic cycle and competes with N_2 reduction, affecting energy efficiency unless managed (Hoffman *et al.*, 2014; Seefeldt *et al.*, 2018). Any device must therefore solve three coupled problems: (a) Low-potential electron delivery, (b) ATP provision or bypass, and (c) near-perfect O_2 exclusion to protect metal cofactors.

Two Architectural Families

A proper analytical framing is to separate biohybrid architectures into:

(a) ATP-coupled systems that preserve Fe-protein cycling and use ATP regeneration modules, and

(b) ATP-independent (ATP-bypassing) systems that deliver electrons directly to MoFe protein via mediators or redox polymers, or through photoexcited electron transfer.

The practical differences are summarised in Table 2.

Table 2: Trade-offs between ATP-coupled and ATP-independent nitrogenase biohybrids

Attribute	ATP-Coupled (Fe Protein + ATP Regeneration)	ATP-Independent or Bypass (Direct MoFe Reduction)
Core benefit	Closest to native mechanism, clearer kinetics or controls	Eliminates the ATP module, simpler energy accounting in principle
System complexity	Higher component count, ATP regeneration must run reliably	Fewer biological components, but electrochemistry may be harsher
Operating potential	Often compatible with biological mediators	Often requires strongly reducing mediators or potentials
Efficiency limits	ATP recycling losses + H_2 evolution	Mediator losses + competing H_2 evolution
TRL profile	More interpretable and incremental	More disruptive but high upside if stabilised
Representative work	Enzymatic fuel-cell concepts coupling current to nitrogenase (Milton *et al.*, 2017)	Redox-polymer wiring enabling ATP-independent $^{15}N_2$-verified NH_3 (Lee *et al.*, 2020)

ATP-Coupled Bioelectrocatalysis: "Bioelectrochemical Haber–Bosch" as a Concept

Bioelectrocatalytic approaches have demonstrated that electrical current can be coupled to nitrogenase turnover under ambient conditions. An influential study framed this as a bioelectrochemical analogue of ammonia synthesis, using electron mediators and ATP regeneration to sustain catalysis and report Faradaic efficiency (Milton *et al.*, 2017). Subsequent reviews and demonstrations emphasise that, while yields remain far from commodity-scale, these systems establish a key principle: Nitrogenase can be integrated into electrochemical hardware with measurable electron-to-product accounting (Rapson & Wood, 2022).

The limitations are equally instructive. ATP regeneration chemistry introduces phosphate accumulation, side reactions, and a second reliability bottleneck; meanwhile, mediators must remain stable and not damage cofactors (Rapson & Wood, 2022). These issues push the field toward either improved ATP recycling modules (e.g., enzyme cascades with longer lifetimes) or ATP bypass.

ATP-Independent Mediated Electron Transfer: Direct Wiring of MoFe Protein

A significant step toward ATP bypass was the demonstration of ATP-independent N_2 reduction using the MoFe protein wired to an electrode through a redox polymer. Lee *et al.* (2020) reported ATP-independent NH_3 formation verified using $^{15}N_2$ labelling, showing that suitably reducing electron delivery can replace the Fe-protein or ATP gating, at least in a proof-of-concept regime. Conceptually, this turns nitrogenase into a more catalyst-like module: Electrons and protons in, NH_3 out, without an ATP subsystem.

But, bypass shifts the difficulty rather than removing it. Direct reduction often requires more negative potentials and careful control of competing hydrogen evolution; mediator stability and enzyme attachment become dominant engineering variables (Lee *et al.*, 2020). The net question becomes: Can the wired enzyme maintain activity for weeks, not hours, and can the hardware deliver a meaningful NH_3 flux without catastrophic losses?

Photobiohybrids: Replacing Biochemical Energy with Light

Photobiohybrids couple nitrogenase to a light absorber that generates photoexcited electrons. In a landmark study, Brown *et al.* (2016) created a CdS nanocrystal–MoFe protein biohybrid that catalysed light-driven N_2 reduction to NH_3 under anaerobic conditions, using isotopic tracing to validate N_2 as the nitrogen source. The result established a core concept, with a sufficiently strong reductant delivered photochemically, nitrogenase can operate with reduced dependence on its native ATP-coupled electron delivery chain (Brown *et al.*, 2016; Seefeldt *et al.*, 2018).

Photobiohybrids raise different engineering questions than electroenzymatic systems. Photon management becomes a design variable: Light intensity, scattering, and absorber stability determine electron flux, while reactive oxygen species or photogenerated radicals can damage proteins and cofactors (Wang *et al.*, 2018). Scaling, therefore depends on illumination, mixing, N_2 mass transfer and photosensitiser stability (Rapson & Wood, 2022).

An emerging direction is whole-cell or microbe-semiconductor biohybrids, in which living diazotrophs serve as self-assembling "enzyme factories" while semiconductor interfaces provide extra reducing power. Other work has demonstrated inorganic-bacterial photoelectrochemical hybrids for solar-driven nitrogen fixation, illustrating how device architectures can exploit both biological maintenance and engineered electron delivery (Zhou *et al.*, 2025). These studies are necessary because they connect laboratory photochemistry to scale-relevant engineering variables.

The Missing Engineering Analysis: Scale, Stability, Separation, and Energy Cost

Four engineering issues dominate whether biohybrid nitrogenase can move beyond laboratory demonstrations.

Long-Term Catalyst Stability and "Cartridge Economics"

Unlike industrial catalysts that operate for years, purified enzymes generally degrade. Nitrogenase contains multiple sensitive metal clusters and is damaged by O_2 and potentially by electrode-adjacent radical chemistry (Hoffman *et al.*, 2014). Immobilisation may improve reuse but can reduce activity by limiting conformational dynamics or substrate access. A credible scale pathway therefore needs explicit "cartridge economics": How often the enzyme module must be replaced, how it is regenerated or recycled, and whether the system can be serviced safely and cheaply?

Continuous Operation, Not Batch Peaks

Most studies show batch or short-run activity. Deployment requires continuous operation with reliable N_2 or NH_3 gas handling, stable pH or ionic strength, durable mediators, and sustained anoxia (MacFarlane *et al.*, 2020; Rapson & Wood, 2022). Realistic "enzyme cartridges" will likely combine O_2 barriers + scavengers with leak-tight flow-cell architectures.

Product Capture and Separation are Not Optional

In any ammonia synthesis route, separation can dominate energy use if NH_3 is produced in a dilute. This is well established in electrochemical ammonia discussions and applies equally to enzymatic systems if product streams are low concentration (MacFarlane *et al.*, 2020; Smith *et al.*, 2020). Biohybrid systems may need integrated capture (e.g., conversion to ammonium salts) to prevent volatilisation and

to produce a usable fertiliser form. The relevant metric is not only "NH_3 produced" but "plant-available nitrogen delivered" per unit energy and cost.

Total System Energy Cost (kWh kg^{-1} NH_3) Versus an Efficient Incumbent

Modern Haber–Bosch plants can be highly efficient and performance varies by hydrogen source and heat integration, this makes the incumbent harder to displace than simplified comparisons imply (Rouwenhorst *et al.*, 2020). Biohybrid systems must account for the energy costs of oxygen exclusion, pumping and compression, mediator cycling losses, and separation. Without these, "ambient conditions" can be misleading as an advantage. For the field to move forward credibly, studies should report full energy and mass balances alongside activity.

From Devices to Deployment: Where Biohybrid Ammonia Could Matter First

Given current performance, purified or biohybrid nitrogenase is more plausible for distributed, low-volume ammonia integrated with local nutrient management than for bulk displacement of Haber–Bosch. Potential niches include controlled environments (seedlings, fertigation, closed hydroponics) where on-demand generation can offset lower throughput and simplify logistics. A promising integration is pairing device-made "starter" nitrogen with engineered diazotrophs for sustained delivery, reducing total inputs without requiring complete substitution (Wen *et al.*, 2021; Martinez-Feria *et al.*, 2024). However, farm deployment demands ruggedness, low maintenance, and safe NH_3 handling, and decentralised economics are highly sensitive to capex, utilisation, and safety requirements.

A more realistic near-term framing is biohybrid devices as niche modules (e.g., small-scale synthesis linked to controlled environments, research platforms, or specialty fertiliser production), with a long-term possibility of distributed deployment only if durability and serviceability are proven.

Protein Engineering and System Co-Design

Nitrogenase relies on metal cofactors and precise conformational changes. Improving protein robustness simplifies requirements across all platforms (Hoffman *et al.*, 2014; Burén *et al.*, 2020). Engineering targets include: (1) Assembly and maturation: Improving expression, solubility, and heterologous compatibility; refactoring nif clusters enables non-native expression, but cofactor biosynthesis remains challenging (Temme *et al.*, 2012; Burén *et al.*, 2020); (2) electron delivery: Optimising ferredoxin or flavodoxin interactions to raise catalytic flux (Seefeldt *et al.*, 2018; Bennett *et al.*, 2023); and, (3) stability and oxygen resilience: Engineering proteolysis resistance, oxygen tolerance, and interfacial stability (Allen *et al.*, 2020; Bennett *et al.*, 2023).

Importantly, protein engineering should be coupled to system-level selection pressures. For biofertilisers, fitness in fluctuating oxygen and carbon environments and the stability of ammonium excretion regulation are as crucial as enzyme turnover (Plunkett *et al.*, 2020; Batista *et al.*, 2021). For plant organelles, constraints include protease cleavage, targeting efficiency, and redox compatibility, motivating designs such as cleavage-resistant NifD variants (Burén & Rubio, 2018; Allen *et al.*, 2020). For devices, selection pressures include interfacial stability, tolerance to mediator radicals or photoexcited species, and retention of activity during repeated cycling (Brown *et al.*, 2016; Lee *et al.*, 2020). Directed evolution and high-throughput screening frameworks discussed in recent synthetic biology reviews provide conceptual routes to impose these pressures experimentally, particularly as biosensors for ammonia and coupling schemes improve (Bennett *et al.*, 2023).

Stability activity trade-offs are common: Cofactor protection or immobilisation can reduce turnover and ATP bypass can require harsher electrochemical conditions that accelerate deactivation (Hoffman *et al.*, 2014; Lee *et al.*, 2020; Rapson & Wood, 2022). Reporting should therefore pair activity with durability under deployment-relevant stressors.

Finally, the right metric for "success" differs by route. A device-optimised nitrogenase might sacrifice maximal turnover for stability on an electrode, while a biofertiliser-optimised nitrogenase might prioritise operation under microaerobic, carbon-limited conditions. Recognising and explicitly engineering for these contexts will help avoid the trap of optimising in vitro metrics that do not translate to field or reactor conditions.

Outlook

Sustainable roadmaps need integrated solutions. Near-term impact will likely come from engineered biofertilisers that fit existing practice but require ecological robustness, field reliability, and containment frameworks (Plunkett *et al.*, 2020; Batista *et al.*, 2021; Wen *et al.*, 2021).

Direct plant nitrogen fixation remains a transformative long-term goal. However, the engineering requirements are complex. Successful implementation requires precise multi-gene expression, cofactor assembly, and organellar targeting. Additionally, researchers must effectively manage oxygen levels and redox states within the plant (Charpentier & Oldroyd, 2010; Burén & Rubio, 2018). An intermediate goal is to engineer plants to better host and regulate diazotrophs. This involves creating microaerobic, carbon-rich niches and specialised signalling frameworks to enhance biofertiliser performance. These strategies provide a realistic path forward while organellar nitrogenase expression continues to mature (Rogers & Oldroyd, 2014; Allen *et al.*, 2020).

Purified enzymes and biohybrids can power nitrogenase using electricity or light. However, the primary challenge shifts from feasibility to long-term performance. Future systems must demonstrate week-scale stability, energy efficiency, and 15N2-validated results within realistic reactors (Brown *et al.*, 2016; Lee *et al.*, 2020). Distributed ammonia production represents the most plausible deployment strategy. Modular devices can produce small quantities on demand near renewable energy sources and local agricultural sites. This colocation minimises risks associated with transport, storage, and nitrogen leakage (Hoffman *et al.*, 2013; Wang *et al.*, 2018). Combining hardware devices with engineered microbes is a strong strategy. These hybrid systems lower the technical demands on any single component. By sharing the functional load, these setups become more viable for real-world use (Wen *et al.*, 2021; Martinez-Feria *et al.*, 2024).

Across routes, three research priorities stand out. First, expand the engineering toolkit for nitrogenase robustness and interface tolerance, with selection pressures aligned to field or device operation (Rapson & Wood, 2022; Bennett *et al.*, 2023). Second, standardise validation and reporting practices, incorporating isotopic tracing and contamination controls to facilitate reliable iterative development. Third, assess technologies based on system-level nitrogen outcomes, such as nitrogen use efficiency and loss reduction, rather than focusing solely on NH_3 production rates. The primary sustainability benefit is derived from reduced emissions and pollution (Galloway *et al.*, 2008; Fowler *et al.*, 2013).

Ammonia is an industrial system, not just a reaction. Production, storage, transport, and farming are optimised for current supply chains; policy often externalises pollution costs (Fowler *et al.*, 2013; Rouwenhorst *et al.*, 2020). Low-carbon pathways must meet safety and

logistics constraints. Decentralised economics depend on utilisation and maintenance, NH_3 safety is non-negotiable. Engineered microbes face regulatory and public scrutiny. Release requires risk assessment, transparency, and monitoring. Governance must specify failure rates, mitigation, and accountability (Rovner *et al.*, 2015; Wen *et al.*, 2021).

Conclusions

Three primary pathways, namely engineered biofertilisers, plant-centred symbiosis or organelle strategies, and renewable-powered purified or biohybrid nitrogenase are currently defining the translational landscape for biological nitrogen fixation (BNF)-inspired fertiliser reduction. Engineered biofertilisers are closest to achieving field-level impact, while plant-centred approaches offer the most significant long-term potential. Biohybrid devices provide a modular engineering platform for integrating nitrogenase with renewable energy sources. In all three approaches, protein engineering and microenvironment design are fundamentally linked. The ability to stabilise nitrogenase and control electron delivery will determine whether nitrogenase remains a scientific curiosity or becomes a viable component of sustainable nitrogen management.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Conflict of Interest Statement

The author declares no conflict of interest.

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