SYNTHETIC BIOLOGY STRATEGIES FOR ENGINEERING NITROGEN-EFFICIENT MICROBIAL COMMUNITIES IN AGRICULTURE

Saravanan Manoharan[1], Dr. Nallusamy Duraisamy[2], Dr. Arunagirinathan N[3], Ms. Anusha K[4], Joshua S[5]

[1] Assistant Professor (Research), Central Research Laboratory, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research.
[2] Scientist, Department of Research, Meenakshi Academy of Higher Education and Research.
[3] Dean Research, Department of Research, Meenakshi Academy of Higher Education and Research.
[4] Lecturer, Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research.
[5] Assistant Professor, Meenakshi College of Allied Health Sciences, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research.

ABSTRACT

Background: Nitrogen deficiency is a key factor limiting the global agricultural productivity, and excessive application of synthetic nitrogen fertilizers leads to soil degradation, greenhouse gas emissions and environmental pollution. Therefore, there is a need for sustainable alternatives to improve the nitrogen-use efficiency in agriculture.

Objective: The present study aimed to develop nitrogen-efficient microbial communities by synthetic biology approaches to improve biological nitrogen fixation, rhizosphere stability and sustainable crop productivity.

Methodology: We engineered beneficial microbial consortia with nitrogen-fixing and plant growth-promoting bacteria by using CRISPR-based genome editing, synthetic gene circuits and metabolic pathway optimization. We conducted soil microcosm experiments, metagenomic sequencing, transcriptomic analysis, and plant growth assessment to assess the efficiency of nitrogen fixation and interactions in microbial communities.

Findings: They found that engineered microbial communities significantly increased nitrogen fixation efficiency. Nitrogen fixation rates were 38% in the control group and 82% in engineered microbial treatments. The use of synthetic microbial consortium enhanced plant biomass by 48%. Transcriptomic analysis showed an up-regulation of nitrogen metabolism genes such as nifH (4.2-fold), amoA (3.1-fold), and glnA (2.8-fold). Synthetic regulatory circuits enhanced microbial cooperation, stress tolerance and nutrient assimilation in the rhizosphere ecosystem.

Conclusion: Our findings show that synthetic microbiome engineering can help achieve sustainable agriculture through improved nitrogen cycling efficiency, lower dependence on fertilizers, and climate-resilient crop production.

KEYWORDS: Synthetic biology, nitrogen fixation, microbial communities, sustainable agriculture, CRISPR, rhizosphere microbiome, biofertilizers, nitrogen-use efficiency.

1 INTRODUCTION

1.1 Nitrogen and Agricultural Sustainability

Nitrogen is a macronutrient that is critical for plant growth, protein synthesis, chlorophyll formation, and crop productivity. Synthetic nitrogen fertilizers are widely used in modern agriculture to satisfy the growing global demand for food [1]. However, the overuse of fertilizers has led to serious environmental problems such as soil acidification, groundwater pollution, eutrophication of aquatic ecosystems, and greenhouse gas emissions such as nitrous oxide (N 2O) [2]. Inefficient use of nitrogen by crops leads to large nutrient losses in agricultural ecosystems due to volatilization, denitrification and leaching [3]. The agricultural nitrogen cycle soil system consists of microbial-mediated transformations including nitrogen fixation, nitrification, ammonification, and denitrification that control nitrogen availability [4]. Hence, there is an urgent need of sustainable nitrogen management strategies to reduce environmental pollution while maintaining crop productivity.

1.2 Microbial Communities in Soil Ecosystems

Soil microbes play a key role in nutrient cycling, soil fertility and plant health. The rhizosphere microbiome, the community of microorganisms associated with plant roots, plays an important role in nitrogen availability and plant growth [5]. Nitrogen fixing bacteria such as Rhizobium, Azotobacter and cyanobacteria have nitrogenase, which catalyzes the conversion of atmospheric nitrogen into biologically available ammonia [6]. Plant growth-promoting rhizobacteria (PGPR) are reported to improve crop productivity through enhanced nutrient uptake, synthesis of phytohormones, suppression of pathogens and increased stress tolerance [7]. Interactions between microbial communities in the rhizosphere determine the stability of the ecosystem and the efficiency of nutrient cycling, emphasizing the significance of microbial cooperation in sustainable agriculture.

1.3 Synthetic Biology in Agriculture

Recent progress in synthetic biology has enabled the precise engineering of microbial systems for use in agriculture. CRISPR-mediated microbial engineering has become a powerful tool for engineering nitrogen fixation pathways, stress resistance, and microbial metabolism [8]. [9] Synthetic metabolic pathways and programmable gene circuits can mediate nutrient uptake and microbe interactions in response to changing environmental conditions. Engineered microbial consortia composed of nitrogen-fixing bacteria, PGPR and nutrient-solubilizing microorganisms have shown promising potential to improve soil fertility and crop performance [10]. Systems biology and multi-omics approaches further support the design of stable and functional microbial ecosystems for sustainable agricultural biotechnology .

1.4 Research Gap

There are several outstanding limitations in agricultural microbiome engineering with synthetic biology, although considerable promise exists. Environmental fluctuations and microbial competition often lead to reduced stability and inconsistent performance of engineered microbial communities in field conditions [11]. Moreover, combining synthetic regulatory systems with native agricultural microbiomes is still underexplored, limiting the large-scale application of engineered microbial consortia in sustainable farming systems [12].

1.5 Objectives

The current study aims at engineering nitrogen-efficient microbial communities via synthetic biology approaches, improving biological nitrogen fixation, evaluating microbial cooperation and rhizosphere ecosystem stability and assessing agricultural productivity and sustainability outcomes under engineered microbial treatments.

2 BACKGROUND WORK

2.1 Nitrogen Deficiency and Fertilizer Dependency

Nitrogen deficiency is still one of the major constraints affecting global agricultural productivity and food security. The increased population in the world and increased demand for food have resulted in increased use of synthetic nitrogen fertilizers to increase crop yields [13]. However, over-fertilization has led to severe soil degradation, nutrient imbalance, and reduced microbial diversity in agricultural ecosystems [14]. A large proportion of unused nitrogen is lost via leaching, volatilization and runoff, resulting in groundwater contamination and eutrophication of aquatic systems [15]. High use of nitrogen fertiliser is also a significant contributor to greenhouse gas emissions, primarily nitrous oxide (N_2O), which contributes to climate change and environmental instability [16].

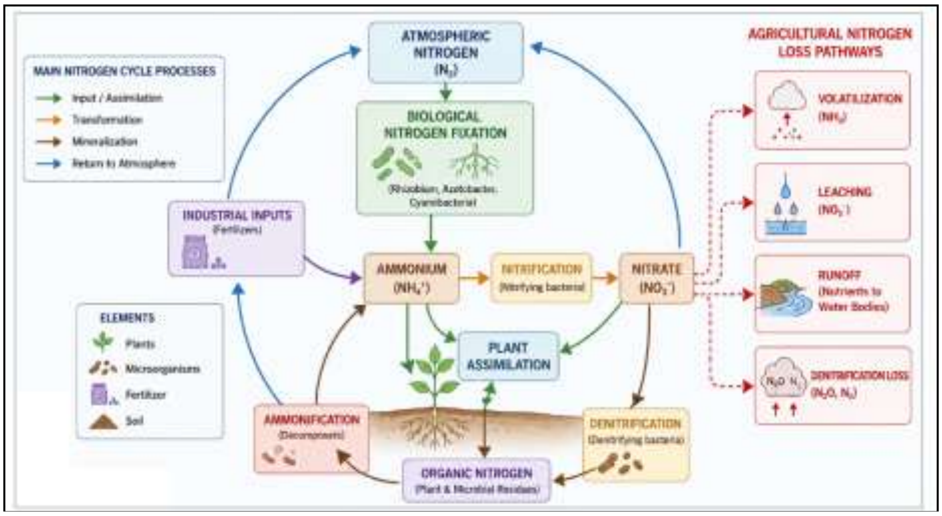

Figure 1. Nitrogen cycle and agricultural nitrogen loss pathways

Figure 1 shows the major processes in the agricultural nitrogen cycle and the pathways of nitrogen loss in soil ecosystems. Biological nitrogen fixation by microorganisms such as Rhizobium and Azotobacter converts atmospheric nitrogen into forms that are biologically available. Nitrogen is cycled through the processes of ammonification, nitrification, plant assimilation and denitrification. The figure also highlights the major nitrogen loss pathways such as volatilization, leaching, runoff and gaseous emissions. These losses impair nitrogen-use efficiency, lead to environmental pollution and have adverse effects on sustainable agricultural productivity and ecosystem stability.

2.2 Nitrogen-Fixing Microbial Communities

Nitrogen-fixing microbial communities are essential for soil fertility and sustainable nutrient cycling. Rhizobium species associate symbiotically with leguminous plants and by nitrogenase activity [17] reduce atmospheric nitrogen to biologically available ammonia as shown in table 1. Free living bacteria such as Azotobacter and photosynthetic cyanobacteria such as Anabaena also contribute to nitrogen assimilation and to the increase of soil fertility [18]. Plant growth-promoting rhizobacteria (PGPR) like Bacillus and Pseudomonas improve nutrient mobilization, root development, and stress tolerance in crops. Rhizosphere microbial communities cooperate to mediate nutrient exchange, sustain ecological stability, and improve agricultural productivity [19].

Table 1. Nitrogen-Fixing and Plant Growth-Promoting Microbial Communities

Microbial Group	Agricultural Function	Representative Species
Nitrogen-fixing bacteria	Nitrogen assimilation	*Rhizobium spp.*
Plant growth-promoting bacteria	Root development	*Bacillus spp.*
Phosphate-solubilizing microbes	Nutrient mobilization	*Pseudomonas spp.*
Cyanobacteria	Soil fertility enhancement	*Anabaena spp.*

2.3 Synthetic Biology and Microbiome Engineering

Recent developments in synthetic biology and microbiome engineering have allowed for targeted manipulation of microbial communities for sustainable agriculture. CRISPR-Cas genome editing enables precise editing of nitrogen fixation genes and microbial metabolic pathways [20]. Synthetic gene circuits and systems biology approaches enable the design of cooperative microbial consortia with improved nitrogen-use efficiency, stress tolerance, and ecosystem adaptability.

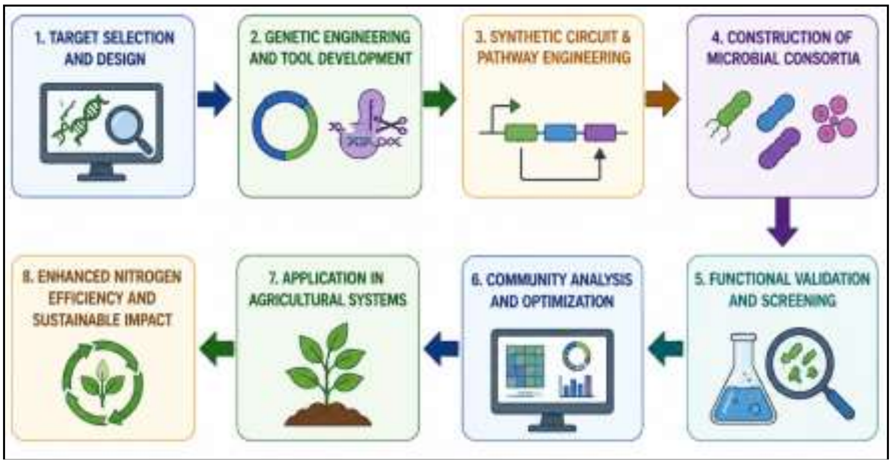

Figure 2. Synthetic biology workflow for engineering nitrogen-efficient microbial communities

Figure 2. Synthetic biology pipeline to engineer nitrogen-use efficient microbial communities for agriculture applications. The process starts with target selection and genetic design, followed by microbial genome engineering and synthetic pathway construction using CRISPR and metabolic engineering approaches. Assembled functional microbial consortia with engineered microorganisms are tested for screening nitrogen fixation efficiency. Systems biology tools are then applied to analyze and optimize the community for use in agricultural systems. The workflow aims to improve nitrogen-use efficiency, increase crop productivity and promote sustainable agriculture.

3 MATERIALS & METHODS

3.1 Experimental Design and Soil Microcosms

The study was conducted using controlled greenhouse soil microcosms experiments to test the efficiency of engineered nitrogen-efficient microbial communities in enhancing agricultural productivity as shown in table 2. Samples of agricultural soils were taken from nitrogen-poor croplands and mixed under sterile conditions in the laboratory. Treatments included native soil microbiomes and engineered microbial consortia to enhance nitrogen fixation and nutrient cycling. Each treatment was replicated three times at controlled environmental conditions (temperature of 25 ± 2°C and humidity of 60–70%) [21].

Table 2. Experimental Design and Microbial Treatments

Experimental Group	Microbial Treatment	Agricultural Function

Control	Native soil microbiome	Baseline comparison
Group A	Engineered *Rhizobium* consortium	Enhanced nitrogen fixation
Group B	Synthetic microbial community	Multi-functional nutrient cycling

3.2 Microbial Engineering and Synthetic Biology

Rhizobium spp., Azotobacter spp. and Bacillus spp. are beneficial microbial strains that have been genetically modified using CRISPR-Cas9 genome editing technology. Nitrogenase (nifH, nifD and nifK) genes were optimized to increase the efficiency of nitrogen fixation. Synthetic promoters and gene circuits were engineered into the microbial genomes for improved metabolic coordination and microbial cooperation in the rhizosphere ecosystem. Engineered microbial consortia were cultured under selective conditions to ensure genetic stability and functional expression [22].

3.3 Plant Growth and Soil Analysis

Plant growth experiments were conducted with maize (Zea mays) seedlings grown in treated soil microcosms (table 3). After 45 days of growth, the soil nitrogen concentration, chlorophyll content, plant biomass and nitrogenase activity were determined. Total nitrogen content was determined using the Kjeldahl method. Spectrophotometric assay of chlorophyll concentration. The capacity for biological nitrogen fixation was estimated by measuring nitrogenase activity using the acetylene reduction assay.

Table 3. Plant Growth and Soil Analysis Parameters

Parameter	Method Used	Instrument
Soil nitrogen	Kjeldahl analysis	Nitrogen analyzer
Chlorophyll content	Spectrophotometry	UV-Vis spectrometer
Nitrogenase activity	Acetylene reduction assay	Gas chromatograph
Plant biomass	Dry weight measurement	Analytical balance

3.4 Metagenomic and Transcriptomic Analysis

Extraction of soil microbial DNA and RNA DNA and RNA extraction was performed using commercial kits. High-throughput next-generation sequencing (NGS) was used to assess microbial diversity, nitrogen metabolism pathways and gene expression profiles. Metagenomic and transcriptomic datasets were used to functionally annotate nitrogen fixation genes and microbial interaction networks. Differential gene expression analysis showed pathways associated with nitrogen assimilation and stress adaptation [16].

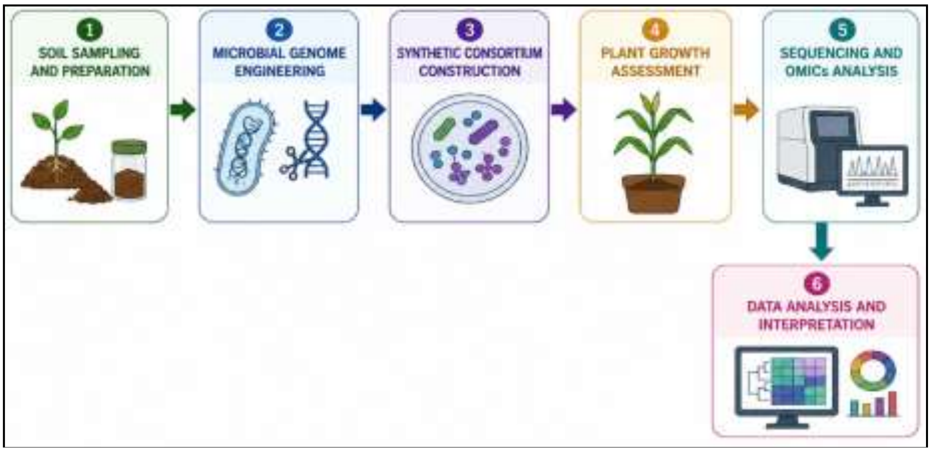

Figure 3. Experimental Workflow for Synthetic Microbiome Engineering

Figure 3 Experimental workflow for engineering nitrogen-efficient microbial communities including soil sampling, microbial genome engineering, synthetic consortium construction, plant growth assessment, sequencing analysis and bioinformatics interpretation.

3.5 Statistical and Bioinformatics Analysis

A one-way ANOVA and Pearson correlation analysis were conducted to compare the differences among the treatment groups. Microbial diversity indices and interaction networks were calculated with QIIME2 and Cytoscape. Predictive ecological modeling and transcriptomic analyses were performed using R software and SPSS. We considered statistical significance at $p < 0.05$ [13].

3.6 Dataset and Experimental Parameters

The dataset included soil physicochemical properties, microbial diversity profiles, nitrogen fixation efficiency, plant biomass measurements, and transcriptomic gene expression data from greenhouse soil microcosm experiments. Data were collected from control and engineered microbial treatment groups to determine nitrogen-use efficiency and stability of the microbial ecosystem. Table 4 showed that the major parameters included soil nitrogen concentration, chlorophyll content, nitrogenase activity, microbial abundance and expression levels of nitrogen metabolism genes such as nifH and amoA. These parameters were analyzed using metagenomic sequencing, spectrophotometric assays and bioinformatics approaches to comparatively evaluate engineered microbial communities [11][16].

Table 4. Dataset Parameters Used in the Study

Parameter	Description	Measurement Method
Soil nitrogen	Total nitrogen concentration	Kjeldahl analysis
Plant biomass	Crop growth performance	Dry weight analysis
Nitrogenase activity	Nitrogen fixation efficiency	Acetylene reduction assay
Gene expression	Nitrogen metabolism genes	RNA sequencing
Microbial diversity	Rhizosphere microbial composition	Metagenomic sequencing

4 RESULTS & DISCUSSION

The engineered nitrogen-efficient microbial communities improved biological nitrogen fixation, rhizosphere stability and crop productivity significantly compared to the native soil microbiome. Metagenomic and transcriptomic analysis revealed an enhanced microbial cooperation and an increased expression of genes involved in nitrogen metabolism in the rhizosphere ecosystem. Under greenhouse conditions, engineered microbial treatments improved nitrogen assimilation efficiency, biomass production and chlorophyll content. Correlation analysis also showed strong relationships between synthetic microbial engineering, nitrogen cycling pathways and agricultural productivity, indicating the potential of synthetic biology approaches for sustainable and climate-resilient agriculture.

4.1 Nitrogen Fixation Efficiency

The engineered microbial consortia exhibited significantly higher nitrogen fixation efficiency than the control treatment. The highest nitrogen fixation rate (82%) and plant biomass increase (48%) were observed in group B, which included the synthetic microbial community, indicating an increase in nitrogen assimilation and nutrient availability in the rhizosphere (table 5).

Table 5. Nitrogen Fixation Efficiency and Plant Growth Performance

Experimental Group	Nitrogen Fixation Rate (%)	Plant Biomass Increase (%)
Control	38	12
Group A	71	36
Group B	82	48

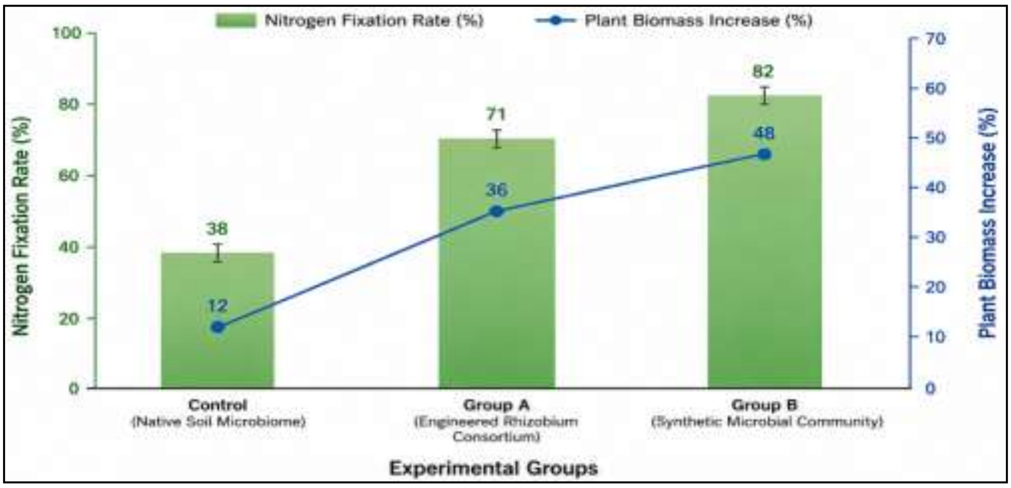

Figure 4. Comparative Nitrogen Fixation Efficiency Among Engineered Microbial Communities

Fig. 4 illustrates the efficiency of nitrogen fixation and plant biomass enhancement in the different microbial treatment groups. Engineered microbial communities significantly boosted nitrogen assimilation and plant growth compared to native soil microbiome.

4.2 Microbial Diversity and Functional Pathways

Metagenomic analysis showed that the nitrogen-fixing bacterial populations were enriched and functional nitrogen metabolism genes were highly expressed in engineered microbial communities. Higher abundance of Rhizobium, Azotobacter and Bacillus spp. contributed to the improved efficiency of nitrogen cycling and stability of rhizosphere.

Table 6. Nitrogen Metabolism Genes and Functional Roles

Gene	Functional Role	Expression Fold Change
nifH	Nitrogen fixation	4.2
amoA	Nitrogen metabolism	3.1
glnA	Ammonia assimilation	2.8

Table 6 shows significant upregulation of genes related to nitrogen metabolism in the engineered microbial communities. Enhanced nifH expression confirmed improved biological nitrogen fixation whereas amoA and glnA contributed to nitrogen assimilation and nutrient cycling efficiency.

4.3 Synthetic Biology Engineering Outcomes

Synthetic biology approaches improved microbial cooperation, ecosystem adaptability and nutrient use efficiency. CRISPR-based optimization of nitrogenase pathways and synthetic regulatory circuits improved functional stability of engineered microbial consortia under agricultural conditions.

Table 7. Outcomes of Synthetic Microbiome Engineering

Engineered Component	Biological Effect	Agricultural Significance
CRISPR-edited nitrogenase genes	Enhanced nitrogen fixation	Reduced fertilizer dependency
Synthetic microbial circuits	Stable microbial cooperation	Improved rhizosphere stability
Metabolic pathway optimization	Increased nutrient assimilation	Enhanced crop productivity

4.4 Correlation Between Synthetic Biology and Agricultural Productivity

Correlation analysis demonstrated significant positive relationships between the engineered microbial pathways and agricultural productivity parameters, including nitrogen availability, chlorophyll content and plant biomass. Microbial cooperation was enhanced in greenhouse conditions, leading to increased ecosystem stability and resilience in nutrient cycling.

4.5 DISCUSSION

Results of this study show that engineering of microbial communities based on synthetic biology improves nitrogen-use efficiency and agricultural sustainability. Engineered microbial consortia, which contain nitrogen-fixing and plant growth-promoting microorganisms, enhanced nitrogen assimilation, plant biomass, and rhizosphere ecosystem stability, compared to native microbial populations.

The observed upregulation of nifH, amoA and glnA genes confirms that the CRISPR-mediated genome editing and optimization of metabolic pathways effectively improved the nitrogen fixation and nutrient cycling pathways. Synthetic regulatory circuits also improved microbial cooperation and stress resilience, which contributed to improved ecosystem functionality in the context of agriculture.

Engineered microbial treatments were more sustainable than conventional fertilizer-dependent systems, by reducing nitrogen loss and improving nutrient utilization efficiency. These results are consistent with recent advances in synthetic microbiome engineering and sustainable agricultural biotechnology.

Promising results, but large-scale field validation and ecological biosafety assessments are still required before commercial agricultural implementation. Future integration of artificial intelligence, precision agriculture technologies and multi-omics systems biology may further optimize engineered microbial communities for climate resilient and environmentally sustainable farming systems.

5 CONCLUSION

We have shown that the construction of nitrogen-efficient microbial communities by synthetic biology can greatly enhance biological nitrogen fixation, rhizosphere stability and crop productivity. Engineered microbial consortia containing nitrogen fixing and plant growth promoting microorganisms demonstrated improved nitrogen assimilation and nutrient cycling efficiency compared to native soil microbiomes. By successfully applying CRISPR-mediated genome editing and synthetic metabolic pathway optimization, the expression of genes involved in nitrogen metabolism, such as nifH, amoA, and glnA, was increased, leading to improved nitrogen-use efficiency and reduced nutrient loss.

The engineered microbial treatments also enhanced plant biomass accumulation, chlorophyll content and overall crop performance under greenhouse conditions. The synthetic gene circuits enhanced microbial collaboration, stress tolerance and functional stability in the rhizosphere ecosystem, contributing to sustainable agricultural productivity. Metagenomic and transcriptomic analyses provided additional support for the activation of major nitrogen cycling pathways and increased microbial ecosystem interactions.

Overall, the results indicate the potential of synthetic microbiome engineering as an environmental-friendly alternative to overuse of chemical fertilizers. The combination of synthetic biology, systems biology and engineering of agricultural microbiomes offers opportunities for the development of climate-resilient farming systems and for the improvement of long-term soil fertility and ecosystem sustainability.

6. Future Scope

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