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Engineering plant–microbe communication for plant nutrient use efficiency

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Nutrient availability and efficient use are critical for crop productivity. Current agricultural practices rely on excessive chemical fertilizers, contributing to greenhouse gas emissions and environmental pollution. Rhizosphere microbes facilitate plant nutrient acquisition and contribute to nutrient use efficiency. Thus, engineering plant–microbe communication within the rhizosphere emerges as a promising and sustainable strategy to enhance agricultural productivity. Recent advances in plant engineering have enabled the development of plants capable of selectively enriching beneficial microbes through root exudates. At the same time, synthetic biology techniques have produced microbes capable of improving nutrient availability and uptake by plants. By engineering plant–microbe communication, researchers aim to harness beneficial soil microbes, thereby offering a targeted and efficient approach to optimizing plant nutrient use efficiency.

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Introduction

Plant roots establish complex and dynamic interactions with diverse microbial communities in the rhizosphere, the soil region near roots. These interactions, which can have beneficial, harmful, or neutral outcomes for the host, are regulated by not only the plant genotype and microbiome composition but also by environmental conditions, plant developmental stage, microbial interspecies interactions, and agricultural practices [1]. This complexity of plant–microbiome interactions presents both challenges and opportunities for improving plant nutrient use efficiency (NUE).

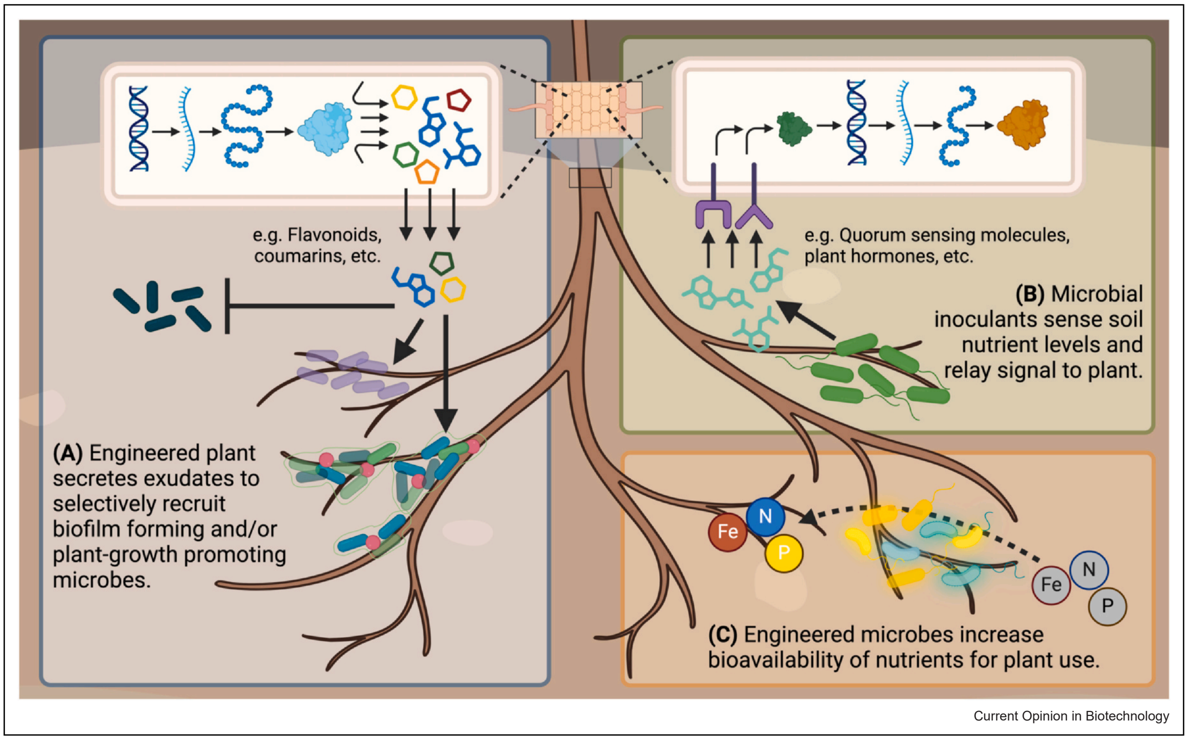
The rhizosphere microbiomes improve plant NUE and growth through increased bioavailability and acquisition of nutrients, altered hormonal signaling in plants, and competitive exclusion of pathogenic microbes [2]. This has led to various approaches of microbiome engineering, from soil amendments and biofertilizers to synthetic microbial communities (SynComs) to improve NUE, growth, and resilience of plants [3–5]. However, molecular mechanisms regulating the microbiome are mostly unclear, especially in non-model plants. Advances in synthetic biology, genome editing, metagenomics, and metatranscriptomics have started to shape the understanding of plant–microbiome interactions and enable targeted engineering of plant–microbiome communication.

Sustainable approaches to improve crop NUE can target the engineering of plants, microbiomes, and/or their interactions. Among these diverse approaches, plant breeding and gene editing are reviewed elsewhere [6,7]. In this review, we focus on communication between plants and rhizosphere microbiomes and survey recent root exudate and microbial community engineering studies that maximize plant nutritional benefits. Symbiotic associations with mycorrhizae and nodule-inducing bacteria are reviewed elsewhere [8–11]. A clear molecular-level understanding of the communication between plants and microbes could lead to improved NUE and crop production, thereby contributing to sustainable global food security.

Root exudates as drivers of plant–microbe communication

Plants use root exudates, which function as nutritional substrates and signaling molecules for microorganisms, as means of chemical communication to modulate the rhizosphere microbiome [12–14] (Figure 1). The wide variety of exuded compounds include sugars, proteins, amino acids, organic acids, fatty acids, and secondary metabolites [15–17]. The composition of root exudates is dynamic and changes with plant genotype, developmental stage, and soil nutrient availability [18,19]. The underlying molecular mechanisms of plant–microbe communication between nitrogen-fixing microbes, phosphate-solubilizing strains, and their plant hosts are discussed elsewhere [20,21]. Here, we only de-

Figure 1



Strategies for plant–microbe communication engineering. **(a)** Plants can be genetically engineered to secrete specific exudate compounds into the rhizosphere, such as flavonoids and coumarins, to promote the recruitment and growth of select beneficial microbial populations or to exclude the certain pathogenic microbes (shown by arrows of → and —|, respectively). **(b)** Sentinel microbes in the soil can sense environmental nutrient levels and inform the plant host through a synthetic communication molecule, such as quorum-sensing molecules or plant hormones, for a rapid genetic response within the plant. **(c)** Microbes can be modified to enhance nutrient solubilization or fixation to increase the amount of plant bioavailable nutrients. This figure was made using BioRender.com.

scribe studies of root exudate properties and responding microbiome under different nutrient availability conditions.

In maize and sorghum, several genetic loci displayed associations with microbiome assembly under nitrogen deprivation [5,20], where exudation of specific flavones in maize promoted colonization of Oxalobacteraceae that influenced lateral root development and promoted nitrogen acquisition [22]. Flavonoids in root exudates are important signals for attracting plant growth-promoting rhizobacteria and enhancing biological nitrogen fixation [23]. Zhang et al. reported that field-grown indica rice exhibited superior nitrogen use efficiency compared with japonica varieties, given the enriched recruitment of bacteria involved in ammonification in the rhizosphere of indica varieties, providing more plant bioavailable nitrogen [19]. They revealed that in indica varieties NRT1.1B, a nitrate transporter and sensor, was associated with the recruitment of these beneficial bacteria, while the indica rice with loss-of-function mutant NRT1.1B or japonica varieties with a natural single nucleotide polymorphism in their NRT1.1B have lower abundance of bacteria with ammonification capabilities in their microbiome [19]. Despite this research providing a direct genetic link between NRT1.1B and the composition of root microbiome, it does not reveal how NRT1.1B at the molecular level is able to enrich these bacteria.

Besides nitrogen, phosphorus solubilization by microbial communities is critical for rendering it plant bioavailable. For instance, Zutter et al. used host-mediated selection under phosphorus deficiency and identified bacterial isolates with high phosphorus solubilization traits to employ as inoculants [24]. Moreover, under iron deficiency, exudation of coumarins is elevated to improve iron availability and to import iron into the roots of many crops [25]. The role of coumarins in shaping the composition of rhizosphere microbiomes under iron deprivation was also demonstrated in the model plant Arabidopsis [26,27]. More such studies are needed for different plants and nutrient types.

A clear understanding of changes in exudate profiles and colonization cues for desirable microbes under different nutrient availability conditions can inform engineering approaches to improve crop NUE.

Recent advances in exudate engineering for improved plant nutrient use efficiency

Manipulating the composition of root exudates is a promising strategy for the targeted enrichment or suppression of specific microbes (Figure 1a). Genetic engineering of plant transporters for secretion of malate in rice and γ-amino butyric acid and citrate in wheat leads to differential exudate and microbiome composition [28]. In addition, the lysine histidine transporter 1 (LHT1) expression in *A. thaliana* represses a plant growth-promoting *Pseudomonas* strain [29]. A few other studies have identified root exudate compounds, such as volatile organic compounds, phenolics, flavonoids, coumarins, and organic acids, which can modulate the rhizosphere and improve plant NUE [27,30–34].

In the context of nitrogen, enhancing root colonization by diazotrophic microbes capable of fixing atmospheric nitrogen offers a promising strategy. In rice, increased apigenin exudation induced colonization and biofilm formation of nitrogen-fixing bacteria, *Gluconacetobacter diazotrophicus*, which led to higher levels of biological nitrogen fixation and improved seed yield compared with wild-type rice [35]. In addition, the plants edited with clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) system had significantly higher nitrogen-fixing bacteria species richness compared with the control plants in all root compartments. This work successfully addressed the complexity of plant–microbe interactions given the initial metabolite screens, selection from multiple biofilm-forming species, and holistic analysis across all root compartments.

An alternative to the modification of native pathways for targeted enrichment is introducing synthetic secretion pathways. After an extensive search for chemical signals that are both recognizable by soil microbes and engineerable into plants, Geddes et al. leveraged rhizopines as a synthetic transkingdom communication molecule between *Nicotiana benthamiana* plants and their microbiome [36]. Rhizopines are carbon sources used by certain root nodule–associated rhizobia and have long been recognized as potential enrichment molecules [37]. Furthermore, the secretion of a rhizopine in barley and its detection by a microbial biosensor were demonstrated [38], which is a key step toward the development of synthetic signaling networks between plants and microbes. More studies are needed to demonstrate the specificity of the signal and desired microbial behavior.

The direction of signaling may also be reversed, where the plant senses signals from its microbiome about environmental nutrient levels, leading to a more fine-tuned nutrient response (Figure 1b) [39]. In a recent proof-of-concept study, a quorum-sensing molecule was employed to develop sentinel microbes to transmit environmental stimuli to plants. This synthetic microbe-to-plant communication system was validated in *A. thaliana* and *Solanum tuberosum* and relayed arsenic contamination to receiver plants grown hydroponically [39]. This work establishes the basis for using sentinel bacteria in the rhizosphere to relay stimuli, such as soil nutrient content, and improve nutrient scavenging in plants (Figure 1c).

Symbiotic microbes simultaneously compete with the plant and other rhizosphere species for limiting nutrients. Therefore, overexpressing certain exudates in plants can come at a metabolic disadvantage. Likewise, the engineering of rhizosphere microbes can reduce their ability to compete. For instance, barley has been engineered to produce *scyllo*-inosamine, a molecule that a nitrogen-fixing cereal endophyte, *Azorhizobium caulinodans*, was modified to have increased sensitivity [40]. Despite the mutant barley having higher colonization by *A. caulinodans*, the engineered bacteria demonstrated little nitrogenase activity, resulting in reduced biomass in the plant. Therefore, engineering exudate secretion must find the optimal expression patterns using thoughtful synthetic circuits in both the plant and the microbial symbiont [41].

Engineering microbial communities for increased nutrient acquisition by plants

Harnessing microbes for improved plant NUE will benefit from microbiome genetic engineering (Figure 1c). Engineering phosphate-solubilizing microbes has focused on the expression of phosphatase and phytase enzymes that release phosphate from insoluble soil sources. For instance, Shulse et al. introduced 82 phytate hydrolysis pathways into three root-associated bacteria [42]. From these, 12 supported *A. thaliana* growth when phytate (poorly usable by plants) is the only phosphorus source. Future studies should test the engineered strains in soil and in other plants to assess the host range. Using multiple microbial strains can increase the plant host range and fill gaps in biochemical processes. In fact, inoculation with microbial consortia improves nutrient acquisition and plant growth promotion [43]. Future work may combine engineered strains into a community that can solubilize multiple organic phosphate sources, including phytate, iron-phosphate, and calcium-phosphate, to maximize release across various soil pH.

In addition to genetic engineering, native microbial SynComs can be leveraged to improve plant NUE. Castrillo et al. showed that a 35-member SynCom upregulated the transcription factor activity of PHR1 in *A. thaliana*, demonstrating the role of the microbiome in plant nutrient signaling [44]. Importantly, the SynCom did not help mutant *Arabidopsis* plants lacking phosphate stress response genes to acquire phosphate, emphasizing the dependency on plant gene modulation by microbes. Levels of available nutrients also affect whether microbes compete with or help the plant. Supporting this, the SynCom increased plant phosphate uptake when transferred from a low to replete phosphate environment.

In terms of nitrogen, Pivot Bio, an agricultural biotech company, developed the first commercially available nitrogen-fixing microbe product for corn [45]. Their product functions in already nitrogen-rich soils and increases the use efficiency of the fertilizer-sourced nutrient. To avoid downregulation of nitrogen fixation due to existing high nitrogen levels in fertilized fields, they used computational tools to identify a suitable strain and further modified it to fix nitrogen regardless of environmental nitrogen levels. Their field-deployable product has been shown to support corn growth.

Considerations for the deployment of biofertilizers in agricultural settings

For translation to field settings, biocontainment is a prominent concern [46,47]. Promising strategies include creating metabolic dependencies [48] and kill switches [49,50] to prevent ecosystem escape. For example, engineered microbes can be designed to require synthetic amino acids and plants may be engineered to supply them [51]. However, these strategies involve plant metabolic interventions to support the microbe's auxotrophy. Kill switches rely on an environmental signal to regulate the expression of toxin-producing genes. In the kill switch 'Deadman,' anhydrotetracycline suppresses toxin expression, and isopropyl β-D-1-thiogalactopyranoside (IPTG) induces it [49]. Halvorsen et al. compared eight different toxin effectors in *Pseudomonas fluorescens* to optimize the tradeoff between kill switch efficacy and evolutionary pressure [52]. Future work should test kill switches in other agriculturally relevant species and reduce the evolutionary pressure on these engineered circuits.

The environmental stability of engineered microbes is an additional challenge for field translation. Many rhizosphere studies are performed in closed systems and under controlled settings. Developing experiments considering the dynamics and heterogeneity of both soil systems and microbiomes is critical [53–55]. In addition, engineered microbes are quickly outcompeted by native communities. Testing the environmental persistence of desirable strains can be aided using SynComs that mimic such communities [11,56]. Developing an organized field trial system, much like clinical trials in the biomedical field, may aid the development and safe deployment of sustainable biofertilizers. This will require cooperation between regulatory bodies, manufacturers, and scientists.

Regulatory challenges further delay the deployment of engineered microbes. Currently, US regulatory pipelines for the use of engineered microbes in open systems are unclear. This uncertainty forces companies to take approaches to prevent the 'genetically engineered' label, such as using endogenous promoters to overexpress native genes [45]. Public sentiment on genetically modified organisms further clouds the prospects for widespread microbial technology use [47]. The use of engineered microbe technologies will depend on the development of clearer policies that rely on scientifically informed guidelines [57,58].

Despite these challenges, the biofertilizer market is expected to grow [59]. Demonstrating greater sustainability and agricultural yields through biofertilizers instead of chemical fertilizers is impeded by a lack of scientific and economic comparison studies. The development of low-cost biofertilizers may encourage the transition to more sustainable agriculture [60].

Future directions & conclusion

Recent studies have demonstrated the promise of not only engineering plants and microbes but also their communication for improving plant NUE. Despite this progress, lab-to-field translation remains a large challenge. Difficulties in engineering non-model food crops combined with regulatory barriers for the deployment of both modified plants and microbes limit progress. Future work to mitigate some of these limitations will rely on finding optimal targets for communication engineering within plants that are highly specific to the target microbe and exploring non-model plant systems. Computational and plant synthetic biology approaches will greatly enhance the search and implementation of plant-specific microbe enrichment. Machine learning to identify promising exudate or gene targets within plants will also aid. Higher efficiency, more facile, and species-independent plant genome engineering methods are still needed, and novel approaches such as nanomaterials are promising [61,62]. Moreover, computational pipelines to survey the whole rhizosphere will reveal how communities affect and contribute to nutrient acquisition instead of focusing on the individual microbes. Synthetic circuit design that integrates information from both the plant and the environment will be critical to this microbe engineering. Lastly, developing microbes that effectively colonize specific plant hosts, compete against native microbes, persist in heterogeneous soil conditions, and meet regulatory demands are key considerations in developing bioinoculants that can assist crops. However, more studies are needed to translate these emerging approaches from lab to field and to more agriculturally relevant cases.

CRediT authorship contribution statement

Catherine Griffin: Conceptualization, Writing – original draft, Visualization. **Tufan Oz:** Conceptualization, Writing – original draft, Visualization. **Gozde S Demirer:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data were used for the research described in the article.

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