

Perspective

Nutrient stress-primed microbial communities improve plant resilience

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Plants live in close association with microbial communities that comprise a diverse array of bacteria, fungi, and protists. These microorganisms thrive in various niches within and around plants, including the rhizosphere (the zone of soil surrounding a plant root), phyllosphere (above-ground plant surfaces), and endosphere (interior of plant tissues). The outcomes of these associations span a broad continuum, ranging from mutualism to commensalism to parasitism. A robust microbial community can benefit its host plant by facilitating nutrient uptake, producing growth-promoting factors, defending against pathogens, and enhancing tolerance to abiotic stresses [1]. Despite significant advancements in understanding the molecular intricacies of individual plant-microbe interactions, our comprehension of how plants establish and interact with their associated microbiota, and the subsequent impacts on plant performance, remains limited. These insights are crucial for developing strategies to optimize host-microbial alliances, thereby improving crop yields and resilience.

As sessile organisms, plants have developed a myriad of mechanisms to adapt and thrive in challenging environments, with soil microorganisms playing a crucial role in alleviating stress. For instance, most terrestrial plants can engage in mutualistic associations with mycorrhizal fungi to facilitate water and nutrient uptake. Similarly, legume plants have evolved to flourish in nitrogen-deficient soils through forming root nodule symbioses with nitrogen-fixing bacteria, known as rhizobia. In addition to these well-characterized symbiotic interactions, plants actively recruit and collaborate with other plant-growth-promoting bacteria within their environment to augment their ability to explore for water and nutrients.

While stress conditions may disrupt the normal succession of root microbiota, plant-soil feedback can reshape soil microbial communities to mitigate stress [2]. Different stressors can selectively promote the proliferation of specific microbial species to maximize their benefits on plants experiencing those stressors. These stress-tailored microbial communities can also confer benefits to subsequent plants encountering similar stresses, a phenomenon often referred to as "legacy effects." A recent study has shed light on this legacy effect by demonstrating that soil microbial communities derived from colder or drier

conditions increased the survival of inoculated tree seedlings exposed to those specific stressors [3]. Likewise, microbiota accustomed to saline conditions, particularly endophytes dwelling within plant leaves, exhibited the capacity to alleviate salt stress in mangrove ecosystems [4]. These findings underscore the potential of harnessing stress-tailored microbiota to bolster plant resilience in adverse environments.

Plants depend on the soil to supply essential mineral nutrients such as nitrogen (N), phosphorus (P), and potassium (K), which can diminish over successive growing seasons, necessitating fertilization to sustain soil fertility. Several studies suggested that plants can respond to nutrient scarcity or imbalance by reprogramming their root-associated microbiome to enhance nutrient cycling in the soil [5, 6]. However, the effects of nutrient stress on the quantitative shifts in microbial communities and their consequent effects on plant growth remain unclear.

In their recent study published [7], Wang et al. explored the dynamics of root-associated bacterial communities throughout various stages of soybean growth in a field subjected to four distinct fertilization treatments. These treatments spanned over four decades, including one with full fertilization (N, P, and K) and three others without N, P, or K supplementation. The experiments revealed significant temporal changes in the composition of root-associated bacteria during soybean development, with a notable increase in bacterial abundance at later growth stages. The absence of N amendment (-N treatment) caused significant divergence in bacterial communities, leading to an increased abundance of several bacterial orders, while the -P treatment reduced total bacterial abundance and hindered the turnover of the rhizosphere bacterial community. Specifically, the -N treatment led to a significant increase in rhizobial abundance in the rhizosphere, whereas the -P treatment resulted in a reduction. This trend aligns with the observed augmentation in both the number and size of root nodules under the -N treatment, contrasting with the decreased nodule count and diameter observed under P deprivation. Relative to the -N and -P treatments, the influence of the -K treatment on microbiome composition is minimal.

In line with observed shifts in rhizosphere bacterial communities, metagenomic analysis unveiled distinct functional adaptations of the rhizosphere microbiome in response to different fertilization regimes, illuminating the intricate interplay between soil nutrient status and microbial activities in soil ecosystems. In the absence of nitrogen fertilization, genes associated with nitrogen mineralization processes were notably enriched, while those linked to nitrogen reduction and denitrification were diminished. This is striking because nitrogen mineralization plays a crucial role in converting organic nitrogen into inorganic forms that plants can readily uptake, and the diminished function of nitrogen reduction and denitrification helps maintain soil fertility by reducing the conversion of nitrate (NO_3^-) to dinitrogen (N_2) and nitrous oxide (N_2O). However, the -N treatment also resulted in a decrease in inorganic P solubilization and K transportation, suggesting negative impacts on P and K absorption or reduced demand for P and K under nitrogen

deficiency. Similarly, the absence of phosphorus fertilization enriched genes associated with inorganic phosphorus solubilization and phosphorus starvation response, while the absence of potassium supplementation enriched genes associated with potassium transport. These observations suggest compensatory mechanisms by the rhizosphere microbiome to sustain the nutrient demands of plants under nutrient-limiting conditions.

To provide a deeper understanding of community dynamics, Wang et al. constructed co-occurrence networks for the core taxonomic groups known as Amplicon Sequence Variants. Their aim was to identify patterns of microbial cohabitation across various treatments throughout different stages of plant development and to define the keystone species potentially pivotal in shaping microbial community structure and function. However, this analysis did not reveal the presence of keystone species in most networks, supporting the idea that it is microbial consortia, rather than individual taxa, that influence the functionality of rhizosphere microbiome [9, 10].

A network analysis of fertilization-induced community changes revealed three distinct ecological clusters (Fig.1). In the first cluster, there was no significant difference in bacterial load between the full fertilization and -N treatments. The second cluster exhibited a significantly increased abundance, defined as a low-nitrogen-enriched cluster. In contrast, the third cluster showed a reduced abundance of the bacterial load.

The researchers took an additional step to evaluate the impact of bacterial community shifts on plant growth. To accomplish this, they isolated over 1,000 bacterial strains from the rhizosphere, representing a diverse set of core clusters, with a majority assignable at the genus level. From these isolated strains, they developed three synthetic communities (SynComs). The first, SynCtrl, served as a control, comprising a bacterial cluster that displayed no significant differences between fully fertilized and -N treatments. The second, SynCom7, included strains from the low-nitrogen-enriched cluster, featuring genera *Rhodococcus*, *Lysobacter*, *Terrabacter*, *Arthrobacter*, *Phyllobacterium*, *Bosea*, and *Aeromicrobium*. To maintain consistency with SynCtrl and prevent potential nodulation induced by *Bradyrhizobiaceae*, SynCom5 was formulated by incorporating the five groups from SynCom7, excluding the *Bosea* and *Aeromicrobium* strains. Soybean plants grown in pots were inoculated with the three SynComs and phenotyped two and three weeks after inoculation. Results indicated that, compared with SynCtrl, both SynCom5 and SynCom7 stimulated plant growth regardless of additional nitrogen application. Furthermore, SynCom7 and SynCom5 exhibited typical traits conducive to plant growth, such as higher levels of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and indole-3-acetic acid (IAA) production.

Modern agriculture is heavily dependent on chemical fertilizers to sustain crop production. However, this widespread practice is not only unsustainable but also contributes to environmental pollution and disrupts ecosystem integrity and function. The revelation that nutrient stress-adapted microbial communities can bolster plant growth presents a

promising strategy for transforming agricultural practices towards greater sustainability. Despite the encouraging results of this proof-of-concept study, several outstanding questions persist and demand further investigation.

The research conducted by Wang et al. focused on soybean, a legume crop. The sustained long-term production of soybean in fields without nitrogen supplementation is primarily attributed to the enhanced nitrogen-fixing root nodule symbiosis. Under this scenario, the absence of nitrogen facilitates nodulation, and the nodulation process subsequently impacts the composition of rhizosphere and endosphere microbial communities [8]. Expanding upon this investigation, it is imperative to comprehend how the different fertilization treatments affect microbial communities in the rhizosphere of cereal crops such as maize and wheat, which are rotated with soybeans in these fields. It will be intriguing to explore whether similar or distinct microbial groups are enriched in the nutrient-deficient soils. If differences exist, understanding potential synergistic or antagonistic effects among these bacterial communities becomes imperative. Furthermore, unraveling the duration and magnitude of legacy effects resulting from nutrient deficiency-adapted microbial communities in the field setting is crucial. Such insights will be invaluable for effectively managing soil microbial communities to benefit multiple crops.

While the study provides valuable insights into bacterial communities, its scope was limited by not including fungi in the analysis, thereby constraining a comprehensive understanding of microbial contributions. Future research should delve into elucidating the intricate interplay between bacteria and fungi to appreciate their collaborative roles in shaping soil microbial networks and nutrient dynamics. Investigating the multipartite interactions involving bacteria, fungi, and plants holds promise for a deeper understanding of ecosystem functioning. Moreover, exploring the effects of nutrient limitations, such as nitrogen and phosphorus, on these interactions could provide crucial insights into optimizing agricultural practices for sustainable nutrient management.

Despite the well-documented shifts in microbial communities under stress conditions, the regulatory mechanisms governing microbe-microbe and plant-microbe interactions, which underlie these changes, remain poorly understood. Stress-induced alterations in gene expression, metabolism, and microbial interactions profoundly impact soil microbial communities' resilience and functioning. Plants modulate these responses through root exudates, rhizodeposition, and symbiotic associations, collectively influencing soil microbial activity, nutrient cycling, and community composition. Integrating multi-omics approaches and advanced computational modeling techniques can further enhance our comprehension of these complex microbial associations and their ecological implications. Addressing these limitations in future studies can lead to more comprehensive and

nuanced perspectives on soil microbial ecology and its implications for ecosystem health and productivity.

Conflict of interest

The authors declare that they have no conflict of interest.

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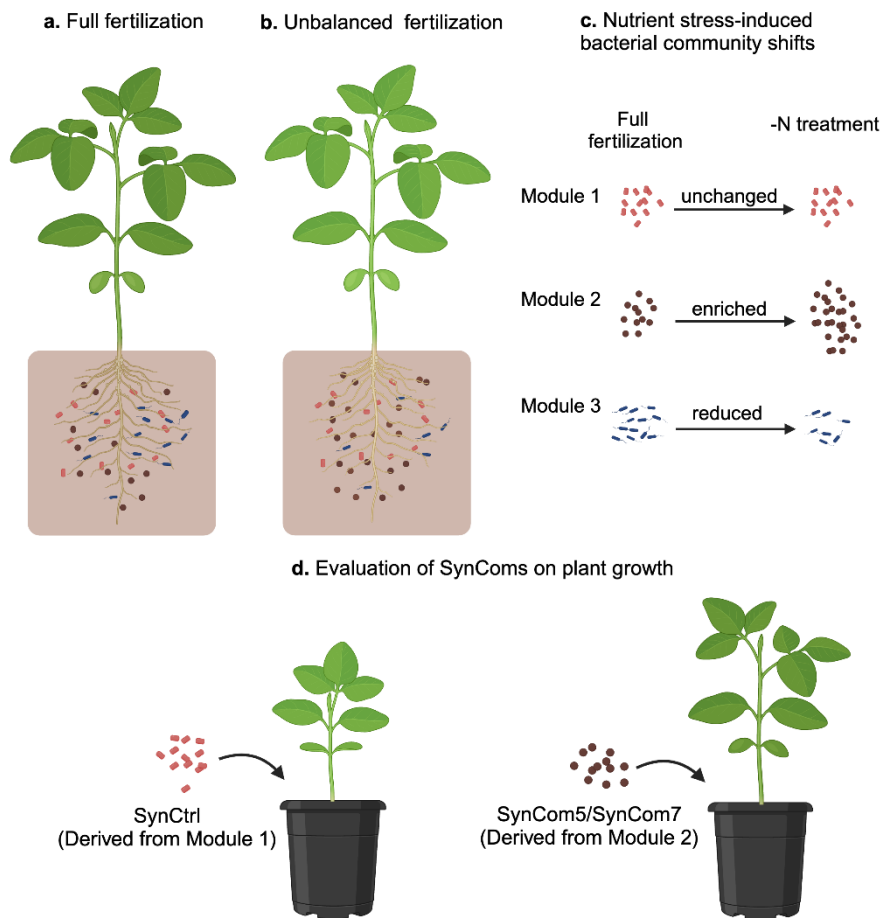


Fig. 1. Schematic illustration of nutrient deficiency-induced bacterial community changes and their impact on plant growth. Soybean plants were grown in soils with full fertilization (a) or unbalanced fertilization (lacked N, P, or K) (b). A network analysis of their bacterial communities revealed three modules (c), including one showing no significant difference between the full fertilization and -N treatments (module 1) and two others with enriched (module 2) or reduced bacterial abundance (module 3) under -N treatment. (d) The two SynComs, SynCom5 and SynCom7, derived from the low-nitrogen-enriched cluster (module 2) enhanced plant growth (right side) when compared with SynCtrl, a SynCom derived from module 1 (left side). This figure was created with BioRender.com.