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# Reductionist synthetic community approaches in root microbiome research

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Synthetic community (SynCom) approaches can provide functional and mechanistic insights into how plants regulate their microbiomes, and how the microbiome in turn influences plant growth and health. Microbial cultivation and reconstruction play pivotal roles in this process, which enables researchers to reproducibly investigate the interactions between plants and a major proportion of plant-associated microbes in controlled laboratory conditions. Here, we summarize the emergence, current achievements, and future opportunities for using SynCom experiments in plant microbiome research, with a focus on plant root-associated bacteria.

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## Introduction

A distinctive microbiome assembles on plant roots, with microbial members originating from soil environments [1••,2,3••]. These microbes interact with plants throughout their lifecycle [4,5], influencing plant growth [6,7••], nutrient uptake [8••,9••], and disease resistance [10••,11,12,13•]. Over the past decade, root microbiome compositions have been extensively investigated in model [1••,3••,14] and crop plants [10••,15••,16–19]. In addition, researchers have cultivated bacteria and established stocks from the microbiomes of a variety of plants, including *Arabidopsis thaliana*, rice (*Oryza sativa*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), clover (*Trifolium pratense*), and sugarcane (*Saccharum* sp.) [8••,10••,18,20••,21••,22,23]. The ultimate goal of culturing these bacteria is to examine the functions and mechanisms of the interactions between root microbiomes and host plants. Here, we discuss the key challenges of using experimental synthetic communities (SynComs), namely microbiome cultivation and reconstitution.

## From holism to reductionism

Research into the interactions between root microbiomes and host plants connects the fields of microbial ecology and plant molecular biology. Ecological studies typically investigate the root microbiome using the holistic approaches, which is critical to reveal the root microbiome status in natural environments. By contrast, studies using the reductionist approaches try to avoid uncontrollable variables, breaking down the interactions of the root microbiome with the plant host into experimentally controllable factors (cultivated microbes, nutrients, plant genotype, and so on) to understand their functions and mechanisms [24•] (Figure 1). Two seminal works pioneered the reductionist concept in root microbiome research [1••,3••]. The authors of these studies grew *Arabidopsis* plants in natural soils under controlled conditions to avoid differences in wind, rain, and temperature while allowing plants to assemble a root microbiome from native soil microbial communities (Figure 1a,b). Using a large number of biological and technical replicates, the two studies concluded that *Arabidopsis* plants assemble a distinctive root microbiome in comparison with the microbiome of unplanted soils. In addition, Bulgarelli *et al.* demonstrated that the major assembly patterns of root microbiomes in natural soils under laboratory conditions were similar to the root microbiomes in natural field sites [1••].

Investigating the assembly of the root microbiome from a natural soil inoculum under controlled conditions became popular for root microbiome studies in species such as rice, *Arabidopsis* and its relatives [15••,25•,26•] (Figure 1b). Reducing environmental variation is critical for meaningful comparative descriptions of microbiomes; however, in order to experimentally manipulate microbiomes, it is essential to dissect the interactions between the root microbiome and the host plants into experimentally controllable factors, such as microbes, plant genotypes, nutrients, and growth conditions (Figure 1c). This requires that researchers cultivate specific microbes [27].





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**Figure 1**

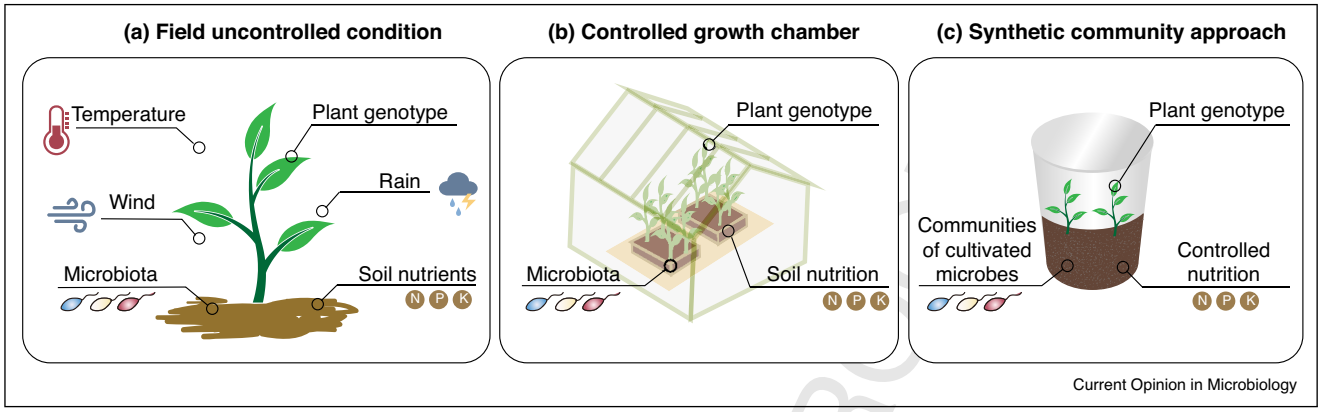


Illustration of microbiota research using field conditions, controlled growth chambers, and synthetic community (SynCom) systems. **(a)** Field experiments are used to explore the assembly and variation of the root microbiome in natural conditions. Environmental factors, such as temperature, wind, rain, microbiota, and soil nutrients, cannot be controlled. **(b)** Experiments using natural soils in controlled growth chambers can be used to avoid environmental variations and provide reproducible results. The microbiota members and soil nutrients cannot be dissected using this technique. **(c)** SynCom approaches use cultivated microbes to provide unique opportunities to obtain fully reproducible results and to investigate the function of the root microbiome in plant fitness.

## Cultivating and dissecting the root microbiome

Approaches that manipulate the microbiota require extensive collections of microbial cultures and axenic systems [20••,28•,29••]. Although thousands of cultivated microbes have been isolated and stored in international stock centers [30], these microbes were obtained from various environments or host species, and may have adaptations to local conditions and to interactions with other components of the native microbiome. Therefore, existing isolates may be genotypically and functionally distinct from the microbes that researchers find in their new samples, thus may not be suitable to dissect the interactions between plants and the root microbiome in a given natural soil.

High-throughput bacterial cultivation provides a solution for capturing and manipulating a bacterial community on a large scale (Figure 2a–d). Recently, Bai *et al.* established a pipeline for high-throughput bacterial cultivation and identification, enabling the characterization of 7943 bacterial isolates from roots and leaves of *Arabidopsis* plants grown in natural soils [20••]. This technique involves the use of tryptone yeast extract glucose, Reasoner's 2A, and related media with the limiting dilution method, complemented with cell sorting and colony picking. The bacterial isolates were characterized using a 454-based protocol, which allowed the authors to identify co-cultivated strains. The approach facilitated the cultivation of more than 50% of the bacterial taxa reproducibly present in the *Arabidopsis* root and leaf microbiomes. More recently, Zhang *et al.* used an improved identification method to characterize 13 512 bacterial isolates from rice roots, covering 70% of the rice root bacterial microbiome members. This new method improved the sequencing depth using Illumina HiSeq2500, and increased accuracy of identification by avoiding chimera formation using two-side barcode labeling and independent library preparation for each isolate [8••]. These methods provide an effective way to dissect a specific root microbiome sample with the advantage of being able to identify co-cultivated bacteria (i.e. bacterial 'isolates' that contain more than one type of bacteria).

To achieve as much diversity as possible, targeted isolation of fastidious bacteria (i.e. bacteria with specific culture requirement not met by the standard media) can complement high-throughput bacterial cultivation. Several approaches based on the 16S rRNA gene sequence or the metagenome can help identify the appropriate specialized isolation media. For example, Oberhardt *et al.* developed a tool to predict the isolation media required using the bacterial 16S rRNA gene sequence [31]. In another example, using a Greengene ID, the BugBase database can be used to obtain detailed bacterial characteristics, such as aerobic or anaerobic growth, which provide guidance for the types of cultivation conditions required [32]. Metagenomic data or bacterial genomes present a second source of information about bacterial carbon utilization and antibiotic resistance genes, which aids the design of isolation media to enriched specific bacteria [33,34]. Guided by metagenomic data, Kwak *et al.* used a marine broth medium containing kanamycin to successfully isolate 22 flavobacteria strains that confer wilt resistance in tomato [10••]. It is important to be careful with the medium preparation technique, however; for example, Kato *et al.* reported that autoclaving phosphate and agar together generates $H_2O_2$,





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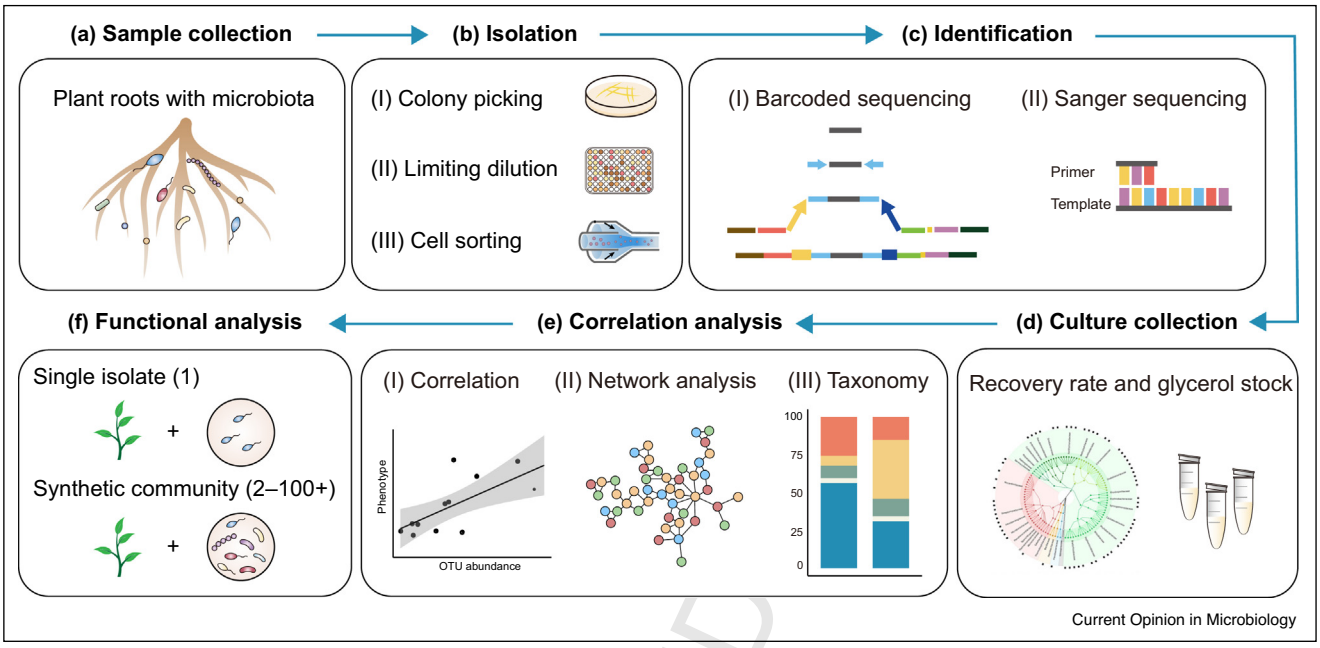
**Figure 2**




SynCom workflow.



which decreases the resulting colony count [35]. Future cultivation effort should be targeted towards gathering 'hard-to-get' taxa (i.e. Acidobacteria, Choroflexi, Deltaproteobacteria and anaerobic taxa), which ubiquitously exist in root microbiota but not well represented in culture collections.

The first-pass characterization and selection of isolates in SynCom experiments often use the 16S rRNA gene sequence of cultivated strains for cross-referencing the microbiota profiling data (Figure 2e). In microbiota profiling, comparing with operational taxonomic units (OTUs) clustering using 97% 16S rRNA gene sequence similarity, amplicon sequencing variants (ASVs, 100% 16S rDNA sequence similarity) obtained in UNOISE, DADA2, and Deblur pipelines provide a higher resolution. ASVs distinguish single nucleotide polymorphisms in the 16S rRNA gene; this allows a more accurate comparison between the cultivated bacteria and amplicon data [36–38]; however, it should be noted that the 16S rRNA gene does not reveal all genomic and functional variations among bacterial isolates, despite being the most widely used taxonomic marker. For example, Karasov *et al.* demonstrated that *Pseudomonas* isolates belonging to the same OTU (based on 16S rRNA gene sequences) showed clear differentiation at the level of genomic content and disease phenotypes [39•]. Selecting bacterial strains with the same 16S rRNA gene sequence but isolated from different roots, representing different colonization events, will therefore increase the functional diversity of the cultivated root microbiome members [8••,20••].

## Comparison of axenic systems

SynComs mainly use three axenic systems: agar-based, clay-based, and FlowPot systems, each system with different advantages and disadvantages. An agar system is highly artificial but many factors, such as nutrient levels and biotic and abiotic stresses, are uniformly controlled [40••,41,42]. Compared with an agar system, a clay-based system better mimics soil conditions because the texture of clay is similar to that of soil. However, the clay matrix influences the nutrient status, making it difficult to deplete specific nutrients such as nitrogen or phosphate, and the clay lacks organic carbon [8••,20••,43••]. The FlowPot system is based on autoclaved and washed soil, and thus contains organic carbon; however, in this system, the nutrient content cannot be controlled [7••,29••]. The choice of systems should therefore be based on the ultimate aim of the experiment.

## Reconstituting root microbiomes using SynComs

Using cultivated pure strains, scientists are able to constitute microbial SynComs in axenic systems based on the natural microbiome patterns, obtained from the correlation between microbes and plant phenotype [10••], the comparison of root microbiomes between wild-type and mutant plants [43••], or network analyses [7••] (Figure 2e). These approaches provide new opportunities to validate the root



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microbiome assembly patterns observed in natural soil conditions, study the effect of the root microbiome on plant fitness under different environmental conditions, and investigate microbe–microbe interactions and microbial gene functions [44,45,46] (Figure 2f).

Using SynComs in axenic conditions provides researchers with a controlled ecological environment in which microbes, nutrients, and plant genotypes are strictly monitored, generating reproducible results that can be used to validate observations of root microbiome patterns in natural soils. For example, Lebeis *et al.* detected alterations in the *A. thaliana* root microbiomes of salicylic acid signaling mutants compared with the wild-type plants grown in natural soils or in a calcined clay-based reconstitution system containing 38 bacterial strains [43••]. Four of the seven bacterial strains showing a difference in abundance between wild-type and mutant plants in the SynCom experiments belonged to families that showed differential abundance between wild-type and mutants in natural soils. Based on the evidence obtained from natural soil and synthetic laboratory conditions, the authors therefore concluded that the salicylic acid-mediated plant innate immunity signaling pathways modulate the assembly of the *Arabidopsis* root microbiome at the family level [43••]. A similar logic was applied by Castrillo *et al.*, who investigated the effect of the phosphate starvation pathways on the establishment of the *Arabidopsis* root microbiome [40••,41]. These authors identified altered root microbiome compositions between wild-type plants and several phosphate starvation mutants in both natural soils and SynCom experiments using Murashige and Skoog agar plates.

In addition, SynCom approaches using sequenced cultivated bacteria provide simple and reproducible systems to study the role of root metabolites (e.g. triterpenes, aromatic acids, coumarin, and camalexin) on the interactions between the root microbiome and the host plants in complex natural soils [47••,48,49•,50,51••]. In summary, experimentation using SynComs under controlled conditions permits the validation of the assembly patterns of the root microbiome and complements studies of the root microbiome in natural soil conditions.

A major benefit of inoculating germ-free plants with SynCom in axenic systems is the ability to study the function of the root microbiome under different environmental conditions. For example, Duran *et al.* inoculated germ-free *Arabidopsis* plants in the soil-based FlowPot system with different SynComs, including bacteria, fungi, and oomycetes, and found that the root bacterial microbiome protects *Arabidopsis* plants against filamentous eukaryotes (fungi and oomycetes) in natural soils [7••]. Castrillo *et al.* inoculated germ-free plants with SynComs under low-phosphate conditions, revealing that bacterial inoculation dramatically increased the plant phosphate starvation responses, and that master regulators of phosphate starvation pathways directly suppress of the plant innate immune system [40••]. In addition, the characterization of a small set of SynComs proved to be sufficient for predicting the effect of the microbiome on the phosphate utilization by *Arabidopsis* [41]. A recent follow-up study revealed that *Burkholderia* competed with plants for phosphate utilization, exacerbating plant phosphate starvation [42]. Using bacteria cultivated from the microbiomes of *indica* and *japonica* rice varieties, Zhang *et al.* revealed that the rice root microbiome facilitates the utilization of organic nitrogen by the plants [8••]. Based on correlation analysis of root microbiomes in wild disease-resistant and susceptible tomato cultivars, Kwak *et al.* successfully cultivated a target flavobacterium and demonstrated that it promoted wilt resistance when inoculated into tomato roots [10••]. Genome analyses of cultivated *Pseudomonas* led to the identification of lipopeptide biosynthesis and quorum-sensing as the major genomic differences between pathogenic and commensal root bacteria [46], in addition to the genes required for root bacteria to evade plant defense or induce plant susceptibility [44,45]. These examples demonstrate the utility of SynComs or cultivated microbes in elucidating the effect of the root microbiome on plant fitness under different environmental conditions.

Cultivated microbes and SynCom approaches provide unique opportunities for the exploration of microbe–microbe interactions and microbial gene functions. Using an agar system and a SynCom consisting of seven bacterial strains cultivated from maize roots, Niu *et al.* showed that the removal of *Enterobacter cloacae* resulted in the complete loss of microbial community structure in the roots of maize seedlings, demonstrating the importance of microbe–microbe interactions and the existence of keystone species in the microbiome [52]. Duran and colleagues performed a large-scale binary assay between cultivated root-derived bacteria, fungi, and oomycetes, revealing that the biocontrol activity of bacteria against filamentous eukaryotes is a redundant trait that maintains the interkingdom balance of the root microbiome [7••]. Levy *et al.* found that the survival of *Escherichia coli* and six leaf bacterial isolates was reduced $10^2$–$10^6$ fold after being incubated with wild-type *Acidovorax* compared with the delta-*Hyde1* and Type VI secretion system (T6SS) *Acidovorax* mutants, suggesting that Hyde and T6SS-mediated antimicrobial activity could be used to manipulate the microbiome [21••]. These studies reveal the value of cultivated microbes in dissecting microbe–microbe interactions.

## Conclusions

Because of the complexity of soil environments, current SynCom approaches can only partially mimic natural conditions. The low complexity of SynCom systems may mean that some important microbiome members or nutrients are lacking; however, microbial cultivation followed by experimentation using SynComs in axenic





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systems permits us to elucidate the causality between the root microbiome and plant phenotypes, and to dissect the interactions between microbiome members under natural soil conditions. More cultivated microbes, genomes, improved axenic systems, and the systematic investigation of different functional microbiome members are needed to further reveal the functions and mechanisms of the root microbiome in plant growth and health.

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