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Community standards and future opportunities for synthetic communities in plant-microbiota research

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Community standards and future opportunities for synthetic communities in plant–microbiota research

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Harnessing beneficial microorganisms is seen as a promising approach to enhance sustainable agriculture production. Synthetic communities (SynComs) are increasingly being used to study relevant microbial activities and interactions with the plant host. Yet, the lack of community standards limits the efficiency and progress in this important area of research. To address this gap, we recommend three actions: (1) defining reference SynComs; (2) establishing community standards, protocols and benchmark data for constructing and using SynComs; and (3) creating an infrastructure for sharing strains and data. We also outline opportunities to develop SynCom research through technical advances, linking to field studies, and filling taxonomic blind spots to move towards fully representative SynComs.

Land plants have evolved in the presence of complex environmental microbial communities for over 500 million years. By providing organic carbon compounds derived from photosynthesis, plants can enrich a subset of these microbes from the surrounding environment. The resulting communities, known as the plant microbiota, provide the host with beneficial functions, such as nutrient mobilization or protection against pathogens[1,2]. Addressing open questions within the field to better understand the molecular, genetic and ecological mechanisms that govern these interactions is pivotal for advancing sustainable agriculture and ecosystem health (Box 1). As we progressively gain insights into these intricate relationships[3], reductionist experimental approaches have emerged as valuable tools[4]. Perhaps the most promising approach centres around assemblies of isolated bacteria and fungi, which can be used to generate synthetic microbial communities of reduced complexity, or SynComs (Fig. 1). By simplifying the vast complexity of natural microbial communities, SynComs offer a controlled

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BOX 1

Open questions in plant–microbiome research that can be addressed using SynComs

- How can we manipulate plant–microbe interactions to improve crop yields, resilience and soil carbon pools?
- How do plants distinguish between beneficial and harmful microbes?
- Do plants communicate and coordinate with beneficial microbes?
- Which plant and microbial genes control root colonization?
- Which molecules drive assembly and other ecological processes in the plant microbiome?
- How are microbial communities functionally structured, for example, into guilds?

approach to dissect and study the interactions between plants and their associated microbes, providing a bridge between established plant–microbe interactions research, the developing field of 'mechanistic ecology', and applied agricultural practices. As the field of plant microbiota research matures (Fig. 1), there is a need to develop a set of community standards and protocols for assembling and employing SynComs. Adopting such standards can enhance the reproducibility of experiments, foster broader community engagement and catalyse collaborative endeavours.

Designing and employing SynComs to study the mechanistic ecology of plant-associated microbiota and their emerging properties involves three steps: isolation of strains and establishment of culture collections (Fig. 2a)[5–11]; the design of simplified communities with representative taxa or functions (Fig. 2b); the formulation of strain mixtures into input inocula and deployment using appropriate gnotobiotic systems (Fig. 2c)[12]. At every step, the establishment of community standards and resources that can be shared between laboratories ensures reproducibility, increases tractability and, at the same time, improves the complexity and versatility of experimental systems. For example, adoption of model SynComs has the potential for generating large quantities of scientific knowledge and creating compatible resources (such as mutant libraries[13,14], reference databases[15] or computational models[16]), and can also improve the ease of adoption by new laboratories. Moreover, adoption of community standards is not without risk: a model SynCom might not be the optimal tool to explore a given scientific question, and even simple gnotobiotic experiments can be technically challenging, especially given reasonable quality checks that may require considerable cost and effort. In the following we outline what we consider to be the key aspects required to establish a set of community standards for plant microbiota research using SynComs.

Importantly, the next stage of plant microbiota research employing reductionist approaches demands tools and methodologies that can capture interaction outcomes at both the macro and micro scales. Technical innovations, such as metagenomic plot sampling by sequencing (MaPS-seq)[17] and spatial host–microbiome sequencing (SHM-seq)[18] are just a few examples of the emerging tools poised to address these challenges. Throughout this Perspective we discuss these developments, emphasizing the transformative potential of adopting community standards in SynCom research, and introduce the technical breakthroughs to deepen our understanding of plant–microbe interplay.

Although some researchers are already benefiting from rich culture collections and specialized SynCom resources, the establishment of reference field sites, universally accessible culture collections and public isolate repositories can further democratize access. This would also ensure that biological and bioinformatic resources can be repurposed and are available to the wider scientific community according to the FAIR principles (findable, accessible, interoperable and reusable)[42]. Standards for metadata collection and reporting can improve the process of replicating, validating and/or building upon existing research, thereby accelerating progress and amplifying collaborative potential.

Establishing microbial culture collections to build SynComs

Microbial culture collections provide the resources required for designing and assembling SynComs. They are typically derived from plants harvested from one or more natural sites or grown on natural soil under controlled greenhouse conditions. Ideally, they should capture a substantial fraction of the complexity and diversity of the natural communities, and incorporate the functional and taxonomic redundancy of their isolates (Fig. 2a). This can be achieved by coupling SynCom development with long-term field experiments (such as Broadbalk at Rothamsted Research) to help translate laboratory and field findings, especially sites that are widely accessible for performing field studies and for recovering isolates[19]. Culture collections include bacteria as well as eukaryotic microbes such as fungi and oomycetes. Importantly, natural communities are shaped to a large degree by microbial interactions, either antagonistic or beneficial, and experiments with SynComs can be used to identify key interactions and 'keystone' species (for example, using drop-out experiments)[20,21]. Culture collections should also be accompanied by the corresponding metadata that are required for SynCom design. These data must include information regarding the original biological material (host species or genotype, developmental stage, disease status and so on) and the corresponding sites of isolation (location, soil properties and other relevant environmental factors). Importantly, data characterizing the natural communities from which the collections are established (typically community composition profiles derived from amplicon sequencing) are essential to design SynComs that maximize taxonomic diversity and are taxonomically representative. Although not currently the standard, it would also be desirable to include information on the functional properties of the natural communities, such as meta-transcriptome or metabolome datasets, to enable the design of SynComs with comparable functional properties[22]. Finally, microbial culture collections can also include the genomic and phenotypic data of isolates, which can be used to generate in silico metabolic models[16,23], predict potential functions and study microbial genome evolution[24]. These genomic databases can also be used to improve the accuracy and resolution of various methods for characterizing SynCom outputs, for instance by providing a reference for amplicon or transcriptome data analyses[25].

The field of mammalian gut microbiome research also has a long history of defining model SynComs, including the eight-strain altered Schaedler flora from mice, the 12-strain oligo-mouse-microbiota, a minimal 15-strain mouse gut community and, most recently, a 104-strain human gut community (hCom1). Although most bacterial strains that constitute these communities are individually available at centres such as the American Type Culture Collection (ATCC) or DSMZ, there is generally a lack of standardized SynCom resources. There are, however, multiple opportunities to learn from this research; for example, depending on the scientific goals, it may be necessary to use more diverse and complex communities, and a lack of standardized collections within public biobanks is an impediment for research with SynComs.

Collections of environmental isolates are only the starting point for SynCom research. As the field matures and reductionist approaches based on engineered communities and gnotobiotic systems progress,

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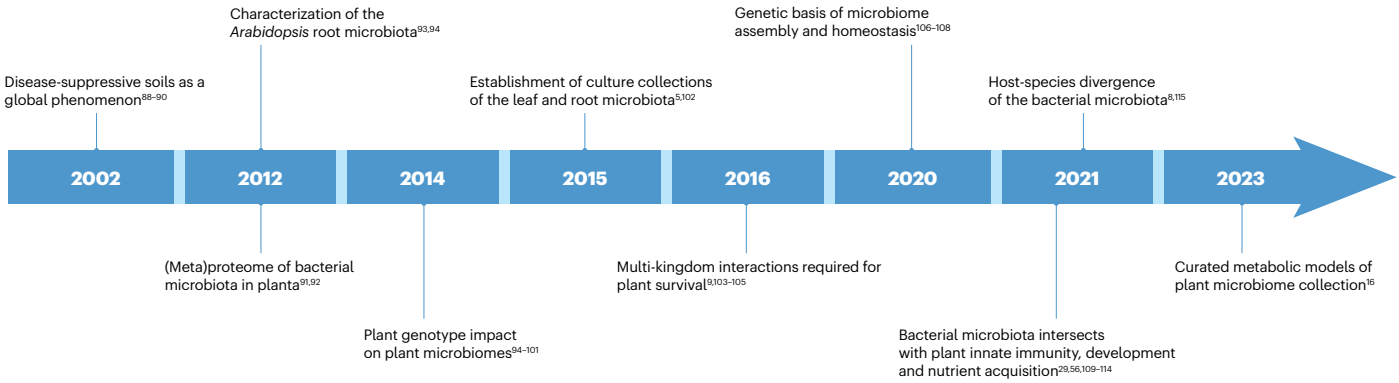

Fig. 1 | Examples of scientific milestones in plant–microbiota interactions. The examples are selected to reflect a diversity of questions and approaches, and the order is based on the earliest cited reference for each group[5,8,9,16,29,56,88–115].

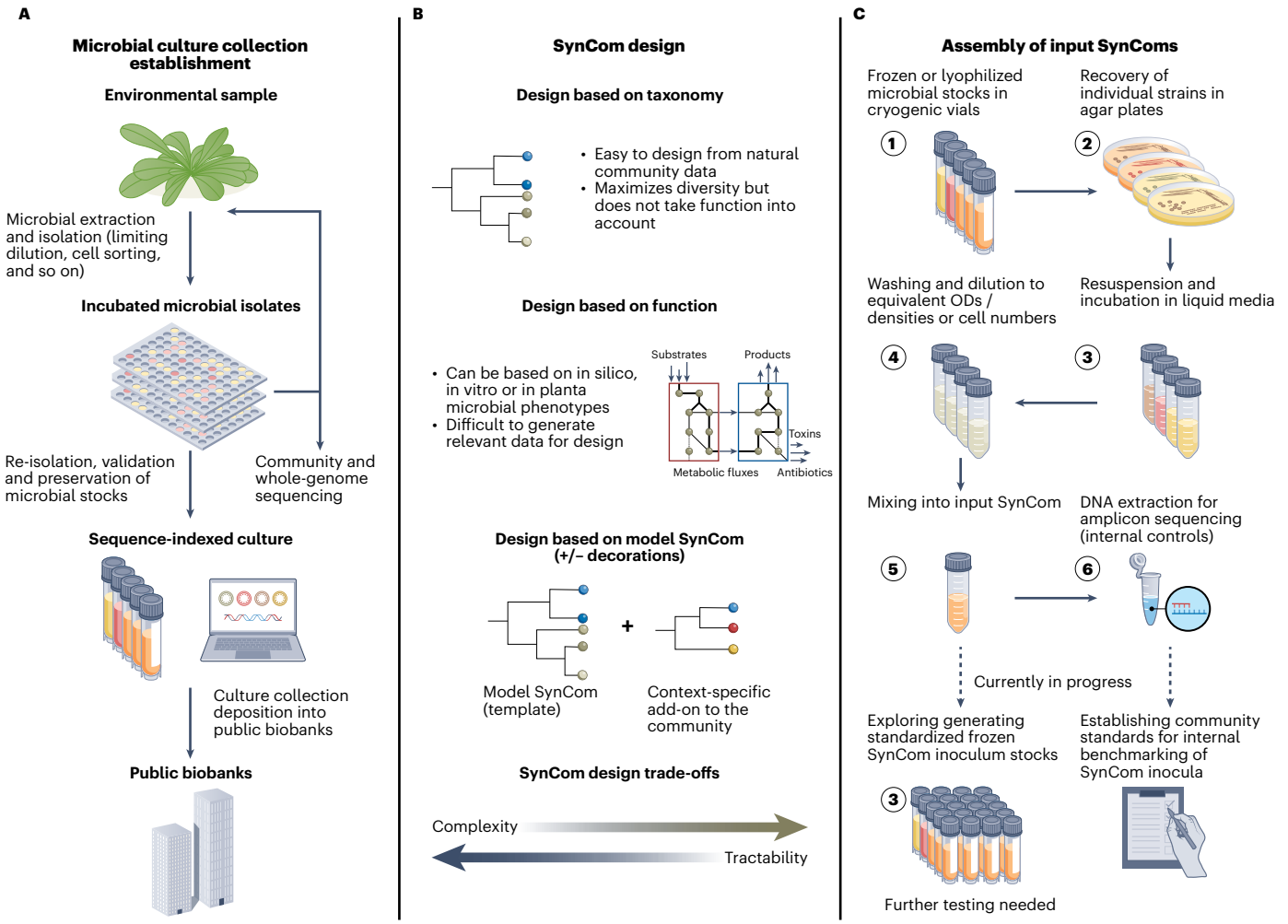

Fig. 2 | Defining standards for SynCom design and assembly using microbial culture collections. a–**c**, There are three major steps in developing a SynCom, from microbial isolation to assembly. First, community members are isolated from environmental samples using high-throughput methods, establishing sequence-indexed culture collections (**a**). Afterwards, SynComs are designed by considering taxonomic, functional and ecological information (**b**). Finally, SynComs are assembled from individual bacterial stocks using standardized laboratory protocols (**c**). OD, optical density. To move the field forward, public biobanks should support large-scale deposition, quality control, redistribution and affordable access to entire collections and SynComs, and best practices for SynCom design should balance simplicity, reproducibility and throughput with more complex, diverse SynComs.

it will increasingly leverage genetic manipulation or engineering to provide mechanistic insights to address questions such as those listed in Box 1. Consequently, novel genetic resources will be generated by research groups, which can ideally be reused and combined, geometrically increasing their usefulness and potential. Examples of such resources include knockout mutants of specific genes or entire mutant

libraries that enable high-throughput forward genetic screens (discussed in more detail in the following), fluorescently labelled strains that can be used for spatiotemporal tracing using imaging approaches, and genetically engineered microbes equipped with novel functions[26]. We believe that coordinating ongoing and future efforts into the generation of compatible resources that can be shared and used for reproducible experimentation is a key aspect in the future development of the field.

Public biobanks for microbial culture collections and derived biological resources

An essential aspect of establishing sequence-indexed microbial culture collections and enabling SynCom research is the need to deposit isolates in public repositories of biological material or biobanks (Fig. 2a). Ideally, this is complemented by the conservation and storage of the whole community, for example, root and rhizosphere samples, as current microbiota culture collections suffer from taxonomic blind spots. This is required to ensure the reproducibility of research and to capitalize on the potential that these large repositories of microbial diversity can provide for fundamental as well as translational research. In addition to enabling reproducible studies across laboratories, these collections will help expand the number and diversity of scientists participating in SynCom research through easy access to strains.

Unfortunately, ensuring the highest standards of purity and integrity in these large collections of environmental isolates while accommodating international legislation regulating the exchange of such material (for instance, the Nagoya Protocol) can pose great logistical challenges[27]. Because of this, most existing institutions and facilities that traditionally act as repositories of microbial cultures are limited in their capacity to receive, curate and subsequently redistribute microbial culture collections. In addition, existing protocols for determining risk groups of microbial isolates often require taxonomic classification or phenotypic characterization without systematically relying on sequencing data, which is often not compatible with the high throughput and large number of isolates that typically characterize these collections.

We believe it is crucial that new protocols and standards are developed, specifically for the deposition and distribution of large culture collections, concurrent with the substantial increases in the pace and scale with which the research community can generate and take advantage of these resources. Given the diversity of plant host species used in SynCom research, our vision is that early successes in creating shared SynCom resources by one community will serve as a blueprint for the development of other host systems. Most importantly, there needs to be increased funding for public biobanks, and this should include the development and application of high-throughput isolation, culturing and characterization of microbial strains needed to develop SynComs for diverse plant species, including key crops in developing countries. We envision publicly available strain collections for plant hosts, for example by building on the framework recently described for the Human Intestinal Bacterial Collection (https://hibc.rwth-aachen.de/) to integrate persistent strain identifiers, culture conditions, taxonomy, data and other metadata[28].

Standardized methods for designing and working with SynComs

One of the central challenges of research with SynComs is designing communities that include ecological or functional properties and interactions relevant to the research question being explored. Usually, this requires balancing a trade-off between complexity and tractability. Working with large and complex SynComs is technically demanding and can impact reproducibility and statistical power, but they can better represent the complexity of the natural communities from which they are inspired. Moreover, small and tractable SynComs may have lower taxonomic and functional diversity but can be more easily and reproducibly assembled (Fig. 2). They can also enable different types of experiment, such as extensive permutations of community composition and comprehensive meta-omics measurements of most, if not all community members[4,29,30].

Ideally, SynCom design should combine ecological, genomic and physiological data, with the goal of capturing as many fundamental features of natural communities as possible (Fig. 2b). For example, to maximize taxonomic coverage, it is common to include at least one representative strain from microbial taxa found to be abundant and/or prevalent in natural communities, whenever available in the corresponding culture collections. Increasingly, microbiome scientists are looking to capture key ecological processes, and this can be accomplished through a variety of methodological approaches, as described by Mehlferber and colleagues, also in this issue[31]. A simple example is including community members with disproportionally positive or negative interactions with other microbes (for example, keystone species). Alternatively, the composition of the SynCom can be fine-tuned by considering genomic or phenotypic data available for individual strains, to design communities with specific genomic or functional characteristics. It is important to consider that, for industrial applications, there is the additional consideration that large-scale fermentation of microbes for agricultural use involves complex and costly fermentation processes that need to be tailored for each microbe. The absence of experience in multi-species fermentation of SynComs, coupled with the prohibitive expense of culturing each strain separately, poses substantial industrial challenges to the large-scale application of SynComs.

In general, the primary goal of SynCom design is to capture the relevant properties of the natural communities while maintaining tractability, but identifying the most relevant criteria can be challenging and highly context-dependent. Although we suggest that SynCom composition should attempt to mirror the corresponding natural community as closely as possible, different research questions and experimental set-ups will require an independent assessment on a case-by-case basis. For example, a SynCom may be required to have specific functions such as nutrient mobilization or enhanced plant stress resilience. In these cases, artificial selection can provide a powerful tool for informing SynCom design[32–34], for example, through sequential transplantation and the selection of microbial communities that promote plant-stress tolerance[32]. Given the prevalence of neutral ecological processes and the high diversity of many natural plant-associated communities, it will be important to validate that the experimental approaches for selecting community members have accurately captured the processes of interest[31].

Another consideration in SynCom design is how community outputs will be measured. Although cultivation-independent approaches such as amplicon sequencing or meta-omics can be applied to most if not all experiments, some limitations need to be considered. For instance, it might be desirable to include strain variation within species within SynComs, in which case identical amplicon sequences could result in a loss of resolution. A way to overcome this limitation is the use of modular bacterial tags (MoBacTags), or chromosomally integrated artificial barcoding sequences, which allow simultaneous tracking of near-isogenic bacterial strains within a community using amplicon sequencing[35,36]. In some cases it may also be desired to determine microbial abundances in absolute terms (that is, cells per gram of biomass). This could be done for small SynComs using a set of selective media and colony morphologies that allows all microbial species to be distinguished when plated after harvest from plants[30]. For larger SynComs, absolute abundance data can also be generated by employing microbial isolate-specific quantitative polymerase chain reaction (qPCR)[37] or spike-ins[38].

The next step after SynCom design includes assembling the input communities from clonal isolates (Fig. 2c). Preparation of the input can be a demanding step, where multiple technical factors can play

an important role in the outcome of the experiment. Such factors include the choice of media, target nutrient and microbial concentrations, the growth stage of constituent isolates, and so on. Currently, there is a lack of community standards and shared protocols for input preparation[39], which we identify as an important need to be addressed by researchers in the near future. Importantly, developing a series of internal standards that can be used for quality control, validation and cross-laboratory comparison will be crucial to establish a baseline for reproducibility and benchmarking. A promising alternative approach is the preparation of standardized frozen or lyophilized SynCom inocula that can be shared between laboratories or generated and distributed by a central biobank[40,41]. Further development and thorough testing of this approach, as well as the necessary infrastructure for bulk and reproducible generation and distribution of frozen SynCom stocks, will be required to make this approach feasible for most research groups. Despite these challenges, such efforts will increase accessibility and lower entry costs for new research groups, while enhancing the comparability of results will present a valuable opportunity for the research field.

Defined minimum metadata standards for depositing SynCom data in public repositories

In addition to relevant data such as physiological properties, genome information and plant colonization attributes that must accompany microbial culture collections, the output of SynCom experiments can provide a wealth of information that can be shared, reused and integrated to provide added value. Among other advantages, deposition and sharing of standardized data has the potential to enable the use of machine-learning approaches by increasing the information and number of available data points. To accomplish this, output sequencing data (for instance, in the form of amplicon community composition profiles) is not sufficient, and additional metadata are required. Such information includes, for example, the identity of the isolates employed in the experiments (and corresponding links to their genomic information and their biological material, deposited in public biobanks), the composition of the input SynCom, as well as information regarding the gnotobiotic system, culture conditions and microhabitat host genotype, to name a few. We suggest that this challenge requires the establishment of a new set of minimum metadata standards, specifically tailored to SynCom datasets to improve replicability and reproducibility, and facilitate sharing and the cross-referencing of results across studies. Ideally, such standards should be the result of joint efforts involving leading data repositories for environmental biomedical research (for example, NCBI, ENA, MDC, KBase and EBI, among others)[42].

Technologies to develop and study representative SynComs

SynComs are the most widely used approach for gaining mechanistic insights into plant-associated microbiomes. However, there are several technical gaps that need to be overcome to achieve the scientific potential of SynComs; for example, to help address open questions in plant–microbiome research (Box 1). These include technologies to isolate representative strains, characterize SynComs and their activities at relevant spatiotemporal scales, and to discover new gene functions (Table 1).

High-throughput methods for isolation

Despite recent advances in the isolation and characterization of representatives of plant microbiota, critical taxonomic gaps remain in current culture collections. Fortunately, emerging technologies can be used to obtain isolates from taxonomic and functional groups recalcitrant to culturing and to study their interactions under relevant conditions. For example, the use of single-cell sequencing and the data from metagenome-assembled genomes in combination with metabolic models will enable the prediction of isolation conditions[43]. Another related and powerful approach is using single amplified genomes and metagenome-assembled genomes to predict membrane proteins and design epitopes for targeted isolation using cell sorting[44]. We anticipate that machine-learning and automation methods such as those developed for human microbiomes will accelerate isolation and characterization. For example, this could be done by extending recently reported methods integrating robotics, imaging, sequencing and machine learning that enabled the isolation of more than 80% of the abundant taxa in a set of faecal samples[45].

Table 1 | Examples of emerging technologies for characterizing plant-associated SynComs

Technology	Capabilities	Limitations
Sterile lab chambers for studying SynComs[9,80,81,83]	Gnotobiotic systems for studying plant–microbiota interactions	Currently limited to small plants and not designed to mimic natural plant environments
Metatranscriptomics including single-cell approaches[46–49]	Determine changes in transcription levels of genes	Transcription is not necessarily equal to gene expression
Metabolomics[85]	Determine identities and quantities of metabolites	Assignment of identity to specific detected masses can be difficult
Metaproteomics[54]	Identify and quantify thousands of proteins and detect gene expression changes	Difficult to detect proteins from low abundant species due to interference from abundant plant proteins
SIP[57,58]	Detect substrate incorporation into biomolecules	Cost of substrates and difficulty of using gaseous substrates
Fluorescence in situ hybridization techniques[50–52,61]	Localization of specific strains with cellular and subcellular resolution	Probe development and staining approaches can be challenging
Spatial metagenomic and meta-transcriptomic sequencing[17,86]	Localization of microbes, genes and gene expression	Relatively high cost and technically challenging to implement
Mass spectrometry and Raman imaging[52,60,87]	Imaging metabolites, proteins and isotopes	Limited ability to identify compounds
RB-TnSeq[13,74]	High-throughput gene functional annotation	Microbes need to be transformable
Robotic cultivation, metagenome guided isolation and cultivation[45]	Filling taxonomic blind spots in SynComs	Remains challenging to isolate many important groups of plant-associated organisms

SIP, stable-isotope probing; RB-TnSeq, random bar code transposon-site sequencing.

Spatial characterization of SynComs

To characterize SynComs, we need to move beyond inferring relationships based on amplicon abundances from bulk samples to include spatial information on microbes, the plant host and environmental parameters. We need technologies to measure interactions at relevant length and time scales to determine microbial localization, interactions and activities.

Fortunately, there are several new technologies that hold considerable promise in this area (Table 1). One such technique is metagenomic plot sampling by sequencing (MaPS-seq), which embeds microbiome samples in a polymeric matrix, which is then fractured using cryo-bead beating[17]. The resulting particles are lysed, sized and encapsulated in

droplets containing barcoded beads. The barcodes are photocleaved and the genomic DNA is released by degrading the polymer matrix for subsequent PCR amplification of 16S rRNA gene and shotgun sequencing to identify colocalized organisms. Spatial host–microbiome sequencing (SHM-seq) is another technique using DNA barcoded probes, in this case immobilized on a glass slide. This allows simultaneous capturing of the polyadenylated (host) transcripts and 16S rRNA from frozen tissue sections deposited on the slide[18]. The related technique spatial metatranscriptomics (SmT) has been developed specifically for studying plant–bacterial–fungal interactions[46]. Recent work shows that using metatranscriptomics in combination with meta-ribosomal sequencing[47] can accurately predict competition and responses to substrate additions. Single-cell sorting and transcriptomic sequencing has been used to classify cells based on their patterns of gene expression and has great potential for studying host responses to SynComs[48,49].

Fluorescence in situ hybridization (FISH) techniques[50] will continue to provide important tools for investigating SynCom structure and dynamics (Table 1). For example, SEER-FISH (sequential error-robust FISH)[51] now enables accurate taxonomic identification in complex communities in the *Arabidopsis thaliana* rhizoplane with single-cell resolution, enabling the investigation of shifts in microbial community in response to plant secondary metabolites. A related technique, SRS-FISH (Raman scattering–two-photon FISH)[52] can detect metabolically active bacterial cells with single-cell resolution. Nanoscale secondary ion mass spectrometry (NanoSIMS) stable isotope probing is another powerful technique for localizing activities with subcellular resolution[53].

Determining substrate uptake, metabolic handoffs and activity

To understand the mechanisms leading to emergent properties of plant-associated microbiota and their reciprocal interactions with the plant, it is critical to be able to study the actual metabolism, physiology, activities and metabolic interactions in these communities. Metaproteomics, metatranscriptomics and metabolomics can be used to measure microbial phenotypes at the molecular level and are particularly powerful when using SynComs (Table 1)[54,55]. Metabolomics is usually challenged by the fact that metabolites cannot be assigned to specific species. Therefore, the ability to drop-in and drop-out specific community members from the SynCom can allow the identification of microbial species that are responsible for specific host interactions, for example, plant hormone production[56]. Similarly, stable isotope probing (SIP) approaches can quantify substrate conversion into biomass by specific species. SIP can also provide evidence for substrate transfer when paired with pulse-chase experiments and/or drop-in/drop-out of specific SynCom species. Substrate uptake or transfer can be detected using different biomolecules that can be assigned to specific microbial species, such as DNA, RNA, protein, metabolites and lipids (DNA-SIP, protein-SIP and so on) as long as relevant labelled substrates can be purchased or be generated in the laboratory[57]. For plant–microbe interactions, simple substrates such as $^{13}CO_2$, $^{15}N_2$ or $^{15}NH_4$ are often used to follow nutrient transfer. This has, for example, been used to show the transfer of plant-fixed CO_2 to plant-associated arbuscular mycorrhizal fungi and subsequently to microorganisms associated with the fungal hyphae[58]. Additionally, SIP approaches allow the detection of substrate-independent changes in microbial activities in response to changes in temperature, pH and lighting and using heavy water ($H_2{}^{18}O$ or D_2O) as a general activity labelling method[59].

Emerging techniques can be used to image metabolites and proteins at single-cell resolution[60] and even subcellular resolution (Table 1)[61]. These approaches can complement data on SynCom localization and gene expression; for example, metabolites can be localized at the root–soil interface[62,63], within root nodules[64] or leaf surfaces[65]. The integration of spatial metabolomics with FISH (metaFISH[66]) by imaging the same tissue sections, first by mass spectrometry (AP-MALDI-MS) and then by FISH, has enabled bacterial submetabolomes to be determined with 3-µm resolution. Another example is the spatial proteomics method, nanoPOTS (nanodroplet processing in one pot for trace samples), which has been used to identify 2,000 proteins with 100-µm spatial resolution from tissue sections[67]. A powerful aspect of these and other MS-based imaging methods is the possibility to integrate these approaches with stable isotopes to study the localization and even rates of biochemical processes[68].

In addition, previous research exploring natural variation in root exudates of the *Arabidopsis* multiparent advanced generation inter-cross (MAGIC) population[69] could be extended to include SynCom interactions. This strategy, coupled with the availability of recombinant inbred lines in crops such as maize[70], presents a promising avenue for high-resolution quantitative trait locus (QTL, the statistical linkage between phenotypes and genotypes) mapping, offering new insights into the genetic factors influencing SynCom assembly and function.

High-throughput discovery of gene functions

Although we are increasingly able to image gene, transcript, protein and metabolite composition in situ (Table 1), determining their functions is another challenge. There are a vast number of computational approaches for improving annotations. A few recent examples include KBase[71], METABOLIC[72] and MetaEuk[73]. These are being complemented by high-throughput genetic techniques, which provide much needed high-throughput methods for determining unknown gene functions for transformable bacteria[74]. RB-TnSeq mutant fitness profiling has been successfully applied to plant-associated bacteria[13,75]. It uses random DNA barcodes, transposon mutagenesis and DNA sequencing to perform genome-wide fitness assays across thousands of mutants in a single assay. Libraries are made so that there is a single barcode insertion in each mutant and the location is known. The fitness of each mutant is assessed by comparing the relative abundance of the barcodes across culture conditions. Lower barcode abundance under a given condition indicates that a particular gene contributed to fitness under that condition, whereas elevated abundance indicates that the loss of that gene improved fitness. One limitation of this technique is that mutants for essential genes will not survive library construction. Fortunately, Dub-seq[76] and CRISPRi libraries[77,78] can also be used to interrogate essential genes. Because many genes presumably only have a function within a relevant ecological context, it is important to have methods for testing gene functions within communities using plant and microbial mutants.

Future perspectives

Using SynComs, we have learned, and continue to learn, about the biology and ecology of plant–microbe interactions. These studies can, for example, shed light on genes and metabolites mediating microbial interactions, which can be extrapolated to natural communities using the technologies described in this Perspective. However, many of the techniques are difficult to deploy in field settings. Thus, bridging the gap between mechanistic studies in the laboratory and native field ecosystems and processes is a major challenge, especially given the high degree of species- and strain-level variation. Constructing SynComs from community-accessible natural field observatories and long-term field experiments will enable the refinement, validation and application of SynComs and use of the derived research results. The rapidly developing modelling capabilities (Fig. 1)[16,71,79] will enable comparisons between laboratory SynComs and native communities, and will also play a critical role in iteratively refining the performance of SynComs. Inter-laboratory comparison studies will also enable the creation and validation of standardized protocols and reference datasets[80].

The eventual vision for SynComs is to create complete 'fabricated ecosystems' that enable the control of important environmental variables, microbes and their interactions, and allow spatiotemporal

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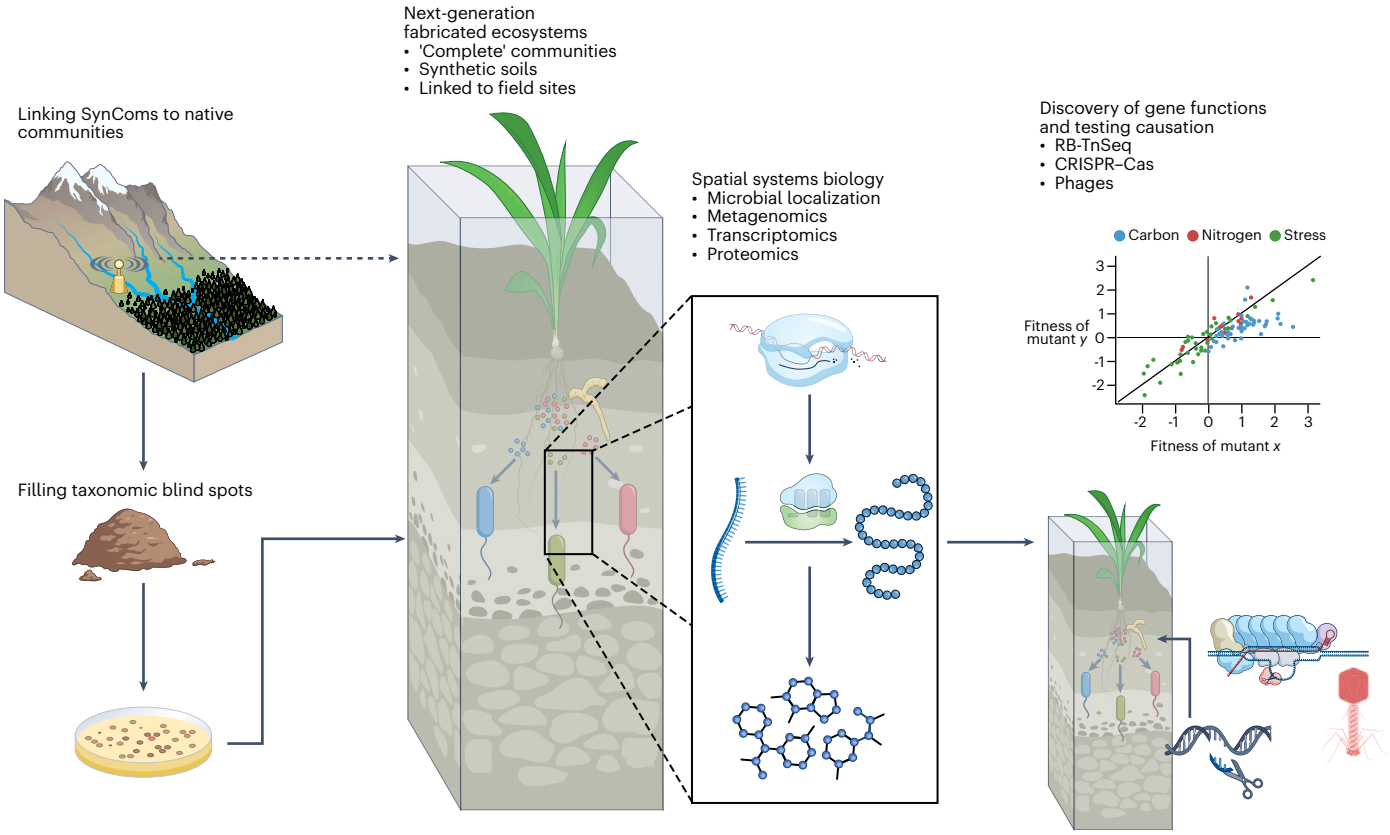

Fig. 3 | Proposed framework for next-generation SynCom experiments and technologies for plant-associated microbes. New technologies for measuring and modelling microbial community composition and metabolic activities with spatiotemporal resolution will help advance mechanistic ecological and molecular understanding of plant microbiomes. Standardization and improved access to field-relevant SynCom strains should be complemented with control of other ecological variables to create 'fabricated ecosystems'. The utility and completeness of the systems and knowledge gained will ultimately depend on the ability to accurately understand and predict environmental processes in native communities, by effectively linking SynComs to natural communities.

analysis (Fig. 3). This will enable them to be used for important applications such as developing more sustainable agricultural practices, increasing crop yields, soil carbon sequestration and the development of climate-resilient crops. Such systems should be suitable for larger plant species and even enable long-term ecosystem-level studies (for example, nutrient cycling, plant communities and so on). Critically, these fabricated ecosystems would have sufficient control to enable interventions to elucidate the mechanisms underlying ecological processes. There are now numerous sterile systems that are suitable for studying plant–microbe interactions. These include simple devices such as the flow-pot[9] and EcoFABs[81,82], among many others[83]. It is important to develop artificial soils that reflect the mineral and organic composition of soils[84]. These would ideally be designed to reflect the soil composition and texture at a reference field site. Longer-term, larger fabricated ecosystems will be needed that more accurately capture field variables (wind, rain, temperature, humidity and so on) and ideally use field sensors to control laboratory ecosystems. Finally, laboratory and digital twin models of field ecosystems would provide a valuable framework for comparing and interpreting results and enabling screening, performance testing and efficacy testing of microbial products in 'closer-to-field' conditions, reducing field-trial costs and providing insights into performance and risks.

The future of sustainable agriculture and ecosystem health hinges on our ability to understand and harness the potential of plant–microbiota interactions. As we have explored in this Perspective, research with SynComs stands at the forefront of this endeavour, offering a controlled and simplified experimental blueprint (Figs. 2 and 3) with which we can study these complex relationships and help address key questions in the field (Box 1). However, as with any emerging field, there are challenges to be addressed and opportunities to be explored. Fortunately, technological advancements are also on the horizon, promising to revolutionize our understanding of the plant microbiota and their interactions with their hosts. From high-resolution temporal and spatial sequencing methods to the establishment of barcoded mutant libraries, the tools at our disposal are becoming increasingly sophisticated. These technologies will allow us to deepen our exploration of the ecological and molecular mechanisms of plant–microbiota interactions, revealing the underlying processes that drive these relationships. Moreover, as we fill taxonomic blind spots and develop methods to study SynCom assembly and dynamics, we move closer to a holistic understanding of these communities.

Technology alone is not sufficient. We need communities of scientists developing common tools and resources. Lack of standardized protocols, methodologies and resources can hinder the reproducibility of experiments and slow the pace of collaborative advancements. By defining reference SynComs, creating internationally funded repositories and infrastructure for the sharing of strains and (meta)data, and establishing benchmark protocols, we can pave the way for more efficient, replicable and collaborative research. Such standards not only ensure the quality and consistency of research but also democratize access to valuable resources, fostering a more dynamic and inclusive scientific community.

In conclusion, the field of SynCom research is at a pivotal juncture. The potential benefits of understanding and harnessing plant–microbe interactions are clear, from boosting agricultural yields to preserving ecosystem robustness and health. However, realizing this potential

requires a concerted effort from the scientific community. By establishing community standards, embracing technological advancements, and bridging the gap between the laboratory and the field, we can set the stage for a new phase of mechanistic ecology in plant–microbiota research.

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Author contributions

T.R.N. and R.G.-O. developed the idea for this Perspective based on discussions with all authors. T.R.N., R.G.-O., T.A., N.G., P.S.-L. and J.A.V. drafted the manuscript, which was subsequently refined through

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Competing interests

T.R.N. is an inventor on several patents held by the University of California related to devices for studying plant–microbe interactions. The remaining authors declare no competing interests.

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