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Review



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Tansley review

Enabling sustainable agriculture through understanding and enhancement of microbiomes

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Summary

Harnessing plant-associated microbiomes offers an invaluable strategy to help agricultural production become more sustainable while also meeting growing demands for food, feed and fiber. A plethora of interconnected interactions among the host, environment and microbes, occurring both above and below ground, drive recognition, recruitment and colonization of plant-associated microbes, resulting in activation of downstream host responses and functionality. Dissecting these complex interactions by integrating multiomic approaches, high-throughput culturing, and computational and synthetic biology advances is providing deeper understanding of the structure and function of native microbial communities. Such insights are paving the way towards development of microbial products as well as microbiomes engineered with synthetic microbial communities capable of delivering agronomic solutions. While there is a growing market for microbial-based solutions to improve crop productivity, challenges with commercialization of these products remain. The continued translation of plant-associated microbiome knowledge into real-world scenarios will require concerted transdisciplinary research, cross-training of a next generation of scientists, and targeted educational efforts to prime growers and the general public for successful adoption of these innovative technologies.

1. Introduction

The 'Green Revolution' increased the world's crop output by an order of magnitude through the use of high-yielding cereal varieties

coupled with higher rates of fertilizers, synthetic agrochemicals and controlled water supplies (Tilman *et al.*, 2002). Poverty and hunger were reduced for millions of people and conversion of new land into cultivation was diminished; however, unintended negative

environmental consequences were later recognized, including increased soil degradation and chemical runoff (Pingali, 2012). With the global population expected to reach 9.7 billion in 2050 (United Nations Department of Economic & Social Affairs, 2019), a new, sustainable revolution is needed that maintains the health of agroecosystems, prevents overuse of inputs and preserves scarce water supplies. Achieving the grand challenges of sustainably increasing food security, enhancing crop resilience to biotic and abiotic stresses, and reducing agriculture's carbon footprint will require unprecedented innovations from many different disciplines and within diverse components of phytobiomes (see Box 1 for a glossary of terms). One promising approach is to harness knowledge of microbiomes to benefit agricultural systems.

The application of microbes to benefit agriculture is not new (Vessey, 2003). In the late 19th century, the practice of mixing 'naturally inoculated' soil with seeds was recommended for legume production in the USA (Smith, 1992), and laboratorygrown cultures of rhizobia were used as inoculants for leguminous crops (Burton, 1979). Harvey Wiley, the first commissioner of the US Food and Drug Administration, emphasized in 1901 the benefits of microbes in agriculture, promoting commercial products containing soil bacteria (later described as rhizobia) that enhanced nitrogen fixation in roots and soils (Wiley, 1902). At the same time, challenges in applying microbes to increase plant performance were recognized; Lorenz Hiltner commented in 1904 that '... apart from some sporadic exceptions, the main goal of our joint effort, to make the research results applicable in practice, has unfortunately not been reached so far. [...] However, I am convinced that soil bacteriology will finally provide results, which are not only of explanatory nature, but that will directly affect and determine agricultural practice' (Hartmann et al., 2008). Although a century old, this statement remains true: the full potential of microbial-based solutions to increase crop production is yet to be harnessed.

Microbes have been applied as biopesticides/biocontrol agents (targeting pests and pathogens by producing a pesticidal effect), biofertilizers (providing plant nutrients) and biostimulants (aiding nutrient assimilation by the plant without being nutrients, pesticides or soil amendments) (Marrone, 2019). The idiosyncratic performance and durability of microbial inoculants led to the realization that the functionality and persistence of microbes are dependent on interactions within the phytobiome, that is with the environment, the host plant and other native microbes (Ojiambo & Scherm, 2006; Bardin et al., 2015). Insights into the complexity of plant-associated microbiomes as well as definitions of their structure, functions and interactions with themselves and their hosts enabled by recent technological breakthroughs are providing a way forward for application of microbiomes and microbial products such as biofertilizers, biopesticides and biostimulants in agriculture (Kaminsky et al., 2019; Trivedi et al., 2020; Singh et al., 2020b). They have spawned considerable interests, efforts and investments (from industry and funding agencies) towards development of targeted microbial combinations to help plants take up nutrients and minerals, cope with stress, or bolster immune responses to suppress pests and pathogens (Singh et al., 2020b). The application of microbiomes extends beyond improving plant

Box 1 Glossary of terms

Agricultural microbiome: (in the context of this review) plant and soil microbiomes that impact plant productivity.

Core microbiome: the group of microbes commonly found within a host's microbiome.

Core functional microbiome: microbes carrying genes that encode essential functions for plant plus microbiota fitness.

Culturomics: an approach allowing an extensive assessment of the microbial composition by high-throughput culturing.

Biological control: reduction in disease or pests through the agency of one or more living organisms other than the host.

Holobionts: plants and their associated microbiota as single entities; 'units of selection' in which plant-microbiome interactions have probably coevolved to maintain host functionality and fitness over ecological and even evolutionary timescales.

Hub microbes: microbes that are highly connected with other microbes in cooccurrence networks and may play a key role in supporting the network structure and orchestrate community-scale processes that underlie plant-microbiome interactions.

Disease suppressive soils: soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soils.

Metabolomics: the comprehensive characterization of the small molecules (metabolites) in a biological specimen.

Metagenome-assembled genomes (MAGs) and complete MAGs (cMAGs): a single-taxon assembly based on one or more binned metagenomes that is a draft representation to an actual individual genome. cMAGs are complete genomes with no gaps assembled from metagenome sequencing.

Microbiome engineering: experimental methods that improve host performance by artificially selecting for microbial communities with specific effects on host fitness. Microbiome engineering applies multigenerational, artificial selection upon hosts that vary in microbiome content affecting the host trait.

Multiomics (integrative omics): an approach to combine sets of different 'omic groups such as the genome, proteome, transcriptome and metabolome to study biological entities in a concerted way.

Phyllosphere: all the aboveground organs of the plants including leaf, flower, stems and fruit.

Phytobiome: relates to a plant (phyto) in a specific ecological area (biome). It includes the plant itself, the environment and all microand macroorganisms living in, on or around the plant. These organisms include microbes, animals and other plants.

Plant growth-promoting bacteria: bacteria that can enhance plant growth and protect plants from disease and abiotic stress through a wide variety of mechanisms.

Rhizosphere: the region of soil in the vicinity of plant roots that is influenced by plant-derived nutrients and oxygen availability. It consists of a gradient in chemical, biological and physical properties that change both radially and longitudinally along the root.

Synthetic biology: a multidisciplinary area of research that seeks to create new biological entities such as enzymes, genetic circuits and cells, or redesign existing biological systems.

Synthetic microbial communities (SynComs): constructed microbial communities with reduced complexity relative to natural systems that allow detailed study and analysis of the fundamental building blocks and processes that compose a microbial community.

health to a more holistic, systems approach that includes human health (Box 2). Systems biology offers a holistic integrative approach to untangle complex microbiome-host interactions that drive the dynamic emergent properties under different environmental conditions (Rodriguez *et al.*, 2019).

In this review, we summarize how the knowledge derived from multiomics techniques has provided a deeper understanding of the structure and function of plant-associated microbiomes. We review different modes and techniques through which microbiomes can be applied to increase plant performance. Finally, we summarize knowledge gaps that impact translation to the field and highlight priority areas in microbiome research to improve agricultural outcomes.

II. Multiomics approaches in understanding plant microbiomes

Integrated multiomics approaches are revealing the composition of microbiomes (through amplicon sequencing and metagenomics), their functions (through metagenomics, metatranscriptomics and metaproteomics) and the networks of interactions in which they participate (through metabolomics). These integrated approaches have provided mechanistic understandings for how individual microbes and microbial communities drive plant–microbiome interactions towards plant health and resilience to environmental stresses. The different techniques and how their applications have advanced our knowledge of the structure and function of plant-associated microbiomes and their interactions with host plants are summarized below, with a focus on characterization of bacterial communities associated with agricultural crops.

1. Amplicon sequencing

High-throughput sequencing of marker gene tags (iTAG) allows profiling and comparing the composition, organization and spatial distribution of microbial communities. These marker gene tags specifically target both taxonomic (usually 16S rRNA for bacteria and archaea and ITS or 18S rRNA for fungi) and functional genes (e.g. amoA for nitrifying bacteria, nifH for nitrogen-fixing bacteria). iTAG sequencing studies have provided an extensive census of the microbiota living on or inside different crops including rice (Edwards et al., 2015), millet (Jin et al., 2017), soybean (Mendes et al., 2014), corn (Walters et al., 2018), barley (Bulgarelli et al., 2015), wheat (Donn et al., 2015), pea (Tkacz et al., 2020a), sugarcane (Hamonts et al., 2018), cucumber (Ofek-Lalzar et al., 2014), citrus (J.Xu et al., 2018) and grapevine (Marasco et al., 2018). In general, these approaches reveal that species richness is highest in soil environments (both bulk and rhizosphere soils) and decreases in the root and phyllosphere compartments. Plantassociated microbiota have a very well-defined and conserved phylogenetic structure with significant overlap between subsets of microbial communities in different plant compartments (Hamonts et al., 2018; Trivedi et al., 2020; Tkacz et al., 2020a,b). Relative to the bulk soil, most plant species harbor an enrichment of bacterial taxa belonging to the phyla Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria; by contrast, Acidobacteria, Verrucomicrobia and Gemmatimonadetes are depleted in plant-associated environments compared to bulk soils (Fig. 1). In the root endosphere, there is an overwhelming dominance of bacteria

Box 2 Interlinked microbiomes

A 'eco-holobiont' concept has been proposed to understand the multikingdom microbial loop, including the effects of plant microbiomes on the human microbiome and human health (Singh et al., 2020a). Plant-associated microbiomes may alter the traits of the human microbiome with consequences for health and wellbeing, but this alteration has largely been overlooked. The major focus on the linkages between the plant and gut microbiomes have been on disease cases and antibiotic resistance genes (Singer et al., 2016: Chen et al., 2019). However, the plant-associated microbiome can have significant contributions towards human health by impacting the quality of the food and/or by indirectly interacting with the gut microbiome though plant-derived chemicals or genetic material. Plant-associated microbiomes play a crucial role in the establishment and maintenance of green roofs that increase urban biodiversity by providing habitat for wildlife, space for urban agriculture, and a more aesthetically pleasing and healthy environment to work and live (Fulthorpe et al., 2018). Although direct linkages have not yet been made, growing up in microbial-rich farm environments can have protective health effects on children (Kirjavainen et al., 2019), suggesting the positive effect of plant-associated microbiomes on immune development. The connections are convincing between plant-indoor microbiomes and the impact of these relationships with human health (Berg et al., 2014). Finally, since diet strongly influences the composition of the gut microbiota, it is likely that the structure and function of gut microbiomes reflect, at least partly, the composition of the plant microbiota and the influence of that microbiota on the plant's biochemistry (e.g. the production of bioactive compounds). This provides compelling approaches for health-directed dietary interventions for consumers that relies on the probiotic capacity of the plant-associated microbiome to influence plant biochemistry.

belonging to the phylum Proteobacteria (Fig. 1). The phyllosphere (aboveground) microbiota is mainly derived from soils (Hamonts *et al.*, 2018; Singh *et al.*, 2019), the vascular tissues of plant or its seed (Barret *et al.*, 2015), and the air or insect vectors (Vorholt, 2012), wherein both host (growth stage, variety) and the environment (seasons, time, site) significantly affect the community assembly.

2. Whole genome shotgun sequencing

Although highly informative, iTAG-based profiling does not provide detailed insights into the genomic potential or functions of plant-associated microbiomes. Shotgun sequencing, which supplies information on the total DNA content, has identified microbial genomic features that are related to plant colonization and plant-microbiome interactions (Ofek-Lalzar *et al.*, 2014; Bulgarelli *et al.*, 2015; J. Xu *et al.*, 2018). We have learned that several microbial traits, including chemotaxis, cellular mobility, stress response, toxin production, secretion and utilization of a range of carbon compounds, contribute to adaptation to plants and that these are under positive selection in the plant-soil environment (Sessitsch *et al.*, 2012; Bulgarelli *et al.*, 2015; J. Xu *et al.*, 2018). Enrichment of genes involved in transcriptional regulation and



Fig. 1 The general structure of bacterial communities from different niches associated with agriculturally relevant plant species grown under field conditions. Bar charts show proportional abundances for major bacterial phyla detected in the bulk soil (Bulk), rhizosphere (Rhizo) and root endosphere (Endo) of pea (Tkacz *et al.*, 2020b), wheat (Tkacz *et al.*, 2020b), maize (Walters *et al.*, 2018) rice (Edwards *et al.*, 2015), citrus (authors unpublished data) and sugarcane (Hamonts *et al.*, 2018). Abundances were estimated by 16S rRNA gene sequencing. Only phyla that represent > 0.5% of the total population in at least one sample are included; low-abundance phyla are grouped as 'Other'. The selected studies used high-throughput sequencing to profile the bacterial communities in field conditions at least at two sites (A, B and C = different sites).

signaling indicates highly coordinated responses in plant-associated microbiomes (Bulgarelli et al., 2015; J. Xu et al., 2018). Plantbeneficial traits, such as those related to P-solubilization, Nmobilization, biocontrol activities and iron-chelation, are enriched in microbes in the plant-associated environment as compared to those in bulk soils (Sessitsch et al., 2012; J. Xu et al., 2018; Carrion et al., 2019; Li et al., 2019). The vast majority of genome-resolved metagenomics have produced uncurated draft genomes (i.e. metagenome-assembled genomes (MAGs) (Zhang et al., 2017; Bandla et al., 2020)), but gaps, assembly errors and contamination from other genomes limit the utility of these assembled genomes to general genomic studies (L. X. Chen et al., 2020). Recent advanced genome curation techniques allow generation of complete (circularized, no gap) MAGs (cMAGs) from very complex microbial communities including soil and sediments (L. X. Chen et al., 2020). The availability of cMAGs from plant/soil environments will permit holistic insights into the evolution and functional significance of plant-associated microbiomes. Although the current metagenomic sequences provide some insight as to the potential functions of the rhizosphere community, assessments of interactions between microbes in plants will only be understood when we have a better understanding of the expression of these traits in situ. This will be achieved by complementing metagenomic approaches with other high-throughput techniques, such as transcriptomics, proteomics and metabolomics, and the study of culturable members of the community.

3. Metatranscriptomics, metaproteomics and metabolomics

Comparative metatranscriptomics has revealed kingdom-level differences in the active rhizosphere microbiome of wheat, oat and pea (Turner *et al.*, 2013). In response to drought stress, the sorghum root-associated microbiome shows increased transcriptional activity of genes associated with carbohydrate and amino acid metabolism and transport; this was largely due to shifts in actinobacterial activity and function (L. Xu *et al.*, 2018). A recent breakthrough involved using metagenomic and metatranscriptomic profiling to identify and develop microbial communities as biosensors of drought stress (Zolti *et al.*, 2019). Although an extremely powerful approach to elucidate the behavior of active members of plant-associated microbiomes, poor correlations between transcription and translation calls for developing proteomic and metabolomic methods to complement transcriptomics.

Metaproteomic analyses have provided direct insights into the molecular phenotypes of microbial communities from the

rhizosphere (Wang et al., 2011; Knief et al., 2012; Moretti et al., 2012; Bona et al., 2019) and phyllosphere (Delmotte et al., 2009) of agricultural plants. These limited studies have demonstrated significant stability with respect to the dominant members of the microbiome and the proteins identified in different plant-associated environments (Delmotte et al., 2009; Knief et al., 2012; Bona et al., 2019). Metaproteomics-inferred in situ physiology of the microbial communities further revealed metabolic processes that allow for the selective colonization and adaptation in the rhizosphere compartments as compared to bulk soil (e.g. phosphorous metabolic process and the regulation of primary metabolic processes were higher in the rhizosphere; Bona et al., 2019) and phyllosphere as compared to the rhizosphere (e.g. transport processes, stress response and methanol-based methylotrophy were higher in the phyllosphere; Knief et al., 2012). These findings support metagenomic-based approaches pointing towards the requirement of common adaptation mechanisms for effective colonization.

Metabolomic approaches are being increasingly used for the diagnosis of plant diseases and their etiological agents, but their application to microbiome science remains limited (Adeniji et al., 2020). Early studies showed that the rhizosphere microbiome alters the phyllosphere metabolome and that these changes are linked to differential insect feeding behavior (Badri et al., 2013). Root metabolome changes can sculpt specialized microbial communities that alter plant performance (Huang et al., 2019) and plantherbivore interactions in the next generation (Hu et al., 2018). Metabolome information is critical for the detection and quantification of small molecules, such as strigolactones and benzoxazinoids that drive plant-microbiome communications and interactions (Leach et al., 2017; Trivedi et al., 2020). In a recent breakthrough, multiomics (metabolome, ionome, microbiome and phenome) and integrated informatics were used to reveal complex interactions between plant traits, metabolites, microbes and minerals in an agroecosystem (Ichihashi et al., 2020). We envision that improved sample preparation (e.g. removal of host sequences for shotgun sequencing and transcriptomics as well as extraction of proteins in a universal manner for proteomics), extended databases for gene, metabolite and protein identification, and the development of algorithms and computational tools for data integration will allow realization of the full potential of multiomics approaches to unravel the genotype-phenotype spectrum in agricultural settings.

4. Culturomics

Harnessing microbiomes for developing commercial inoculants requires cultivated bacteria (Sarhan *et al.*, 2019). Further, bacterial cultures are required for experimental validation and to provide reference bacterial genome sequences for interpreting metagenome datasets and guiding functional analyses (Forster *et al.*, 2019). Unlike for bulk soil, plant-associated microbiomes consist of a relatively high fraction of culturable microbes, particularly bacteria, and few large-scale culture collections have been established from plant-associated environments (summarized in Finkel *et al.*, 2017). The power of these collections is clear: comparative genomics from

large collections of isolates have identified homologs of known bacterial genes involved in colonization, pathogenesis or provision of nutrients to plants (Levy *et al.*, 2018). An interesting discovery was the identification of protein domains in plant-associated bacteria that mimic plant protein domains; the bacterial proteins are postulated to interfere with or mimic host cell signaling (Levy *et al.*, 2018). The availability of cultured members also allows for validation of candidate genes by complementary molecular approaches, including mutagenic and bioreporter expression systems (Cole *et al.*, 2017; Pini *et al.*, 2017).

Despite recent progress in culturomics, we are just at the tip of the iceberg as genomic diversity in cultured bacterial isolates is not nearing saturation (Bai et al., 2015). For example, of the more than 70 000 bacterial genome sequences available at Joint Genome Institute Integrated Microbial Genomes (https://img. jgi.doe.gov/), < 3000 are plant-associated and these are mostly skewed towards plant pathogens. The development of unbiased, sequenced culture collections from a variety of crops/plants and soil types along with mechanisms to curate, share and standardize metadata for strains from these collections is needed for future research into the functional potential of plant-associated microbes (Finkel et al., 2017). Isolation efficiency can be enhanced by the use of microdroplet and microfluidics technologies adapted as high-throughput culturing platforms (Kaminski et al., 2016) to allow for large-scale isolation, genome sequencing and functional screening of microbial isolates. To improve success of culturing previously uncultured microbiome members, single-cell amplified (SAGs) and metagenome-assembled (MAGs) genomes together with genome-based metabolic reconstructions are being used to guide formulation of specific media recipes (Kwak & Park, 2018).

III. Core and hub microbiota

Several studies have identified a 'core microbiome', that is a group of microbes commonly found within a host's microbiome, through the integration of high-throughput sequencing-based studies of plants growing at different locations with persistence of association as selection criteria (Blaustein et al., 2017; Hamonts et al., 2018; L. Xu et al., 2018; Compant et al., 2019; Simonin et al., 2020; Singh et al., 2020b). Interestingly, there is significant overlap between the members of the so-called 'core microbiome' across multiple accessions of one plant species and across phylogenetically distinct plants, raising the possibility that certain bacterial groups have had a long association with plants (Blaustein et al., 2017; Hamonts et al., 2018; J. Xu et al., 2018; Simonin et al., 2020). Studies also have shown that a set of microbial communities form stable associations with particular hosts across temporal and geographic scales (Blaustein et al., 2017; Hamonts et al., 2018; J. Xu et al., 2018). In addition to being persistent and prevalent, these microbes are highly abundant. In wheat (Simonin et al., 2020) and sugarcane (de Souza et al., 2016) the 'core' bacteria represent only a small fraction of microbial richness (3% and 20% for wheat and sugarcane, respectively), but they account for a significant portion of the relative microbial abundance (50% and 90% for wheat and sugarcane, respectively). Core genera include bacteria belonging to Pseudomonas, Agrobacterium, Cupriavidus, Bradyrhizobium,

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Rhizobium, Shinella, Mesorhizobium, Burkholderia, Cellvibrio, Sphingomonas, Variovorax, Paraburkholderia, Dyadobacter, Novosphingobium, Devosia and Ensifer (de Souza et al., 2016; Hamonts et al., 2018; L. Xu et al., 2018; Simonin et al., 2020). Groups of microbes within the 'core microbiome' are postulated to perform roles critical to plant colonization, and individual core group members possess plant growth promotion traits (Dai et al., 2020). Therefore, exploring the 'core functional microbiota' as a basic component may provide a path to harnessing plantmicrobiome interactions (Lemanceau et al., 2017). However, while microbial functional traits can directly affect host physiology and performance (Trivedi et al., 2020), there is little evidence that the occupancy frequency of core microbiomes provides benefits to the host. Also, a close association of core microbiomes with their host does not indicate the involvement of coevolutionary processes during selective enrichment. Although the identification of a 'core microbiome' is a useful first step in reducing the complexity of the plant-associated microbiome, systematic reductionist efforts that incorporate deconstruction (establishment of a culture collection of core members) and reconstruction (core microbiota reconstitution and experiments using plant systems) phases are needed to test the significance of 'core microbiomes' on plant fitness parameters.

Bioinformatics tools that infer microbial cooccurrence networks have revealed 'spatially distinct and highly connected' modules within the microbial networks (Banerjee et al., 2018). Within these networks, a few microbes, called 'hub' microbes (analogous to a keystone guild), are highly connected with other microbes and may play a key role in supporting the network structure and orchestrating community-scale processes important for plantmicrobiome interactions (Banerjee et al., 2018). These hub microbes control, negatively or positively, the abundances, and possibly functions, of other microbes and transmit the effect of the host onto the network of the plant-associated microbiome (Hamonts et al., 2018; Roman-Reyna et al., 2019). As a result, perturbation of hubs can have significant downstream effects on the wider network, resulting in the loss of plant-microbiota-mediated functions. For example, removal of the hub species Enterobacter *cloacae* from a seven-member microbial community led to dramatic changes in the community composition, with near complete extinction of other members except Curtobacterium pusillum (Niu et al., 2017). While beneficial keystones increase the overall diversity (Herren & McMahon, 2018), pathogenic keystones tend to reduce diversity (Agler et al., 2016). The obligate biotrophic oomycete Albugo laibachii acts as a keystone species and stabilizes the postinfection community composition, thus reducing secondary infection from other pathogens in Arabidopsis thaliana. If these hub organisms are crucial to sustaining plant health (Agler et al., 2016), they could represent prime targets for novel crop management strategies. Therefore, their identity, functional role and how they perform under stress conditions need to be elucidated.

IV. Impact of microbiomes on plant functions

The microbiome of plant roots is analogous to the microbiome of the human gut: both provide similar functional roles to their hosts,

including nutrient uptake, growth promotion and disease suppression (Berendsen et al., 2012). Microbiomes may facilitate plant growth and health by direct or indirect mechanisms (Glick, 2012). Direct mechanisms involve: essential nutrient acquisition, such as nitrogen, phosphorus and iron (Calvo & Friston, 2017); hormonelevel modulation through the synthesis of one or more phytohormones, such as auxins, cytokinins and gibberellins (Bhattacharyya & Jha, 2012; Egamberdieva et al., 2017); and stress relief through production of enzymes such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, that cleaves the compound ACC which is the immediate precursor of ethylene (Glick, 2005, 2012). Indirect mechanisms include decreased plant damage in response to pathogen infection (Compant et al., 2005; Ryan et al., 2008) via: direct antagonism through antibiotic production (e.g. 2,4diacetylphloroglucinol), proteases, chitinases, bacteriocins, siderophores, lipopeptides (e.g. iturin A, bacillomycin D and mycosubtilin) and volatile organic compounds; niche exclusion by competition; predation and/or direct parasitism; and/or microbiota-modulated immunity (MMI) wherein microbes could stimulate or inhibit innate immune responses (Vannier et al., 2019). The aforementioned mechanisms have been extensively studied and several reports show the potential of individual microbes (also referred to as plant growth-promoting bacteria) to restrict growth of phytopathogens and promote plant growth (Fig. 2; see references above). Here we focus on recent studies that have taken a community perspective to explore the microbiomefacilitated beneficial effects. We highlight studies that have applied synthetic microbial communities (SynComs) to understand microbial-host relationships and the contribution of microbiota towards improved plant performance.

1. Nutrient mobilization

In agricultural systems, macronutrients are provided through the application of mineral fertilizers. However, diminishing rock phosphate reserves, energy-extensive N-fertilizer production and environmental problems caused by the unsustainable application of inorganic fertilizers have prompted interest towards finding alternative methods of sustaining plant nutrition (Jacoby et al., 2017). Microbial nutrient transformations are key drivers of plant growth and can sometimes be the rate-limiting step in ecosystem productivity (Schimel & Bennett, 2004). There is fine tuning between the host genetic response and activities of associated microbiomes that drive plant-microbiome interactions to facilitate nutrient uptake (Castrillo et al., 2017). For example, to maximize orthophosphate (Pi) use efficiency, plants possess adaptive phosphate starvation responses (PSRs) that occur in the presence of their associated microbiome (Castrillo et al., 2017). The genetic network for PSR signaling influences the structure of the plant-associated microbiome by repressing microbial-driven plant immune system outputs (Castrillo et al., 2017; Finkel et al., 2019). Using a 35member SynCom, microbiome-driven PSR activation was demonstrated in response to low Pi conditions (Castrillo et al., 2017). Using 185-member SynComs across a wide range of P concentrations, Finkel et al., (2019) demonstrated that under Pi-stressed conditions selective recruitment of latent opportunistic



Fig. 2 The assembly of plant-associated microbiomes and beneficial plant–microbiome interactions. Microbial composition of any plant organ is influenced by a range of biotic and abiotic factors. Environmental factors (UV, light, precipitation) mainly influence the aboveground microbiome, whereas farm management and soil factors (e.g. pH, soil type, soil nutrients and moisture) are most relevant for belowground plant parts. Multiple and complex plant–microorganism and microorganism–microorganism interactions are required for the selective assembly of plant-associated microbiomes. (1) Bulk soils act as banks for the selective recruitment of plant-associated microbiome assembly is synchronization between plant exudation patterns with the genetic machinery of microbes to import and utilize distinct metabolites. (2) Plant microbiome assembly is further shaped by intense microbe–microbe interactions mediated via strain-specific production and perception of molecules with dedicated antimicrobial or probiotic activities. (3) Genes involved in motility, chemotaxis, adhesion and biofilm production contribute to plant (green color) through multiple direct or indirect mechanisms leading to growth promotion (phytohormone production), nutrient uptake (e.g. uptake of Pi, Zn, Fe); stress tolerance (production of ACC deaminase, osmoprotectant, or enzymes that detoxify ROS) and resistance to pathogens (production of antibiotics, VOC, HCN, bacteriocin, or by induction of ISR). Abbreviations: IAA, indole-3-acetic acid; GA, gibberellic acid; CA, cytokinin; VOC, volatile organic compounds; HCN, hydrogen cyanide; SAM, S-adenosyl methionine; ACC, 1-aminocyclopropane-1-carboxylate; ROS, reactive oxygen species; ISR, induced systemic resistance.

competitors can exacerbate P-starvation. In rice, differences in the nitrogen use efficiency (NUE) of *indica* (superior NUE) and *japonica* varieties were correlated with differences in the root-associated microbiomes (Zhang *et al.*, 2019). Microbiomes associated with *indica* varieties were highly diverse and had a greater abundance of bacteria with traits related to nitrogen metabolism as compared to *japonica* varieties. The contribution of organic nitrogen-related functions of *indica*-enriched bacteria

towards higher NUE in the *indica* varieties was confirmed by using a 16-member SynCom.

2. Biocontrol

Introduction of pathogens can induce shifts in the plant-associated microbial community resulting in the selective recruitment of microbes with biocontrol traits. These traits include production of chitinases (fungal cell-wall-degrading enzymes) and secondary metabolites, such as phenazines, polyketides and siderophores (Mendes et al., 2011; Cha et al., 2016). Notably, the endophytic microbial communities of sugar beets growing in soils that promote resistance to Rhizoctonia solani wilt disease were enriched for chitinase genes and various unknown biosynthetic gene clusters that encode the production of nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs), enzymes potentially involved in the synthesis of inhibitory secondary metabolites (Carrion et al., 2019). A 'minimal' consortia of two endophytes that were enriched in the endosphere of sugar beet rescued plants from damping-off disease (Carrion et al., 2019). A simplified SynCom consisting of seven bacterial strains representing three of the four most dominant phyla in maize roots not only promoted maize growth but also inhibited the pathogen Fusarium verticillioides (Niu et al., 2017). The disease resistance traits of plant varieties are also a function of the associated microbiome (Kwak et al., 2018). Comparative analysis of rhizosphere metagenomes from tomato varieties Hawaii 7996 (resistant to Ralstonia solanacearum) and Moneymaker (susceptible to R. solanacearum) demonstrated that bacteria belonging to Flavobacterium are correlated with disease resistance. Assembly of high-quality MAGs allowed cultivation of Flavobacterium sp. TRM1, a strain that efficiently suppressed R. solanacearum disease development in susceptible plants (Kwak et al., 2018). A complex SynCom (148 bacteria, 34 fungi and nine oomycetes) for biocontrol used to demonstrate inter- and intrakingdom interactions in root-associated bacterial communities maintained a microbial balance that favored plant health (Duran et al., 2018).

V. Rules of microbiome assembly

Successful application of microbiome treatments in the field requires a thorough understanding of the ecological processes that govern the establishment, stability and local adaptation of the plant-associated microbiome. However, until now, most microbiome studies have focused on 'who is there?', 'what do they do?', and 'when, where, and which microbiome features are related to a particular host phenotype?'. We do not yet have a conceptual framework to understand how well-defined ecological processes govern the assembly and functionality of the host microbiome (Dini-Andreote & Raaijmakers, 2018; Cordovez et al., 2019). Knowledge of the interplay of ecological processes to generate and maintain variations in the plant-associated microbiomes is crucial for developing models that will allow prediction of the factors that promote successful colonization of introduced microbial strains in the context of the recipient native microbiome. Both ecological (Fierer et al., 2012) and metacommunity theory (Miller et al., 2018) posit that microbiomes and their associated hosts should be viewed as ecological systems wherein the interplay between four main coevolutionary processes – dispersal, diversification, selection and drift - are responsible for multispecies assemblages. Among these, the input of organisms is driven by dispersal from the regional species pool and in situ diversification while selection and drift modulate the relative abundance of different species. The effects on community assembly are governed by complex interactions

between these processes along with the ecological characteristics (e.g. resistance, resilience and functional redundancy) of the microbial communities that are required to maintain community stability. The relative importance of these processes changes with plant growth stages, where dispersal and drift are more important during the seed and emerging root systems while selection has a greater influence in community assembly at later stages (Paredes & Lebeis, 2016; Dini-Andreote & Raaijmakers, 2018). The arrival order (also known as 'priority effects') can impact both the assembly and the functionality of plant-associated microbiomes (Fitzpatrick & Schneider, 2020). For example, rare taxa could drive priority effects in microbiome assembly during the early stages of plant development, either leading to more suppressive bacteria or by priming the plant immune system (Wei et al., 2019). A SynCom of 62 bacterial strains was used to test the importance of priority effects on structuring the microbial community composition of the phyllosphere (Carlstrom et al., 2019). The results showed that the founding taxa have a persistent influence on microbial community assembly and, as such, are resistant to invasion by latecomers. This is important as it demonstrates that, to be effective, microbiome inoculation should be targeted at the early stages of plant growth. Metacommunity theory also posits that community assembly is driven by specific interdependent processes that occur at a local scale, including ecological interactions between and evolution of community members, abiotic factors, immigration from other niches/ecosystems, and community history (Miller et al., 2018). It is important to view and investigate microbial communities as dynamic entities that morph and evolve constantly. In addition, research focusing on the arbuscular mycorrhizal fungus Rhizophagus irregularis provides cues to recognizing the importance of genetic variability among isolates of a microbe species and their potential effects on host-plant fitness and plant community structure (Koch et al., 2006; Savary et al., 2018).

VI. Roadmap for translation

The huge volume of data obtained from characterization of microbiomes is clearly calling us to move beyond a 'discovery phase' towards the 'translational phase' where the microbiome-based solutions are applied *in situ* for promoting plant growth under a range of environmental conditions and increasing resilience to biotic and abiotic stresses. However, technical, translational and policy challenges must be addressed first. Here, we discuss different ways through which microbial communities can be tailored to carry out specific functions that lead to increased plant performance.

1. Plant-optimized microbiomes: host-mediated microbiome engineering

The ability to assemble microbial communities with host-beneficial traits is a critical step towards successful application of microbiomebased solutions to increase plant performance. The application of native microbiota that are well adapted to the environmental conditions, soil types and/or plant niche (e.g. rhizosphere or phyllosphere) provide a greater chance of establishment and manifestation of beneficial traits compared to alien microbiota (Gu

et al., 2020). Through an experimental evolutionary approach, it is possible to select for a robust, plant-optimized microbiome that is resistant to random invasion (Morella et al., 2020). These evolved microbiomes can survive in, on and around plants for multiple generations and can provide long-term benefits (Arias-Sanchez et al., 2019). Host-mediated microbiome engineering based on multigenerational selection of beneficial host-microbiome interactions has been employed successfully to assemble microbial communities that are well adapted to the plant and that perform a particular function (Panke-Buisse et al., 2015, 2017; Mueller et al., 2016; Jochum et al., 2019). For example, specialized microbial communities can be assembled through artificial selection that alter the flowering time of plants in a highly reproducible manner (Lau & Lennon, 2012; Panke-Buisse et al., 2015). Accelerating flowering time is an evolutionary strategy adopted by plants to maximize the chances of reproduction under multiple stress conditions (Kazan & Lyons, 2016). In this context, optimizing the plant microbiome through artificial selection can lead to the development of stress-tolerant phenotypes with increased productivity under changing climates. Artificial selection strategies have also been used to engineer plants with drought (Jochum et al., 2019) and salt tolerance (Muller et al., 2016).

2. Plant-optimized microbiomes: plant engineering

Metaanalysis studies have shown taxonomic shifts in root microbial communities due to domestication and highlight the need to understand the role of missing microbes that may confer competitive ability to wild species compared to their cultivated counterparts (Perez-Jaramillo et al., 2018). High-yielding semidwarf wheat cultivars developed during the Green Revolution resulted in the selection of less complex rhizosphere bacterial communities compared to pre-Green Revolution tall wheat cultivars (Kavamura et al., 2020). Further, host genetics influence the root microbiome composition, indicating the potential of a microbiome-based trait for selection in plant breeding (Taye et al., 2020). Current studies indicate heritability of microbiomes as well as their association with agronomic characteristics. For example, rhizosphere microbiome data obtained at flowering stage from 16 diverse canola genotypes attributed 37-59% of variation in the number of different microbial species to genetics (Taye et al., 2020). Likewise, field studies show that certain taxa (Amycolatopsis sp., Serratia proteamaculans, Pedobacter sp., Arthrobacter sp., Stenotrophomonas sp., Fusarium merismoides and Fusicolla sp.) are positively correlated with canola yields (Lay et al., 2018). Community-level physiological profiling and metatranscriptomic studies also indicate that breeding for disease resistance has led to unintentional changes in rhizosphere communities with increased bacterial taxa that metabolize compounds conferring protection against root pathogens (Mendes et al., 2019). Despite growing knowledge that the microbiome affects host performance, a deeper understanding of genotype × environment × microbiome × management interactions is needed for successful integration into breeding programs (Busby et al., 2017). This can be realized if future studies to detect plant genes that have true effects on microbiome composition involve multilocation, multiyear field $\ensuremath{\text{Box 3}}$ Synthetic biology to enhance microbial application in agriculture

Both plants and their associated microbiomes produce a variety of specialized metabolites to communicate and interact with each other. Sometimes the genes involved in the production of these metabolites are arranged in regions called biosynthetic gene clusters (BCGs). New computational algorithms now allow us to discover novel BCGs from environmental samples (Blair et al., 2018; Sugimoto et al., 2019). Using synthetic biology tools (e.g. CRISPR/Cas 9 systems and rapid methods to assemble DNA fragments), BCGs can be expressed in heterologous hosts to produce novel compounds in vitro (Sugimoto et al., 2019). Furthermore, new synthetic platforms that employ a recombinase-based gene integration method for rapid and efficient insertion of large DNA fragments into eukaryotes allow commercially feasible scale-up in the production of novel compounds (Cao et al., 2018). Large-scale systems-biology enables investigations that combine multiomics and computational modeling approaches to identify key microbes and molecules that drive metabolic and signaling interactions in plant-microbe interactions. Using this information, synthetic biology tools can be employed to engineer multifunctional regulatory gene repertoires for efficient assembly and regulation of collective functions in microbial consortia. Furthermore, synthetic biology tools can be used to engineer inducible circuits that can increase plant performance. This could involve engineering of signaling mechanisms that enable host-microbe (interspecies) communications using plant-derived signals, or microbe-microbe (intraspecies) communications such as bacterial quorum sensing (QS) cross-talk.

trials, preferably in natural field soils that are managed using best practices (Wille *et al.*, 2019). Thus, a key aspect to translation is a thorough understanding of the target field environment as well as the crop of interest (Saad *et al.*, 2020).

Plants themselves can be engineered through gene manipulation for specialized microbiome assembly or enhanced microbialmediated functions. For example, poplar plants genetically modified for downregulation of cinnamoyl-CoA reductase (CCR) influence the structure and function of the root endophytic community without influencing the rhizosphere microbiome (Beckers et al., 2016). The changes in the endophytic communities were driven by the differential accumulation of compounds in the xylem of CCR-deficient poplar trees. Several genome-wide association studies (GWAS) have identified candidate plant genes that influence the assembly of plant-associated microbiota (Walters et al., 2018; Beilsmith et al., 2019; Roman-Reyna et al., 2019; Deng et al., 2020). These genes are shared between different plant varieties and are correlated highly with the abundance of specific subsets of the rhizosphere microbiome (Walters et al., 2018; Deng et al., 2020). Manipulation of these candidate genes can provide a way forward to generate designer plants with defined microbiomes and their associated benefits (Box 3). For example, in rice, genes associated with stress responses and carbohydrate metabolism influence the phyllosphere microbiome (Roman-Reyna et al., 2019). Overexpressing lines with increased callose deposition and salicylic acid production reshape the composition of phyllosphere microbiome in a highly controlled fashion (Jochum et al., 2019). It

must be noted that although GWAS can provide important information on the genetic basis of microbiome-host interactions, it may also represent spurious associations and do not necessarily pinpoint causal relationships. A single mutation in a rice nitrate transporter and sensor NRT1.1B (amino acid 327 is methionine in indica vs threonine in japonica) is related to the recruitment of distinct microbiota that are responsible for higher NUE of *indica* as compared to *japonica* varieties (Zhang et al., 2019). Thus, mutation of NRT1.1B possibly can lead to selection of a microbiome for increased NUE in japonica varieties. Medicago trunculata and Hordeum vulgare engineered with a synthetic pathway producing a bacterial-derived rhizopine (chemical signals involved in rhizobium-legume symbiosis) now selectively recruit nitrogen-fixing rhizobia (Geddes et al., 2019). Plants can be genetically modified to excrete secondary metabolites such as coumarin (an iron-mobilizing phenolic compound) (Stringlis et al., 2018) or plant hormones such as salicylic acid (Lebeis et al., 2015) that are known to sculpt root microbiota under stress conditions.

3. Management practices to optimize the microbiome

Disease suppressive soils can be developed through certain management practices such as tillage and crop rotation (Peters et al., 2003), while disease suppressive properties can be transferred by mixing small amounts of suppressive soils with conducive soils (Mendes et al., 2011; Raaijmakers & Mazzola, 2016). Thus, the beneficial microbial effects of certain management practices can be transferred to steer agroecosystems towards healthier states. An elegant management mechanism wherein gaseous signal exchange between cassava and peanut under intercropping results in the reassembly of the peanut-associated microbiome was elucidated by Y. Chen, Y et al., 2020). This restructured microbiome increases the fitness of peanut plants, leading to greater seed production in intercropping as compared to monoculture. The legacy effect of management practices on the assembly of soil microbiomes influences plant physiology by impacting the expression of several genes involved in plant hormone production (Li et al., 2019). Interestingly, a new study demonstrates that soil microbiomes can be directed through plant-soil feedbacks to induce resistance against aboveground herbivorous insects (Pineda et al., 2020). Understanding that some insects, such as the Russian wheat aphid, depend on their microbiome for full virulence to their host (Luna et al., 2018) opens new strategies for pest control. Management practices such as organic farming and conservation tillage practices have been shown in some cases to improve soil nutrient cycling while maintaining plant yields through their influence on the structure and activities of the soil microbiome (Hartman et al., 2018; Trivedi et al., 2020). However, management decisions are based on several factors, including nutrient applications, site characteristics, profitability, use of machinery and varieties/hybrids of crops. More studies are required to understand how different management practices influence the relevant soil microbiota and whether modified management practices and microbiome engineering could contribute to more sustainable agricultural production in the long term.

4. Optimized microbiomes: genetically modified microbes

Genome-based approaches have identified various genes in microbes involved in mediating plant-microbe interactions (Blair et al., 2018; Levy et al., 2018). Engineering microbial genes that facilitate beneficial traits holds tremendous potential for the development of superior microbial inoculants. In a recent breakthrough, the symbiotic bee gut bacterium Snodgrassella alvi was genetically modified to produce double-stranded RNA that triggers RNA interference (RNAi) in bee hosts (Leonard et al., 2020). Activation of RNAi via the engineered bacterium protects honeybees from viral infection and parasitic mites. Similar approaches can be used to engineer bacterial endophytes that trigger plant defense responses against pathogens and pests. Genome editing technologies, including site-directed nucleases such as the highly efficient CRISPR and CRISPR/Cas 9 methods, have opened new possibilities to edit genes and genomes to enhance the beneficial traits of plant-associated bacteria. These technologies allow the design of inducible regulatory circuits that can enable controlled induction of beneficial traits in response to signals derived from the plant host or target organisms (in the case of biocontrol) (Farrar et al., 2014).

Significant efforts are underway to redesign microbial circuits (novel gene networks designed through synthetic biology tools) and biosensors for application in agriculture (Goold et al., 2018). One product, BananaGuard, has been produced and consists of a Pseudomonas putida strain genetically modified to control Fusarium oxysporum which causes Panama disease of banana (Kemal et al., 2016). This engineered bacterium produces fungal growth inhibitors after sensing F. oxysporum-produced fusaric acid and has a kill-switch that induces self-destruction when the fungus is no longer detected. In a recent breakthrough, naturally occurring epiphytic or endophytic bacteria of cereal crops were engineered for improved nitrogen fixation (Ryu et al., 2020). The engineered N2fixing strains were further modified to express N2-fixing genes in response to chemical signals present in the rhizosphere and seeds that are known to remodel plant-associated microbiomes. With the advances in targeted genome editing, it is possible to develop designer genetic circuits in microbes that are responsive to plantderived signals for fine-tuning host-microbe interactions. It is particularly exciting that these new tools are not only able to modify the genomes of cultured bacteria, but they can also be used to modify a complex microbiome (Sheth et al., 2016). For example, broad-host-range plasmids that can be transferred to 11 different bacterial phyla in a complex microbial community are now available (Klumper et al., 2015). Despite regulatory uncertainty in some jurisdictions and potential public concern, gene editing technologies can be instrumental in engineering effective microbial inoculants or modifying microbiomes in situ. The public may be much more accepting of these technologies if, in most cases, their deployment will reduce the environmental footprint of agriculture. For example, several projects underway are aimed at increasing nitrogen fixation in crop-associated microbes which, if successful, will reduce the use of fertilizer and concurrent run-off that impacts waterways negatively (Goold et al., 2018).

It is postulated that inoculation with exotic microbial strains may have consequences for natural systems, particularly on native microbial and plant biodiversity (Schwartz *et al.*, 2006). However, the unintended consequences of microbial inoculants in natural systems are not realistic (Köhl *et al.*, 2019) when one takes into consideration: the general principles of microbial population dynamics; that pathogenic microbes are immediately excluded from products by developers and by regulations prohibiting their use; the environmental fate of decades-long experience with nonpathogenic microbes has never given rise to uncontrollable situations; and that many efficacy trials fail because after application the densities tend to decrease rapidly.

7. Product commercialization

While the above-mentioned approaches provide promising routes to harness microbiomes for increased plant productivity, another feasible and economical option is to develop microbial inoculants. Although microbes have long been applied as inoculants for biocontrol or biostimulation in agricultural systems, their efficacy varies with climate, soil type and other environmental factors (Kaminsky et al., 2019). Unpredictable establishment of the microbes which leads to ineffective delivery of desirable benefits is a major constraint for translating results from the lab to field. Despite this, the advances in our understanding of plant-microbiome interactions linked with the high-throughput culturing and screening techniques described above (Lagier et al., 2012; Pham & Kim, 2012; Sarhan et al., 2019, 2020) has renewed interest in developing microbial inoculants for agricultural application. The clear recognition of the commercial potential of the microbial inoculants is evident from the fact that the microbial soil inoculants market is expected to grow at a compound annual growth rate of 9.5% and reach US \$623.51 million in 2023 as compared to \$396.07 million in 2018 (https://www.marketdataforecast.com/ market-reports/microbial-soil-inoculants-market).

1. Feature select (SynComs vs individual strains)

Basic research focused on identifying beneficial microbes or microbial consortia is being translated to field studies. For example, trials conducted in Senegal on grower fields demonstrated the ability of Aflasafe SN01, a biocontrol product consisting of a blend of four atoxigenic Aspergillus flavus strains native to Senegal, to be effective in reducing aflatoxin (a carcinogenic secondary metabolite) contamination in groundnut and maize (Senghor et al., 2020). Both single-strain (Afla-Guard or AF36 Prevail) or multistrain (Aflasafe, Aflasafe SN01) atoxigenic A. flavus strains successfully reduced aflatoxin contamination in crops such as groundnut, cotton and maize (Dorner, 2004; Bandyopadhyay et al., 2016). Bacillus amyloliquefaciens strain NJN-6, isolated from suppressive soils, suppressed banana Panama disease (Xue et al., 2015). More recently, Trivedi et al. (personal communication) developed an optimized plant microbiome-based sustainable disease management strategy to manage Citrus Huanglongbing (HLB) disease. A 23-member SynCom of bacteria applied as liquid formulation under field conditions showed a 10-fold decrease in the titers of HLB pathogen Candidatus Liberibacter asiaticus 1 month after SynCom inoculation of citrus trees compared to no significant pathogen titer changes in controls (trees inoculated with the media without bacterial SynCom). Similarly, other studies showed positive effects of microbial consortia (Castrillo et al., 2017; Niu et al., 2017; Carrion et al., 2019). There is a greater chance for cocktails of more than one beneficial microbe to establish and provide functional benefits than a single-strain inoculant, yet it remains unknown how consortia will perform in diverse environments (Kaminsky et al., 2019). Soil microbial inoculant development programs should focus not only on functional traits but also on the ecology or establishment/survival traits of the microbe (Kaminsky et al., 2019). Besides the scientific quest to understand the mechanisms that underpin the success and stability of microbial consortia, what matters most from a practical perspective is the need for solutions to grower problems. The litmus test for either a single strain or a consortium of microbes lies in the ability of the microbial inoculant to provide the expected agronomic benefits in the growers' fields.

2. Formulations

Formulation is critical to the success of a microbial inoculant, particularly one containing Gram-negative, nonspore-forming bacteria as it determines the stability and viability of microbes during various stages of a production pipeline until application (O'Callaghan, 2016; Berninger et al., 2018). The importance of formulation in product development is further evident as companies develop their own proprietary formulations (O'Callaghan, 2016). In general, formulations can be dry or wet. Wettable powders, water-dispersible granules and dry granules are common forms of dry formulations in which the microbe of interest is mixed with carriers such as peat, clay, talc, milk powder, silica, ground corn cobs and coconut shells (Bashan et al., 2014; Jackson, 2017). Liquid formulations are produced as suspension concentrates or emulsifiable concentrates. Although the production costs are high compared to conventional formulation technologies, encapsulating microbes in a polymer matrix such as alginate beads promotes the slow release of microbes and protects them from the soil environment as well as other competing soil microbes (Young et al., 2006; John et al., 2011; Bashan et al., 2014). For example, Hirsutella rhossiliensis, a nematophagous fungus, formulated in cellulose-based microcapsules with 3% baker's yeast resulted in 90% parasitism of Heterodera schachtii juveniles compared to 37% with nonformulated application of fungal mycelia to soil (Hallmann et al., 2019). More recently, amidated pectin beads were shown to be an effective formulation strategy for colonizing radish endosphere by a Gram-negative endophytic bacterium (Barrera et al., 2020). For comprehensive reviews on various formulation techniques, see Herrmann & Lesueur (2013), Bashan et al. (2014) and Berninger et al. (2018).

A major bottleneck in the development of formulations, especially with nonspore-forming bacteria, is drying stress. A recent review focuses on methods and factors to mitigate this stress (Berninger *et al.*, 2018). Market needs and intrinsic microbe characteristics both play a vital role in the formulation of a product

(Jackson, 2017) and must be considered on a case-by-case basis especially when combining multiple species in SynComs. For example, one of the reasons for the commercial availability of several *Bacillus*-based products is the ability of microbes in this genus to produce dormant spores capable of surviving adverse environmental conditions (Cawoy *et al.*, 2011). Spores facilitate the production of easy-to-handle powder formulations with a longer shelf life, attributes required for successful commercialization.

3. Delivery, efficacy, monitoring and storage

Microbe delivery methods vary for different agricultural systems, such as field or horticultural crops. Several commercially available microbial products are available for soil application or foliar sprays. Broadcasting of carrier material, such as sorghum seed coated with beneficial microbes, is also being practised (Senghor et al., 2020). Seed treatment provides a localized conduit to deliver microbes to the rhizosphere and requires less inoculum compared to soil application (Wilson & Jackson, 2013). An analysis of 191 published studies between 1960 and 2019 on microbial seed coating revealed that about 41% of these studies were developed in the last decade, thereby indicating the increasing popularity of this delivery method (Rocha et al., 2019). A further development in seed inoculation that facilitates ease in product application includes stacking microbes with chemicals on crop seeds (Marrone, 2019). Another mode of delivery, root dipping of seedlings, is being used to manage diseases such as sheath blight and bacterial leaf blight of rice (Jambhulkar et al., 2016). Seed treatment, soil application and root dipping are considered as some of the main delivery routes for the application of biofertilizers (Soumare et al., 2019). Endophytes have developed an intricate association with their hosts, providing a unique opportunity for microbe delivery. For example, the plant-growthpromoting endophyte Paraburkholderia phytofirmans can be integrated stably into seeds by spray-inoculating flowers of crops such as wheat, maize, soybean and pepper (Mitter et al., 2017). In this case, in addition to endophyte introduction, changes in seed microbiome composition were observed. Such an approach, where the microbe of interest is protected inside the seed, provides enhanced protection from environmental stresses as well as from early competition from soil microbes (Mitter et al., 2017).

For horticultural crops, the choice of microbial product delivery method to protect trees against pests and diseases (foliar sprays, soil drench or trunk injection) depends on the affected tree parts (leaves, roots or stems) (Rabiey *et al.*, 2019). Recent *in vitro* proof-of-concept studies showed that encapsulation of beneficial fungal spores in a biodegradable lignin shell, the release of which is triggered by the secretion of lignindegrading enzymes by a pathogenic fungus, has the potential for delivery via trunk injection as a curative treatment for plants affected by trunk diseases (Peil *et al.*, 2020). Even though considerable microbial losses can occur due to improper delivery methods, there are currently limited published research efforts focused on developing efficient delivery strategies (Qiu *et al.*, 2019). The crux in developing an effective delivery mode

Box 4 Outstanding research and educational needs

- Comprehensively determine the makeup and genetic potential of plant-associated microbiomes to unravel complex interspecies ecological interactions and metabolic networks.
- Ensure accuracy and reproducibility in experimental and field settings to move beyond correlation to causation.
- Model and predict host genotype, microbiome genotype, environment, and management ($G_H \times G_M \times E \times M$) interactions to tailor microbial formulations.
- Develop and adopt standardized procedures for collecting and reporting consistent and well-annotated metadata (Dundore-Arias *et al.*, 2020).
- Develop globally accepted standards for plant-associated microbial products to ensure rapid translation of microbiome innovations in real world conditions.
- Test and develop cost-effective, in-field sequencing platforms for diagnostics and increased understanding of plant-associated microbiomes and farm management practices.
- Develop stabilization methods for Gram-negative bacteria to improve microbial product performance for growers.
- Educate and train the next generation of researchers and extension specialists in the highly diverse field of microbiome science that requires collaboration, skill integration and sharing of best practices across many different disciplines, including microbiology, plant pathology, plant and soil science, ecology, agronomy, bioinformatics, mathematics and biochemistry.

of the target microbes is that the product should be in a form that can be easily integrated with farm operations and current production technologies while also being cost-effective for the developer as well as growers.

Standards are needed to ensure that the appropriate load of microbial inoculum is delivered with the product. Even with the long-used practice of treating legume seed with rhizobia, concerns regarding the survivability of inoculum on preinoculated seed have been noted (Gemell et al., 2005). For example, survival of rhizobia on seed is affected by water quality and the type of polymer adhesive used in the process of inoculating seed (Hartley et al., 2012). In some instances, governmental regulations are in place that set minimum standards for the number of rhizobia to be delivered per seed (Lupwayi et al., 2000), although unified internationally accepted standards are lacking (Penna et al., 2011). Universally accepted efficacy standards for individual strains or SynComs that are host-specific would be ideal, but are unlikely in the near future. Methods such as counting colony-forming units, absorbance spectrophotometry, flow cytometry and quantitative PCR using DNA intercalating dyes are in place to enumerate viable cells and can be utilized to conduct quality checks (Berninger et al., 2018). Still, relatively few peer-reviewed studies focus on the shelf-life of microbial inoculants, which is influenced by the type of formulation and storage conditions (high temperature, humidity, light and type of packaging material) (O'Callaghan, 2016; Berninger et al., 2018). In general, the microbial inoculant shelf-life varies from 2 months to 2 years when stored at lower temperatures (Berninger et al., 2018).

4. Regulatory framework

Regardless of the successful development of a microbial product for agriculture, it is meaningless unless it can be commercialized and this requires an understanding of the regulatory framework within which the product can be distributed. In almost every legal jurisdiction, microbial products are categorized by regulations as biopesticides/biocontrol agents, biofertilizers and biostimulants (Leahy et al., 2014; Du Jardin, 2015; Marrone, 2019). The regulatory framework, registration and commercialization of each of these types of microbial products is complex, varies by country and, in the USA, by state. There are several nonidentical but somewhat consistent and relevant definitions of biopesticides and biofertilizers as well as various, at times inconsistent, definitions of biostimulants (Marrone, 2019; Du Jardin et al., 2020). Depending on the type of product, every political jurisdiction has its own set of compliance programs for their registration based on legal mandates which underpin the specific standards, fees and other regulatory requirements (Du Jardin, 2015). The regulatory regime in most jurisdictions for biopesticides is fairly well defined as it, generally, follows the rules established for synthetic pesticides although, fortunately, the development of dossiers tends to be considerably cheaper for biopesticides than for synthetic chemicals (Leahy et al., 2014; Frederiks & Wesseler, 2019). International cooperation is increasing and the USA and Canada review biopesticides jointly to increase registration efficiency and grower access to more sustainable pest management solutions (Leahy et al., 2014). Even in cases where the regulatory requirements are clear, the pest and environmental risk assessments for gaining approval to commercialize a microbial biocontrol agent/biopesticide may be cost-prohibitive (Köhl et al., 2019) and this has hampered innovation in some jurisdictions, most notably in the EU (Frederiks & Wesseler, 2019; Vekemans & Marchand, 2020). In jurisdictions that are 'claims based' (i.e. the requisite regulations depend on the marketing plans for the product), developers try to avoid pesticidal claims to bypass more rigorous, extensive data requirements. Biofertilizer regulations have been implemented in many US state jurisdictions and in several countries as part of the general fertilizer registration requirements. In some cases, the goal of these regulations was to provide clarity and assurances to growers regarding the efficacy of the product while, in other cases, the goal was to accommodate organic agriculture (Malusá & Vassilev, 2014; Marrone, 2019). Regulation of microbial biostimulants is extremely complex as in most jurisdictions they have been commercialized through fertilizer regulations or through pesticide or plant protection product regulations (Du Jardin, 2015). Currently, there is no universally recognized and agreed upon definition of biostimulant in academia or by regulatory agencies (Du Jardin et al., 2020). While the field is in flux, efforts are underway to establish legal definitions and national or international standards and guidelines for their commercialization. The EU recently implemented a new regulation which defined plant biostimulants as a '... fertilizing product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the of the following characteristics of the plant or the plant rhizosphere: (1) nutrient use efficiency, (2)

tolerance to abiotic stress, (3) quality traits, or (4) availability of confined nutrients in the soil or rhizosphere' (Rouphael & Colla, 2020). The United States Congress, in the Agricultural Improvement Act of 2018 (Public Law 115-334), defined biostimulants as 'a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield' and directed the USDA to review the definition and report on whether new legislation would be needed to facilitate biostimulant commercialization. The US Environmental Protection Agency (EPA), shortly thereafter, released a draft guidance for plant growth regulators, including plant biostimulants, for public comment in 2019 (EPA, 2019). It remains to be seen how quickly the US regulatory regime will be finalized and how any national structure will fit within the currently existing state regulations.

Although complex, the regulatory cycle for the development and commercialization of microbial-based products are generally streamlined and well articulated (Parnell et al., 2016). While agencies are working on improving the regulatory framework for biostimulants, there remains the problem of inconsistent labeling and product performance. Use of biostimulants that are of low quality or not suited to environmental conditions or grower management practices can reduce profitability and erode public trust. On the other hand, trust can be built from ensuring product quality and developing new products based on beneficial attributes specifically linked to positive outcomes. Ricci et al. (2019) provided general guiding principles to follow when justifying biostimulants claims for labeling and regulatory purposes. In the rapidly developing field of microbiome and microbial science, we expect to see the emergence of novel technologies that might not fall into preexisting regulatory categories. A general rule to consider on any occasion that regulatory agencies will be involved in product commercialization is to establish an early dialogue with the appropriate agency to facilitate the registration process.

8. Conclusion: an integrated approach is still the golden rule

Consider a scenario in which a microbe or microbial consortia identified by one of the several approaches outlined earlier was successfully formulated, delivered and shown to have field efficacy (Fig. 3). Such an approach is not a panacea that will enhance crop productivity, as there are several edaphic, environmental and microbial community factors influencing the successful performance of a microbial inoculant upon introduction in the field (van Veen et al., 1997; Krober et al., 2014; Schreiter et al., 2014). Drawing inferences from studies where biocontrol products are considered as the core for disease management (Bandyopadhyay et al., 2016), new generation microbiome-based inoculants should be used in unison with existing integrated management practices. Top-down approaches using designer plants that can selectively recruit microbes from the bulk soil can be developed to tailor functional microbiomes appropriate for the purpose. Clearly, a holistic approach towards the use of microbial products, wherein the products are targeted for a specific field environment and crop



Fig. 3 Proposed technical flow for artificial construction of a synthetic microbial community (SynCom) to augment plant fitness and productivity. Microbiomes from sites with disease suppression or better plant performance are characterized using multiomics techniques. The beneficial microbiome is selected through construction of an interaction network and potential key microbes are identified based on structural properties and/or functional modules. Individual microbial members are cultured using high-throughput platforms and characterized through genomic, metabolic and physiological analysis. The isolates are screened first for beneficial traits and then in binary microbe–microbe and in plant–microbiome interactions using microfluidics-based platforms. Desirable functions for microbes destined for inclusion in SynComs may be improved through synthetic biology. SynComs with different complexities are designed through predictive modeling that evaluates trait redundancy, dominance, modularity, interactions and assembly. SynComs are further validated for their plant growth promotion abilities in the glasshouse or using standardized fabricated ecosystems or microfluidic platforms for reproducible interrogation of beneficial traits. The most promising SynComs are applied to fields where a variety of sensors mounted on unmanned aerial vehicles coupled with mobile DNA sequencers will allow automated monitoring of the plant response and microbial community structures. Integration of microbiome–phenome–environment datasets will be used to forecast microbial dynamics and enable scaleup of SynCom application in smart farming systems.

genotype (Saad *et al.*, 2020), will be needed to realize the full potential of microbiome enhancement. Significant gaps remain to be addressed (Box 4), but the trajectory is strong towards a phytobiomes-based approach that provides growers with all of the tools necessary for site-specific, highly sustainable and efficient agricultural production.

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