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Supplementary information

Plant–microbiome interactions: from community assembly to plant health

In the format provided by the authors and unedited

S1. Host and environmental drivers of the assembly of the plant-associated microbiome

Host Genetics

Host plant genotypes, along with external environmental conditions and plant developmental stages, are important factors determining microbiome structures. Heritability studies show that the host genome does influence microbiome composition, and many heritable taxa belong to the core microbiome suggesting positive plant-microbiome feedback over evolutionary timescales. Based on genome-wide association studies (GWAS), several quantitative trait loci (QTL) have been identified that underlie variation in the microbiome traits, species richness and community structure in roots and phyllosphere. The top gene ontology (GO) categories associated with fungal and bacterial taxa were involved in host defense[1, 2] and the top GO enrichment category for species richness was regulation of viral reproduction. Genes for root development, cell wall components, and aging, also part of defensive barriers were also implicated in the GWAS. Interestingly, GWAS results connected to bacterial and fungal richness showed little overlap suggesting that bacterial and fungal communities are influenced by different plant genes[1]. For example, the biological processes associated with bacterial richness related to cell-wall modification, sugar processing, and cellulase activities, while processes related to the epidermal cell layer and programmed cell death underlie variation in fungal richness. A recent GWAS study conducted on 3,024 rice accessions growing in two different environments showed that QTL associated with biotic and abiotic stresses or morphological and physiological traits could modulate the assembly leaf microbial community[3]. Similarly, host genetic factors involved in these same processes were linked to leaf microbiome assembly in *Arabidopsis*, *Nicotiana,* and maize[1, 3, 4]. This makes it likely that allelic variation in certain host genes influences the abundance of hub genera and this controls the composition of the plant-associated microbiomes. Although studies conducted in a single environment identified plant genes that influence microbiome assembly, it remains unclear if genes tested in a single setting are also influential across a range of environments, or if gene-by-environment interactions have an overriding effect on the microbiome.

Morphological characteristics

Plant genetic variation affects morphological characteristics including root architecture, growth and exudation patterns (see below), which in turn can significantly impact the assembly and functionality of the associated microbiome. The root's genetically determined traits (aka phenes) and root architecture result in the selective filtering and recruitment of different microbial communities[5, 6-8]. These microbial communities perform critical functions for that plant, which might provide a selective advantage for performance of a particular rootstock in a given set of environmental conditions[6-8]. Root genotypes control uptake of water and nutrients as well as transport of phytohormones or signalling molecules, thus affecting leaf physiology and subsequently influencing compositions of endophytic and epiphytic microbial communities in above-ground plant tissues[9]. Plant features modulate community assembly belowground, but

plant attributes such as taxonomic identity, phylogeny, growth and mortality rates, wood density, leaf mass per area, and leaf nitrogen and phosphorous concentrations are correlated with bacterial community structure on leaves[10].

Immune response

Plants encounter a myriad of microbes during their lifetime and the establishment of a homeostatic microbiome requires the ability to discriminate between beneficial and pathogenic members. Achieving this balance requires the perception of signals followed by the activation of either symbiotic responses that promote microbial colonization or immune responses that limit it[11]. It has been suggested that different selection pressures in the immune response, resulting from contrasting fitness outcomes, lead to the controlled assembly of the beneficial bacteria and selective removal of pathogens[12]. Diversification of MAMPs (e.g. flg22, peptidoglycan, or cold shock proteins) and their pattern recognition receptors (PRRs) can play a fundamental role in the evasion of MAMP triggered immunity (MTI) surveillance, thus accommodating the population of beneficial bacteria[12-14]. Different hosts may have evolved specificity to different elicitors and consequently the marked expansion and diversification of elicitor variants allow the assembly of distinct plant-associated microbial communities in natural plant populations in response to local environments[13]. Exposure of plants to habitat-specific MAMP repertoires stimulates environment-adapted plant growth responses to maximize overall plant fitness at the local scale through the sculpting of a distinct microbial community[12] as also suggested by various GWAS studies[1, 2, 3]. As immune response output upon MAMP/DAMP (danger associated molecular patterns) exposure are dose dependent, it has also been postulated that the plant innate immune system has evolved to tightly monitor the load of associated microbiota by quantitative and transient adjustment of MTI and plant growth responses[12].

Phytohormone signalling canonically mediated by salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) is central to plant defense responses. Whereas SA mediates systemic acquired resistance (SAR) and defense against biotrophic and hemibiotrophic pathogen attack, JA and ET mediate induced systemic resistance (ISR) and defense against necrotrophs and insects[15]. Although these are seemingly distinct classifications, however, it is clear that hormone signalling is highly integrated, and multiple hormones influence any process of interest[16, 17]. Accordingly, phytohormones are also significant for the bi-directional communication between plant and microbes. Several studies have reported the direct and indirect effect of SA[18], JA[19, 20] and ET[21, 22] on the assembly of plant-associated microbiomes. A recent study illustrates the importance of active balancing of stress-response trade-offs for plant fitness maintenance and for interaction with the plant microbiota through cross talk between stress (abscisic acid) and defense (SA) hormones[23]. Although interplay between different plant hormones has been described, little has been done to understand the capacity of pathway cross-talk to manipulate and shape the microbiome.

Root exudates

The recruitment, shaping and tuning of the plant-associated microbiome particularly in the rhizosphere and root region is primarily modulated by root exudates[24-26]. The chemistry and blend of the root exudates is not static and varies depending on plant species, growth stages, stress conditions, nutrition and soil types, and root traits, among other factors[26, 27]. Comparative genomics and exometabolomics-based analysis demonstrated that metabolic synchronization between plant exudation traits and microbial substrate utilization traits resulted in the predictable temporal patterns of microbial community assembly observed in the rhizosphere of different plant species[27-29]. Plants can recruit beneficial rhizosphere bacteria in response to exposure to aboveground pathogens via alterations in the root exudation profiles; this results in long-term changes of benefits to subsequent plant generations[29]. For example, benzoxazinoids (tryptophan-derived secondary metabolites released by roots of wheat and maize) not only have a direct influence on the assembly of the plant microbiome, they also act as endogenous plant signalling regulators of root metabolism that induce the production and release of a wider set of rhizosphere active semiochemicals thus indirectly recruiting selected microbes[29, 30]. These belowground root exudate mediated alterations in the plant microbiome can alter aboveground plant responses, such as suppression of herbivore performance, by impacting plant hormone signalling and plant defense response[31, 32]. Under iron stress, regulation of the root-specific transcription regulation factor MYB72 along with its transcriptionally regulated counterpart β-glucosidase BGLU42 influence the assembly of the root microbiome in a way that favors plant beneficial bacteria and inhibits plant pathogenic fungi; this occurs through regulation of the biosynthesis and exudation of the coumarin scopoletin into the rhizosphere[32]. Both MYB72 and BGLU42 are a part of the circuitry that controls the onset of ISR as well as coordinated responses to optimize iron uptake and mitigate nutritional stress[32, 33]. In a recent breakthrough, it was demonstrated that root-specialized metabolites such as triterpenes play important roles in tuning the rhizosphere for assembly and maintenance of host-specific microbiome[34]. Overall, recent work has provided strong evidence that metabolic diversification within the plant kingdom may provide a basis for communication and recognition that direct the assembly and maintenance of custom-made microbiota tailored to the need of host. The next frontier will be to identify mechanisms by which bioactive exudates interact with each other in field settings and how we manipulate the patterns of root exudation in natural settings to customize beneficial microbiome.

Abiotic stresses

Plants can "cry for help" to assemble a specific microbiome that alleviates the impacts of a plant stress[35-37]. The prime example for the "cry for help" hypothesis is the recruitment of nutrient-delivering AM fungi and nitrogen-fixing rhizobia when plants are grown under low phosphate or nitrogen conditions[38, 39]. The phosphate (Pi) starvation response (PSR) influences the assembly of distinct bacterial[40] and fungal[41] communities in the rhizosphere of Arabidopsis. This trait is likely to be relevant for plant performance as Pi limitation interacts with the plant immune

responses through the master transcriptional regulator of PSR, PHR1, which represses salicylic acid-dependent responses and induces JA biosynthetic genes[40]. The PSR regulatory network includes genes involved in the production of antimicrobial plant secondary metabolites such as glucosinolates[42] and organic acids[40] that are responsible for selective enrichment of a Pi responsive microbiome. However, different plant species have evolved different microbial associations under the selection pressure of low Pi availability, suggesting host mediated control of the assembly of a "stress tolerant microbiome". Iron starvation also triggers an adaptive root exudation pattern induced by beneficial root-colonizing bacteria resulting in the secretion of plant derived scopoletin that not only increases iron mobilization and uptake by roots, but also has antimicrobial activities against pathogens[32].

Recent work clearly demonstrated that drought induced compositional shifts of the plant-associated microbiomes are correlated with drought tolerance across host plant species[43-45]. In general, drought leads to reduced microbial diversity within the root-associated microbiome and increases the relative abundance of nearly all monoderm bacteria (i.e. Actinobacteria, Firmicutes) which lack an outer membrane and typically have thicker cell walls as compared to diderm bacteria (i.e Proteobacteria, Bacteroidetes). Interestingly, multiple stresses induce a common response in the microbial community structure that is best explained by the influence of the stressed host plant[44]. For example, while plant responses to stresses such as water limitation, light limitation, and metal toxicity varied in terms of growth, photosynthesis, gene expression and metabolite profiles, a core set of bacterial genera that change in abundance in response to host stress were identified[44]. The selective enrichment of the "stress tolerant microbiome" is driven by differences in plant metabolism and immune-associated traits[27, 32]. This reinforces the functional links between immunity and nutrition and suggests that plants may repurpose the same stress pathways to effectively respond to biotic and abiotic stresses.

Biotic stress

Disease incidence often correlates with microbial community shifts in different plant compartments, including seeds[46], rhizosphere[47]; roots[48, 49],[50] and leaves[51, 52]. Significant associations between the composition of native microbiome with disease intensity suggest that plant-associated microbiomes can enhance or attenuate disease severity in several cases[52-54]. Generally, pathogen infection causes clear reduction in the diversity and abundance of non-pathogenic bacteria that could potentially affect the likelihood of secondary pathogen invasions during following crop seasons as less diverse microbial communities are also often less resistant to invasions[46, 49, 50]. In some instances, "helper microbes" can interact with the incoming pathogen through metabolic, signalling, and genetic exchange and assist disease incidence[55, 56]. Both belowground and aboveground herbivory influence the assemblages of plant-associated microbiota by altering the production of plant secondary metabolites and root exudates[57, 58].

Crop management

In general, low-input farming systems promote higher abundance and diversity of most organisms[59]. Changes in the microbial seed banks in bulk soil with long-term intensive crop management practices such as heavy fertilization[60], tillage[61], or organic farming[62] impact the assemblages of the plant-associated microbiome. In grasslands land use intensity, rather than plant functional identity, is reported to shape the plant-associated microbiome[63]. However, opposite trends have been reported from agricultural systems[64]. The effect of land management practices is specific for different microbiomes. For example, in wheat roots, management type was the most influential factor for root bacteria, while tillage intensity explained most of the variation in the root fungi[65]. It is possible the plant-associated microbiome is responsible for the reprogramming of plant gene expression and physiology that results in differences in the host response under different land use[66, 67]. Decrease in the the network connectivity and the abundance of keystone taxa has been observed in intensively farmed systems than those managed by low intensity practices such as organic farming[65, 68]. It should however be noted that that even if network indices and keystone abundance are changed, it is still an open question how these parameters affect community functions. Our understanding of the interactions between various land practices and the dynamics of the microbial ecosystem has significantly advanced. However, the effects of agro-management and other factors such as the environment are highly complex, and better understanding is required of the abundance and spatio-temporal pattern of the cropping sensitive microbes for developing microbiota management strategies for smart farming.

Multi-kingdom interactions

Given the diversity of microbes that colonize plants, an emerging focus in the field of the plant microbiomes is the role of multi-kingdom interactions in shaping microbial communities (Box 2). Although much remains to be elucidated on how microeukaryotes can support bacterial diversity, evidence supports that multitrophic network stability is highly dependent on microbial "hubs," which, via microbe–microbe interactions, transmit the effects to the microbial community[69, 70]. Within the root microbiome, positive correlations dominate within bacterial, fungal, and oomycete communities; however negative correlations dominate between bacteria and the filamentous eukaryotes[71]. On the other hand, that both intra- and inter-kingdom correlations between bacteria and fungi exhibited a slight positive skew in the leaf and root microbiome[69-71]. Interkingdom interactions can potentially modulate community structure and influence disease incidence by disturbing the microbial balance resulting in disease[69]. As potential ecosystem engineers, the loss of larger-sized predator organisms such as *protists*, likely exerts a significant impact on smaller prey organisms such as bacteria. Use of the stable isotope probing (SIP) approach demonstrated that rhizodeposits are competitively utilized by specific sub-populations across microbial kingdoms and are dynamic in nature[72]. These developments are particularly exciting from an ecological perspective, as there is a great deal of theory pertaining to multi-trophic communities that may be applicable to our understanding of these systems.

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S2. Proposed technical flow for artificial construction of a synthetic microbial community (SynCom) to augment plant fitness and productivity.

Site selection

Initial selection depends on the end purpose of the SynComs (e.g. disease suppression, nutrient support, or stress tolerance) and the niche where they are intended to work (e.g. rhizosphere, endosphere, phyllosphere). It is expected that the microbiome from high-quality crops will be an ideal origin for SynCom meant to confer better growth and quality to the same plants. Escape plants that survive in disease infested areas or extreme stress can also act as hotspot for beneficial members[1]. Moreover, wild plants are known to support populations of beneficial microbes that confer traits such as high nutrient use efficiency and stress tolerance to the host[2].

Microbial characterization and reconstitution

Once the hotspots of isolation are identified, multi "omics" in combination with metagenome-wide association analysis, network analysis, genome mining and reconstruction of transcriptional and regulatory networks, may be used to identify the members and predict functional traits enriched in the presence of a beneficial microbial community. Other metadata including site characteristics, plant organ, management history, and environmental factors will be helpful in selection according to the chosen criteria[3]. Statistical modelling approaches such as Structure Equation Models can further be used to integrate different forms of data to select for "non-native" communities to investigate their succession ability[4].

It is clear that SynComs will not act as 'one size fits all', and therefore different strategies are being explored to select for the members of SynComs for reconstitution experiments[3]. Selection for core "functional" vs the core "taxonomic" microbiome for SynComs has been emphasized (Box 3). Within this concept, functional keystone species can be predicted through the topological networks derived from interactions and metabolic models. This approach provides a pathway to maximize SynCom persistence and trait expression success in natural

settings by identifying possible points of control for manipulating microbial diversity and microbe-host interactions[5].

High-throughput culturing and screening for beneficial traits

Although we can predict community function from multi-omics data alone to some extent, validation of interactions requires the complementary work with cultured isolates that can be interrogated in the laboratory. While some microbes clearly are difficult to culture *in vitro*, new advances in high-throughput isolation and enrichment techniques as well as an embrace of co-culturing approaches now make it possible to capture a much greater proportion of the existing microbial diversity than previously thought possible. Recently developed microfluidics and microfabricated arrays can be used to cultivate a broad diversity of taxa which can then be randomly assembled into consortia from experimental systems either through top-down community reduction or bottom-up assembly from isolates[6, 7]. These high-throughput platforms enable gene discovery, assessment of physiological traits and ecological strategies, and reveal network configurations that will lead to robust microbiomes. Information derived from metagenome assembled genomes (MAGs)[8, 9] can be used to design media using web-based platforms, such as KOMODO (Known Media Database)[10] to culture ecologically relevant microbes. can be used to predict the media components for culturing the target microbes. Once isolated, individual strains should be pre-screened for potential beneficial traits for which the SynComs are assembled[11]. For this purpose, genome markers might provide a faster and less-labor intensive alternative to physiological *in-vitro* screening, while also providing the opportunity for the discovery of correlated and novel beneficial traits. Key aspects of fundamental ecological process of microbial colonization- which includes invasion, establishment, activities and phenotypic changes should be identified by high-throughput phenotyping systems.

Predictive modelling

Ecological models are useful tools for unraveling fundamental principles governing microbial assembly for SynCom development and to translate insights gained from omics approaches, experiments, and *in-situ* sensors into testable predictions. Several complementary modeling approaches have strong potential to enable a predictive framework for the physiology, taxonomic structure, and the spatio-temporal dynamics of microbial consortia. Some of these approaches are based on a mechanistic understanding of how individual genes and molecules collectively give rise to cell- and ecosystem-level fluxes. These flux-based models translate genomic information into predictions of metabolic phenotypes, including growth capability, intracellular reaction rates, and uptake/secretion fluxes[12]. While genome-scale models of metabolism were initially developed for the study of individual microbial species (mainly in the context of metabolic engineering applications), these approaches have been now extended to multiple species to predict competition or synergism based on molecular exchanges microbial communities. Metabolic networks of members in a consortium can also be studied in a variety of real-world

contexts with elementary mode analysis, which breaks down each metabolic system into a series of biochemical pathways that satisfy steady state conditions[13]. Modern agent-based models can predict cellular morphologies and physical interactions between individual members in the consortium and can even be adjusted to account for complex conditions, such as environmental signals, nutrient uptake, and spatial separation between cells[14]. In parallel to these mechanistic models, it is possible to employ data-driven approaches, such as statistical inference, neural and machine learning and reinforcement learning to identify patterns in large datasets and classify the expected properties of microbiomes based on a large number of observations[15].

Design and validation

Model predictions need to be tested experimentally, providing feedback that can be used to refine knowledge, formulate new hypotheses, and redirect research plans. Microfluidic systems that allow the manipulation of natural aspects in a controlled laboratory environment are desirable for such validations. Microfluidics platforms have been used to study heterogenous environments[16], and currently these important devices are designed to build microbial communities and visualize plant-microbe interactions in real time[17]. Recently a modular growth system, the EcoFAB (Ecosystem Fabrication) was developed to facilitate the evaluation of root morphology and exudation of various plants over the course of several plant developmental stages up to several weeks[18]. Other strategies using micro-contact and 3-D printing can be used to assemble spatially defined microbial communities by organizing the physical and chemical environment and/or pattern specific community structure[19]. In addition, the application of transparent soil represents an innovative approach to study and live-image microbes on plant roots in an environment which mimics different soil textures[20].

Synthetic biology:

Using existing synthetic biology approaches, including computational models and modular DNA "parts", we can now rationally engineer cell populations for the construction of defined SynComs[21]. Advancements in genetic engineering (including CRISPER/Cas systems) and rapid methods to assemble DNA fragments allow construction of modular components with interconnected metabolic pathways and biological circuits to control cell behavior within microbial consortia[22]. The assembly and control of SynComs can be facilitated by using synthetic biology tools to engineer communication pathways through which specific population behaviors can be controlled and codependent networks can be built through syntrophic interactions. QS-based systems can be linked to the expression of other genes such as those encoding biosynthetic enzymes for toxins (e.g. bacteriocins or antimicrobial peptides) and biofilm formation either to modify communications between organisms in consortia or to facilitate plant colonization by responding to plant-derived chemical cues[23]. Multiple non-interfering cell-to-cell communication channels can be constructed for bidirectional communication systems that, when interfaced with genetic circuits, can reliably control population levels within SynComs. Furthermore, intracellular signaling mechanisms between

cells and exogenous inputs to control gene expressions can be used to form distinct spatial patterns and morphologies in SynComs by activating the expressions of adhesins, ligand receptors, or other polymers.

Evaluation and refinement

Ultimately, SynComs will need to be operative in the field where they need to invade, grow, persist and act in the direct contact with the indigenous microbiome and local abiotic conditions in variable settings. This lab-to-field transfer faces multiple challenges due to inherent conflicts created by the long and varied stages between formulation development and application[24]. The challenges associated with the development and delivery of SynComs as commercial formulations can by tackled by using alternative delivery approaches such as introduction into the seed microbiome either by seed inoculation or by spraying on flowers so that the SynComs can be established into the progeny seeds[25]. Measuring persistence and activities of SynComs in soil poses technical difficulties. The use of high-resolution tools (e.g., *in situ* sensors, omics analyses) can be used for measurement of diagnostic chemical species (e.g., root exudates or volatiles) or microorganisms that indicate the presence of desired taxa and reactions. Hyperspectral sensors on drones can be used to measure changes in the intended plant phenotype due to successful plant colonization of SynComs[26]. A discovery-driven planning approach, wherein systematic measurements, data integration, model generation, hypothesis testing, and new ecological hypothesis follow each other interactively[3, 27], should cumulate in the development of new computational tools and host/microbiome models that enable plant breeders and plant ecologists to predict beneficial interactions to achieve improved yields and plant resilience in changing environments.

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