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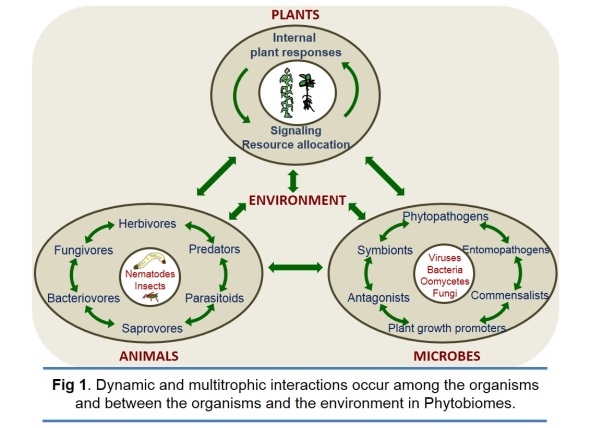
**Phytobiomes 2015**

**Designing a New Paradigm for Crop Improvement**

**Discussion Draft I: A Phytobiomes Research and Translation Roadmap**

**DEFINING THE PHYTOBIOME**

Plants grow in association with complex communities of other organisms, many of which can influence plant health, productivity, and responses to pathogens, pests and environmental stresses. Phytobiomes thus consist of other plants, animals (insects and nematodes), and a wide diversity of microbes (viruses, bacteria, fungi, oomycetes, amoeba, and algae).  Phytobiomes also include the non-living components of the environment, such as the soil and climate. Due to the diverse and dynamic processes carried out by biome members in their interactions with each other and the environment, phytobiomesplay an important role in the sustained health and productivity of plants, plant ecosystems, and consumers of plants and plant products. Phytobiomes can also impact environmental quality via their contribution to pollutant degradation, habitat health due to their key role in restoration and maintenance, bioenergy from biofuel crops, and any industry utilizing plants and plant products. Finally, human and animal health can also be affected by phytobiomes of food and feed crops due to crop nutrient composition, availability and quality, digestibility of plant tissues, and potential to contain plant-associated human pathogens.

**CURRENT STATUS OF PHYTOBIOME KNOWLEDGE**

Our current paradigm for how plants and other organisms such as microbes interact with plants has grown from a broad community of researchers. For example, plant pathologists and plant physiologists have elucidated how pathogens induce and manipulate plant defense pathways, whereas entomologists and chemical ecologists have highlighted how microbes can manipulate plant responses to insects and even modulate insect behavior. Bacteriologists and mycologists have detailed sophisticated developmental processes by which symbiotic nitrogen-fixing bacteria and mycorrhizae collaborate with plants to create structures that dramatically enhance plant access to water, usable nitrogen, and phosphorus. The exponential increase in studies profiling the composition of plant microbiomes using sequencing approaches is adding to this substantial foundation of knowledge on plant phytobiomes, and this knowledge is poised to rapidly increase with the availability, affordability, and application of other '-omics’ technologies to phytobiome-centered questions.

The plant microbiome is a prominent component of the phytobiome and will benefit from advances made in microbiome studies in other systems. Plant microbiome research, however, presents distinct challenges from human microbiome studies, many of which are focused on the gut. A key challenge for understanding the plant microbiome is the open and dynamic nature of plants and their habitats compared to the humangut. Whereas dysbiosis may be recognizable in a gut microbiome as a disruption to homeostasis, this concept may not be appropriate for plant microbiomes. Likewise, human microbiomes generally exist in relatively buffered and host-dominated environments while phytobiomes are complex ecosystems that involve interactions among microbes, animals, plants and diverse environments with fluctuating conditions (Fig 1). Thus, understanding phytobiomes requires systems-level approaches that encompass a broader range of system components than most human microbiomes. This presents challenges for model development and data integration in phytobiome research that are different from those faced in human or animal microbiome studies.

For phytobiomes knowledge to be useful, it must be placed in the appropriate context. In agriculture, placing the data in context is well underway. Beginning in the mid-1990s, global positioning systems (GPS) laid the foundation for precision agriculture, and ushered in improvements in crop, forage, and forest management sustainability and productivity. Application of advanced technologies for yield monitoring, variable rate nutrient application, active farm sensors, geographic information systems, and remote irrigation control is allowing farmers to collect, analyze, and act upon their own field data. Phytochemicals are providing precision nutrition targeted to the needs of individual crops. Integration of instant weather data and soil and moisture-sensing technologies with irrigation management scheduling as well as real time data assimilation and modeling are on the horizon. Future agricultural products couple agronomic modeling with weather simulations and predictions for improved crop management and adaptation to changing climatic patterns. Unmanned aerial systems (i.e., drones) are expected to be adopted rapidly by farmers to further enhance precision planting and management.

Biologicals, products based on biological modes of action for protecting plants against pathogens, pests, and nematodes, or enhancing microbial communities in crop phytobiomes for yield improvements, are positioned to change precision agriculture even further as our understanding of the interactions between genetics (e.g., seed variety/hybrid), environment, and the plant microbiome expand. To design the optimal crop and management practices for a given field, however, we will need to move to next generation precision agriculture that will take into consideration the interaction of all components of the entire system in real time and provide farmers with analytical data that predicts more accurately future conditions (e.g., weather patterns, subsoil moisture retention, disease patterns, etc.) as well as outcomes.

**UNANSWERED QUESTIONS IN PHYTOBIOMES**

We are poised to make rapid advances in understanding the fundamental ecological and physiological interactions within and between components of the phytobiome. To help establish and move beyond this foundation, *Phytobiomes 2015* participants are encouraged to consider the many important questions that must be answered to advance our systematic understanding of phytobiomes and translate this knowledge into improved crops and new, sustainable crop production and management systems. These questions may address gaps in many areas, including our conceptual knowledge, our technological abilities, our infrastructure for research or translation/application, and our training needs to generate a workforce that will carry this field into the future. Some initial questions that address gaps in these key areas are:

**Knowledge Gaps**

1. ***Phytobiome composition***

* Is defining the phytobiome to include organisms beyond the microbiome useful for advancing a phytobiomes’ research agenda? What are the benefits and costs of this definition?
* Given the complexity of the microbiome, in what time-frame will we have the ability to be comprehensive in our understanding of all organisms in the phytobiome?
* Is it possible to identify true keystone species for the establishment of microbial communities on plants and to what extent do different plant species chair keystone microbial colonists?
* How much diversity is present within species and what are the functional impacts of this diversity?
* How interchangeable are components of the phytobiome?

1. ***Processes driving phytobiome composition and dynamics***

* Can we identify universal principles underlying the development and compositional changes in phytobiomes in response to the environment? Do such universal principles exist? That is, are there general principles of assembly of phytobiome constituents that are operative over all agricultural systems?
* How idiosyncratic are the drivers of the assembly of phytobiome constituents for a given plant species or environment?
* To what extent do plants live in an “open” environment from which the phytobiome is locally selected?
* To what extent is the assembly of phytobiome constituents subject to a “legacy affect” where the order of assembly influences final community composition?

***B1. Impact of host genotype and phenotype***

* What progress has been made in linking particular taxa or the genes or traits that they possess with distinct plant phenotypes?
* Are there genetic loci in the host that shape the function and composition of the associated animal and microbial flora?
* Will there be a strong association of a particular phenological state of a plant with the effects of a phytobiome constituent – e.g. How commonly will persistent effects of the presence of a particular phytobiome constituent on the plant be seen from even the transient presence of a partner?
* Are there differences in the relative magnitude or qualitative differences in the effects of associations of phytobiome constituents on perennial versus annual plants?

1. ***Phytobiome function: understanding impacts on plant health and productivity***

* How do phytobiomes affect plant performance?
* What are the functional capacities of diverse strains within taxa, individual taxa, and simple communities to alter plant productivity?
* Are the net effects of the microbial communities on a plant different from the effects of individual microbes?
* Will rare members of the phytobiome have large effects on plant productivity and how can they be studied and manipulated?
* What is the extent and predictability of interaction of various taxa in the phytobiome as they might influence plant health and productivity?
* How can universal metrics to identify “soil health” be identified?
* Is the effect of phytobiome constituents on plant responses greater for foliar or subterranean plant parts?

**D. *Integrating data into meaningful and predictive models and networks***

* How do we successfully integrate data to capture the complex beneficial, synergistic, antagonistic, exploitative, parasitic, and pathogenic associations that cascade through complex, real-world phytobiomes?
* How do we relate an explicit body of understanding, predictions, and principles in phytobiome ecology, evolutionary biology, and function to plant productivity?
* Can we exploit predictive analytics to design seeds and ecosystems adapted to future environmental challenges?

**E. *Managing phytobiomes for maximizing plant health and productivity***

* What can we learn from past and current efforts to manipulate phytobiomes to achieve disease control or increased plant productivity?
* How can we design novel approaches for effectively and reproducibly managing phytobiomes to optimize plant productivity?
* How do common cultural practices used in agriculture alter the phytobiome? Can they be used for directed modification?
* Are “founder affects” of initial colonists of plants sufficiently robust that permanent shifts of microbiome constituents can be achieved from simple manipulations of early colonists?
* Are there particular phenological stages of plants for which the phytobiome is most easily manipulated and/or to which the plant is most responsive?
* To what extent are the phytobiome constituents subject to vertical transmission, and hence amenable to manipulation by seed treatment?

**F. *Translating phytobiomes knowledge into strategies to enhance crop productivity for sustained global food security and bioenergy production***

* How can the phytobiomes of crops be manipulated to increase agricultural production in an environmentally sound manner?
* How can we build a knowledge base that will empower the development of crop varieties, management practices, and nutrient inputs that are adapted to the environmental conditions and organismal composition of a specific site? How can we best disseminate this knowledge base?
* How can the extensive infrastructure for breeding and crop management that has evolved with the development of modern agriculture facilitate the translation of knowledge of phytobiomes for agricultural improvements?

***F1. Strategies that target cultural practices (crop or soil management, water, inputs, tillage,...)***

* How can we correlate specific management practices to predictable changes in the phytobiome?

***F2. Strategies that target microbial management (inoculants, selection of indigenous microbes,…)***

* How can exogenous microbial signaling molecules or secondary metabolites be used to induce predictable shifts in microbial communities?

***F3. Strategies that target plant selection/plant genetics***

* How can collaborations between plant breeders and microbiologists shift plant breeding practices to better incorporate the contributions of phytobiomes, such as to maximize the impact of beneficial microbes?
* Will useful plant traits to optimize phytobiome composition be readily selectable?

**G. *Broader impacts of phytobiomes knowledge***

* What useful organisms, genes, and products can be mined from phytobiomes?
* How can we exploit knowledge of crop phytobiomes to increase total factor productivity sufficiently to double overall production in a sustainable manner?

**Technical Gaps**

1. ***High throughput sequencing limitations***

* How can we improve the phylogenetic resolution of high throughput sequencing techniques for bacterial and fungal community profiling?

**B. *High throughput phenotyping limitations***

* Utilizing knowledge of crop phytobiomes, can we design high-throughput phenotyping in the field that is non-destructive? What traits/components need to be measured?
* How can drones be adapted for real-time, high-throughput phenotyping?
* How can sensor technologies be optimally deployed for real-time, high-throughput phenotyping?
* What breakthroughs will be needed in high-throughput phenotyping of plant traits to enable connections to be made with the presence or activity of phytobiome members?

1. ***Analytical limitations***

* What statistical tools are needed to identify taxa differences among microbial communities in multifactorial experiments?
* How can computational tools be developed to better characterize, analyze, and model species (genomic, phenotypic) interactions within complex communities?

**Infrastructure Gaps**

1. ***Databases***

* We need data and the corresponding metadata on phytobiomes representing a much wider range of plant hosts and habitat types.
* Can these data be integrated in a single database, such as in an iPhytobiome extension to the iPlant Collaboration?

**Training Gaps**

* We need to promote the training of students and postdocs in disciplines that support an increased understanding of phytobiomes and their applications. This includes developing a workforce that nimbly integrates knowledge spanning from ‘-omics tools, multi-scale modeling, network analyses, to plant breeding, plant pathology, entomology and agronomy.
* We need to promote broad training of students and postdocs, including training in the field, to prepare them for industry jobs that will arise from exploitation of crop, forage, and forest phytobiomes.
* We need to promote community education, appreciation, and public support for generating and translating phytobiome knowledge for societal benefits.

**LOOKING TO THE FUTURE – STEPS TO BEGIN TO ADDRESS THESE GAPS**

Coordinating efforts is important for meeting the challenges and exploiting the opportunities in phytobiomes research to best generate and use knowledge of phytobiomes to build the foundation for improving the production of food, feed and fiber by 2025. Many issues in coordination thus must be addressed such whether or how best to:

* Forge international and public-private collaborations in foundational and translational phytobiomes research.
* Develop standards and protocols for techniques, analyses, field study, and reporting of phytobiome data and research, and identify effective mechanisms for disseminating this information.
* Coordinate and leverage with existing studies of diverse hosts and ecosystems, including microbiome studies in animal- and plant-based agriculture, and soil health studies.
* Enhance education and outreach to the public highlighting the benefits of understanding phytobiomes.
* Identify funding mechanisms to support a diverse range of phytobiomes studies.
* Advance training of students and postdocs to better prepare them for phytobiomes research and the downstream application of phytobiomes knowledge for crop and forest improvement.

**FUTURE DEVELOPMENT OF THE *Phytobiomes Research and Translation Roadmap***

The *Phytobiomes 2015* workshop is expected to make considerable progress in identifying gaps and needs for advancing phytobiomes research and translation, as well as strategies for addressing these needs. A follow-up workshop with a smaller number of participants will be held to summarize this information and develop actionable initiatives in the form of a *Phytobiomes Research and Translation Roadmap.* The participants of *Phytobiomes 2015* are encouraged to provide suggestions, comments, and questions such as the following to help develop these actionable initiatives:

* What research initiatives should be advanced to promote a phytobiomes systems approach that will enhance our fundamental understanding of phytobiomes and address major problems in agriculture?
* What public and private partnership opportunities will advance phytobiomes knowledge and its applications?
* Are there disciplines that were missing from these discussions?
* How can the broad community be engaged effectively in implementing knowledge of phytobiomes?
* How can the results of this workshop be applied to interest policymakers in new challenges?
* What initiatives should be advanced to increase the pipeline of phytobiomes expertise and help coordinate synergistic efforts in phytobiomes research?
* What follow-up activities are needed to sustain engagement and progress?
* How can we best promote synergies between public and private entities to ensure measurable gains in crop, pasture, and forest productivity and health?

**Goals for Phytobiomes Technical Needs Translational Outcomes**

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| 1. Define who is present and the relationships of phytobiome composition to plant productivity. | * High throughput metagenomics approaches, including functional metagenomics * Efficient approaches for meta-analyses of large numbers of genomic datasets | * Identify characteristics of `healthy' vs. `nonadaptive' phytobiomes * Define a core phytobiome associated with consistent plant productivity, or with poor productivity * Determine targets for inoculant development, or for populations to suppress |
| 1. Identify key drivers of phytobiome composition and dynamics. | * Extensive (enormous?) correlative data and systematic experimental data from diverse habitats and environmental conditions * Efficient approaches for integration and analysis of complex community data across diverse systems | * Identify potential suite(s) of factors that may be targeted by field management |
| 1. Determine the functional capacities of individual taxa and simple communities to alter plant productivity. | * High-throughput plant phenotyping * High-throughput, in vivo/in planta microbial functional analyses and transcriptomics | * Identify potential inoculant or inoculant mixtures * Identify unexpected functional outcomes, and correlates of unexpected outcomes (e.g. taxonomic or functional characteristics of individual taxa or combinations of taxa that lead to greater-than-expected, or poorer-than-expected plant outcomes) |
| 1. Characterize the emergent properties of phytobiomes. | * Systems-biology approaches for analysis and prediction of complex community effects on individual microbial properties/phenotypes and on plant phenotypes * High-throughput microbial and plant transcriptomics | * Develop core principles for designing optimal synthetic phytobiomes for maximizing plant productivity |
| 1. Design novel approaches for managing phytobiomes to optimize plant productivity. | * Efficient, reproducible, high-throughput field testing platforms | * Integrate information from goals A - D to propose broad-based management solutions for agriculture |

**All goals should be accomplished for diverse plant hosts across a broad range of habitats and environmental conditions.**

**Every goal addresses multiple specific questions as elaborated throughout the text.**

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