PMI Performance Metric for FY18: Using genomics-based techniques, develop an approach to explore the functioning of plant-microbe interactions.

Summary of progress on understanding plant-microbe interactions

Introduction
The Plant-Microbe Interfaces (PMI) project is a Scientific Focus Area directed towards understanding the dynamic interface that exists between plants, microbes and their environment. Project efforts are focused on characterizing and interpreting systems comprising the poplar tree (*Populus*) and its microbial community, in the context of favorable plant microbe interactions. We seek to define the relationships among these organisms in natural settings, dissect the molecular signals and gene-level responses of the organisms using natural and model systems, and rebuild the complexity of these systems using sequence characterized plants and microbes. *Populus* is an ideal host system for examining interfaces between plants and microbes and a leading candidate for bioenergy production. It is a dominant perennial component of many North American temperate forests and among only a few plant species that host both endo- and ectomycorrhizal fungal associates. Numerous other types of microorganisms can be found within, or closely associated with, various *Populus* tissues, and these organisms may range from highly beneficial to pathogenic with respect to effect on host fitness. Ultimately, an improved fundamental understanding of plant-microbe interfaces will enable the use of indigenous or engineered systems to address challenges as diverse as bioenergy production, environmental remediation, and carbon cycling and sequestration.

This report highlights genomics-based techniques exploring the functioning of plant-microbe interactions and summarizes the material found in previous reports on:

- **The use of “omics” techniques to understand the functioning of microbial communities in association with plants.** “Omics” (a shorthand term used to refer to techniques involving genomics, transcriptomics, proteomics, and/or metabolomics, applied either individually or in combination) approaches are central to PMI efforts aimed at understanding the functioning of microbial communities in association with *Populus*. Diverse techniques have been central to defining the composition and functional contributions of the *Populus* associated microbiome.

- **The progress being made towards understanding fungal relationships with plants.** The investigation of *Populus*-fungal relationships has focused on understanding the key molecular factors, mechanisms, and gene networks involved in symbiosis formation and function using the *Populus-Laccaria bicolor* model system. The knowledge acquired from the *Populus-L. bicolor* interaction is being extended to other *Populus* associated fungi to better understand such beneficial associations and its connections to ecological processes in forest ecosystems.

- **The latest techniques that are being used to detect and understand signaling between plants and microbial communities.** Specific biochemical events lead to particular associations between *Populus* and its microbial partners (microbiome). A key focus of PMI is to gain a genomics-based understanding of the metabolites and signals responsible for shaping these plant-microbial interactions. Tractable bacterial model systems and various analytical
technologies for real-time imaging of plant-microbe interactions and temporal sampling of metabolites have been advanced for understanding microbial community assembly.

- The latest computational approaches that are being used to interpret the functioning of plant-microbe interactions. A deep understanding of the molecular and cellular events involved in establishing and maintaining plant-microbe interactions requires multiple disciplines and advanced experimental and computational tools to analyze and reconstruct these complex relationships. Computational analyses that take advantage of ORNL’s advanced computing facilities are being leveraged towards the goal of understanding the complexity of the plant-microbe interface. This powerful approach is helping to detect genes, genotypes, environmental drivers and microbiome taxa that control these interactions.

The use of “omics” techniques to understand the functioning of microbial communities in association with plants. The PMI SFA project at ORNL creates, adapts and applies techniques from genomics, transcriptomics, proteomics, and metabolomics either individually or in combination to understand the functioning of the microbiome associated with *Populus*. These omics tools are implemented in order to understand the complex multiscale system represented by *Populus* and its microbiome.

Studies conducted in PMI have shown that overall microbial communities (bacteria, fungi and archaea) vary significantly in the endosphere of different plant species. Within *Populus*, we have shown that although soil and environmental drivers tend to be stronger determinants of community composition, plant host genotype often played a role in structuring these communities in wild populations. Host tissue level populations also differ dramatically (Figure 1), salicylate chemistry (Veatch et al. in revision), host stressors of drought, shading and heavy metals, and can be modulated by host cell wall chemistry.

From these studies of both natural and common garden *Populus* trees, an unprecedented number of bacterial (>3800) and fungal (>1800) members of this community have been isolated in pure culture, including species new to science. Genomic sequence based comparisons show many shared genomic features across microbiome members associated not only with *Populus* but other commonly studied hosts such as *Arabidopsis* and *Corn*. The latter include members of 4 common bacterial phyla. In addition to traditional

![Figure 1. Summary of tissue level microbial community composition across the major habitat zones of *Populus deltoides* versus hybrid (*trichocarpa x deltoides*) trees.](image)
cultivation approaches members of several unique phyla level groups of Acidobacteria, Armatimonadetes (former OP10), Verrucomicrobia have been characterized via targeted flow cytometry based single cell genomic approaches\(^\text{15}\) and now several new pure cultures isolates were recently obtained.

**The progress being made towards understanding fungal relationships with plants.**

The fungal community associated with *Populus* has been extensively characterized using both traditional mycological approaches as well as molecular-based surveys. The PMI team has developed a collection of over 2000 fungal strains representing multiple functional groups (guilds) which interact with *Populus*: these include arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (EMF), endophytic fungi (ENF), as well as pathogens. AMF and EMF both form mycorrhizal structures that provide the plant host with nutrients and protection from pathogens in exchange for photosynthetically fixed carbon. Our environmental metagenomic surveys of *Populus* species and genotypes have shown that fungal communities vary considerably within different rhizosphere compartments,\(^\text{5,6}\) across different plant habitats within the tree\(^\text{1}\) and by tree species/genotype.\(^\text{1,4}\) Not surprisingly, across *Populus* species, susceptibility to fungal pathogen infection plays a large role in structuring aboveground fungal leaf communities (Figure 1).\(^\text{1}\) Furthermore, native leaf fungal endophytes have also been shown to modify plant disease severity.\(^\text{16}\)

The basidiomycete *Laccaria bicolor* is a common ectomycorrhizal fungus (ECM) that is a mutualist with many northern temperate forest trees including *Populus*. *Laccaria bicolor* has been used extensively in both basic and applied research. The physiological ecology of *L. bicolor* is well studied among ectomycorrhizal taxa, because it grows rapidly in culture and its mycorrhiza are easily established with tree roots under laboratory conditions.\(^\text{17}\) *L. bicolor* is commonly used in microcosms and *in vitro* experiments in dual culture with *Populus* or conifer seedlings\(^\text{18}\) and has become a model organism to investigate ectomycorrhizal symbiosis. Within the PMI project, *L. bicolor* has been used to investigate molecular cross talk with *Populus* as the host plant, particularly within the complex between plant protein receptors and symbiotic effectors, which enables the formation of an ectomycorrhizal and mutualistic relationship. We have demonstrated that *L. bicolor* MiSSP7 and MiSSP8 are required for symbiosis establishment and development (Plett et al., 2011).\(^\text{19}\) MiSSP7 is a 7kDa protein that accumulates in the hyphae and is secreted into the extracellular environment after sensing of diffusible plant signals. Secreted MiSSP7 is imported into root cells, where it accumulates rapidly in root cell nuclei. In the host nuclei, MiSSP7 interacts with the transcriptional repressor, JASMONATE ZIM DOMAIN protein 6 (PtJAZ6), which is a master regulator of the jasmonate signaling pathway.\(^\text{20}\) The interaction between MiSSP7 and PtJAZ6 prevents the proteasomal degradation of PtJAZ6 that would otherwise be activated by the accumulation of jasmonate triggered by fungal colonization. This stabilization of PtJAZ6 maintains repression of part of the jasmonate-signaling pathways, allowing fungal colonization of the root apoplastic space.\(^\text{20}\) Efforts in the PMI project have also highlighted the potential role of plant SSPs in plant-fungi symbioses. We performed RNA sequencing of *Populus trichocarpa* roots in mutualistic symbiosis with the ectomycorrhizal fungus *Laccaria bicolor*. Through computational analysis of the RNA-seq data, we identified 417 plant-encoded putative SSPs that were significantly regulated during this interaction. The predicted secretion of poplar SSPs was tested using a yeast complementation
assay, in which the survival of a *Saccharomyces cerevisiae suC2* mutant depends on the secretion of a truncated SUC2 protein that lacked its native secretion signal and was fused to the poplar SSP candidates. We found that on average, 15 of the 40 (38%) poplar SSPs that were tested, complemented the suC2 mutation. Furthermore, we demonstrated that four of the five poplar SSPs tested in an *in-vitro* feeding experiment could enter *L. bicolor* hyphae and accumulate in the nucleus. Additionally, two of these poplar SSPs significantly affected hyphal growth and morphology. These results indicate that plants encode proteins that function as effectors that may regulate symbiotic associations.

In parallel, coupling QTL mapping, transcriptomic and whole-genome resequencing analyses, we have identified a whole-gene deletion in *P. deltoides* involving a G-type lectin receptor-like kinase (PtLecRLK1), which segregated consistently with *L. bicolor* colonization efficiency across the pedigree. The presence of the functional *P. trichocarpa* copy conferred approximately 2x the colonization efficiency when compared to *P. deltoides* deletion variants. The role of the G-type lectin receptor-like kinase in mediating colonization by *L. bicolor* was validated using heterologous expression in *Arabidopsis* which converted a non-host into a host for the fungal symbiont. We have also demonstrated that this PtLecLRK1 functions by suppressing the host-defense pathways to allow compatible interactions.

Finally, over the past few years, we have gained an extensive collection representative of fungal endophytic associates that we recently extended with a focus on ectomycorrhizal communities associated with *Populus trichocarpa* across its range in the Pacific Northwest. This effort resulted in over 300 collections of EMF, including pure cultures, spore prints and bulk soil that we further used in bioassay studies with different *Populus* genotypes (Figure 2). The PMI SFA project at ORNL has pioneered the investigation of *Populus*-fungal relationships. These new model systems represent the diverse types of fungi that interact with *Populus* and will serve to deepen our understanding of *Populus*-fungal relationships and the functioning of *Populus*’ microbiome in effecting the host plant’s performance.

**The latest techniques that are being used to detect and understand signaling between plants and microbial communities.**
Specific biochemical events lead to particular associations between *Populus* and its microbial partners. These interactions are driven by secretion and detection of chemical exudates and small molecule signals, which are produced by both plant and microbes (reviewed in 22-24). Defining these chemical features and the molecular basis for host- and community-directed assembly of the system represent key challenges in understanding the plant-microbiome interface. To address these challenges, we are advancing technologies, expanding measurement capabilities, and developing tractable microbial model systems selected from our large inventory of genome sequenced isolates. These advances will help to define the metabolite exchange and signaling events underpinning the molecular, spatial and temporal dynamics of microbial community assembly with the *Populus* host and are likely applicable to understanding other host-microbe systems.

Metabolite exchange between *Populus* and its microbiome.

The organization and behavior of plant-microbe communities are dictated by a complex network of physical and chemical interactions between organisms within dynamic environments. *Populus* produces a wide diversity of metabolites that vary based on genotype, age, and environmental conditions. 25-29 We also demonstrated that the PMI bacterial and fungal isolates produce and secrete an array of compounds that influence their associations with other microbes and with their *Populus* host. 3,30,31

In order to define niche preferences, temporal behaviors, and patterns of microbe migration in the proximity of the plant root, we developed two technologies: a 3D-printed imaging chamber (US Patent D754,871S) and a plant-on-a-chip microfluidic platform in which we grow and maintain seedlings for 7-10 days. 2 These systems have been used to quantify *Pantoea* colonization, which displays a preference to colonize near the root tip, and compare it with that of *Variovorax* sp. CF313, which displays a relatively even distribution along the plant roots (Figure 3). 2 These approaches provide context for understanding plant-microbe interactions during the early stages of plant development and provide a platform for integrating real time sampling of the local fluid environment to better understand the chemical drivers that shape these colonization patterns.

![Figure 3](image)

**Figure 3.** Two bacterial isolates inhabit specific spatial niches along *Arabidopsis* roots as imaged using engineered habitats. (top panel) *Pantoea* sp. YR343 (green) prefers to colonize newly developed root tissue (scale bar 100µm). (bottom panel) *Variovorax* sp. CF313 (red) exhibits a uniform distribution along *A. thaliana* roots (scale bar 100µm; Adapted from 2).

Inventory of potential microbial natural products.

To examine how microbial metabolites influence bacterial community structure, cell communication, and plant health, we evaluated the diversity and uniqueness of biosynthetic gene clusters (BGCs) in 339 sequenced genomes of bacteria isolated from *Populus*, as well as in *Populus* bulk soil, rhizosphere, and endosphere metagenomes. 32 From these analyses, we identified approximately 3400 individual BGCs, including many potential antimicrobials and signaling compounds. These BGCs are diverse across natural product type and also distinct from known natural product clusters as only ~1% of
all clusters matched a previously characterized BGC, suggesting a great opportunity for the discovery of novel natural products involved in host and/or microbe communication or microbiome structure and stability. Current efforts are focused on developing additional analytical tools (MALDI MS-imaging and LC-MS/MS) for detection of natural products in laboratory cultures and in planta.

Signaling among and between microbes and their plant host.
Plant-associated microbes, both beneficial and pathogenic, produce and detect a wide variety of chemical signals that contribute to their ability to colonize plant hosts.33,34 Our work has established that AHL-type quorum sensing signaling systems are prevalent among members of the Populus microbiome and are enriched in metagenomic DNA libraries from the Populus endosphere, relative to rhizosphere and soil libraries.32,35 This suggests that QS pathways are important for structuring the Populus bacterial microbiome. Some of the LuxR homologs identified in Populus-associated bacteria belong to a widespread, but understudied, subfamily called PipR (OryR) homologs (Figure 4). PipR receptors do not detect AHL signals, instead they have evolved to detect a previously undefined in planta signal to control gene expression. We defined a PipR system in the root endophyte Pseudomonas sp. GM79 3 and recently discovered that the GM79 PipR signal is a spontaneously formed degradation product from ethanolamine.36 Our discovery sets the stage for new questions as to the role of this signal in bacterial-plant interactions.

Signaling in fungi: the role of lipochitooligosaccharides (LCOs) and terpenes.
We are also studying how fungal metabolites mediate mutualistic interactions, including chitin-derived lipochitooligosaccharides (LCOs) and terpenes. Mycorrhizal fungi produce LCO-type signaling molecules (named Myc-LCOs), which have been demonstrated in arbuscular mycorrhizal fungi (AMF)37 and are involved in the molecular cross-talk with plants and mediate the symbiotic accommodation of the microbe. Despite the functional importance of these molecules in mediating plant-AMF interactions, little is known about the role of similar molecules in ectomycorrhizal fungal (EMF) groups. To examine this, we surveyed the genome sequences of diverse EMF and endophytic fungi for LCO gene synthesis, validated LCO production in several fungal isolates (Russulaceae

**Figure 4.** Model for PipR interkingdom signaling in the PMI isolate Pseudomonas GM79. The Populus signal (bright green star) requires a transporter for entry; it enters the periplasmic space where it is bound by the periplasmic binding protein (PBP), which delivers the signal to the other components of the ABC-type transporter (4-component lavender complex). Once inside the cell, the signal can bind PipR (red circle), converting it to a form capable of binding the pipA (and other genes) promoter region to activate transcription. We hypothesize that the PipA and aapA peptidases act on the signal to reduce activity, thus creating a negative-feedback loop. The GM79 PipR signal is spontaneously produced from a well-known plant metabolite.
members), and characterized their effects on the plant host. These results suggest LCO production capabilities are widespread across EMF; however, only a subset of LCOs from mycorrhizal fungi affect *Populus* roots. We have also analyzed the genome sequences of diverse EMF and endophytic fungi for terpene synthase genes and validated their production in ten Russulaceae isolates. We expect that defining the function of these fungal metabolites in *Populus* interactions will broaden our understanding of plant-fungal interactions.

The foundational work aimed at understanding metabolite exchange and signaling in the *Populus* microbiome has resulted in definition of key signaling molecules and has poised the PMI project to continue to make exciting discoveries. We expect that these studies and tools will advance methods for monitoring complex communities and provide fundamental insights into how the microbiome is organized and proportioned in response to local environmental perturbations. This knowledge could help develop novel strategies for promoting plant performance.

**Computational approaches for interpreting the functioning of plant-microbe interactions.**

**Comparative genomic analysis** provides a powerful tool to understand variation among organisms and help identify the genes that are both conserved and divergent among species, leading to their similarities and differences. Leveraging our collection of sequenced bacterial isolates, we used comparative genomics to identify genes which are enriched in the bacterial genomes relative to non-plant associated microbes,\textsuperscript{11,12,14} to uncover the environmental and functional diversity *Pseudomonas* spp.,\textsuperscript{11,14} and characterize the function of bacterial plant-associated genes vs. bacterial root colonization genes from new bacterial isolates from the roots of Brassicaceae, maize, and poplar trees.\textsuperscript{12}

**Metagenomic analysis and microbial community structure** allows us to identify the genes involved in specific *Populus* associations. Extensive analyses of bacterial community structure and functional potential were carried out using metagenomics data, assembly, gene calling and annotation (JGI). The resulting annotation was used for functional enrichment and differential analyses across compartments (soils, rhizosphere and root endosphere) and host species (*P. deltoides* vs. *P. trichocarpa* x *deltoides* hybrids). ParaKraken was used to parallelize genome database matching for taxa identification, and Proportional Similarity Indices were used to define community structures and perform comparisons across compartments and host species, allowing us to identify taxa and functions significantly enriched in endosphere relative to soil and rhizosphere. Mapping of related genes to our reference genomes has allowed us to prioritize strains for hypothesis testing. We also analyzed sequenced genomes of isolates from the *Populus* root microbiome to characterize organisms for the development of natural products (NP) potential and uncovered a wealth of novel chemical signatures in the *Populus* rhizosphere.\textsuperscript{32} The sequenced collection captures a broad phylogenetic diversity of the *Populus* microbiome that could lead to the discovery of compounds involved in communication and control of key plant-microbial interactions.

Effects of drought and differential gene expression between host and the phytobiome in poplar were determined through co-differential expression/abundance and associations created by DUO (Figure 5). Changes that occur during cyclic drought suggest that these plants are better acclimated to drought compared to those subjected to a progressive acute drought.
Figure 5. Network analysis of DUO associations between taxa and GO terms. *Rhizophagus* and *Trichinella* are associated with poplar host ROS metabolism, supporting the hypothesis that *Rhizophagus* may be mitigating drought stress and *Trichinella* may be modulating the host immune system. Additionally, *Streptomyces* and *Pseudomonas* are positively associated with disease resistance genes, which may help protect plants from disease during drought stress. Also, acute drought and cyclic drought have non-overlapping associations of disease resistant genes and taxa suggesting that both conditions are experiencing different types of disease stresses.

High-Performance Computing Applications

*Artificial Intelligence – Genome Wide Association Phytobiome Analysis (AI-GWAPA).* Machine learning, deep learning and general artificial intelligence (AI) techniques help to elucidate the interactions between microbial and viral constituents of the *Populus trichocarpa* GWAS population. With more advanced metrics, such as DUO, we see differences in the types mutualistic/antagonistic relationships when comparing leaf and xylem. With sample specific networks we can start to understand the genotypic effect on these relationships. By further using a deep learning-based protein interaction model we can work towards a protein level understanding of this dynamical system.

*Annotation-based Lines of Evidence (LOE) Network Mining* helps deconstruct elements of complex polygenic phenotypes of importance to PMI: microbial recognition; signaling; disease susceptibility/resistance and defense response; apoptosis; cell wall regulation; signaling; biotic stress; etc. By scoring data derived from different data layers, the number of lines of evidence linking genes to target functions can be quantified.

*Co-evolutionary networks for understanding host and symbiotic systems* uses the computational power and scaling of Titan and Summit to calculate the correlation between mutations (SNPs) across a population of individuals that can identify groups of SNPs with population-level or phenotypic associations. Custom Correlation Coefficient (CCC) also considers genetic heterogeneity and finds correlations. The resulting sets extracted from CCC network topologies
represent higher-order combinatorial sets that allow for much more complex interactions to be tested than simple SNP pairs tested by extant methods.

**Proportional Similarity metric for pleiotropy discovery.** Our overall research project in each of the species that we are studying involves the creation of extensive systems biology models of each species that capture the molecular interactions in the cell that lead to emergent properties and complex, organismal-scale phenotypes. This goal includes the determination of genome-variant to phenotype relationships for all available phenotypes. For some species we already have collected thousands of phenotypes (160,000 phenotypes in the case of our bioenergy project with *Populus trichocarpa*). As such, pleiotropy is an important factor that needs to be captured in these extensive models. Pleiotropy is the phenomenon in which a gene is involved in multiple phenotypes.\(^{42}\)

**Explainable AI with iterative Random Forests (iRF)** – Recent developments include random intersection trees (RITs)\(^{43}\) and iterative RFs (iRFs)\(^{44}\) that we have re-written and scaled up to run on ORNL supercomputers such as Summit. These approaches use density estimates (or response surfaces) that can be mined not only for feature importance but also for interactions between features. It is now possible to identify interactions of any form or order at the same computational cost as main effects.\(^{43,44}\)

**From Matrices to Cubes to Polytopes.** Plant-microbe systems contain many layers of data/molecule types (genome, epigenome, chromatin structure, transcriptome, proteome, metabolome, lipidome, microbiome, etc.) and various layers of emergent phenotypes that range from localized mechanisms up to whole organism scales. We are interested in finding associations within and across each of these layers which can be represented as matrices. As such, there is a need for algorithms that can find higher order associations within and across an arbitrary number of polytopes of differing scales. Algorithms that we are currently developing, such as Tensor iterative Random Forests (TiRFs) are aimed at this problem.\(^{43-45}\)

**Summary**

The PMI SFA project at ORNL applies genomics, transcriptomics, proteomics, and metabolomics either individually or in combination to understanding the functioning of the microbiome associated with *Populus*. These omics tools are implemented in order to understand the host-microbe relationship and the component organisms. The PMI SFA project at ORNL has pioneered the investigation of *Populus*-fungal relationships. Key fungal derived molecular factors have been identified, gene interaction networks have been defined, and, critically, knowledge on how to manipulate these complex systems has been gained. Furthermore, foundational work aimed at understanding metabolite exchange and signaling in the *Populus* microbiome has resulted in definition of key signaling molecules and has provided fundamental insights into how the microbiome is organized and proportioned in response to local environmental perturbations. With novel computational tool development and advanced analytical infrastructure, we are poised to determine the commonalities and genetic underpinnings of bacterial and fungal recognition, discover new regulators, and define molecular mechanisms underlying selectivity and reveal signaling cascades leading to root colonization.
Further details on PMI project efforts can be found in our research publications. A complete listing of PMI project publications is available at https://pmiweb.ornl.gov/portfolio/

**Literature Cited**


Joubert, W. J. *et al.* in *SC18*.

