REVIEW

Plant–Microbe Interactions: From Genes to Ecosystems Using *Populus* as a Model System

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ABSTRACT

Plant–microbe symbioses span a continuum from pathogenic to mutualistic, with functional consequences for both organisms in the symbiosis. In order to increase sustainable food and fuel production in the future, it is imperative that we harness these symbioses. The tree genus *Populus* is an excellent model system for studies examining plant–microbe interactions due to the wealth of genomic information available and the molecular tools that have been developed to manipulate *Populus*–microbe symbioses. In this review, we highlight how *Populus* can serve as a model system to explore plant–microbe interactions. Specifically, we highlight research linking *Populus*–microbe interactions from the gene to the ecosystem level. We explore why *Populus* is an excellent model for perennial plant systems, the molecular underpinnings of *Populus*–microbe interactions, how host genetics

influence microbial community composition, and how microbial communities vary at fine spatial scales and between *Populus* spp. Furthermore, we explore how changes in the microbiome may affect ecosystem-level functions in managed and natural ecosystems. Understanding and manipulating these interactions in *Populus* has the potential to improve plant health and affect ecosystem sustainability and processes because *Populus* trees function as foundational species in many natural ecosystems and are also deployed in managed ecosystems for various agroforestry applications.

Keywords: agriculture, ecosystems, endophytes, genomics, microbiome, mycology, natural habitats, plants, rhizosphere and phyllosphere, soil ecology

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A basic premise of systems biology is that biological units (e.g., organisms, cells, and molecular signals) do not exist in isolation and that interactions among these units at multiple scales affects the functioning of the entire system (Kitano 2002). Although the importance of the interactions between plants and mycorrhizal fungi has been recognized for over a century (Berch et al. 2005), we have only recently begun to understand the true complexity of plant–microbe interactions because plants simultaneously associate with a myriad of microbes, including archaea, bacteria, fungi, viruses, and microeukaryotes (Cordovez et al. 2019). Because plant-associated microbes have both positive and negative consequences for the host plant (Hirsch 2004), a greater understanding of the plant holobiont (i.e., the plant and its associated microbes) may have important agricultural, economical, and environmental implications.

Studying plant–microbe relationships at multiple biological scales gives insights into the molecular mechanisms that mediate the interaction, the genes that regulate these mechanisms, and

whole-organism and ecosystem consequences of these interactions. Ultimately, we wish to understand how a particular interaction yields observed functional consequences, or to predict how changes to an interaction might affect the outcome of these functions across scales. Significant progress has been made in understanding the effects of plant–microbe interactions at multiple biological scales in *Populus* spp. (Hacquard and Schadt 2015). However, the broader goal of manipulating these interactions will require focused gene- to ecosystem-scale efforts to integrate this knowledge across scales, from the molecular and genetic levels to organismal phenotypes and ecosystem effects (Wymore et al. 2011).

The field of ecological genetics and genomics posits that “extended phenotypes” emerge as individual genotypes have community- and ecosystem-level consequences (Whitham et al. 2003). For example, genetic variation within a keystone species can affect the way in which an organism interacts with other species. Given that microbes influence nearly all of Earth’s biogeochemical cycles, plant-mediated changes in microbial functioning could influence the nutritional status of the entire ecosystem (Schweitzer et al. 2004). *Populus* is an excellent model plant for exploring these gene–ecosystem interactions because (i) *Populus* spp. can be clonally propagated using vegetative cuttings (Whitham et al. 2003) and callus tissue culture techniques ((Tuskan et al. 2018) that enable robust experimental designs that control fully for genetic effects, (ii) the genus *Populus* is largely undomesticated and its outcrossing nature combined with a propensity for hybridization yields immense genetic and phenotypic diversity, (iii) *Populus* spp. are important foundational species in riparian ecosystems, and (iv) variation in the *Populus*–microbe system results in altered ecosystem process rates such as primary productivity.

Generally, all *Populus* spp. and hybrids can be vegetatively propagated with ease from dormant cuttings (Merkle and Nairn 2005), making clonally replicated experimental designs a common feature of many *Populus* experiments. The *Populus* genotype 717 has been used for decades as the standard genotype for in vitro propagation and transformation experiments (Chupeau et al. 1993), and numerous transgenic lines have been created for *Populus* using genotype 717, including transgenics with varied phenotypes and responses to microbial inoculations. CRISPR genome editing systems have also recently been developed and deployed for *Populus* (<http://aspendb.uga.edu/s717>) (Bewg et al. 2018), and web-based RNA resource guides are available (<https://chopchop.cbu.uib.no>), which allow for precise targeting of genes of interest. This targeting eases the process of defining the functioning of plant–microbe interactions and other biological features (Tsai et al. 2020).

There are currently five published reference genomes for the genus *Populus*, which include male and female genotypes across three different *Populus* spp. and a hybrid poplar (Tuskan et al. 2006; Zhang and Gao 2016) (<https://phytozome.jgi.doe.gov/pz/portal.html> and <https://popgenie.org/aspseq>). As an opportunistic byproduct of these plant genomic sequencing efforts, the first microbial metagenome associated with any plant was identified within the sequenced plant material and published as part of the Nisqually-1 reference genome paper (Tuskan et al. 2006). These unique genetic resources provide useful molecular and laboratory experimental systems for research efforts using *Populus*.

Interspecific quantitative trait loci (QTL) mapping pedigrees with high-density genetic maps of *Populus* are available and have been used to successfully map genomic regions that confer species-specificity in host–microbe interactions. Complementing these resources, an intensively characterized genome-wide association study (GWAS) mapping panel of >1,000 diverse *Populus*

trichocarpa genotypes from throughout its native range in the Pacific Northwest of the United States and Canada has been established in greenhouses and several common gardens, allowing for controlled assessment of phenotypic variation across the species (Evans et al. 2014). Whole-genome resequencing of this population yielded >28 million single-nucleotide polymorphisms that are routinely used in genotype-to-phenotype correlations (<https://cbi.ornl.gov/data>) and, from these genotypes, a pan-genome for *P. trichocarpa* has been assembled and annotated (Chhetri et al. 2019; Pinosio et al. 2016), adding an additional 24,000 gene models that have been identified over the original 41,335 identified in the Nisqually-1 reference genome. These GWAS resources have been used to identify numerous genes linked to disease resistance (Abraham et al. 2019), pathogen-defense related secondary metabolite biosynthesis (Zhang et al. 2019), cell wall properties (Fahrenkrog et al. 2017; Li et al. 2018; McKown et al. 2014; Muchero et al. 2015; Porth et al. 2013), and overall growth and phenological traits (Chhetri et al. 2019; Evans et al. 2014).

As the *P. trichocarpa* Nisqually-1 genome was nearing completion, an effort was put forward to also sequence several important *Populus* fungal symbionts of various types, including the ectomycorrhizal (ECM) species *Laccaria bicolor*, the arbuscular mycorrhizal (AM) species *Rhizophagus irregularis*, the rust pathogen *Melampsora larici-populina*, and several other species (Martin et al. 2004). These resources allowed the development of a “mesocosm” of model species across kingdoms for understanding the genetic basis of plant–microbe interactions and the linking of specific plant genes to associated microbial genes. Currently, there are over 5,000 isolates in culture that are representative of the *Populus* microbiome. Members include key bacterial (>3,000 isolates) and fungal (>2,000 isolates) (Bonito et al. 2016) groups associated with wild and field-grown trees.

From these recent microbial collections, over 550 *Populus* bacterial and 65 fungal isolate genomes have been sequenced, largely in collaboration with the Department of Energy–Joint Genome Institute (Blair et al. 2018; Bonito et al. 2016; Brown et al. 2012a,b; Klingeman et al. 2015; Levy et al. 2018; Looney et al. 2018; Taghavi et al. 2009; Uehling et al. 2017), and the genome sequences in this escalating resource are representative of the current *Populus* microbiome. Furthermore, they are an expansion of the original model fungal symbionts, including the ECM fungus *L. bicolor*, which has largely served as a research platform for understanding the genetic basis of ECM symbioses (Martin et al. 2004; Pellegrin et al. 2019; Plett et al. 2011). These isolates and genome sequences are now beginning to facilitate broader constructed community experiments (Bible et al. 2016; Timm et al. 2016) and comparative genomic analyses (Jun et al. 2016; Levy et al. 2018; Martino et al. 2018; Pereira et al. 2018; Schaefer et al. 2013; Timm et al. 2015).

Populus spp. and their microbes constitute one of the most widely studied perennial plant systems and, therefore, can facilitate understanding of the genomic underpinnings of other plant–microbe systems in the context of important ecosystem functions. Various *Populus* spp. serve as keystone organisms across globally distributed ecosystems, especially in biodiverse riparian habitats, making them relevant for ecosystem-level studies examining how plants interact and associate with microorganisms (Whitham et al. 1999). Additionally, the long-lived and clonal nature of *Populus* spp. (Mock et al. 2008) may lead to essential, functional relationships with microbes that may not be well developed or are absent in many annual model plant species that typically have served as model organisms. Given these factors, we highlight recent work examining *Populus*–microbe interactions from the gene to the ecosystem scale.

GENETIC MECHANISMS UNDERPINNING POPULUS–MICROBE INTERACTIONS

Although there have been significant advances in characterizing plant-associated microbial communities, there are still many unanswered questions about how these interactions are selected, developed, and maintained throughout the life of the plant at the genetic level. Studies of model microbial associations in *Populus* and large isolate collections are allowing us to begin uncovering characteristics of host–microbe selectivity, and aid in the identification of the molecular mechanisms underlying community assembly and function.

Among the various identified signaling molecules in fungi and bacteria, chitin-derived lipo-chitooligosaccharides (LCOs) and terpenes have been reported to mediate mutualistic interactions (Lerouge et al. 1990; Maillet et al. 2011; Rasman and Turlings 2016). LCOs have been studied extensively in AM fungi, where they are involved in the molecular crosstalk with plants that mediate the symbiotic accommodation of the fungus. In AM fungi, plant-secreted exudates can stimulate hyphal branching (Labbé et al. 2014). The secretion of plant signaling molecules (specifically, Myc factors and LCO molecules) can lead to a signaling cascade that allows for symbiotic AM associations at the root (Camps et al. 2015). Genomic analyses of diverse *Populus* root-associated fungi demonstrate that LCO production is not confined to the AM fungi in the order Glomales but, indeed, is widespread in taxonomically and functionally diverse symbionts within the fungal kingdom (Cope et al. 2019). The importance of these molecules in mediating plant–AM fungi interactions highlights the need to study the role of these molecules in other fungal associates.

Characterization of the interactions of *Populus* with the ECM fungal genus *Laccaria* has led to the discovery of small secreted proteins (SSPs) from both the host and fungal associate that act as signaling molecules and facilitate mycorrhization (Pellegrin et al. 2019; Plett et al. 2011). Many of the SSPs encoded by either partner move across host plant and fungal membranes and some fungal SSPs (e.g., *Laccaria* MISSP7) have been shown to localize in the nucleus of the plant symbiont (Plett et al. 2011). These molecules subsequently affect gene expression, defense signaling, host tissue and organ development, and the downstream phenotypes of both the plant host and fungal partner (Plett et al. 2014). Genetic knockout and knockdown experiments have shown that the lack of SSP signaling can eliminate the ability of ECM fungi to effectively colonize *Populus* roots and form the characteristic symbiotic interface of these interactions (i.e., the Hartig net) (Pellegrin et al. 2019). Although some SSPs are homologous and widespread within diverse plants and fungi, others seem to be species specific (Pellegrin et al. 2015; Tsuzuki et al. 2016), suggesting that they may play a role in foundational ECM development processes, as well as in species-specific recognition that has not been fully defined.

Terpenes and volatile organic compounds also play important roles in microbial interactions and communications with the host plant at larger spatial scales than LCOs and SSPs (Bitas et al. 2013; Blom et al. 2011; Schulz and Dickschat 2007). Using *Populus*-derived genome sequences of the abovementioned ECM and endomycorrhizal fungi, researchers have measured terpene production in various *Populus* root-associated fungi and identified bacterial terpene synthase-like genes in several pathogenic fungi that originated from bacteria by horizontal gene transfer (Jia et al. 2019). Such compounds appear to be involved in broader plant–fungus interactions. Monoterpene emission in roots has been shown to have inhibitory effects on the growth of *Phytophthora cactorum* (Oomycetes), an important plant pathogen (Lackus et al. 2018). The exploration of these microbial signaling molecules and their effects in *Populus* systems and other plants is advancing rapidly but the

broader effects and extent of these interactions are still poorly understood.

As with fungi, the ability of bacteria to exert effects on plant hosts is mediated through chemical and physical associations. Many bacteria use chemotaxis signaling pathways to sense and move toward compounds found in plant root exudates (Currier and Strobel 1976; de Weert et al. 2002; Feng et al. 2018; Gaworzewska and Carlile 1982). Some bacterial species produce acyl-homoserine lactones (AHLs) that are involved in quorum sensing, a method of communication between bacteria that results in cell-density-dependent changes in gene expression and behavior that can promote plant colonization (Danhorn and Fuqua 2007; Hibbing et al. 2010). Indeed, genomic analyses have shown that AHL-based signaling systems mediated by the *luxI-luxR* regulatory gene families are prevalent in the microbiome of *Populus* (Schaefer et al. 2013). Interestingly, some plant-associated bacteria encode a subfamily of LuxR homologs that respond to plant-derived signals rather than bacteria-derived signals (González and Venturi 2013; Subramoni and Venturi 2009). This interkingdom signaling system was first described for the LuxR homologs XccR from *Xanthomonas campestris* pv. *campestris* and OryR from *X. oryzae* pv. *oryzae*, which regulate virulence by responding to unidentified signals from cabbage and rice, respectively (Zhang et al. 2007). A *Populus*-derived signal that activates the LuxR homolog PipR in the *Populus* root endophyte *Pseudomonas* sp. GM79 was recently identified as the ethanolamine derivative, *N*-(2-hydroxyethyl)-2-(2-hydroxyethylamino)acetamide (Coutinho et al. 2018; Schaefer et al. 2013). Ongoing efforts to better understand these signaling systems and their distribution across diverse host–symbiont interactions are poised to shed light on how bacteria sense and respond to their host environments.

Comparative genomic analyses of diverse plant-associated bacteria have identified common candidate genes. Specifically, genes encoding carbohydrate metabolic functions that are enriched in plant-associated bacteria when compared with nonplant-associated bacteria allow exploration of important molecular mechanisms that drive microbial colonization on plants (Levy et al. 2018). The generation and characterization of mutants in genetically tractable microbes such as *Pseudomonas* spp. has also revealed genes and pathways that are required for efficient plant colonization (Cole et al. 2017; Lugtenberg et al. 2001). In *Pantoea* sp. YR343, a bacterial isolate from poplar, a mutant defective in carotenoid production showed defects in root colonization and motility, secretion of indole-3-acetic acid (IAA), and increased sensitivity to oxidative stress (Bible et al. 2016). These defects can be explained, at least in part, by alterations in membrane lipid as well as protein composition and organization resulting from the absence of carotenoids that likely influence signaling and transport (Kumar et al. 2019). In contrast, a *Pantoea* sp. YR343 strain with a deletion in the indole-3-pyruvate decarboxylase gene (*ipdC*) shows a significant reduction in IAA production. This strain is still able to colonize plants, although loss of this protein significantly affected the ability of *Pantoea* sp. YR343 to stimulate lateral root formation (Estenson et al. 2018). Studies such as these suggest that the continued development and refinement of molecular and genetic tools that manipulate phylogenetically diverse plant-associated bacteria will accelerate the discovery of genetic factors that drive plant colonization and may ultimately enable the strategic use of microbial communities to promote desired plant traits.

FINE-SCALE SPATIAL VARIATION IN POPULUS–MICROBE INTERACTIONS

Within the genus *Populus*, microbial diversity differs between plant-associated tissue types. Leaves, stems, and roots often contain

common endophytic bacterial and fungal phyla that are broadly comparable with those of other plant hosts (Ottesen et al. 2013; Peay et al. 2016; Pinto et al. 2014). However, within these broad tissue types, there is also significant variation in the *Populus* microbiome at finer scales (e.g., distinct community differences are seen between developing and mature leaf tissues) (Cregger et al. 2018). In internal plant compartments such as root and leaf tissues, fine tuning and adaptation of the microbiome is clearly evident (Beckers et al. 2017) but the specific factors controlling local microbial assembly and stability is still unclear.

Emerging microfluidic and engineered habitats enable high-resolution optical and chemical imaging studies that can determine plant–microbe interactions at finer spatial and temporal scales than traditional amplicon, metagenomic, and cell-counting assays (Aufrecht et al. 2017). Such platforms have been used to characterize microbial colonization, including microbial recruitment,

assembly, and maintenance of the symbiotic interaction (Morrell-Falvey et al. 2019) (Fig. 1). These systems have been used to examine the colonization behavior of green-fluorescent-protein-expressing *Pantoea* sp. YR343, as well as other genetically tractable *Populus* microbial isolates such as species of *Variovorax*, *Methylobium*, and *Caulobacter* (Fig. 2). Additionally, more controlled microfluidic platforms have recently been developed and are emerging as a powerful platform for visualizing and quantifying plant–microbe interactions (Aufrecht et al. 2017, 2018; Massalha et al. 2017), fungal–microbe interactions (Millet et al. 2019; Uehling et al. 2019), and microbial community development (Hansen et al. 2016; Timm et al. 2017; Wilmoth et al. 2018).

Using these platforms, the colonization behaviors of two *Populus* isolates, *Pantoea* sp. YR343 and *Variovorax* sp. CF313, have been compared and quantified (Aufrecht et al. 2018). The impact of cocolonization was examined, and it was noted that these two

Fig. 1. Growth of *Arabidopsis thaliana* in a two-layer microfluidic platform. **A**, Device consists of two layers to confine root hairs to the same imaging plane as the main root (scale = 1 mm). Seedlings may be **B**, contained in a gnotobiotic environment constructed of poly-dimethylsiloxane (PDMS) and **C**, monitored for growth for the duration of coculture experiments (scale bar = 250 µm).

bacterial species form spatially distinct associations along developing root tissues. Although there did not appear to be any exclusion or colocalization effects, there was a clear impact of the faster-growing *Variovorax* sp. CF313 on the ultimate colonization patterns of *Pantoea* sp. YR343 (Aufrecht et al. 2018). Moreover, changes in root phenotype from these bacterial treatments depended on both the initial concentrations and the species of the bacterial population. Such distinct colonization patterns suggest that chemical and physical cues, which promote microbial recruitment, attachment, and development, occur at fine scales within the host–symbiont interface.

This fine-scale spatial variation within the root microbiome can play an important role in maintaining beneficial plant–microbe interactions. In a phenomenon known as Simpson’s Paradox (Chuang et al. 2009), the abundance of mutualistic strains in a system can increase globally, even if mutualists are locally out-competed in all subcommunities. In a synthetic microbiome, altruism was selected for at the population level as producers generated common goods despite production costs at the individual level (Chuang et al. 2009). This effect requires local heterogeneity in community composition, rewards for community cooperation that accumulate within the local microbial community, and periodic redistribution (but not homogenization) between local communities. Each of these conditions is plausible in the *Populus* rhizosphere but further research is necessary to examine these hypotheses.

MICROBIOME VARIABILITY ACROSS *POPULUS* SPP.

Over the last decade, expansive efforts have been made to characterize variation in microbial communities among plant genotypes, amid plant species within genera, and across the broader diversity of plants (Bodenhausen et al. 2013; Lundberg et al. 2012; Wagner et al. 2016; Zarraonaindia et al. 2015). Recently described efforts have thoroughly characterized the microbiomes of numerous plant species, including *Populus* (Cregger et al. 2018), *Arabidopsis* (Bodenhausen et al. 2013; Lundberg et al. 2012), rice (Edwards et al. 2015), grape (Bokulich et al. 2016; Zarraonaindia et al. 2015), eucalyptus (Santiago et al. 2015), sugarcane (Armanhi et al. 2016), legumes (Hartman et al. 2017; Zgadzaj et al. 2016), mustard (Wagner et al. 2016), pine (Carper et al. 2018), maize (Peiffer et al. 2013), and several other angiosperm species (Fitzpatrick et al. 2018). These efforts reveal high-level commonalities regarding root microbiome structure. For instance, while *Populus* spp. harbor

a great deal of microbial diversity, four bacterial phyla—Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes—have been shown to represent roughly 40 to 50, 15 to 20, 5 to 10, and 1 to 3%, respectively, of the bacterial community across multiple *Populus* spp. and genotypes (Bonito et al. 2014; Cregger et al. 2018; Shakya et al. 2013) and are usually the most common within these other plant species.

Contrastingly, at lower taxonomic levels, significant differences in microbiome constituency are evident and can depend on environmental factors, host habitat, and host genotype. *Populus* spp. selectively recruit fungal and bacterial associates from soil microbiomes that are different from other plant species (e.g., *Quercus* or *Pinus*) when grown with common inocula under greenhouse conditions (Bonito et al. 2014), where oak and pine were dominated by only a few ectomycorrhizal taxa and *Populus* contained a more diverse endophytic array of taxa. Furthermore, individual genotypes from closely related species such as *P. trichocarpa*, *P. deltoides*, and their hybrids show significant differences in their microbial composition when grown in common field environments. Interestingly, *P. deltoides* had higher fungal diversity relative to hybrid trees (Cregger et al. 2018). These observations of host-dependent differences in microbiome structure seem common across study systems, with comparable microbiome observations in animal systems (Goodrich et al. 2016; Lloyd-Price et al. 2017).

Variation in the *Populus* microbiome across *Populus* spp. and genotypes may be driven by differences in defense-related traits such as biosynthesis of phenolic glycoside and other plant metabolites (Lindroth and St. Clair 2013). Recent research shows that these traits may have some influence on fine tuning microbiome composition in *P. trichocarpa* (Veatch et al. 2019) and fungal endophyte colonization in *P. fremontii*, *P. angustifolia*, and their hybrids (Bailey et al. 2005). Within *P. deltoides*, salicylic acid concentrations have been shown to fluctuate in response to abiotic stress (Jung et al. 2009; Tschaplinski et al. 2019). Furthermore, these defense compounds have been shown to fluctuate across *Populus* genotypes, with implications for the ability to defend against herbivores (Lindroth and St. Clair 2013). Although it is clear that changes in plant traits alter microbiome composition, it is unclear how these fine-scale processes result in variation in plant–microbe interactions across larger spatial scales.

Variation in microbiome composition within *Populus* spp. is often apparent across landscapes and species’ native ranges (Barge et al. 2019; Shakya et al. 2013; Ware et al. 2019). Variance partitioning approaches have shown that, whereas the majority of community composition remains unexplained, soil chemical and physical characteristics, geographic distance, climatic gradients, and season of sampling explain greater variance in microbiome composition than tree genotype within *P. deltoides* species (Fig. 3) (Barge et al. 2019; Shakya et al. 2013). These patterns of relatively low intra- versus interspecific local variation and higher intraspecific variability across landscapes have also been shown in other plant species and ecosystems (Redford et al. 2010). Interestingly, interspecific variability in microbiome traits is more obvious in controlled greenhouses and common gardens (Busby et al. 2016a,b; Newcombe et al. 2018). These environments provide a unique opportunity to understand genetic factors underlying these interactions using host comparative genomics and QTL mapping pedigrees segregating for species-specific colonization efficiency, as discussed elsewhere in this review. Although phylogenetic surveys accomplish a critical first step in revealing who is present in the microbiome in natural and controlled conditions, and show that composition can depend on and respond to environmental conditions, host genotype or phenotype, and environmental stressors, a

Fig. 2. Visualizing the dynamics of microbial colonization. **A**, *Populus trichocarpa* cutting growing in a three-dimensional printed imaging chamber. **B**, *Pantoea* sp. YR343 expressing green fluorescent protein (green) was imaged during colonization of *P. trichocarpa* (red) using the imaging chamber. The plant root was imaged using autofluorescence in the red channel (scale bar = 10 μ m).

challenge remains in determining how particular microbes are selected and how they collectively function in concert with *Populus* spp.

ECOSYSTEM CONSEQUENCES OF *POPULUS*-MICROBE INTERACTIONS

Plants exchange carbon (C)-rich photosynthates for microbially derived nutrients and the potential for enhanced defenses against pathogens and herbivores. The costs and benefits of these complex species interactions are dynamic and context dependent (Chamberlain et al. 2014), and, as noted above, changing abiotic or biotic conditions can convert mutualisms into commensal or even antagonistic interactions (Johnson et al. 1997; Simms et al. 2006). For a plant host, the cost of participating in mutualistic interactions is dependent upon the supply of fixed carbohydrates from photosynthesis (Clark et al. 2019; Friel and Friesen 2019; Kaschuk et al. 2009; Lau and Lennon 2012; Timm et al. 2018). This supply changes due to differences in plant genetics and varying environmental conditions that fluctuate on a diurnal, seasonal, and annual basis. Such variability can undermine species interactions; for example, when photosynthetic limitations force the plant to redirect C from mutualists to other plant C sinks such as growth, respiration, and storage (Pringle et al. 2016). Thus, how plants interact with their associated microbial symbionts in a given environment can be determined, in part, by C fixation potential (i.e., photosynthetic rates) and C allocation patterns. At the same time, the rate of C fixation within a plant can be altered by the microbial

constituents living on and within the plant, highlighting the bidirectional nature of this symbiosis.

The mechanisms of C exchange may provide an opportunity for stabilizing the plant-microbe relationship against evolutionary challenges. Some plants have been shown to use sanctions on mutualist partners, modulating the C provided in response to the nutrients returned by the microbiome (Kiers and Denison 2008). This strategy has previously been reported in soybean root systems, where underperforming microbial nitrogen fixation nodules were starved by their plant host (Kiers et al. 2003). Similar dynamics can also be initiated by symbionts, as shown by AM fungi that transfer nutrients only to *Medicago truncatula* roots that, in return, provide C exudates (Kiers et al. 2011). A similar system of local detection and sanction may be present in the roots of nonnodulating plants such as *Populus* spp. but further study is required.

Plant genetic control of root exudate profiles and litter chemistry can also have significant impacts on the soil microbial community and on resulting ecosystem functions such as decomposition and nutrient cycling. For example, condensed tannin concentrations in *Populus* alter the composition of the soil microbial community and, subsequently, rates of decomposition (Bailey et al. 2005; Schweitzer et al. 2004, 2008). Similarly, genotypic variation in *Populus* leaf senescence and phytochemistry alter decomposition rates (Lindroth et al. 2002). Often, *Populus* genetic diversity in mixed stands influences soil microbial community composition and exoenzyme activity (Schweitzer et al. 2011). Although these studies clearly show higher-order consequences of the interactions, fingerprinting methods used at the time did not allow a robust

Fig. 3. Variance partitioning of **A**, bacterial and **B**, fungal communities from the root endosphere and soil rhizosphere of *Populus deltoides* studied in two river systems in Tennessee and North Carolina. Colors represent covariates in the model: soil properties (brown), host properties (yellow), geographic or spatial distance (orange), host genotype (purple), season of sampling (pink), and β diversity of corresponding bacterial or fungal community (green). Each bar represents total variance partitioned into main effects or the interaction of two or more factors. Figure from Shakya et al. (2013).

understanding of the phylogenetic microbial community changes associated with them. How these plant level controls scale, through the microbial community, to alter complex ecosystem functions such as nutrient cycles across diverse ecosystems clearly requires further investigation.

CONCLUSION

The enormous wealth of information available about the genus *Populus* and its microbiome provides an ideal test bed to examine complex plant–microbe interactions from the gene to ecosystem levels. As highlighted, variation in the genes of *Populus* spp. and their symbionts alters which relationships establish and persist which, in turn, has consequences that can scale to ecosystem-level functions. Although *Populus* may be an excellent model to address many of these gene to ecosystem questions, we recognize that there are caveats to keep in mind when using this system. For studies requiring rapid turnaround times, *Populus* may not work well, because growing trees to maturity for experimentation can take years. Furthermore, our understanding of how conclusions drawn from young trees translate as the tree ages is incomplete. Even with the prior research efforts highlighted in this review, there are numerous unanswered questions at each biological scale, and between scales, that will need to be tackled to fully understand how plants and microbes interact within ecosystems and how these interactions alter ecosystem structure and function. Do microorganisms influence plant establishment and persistence in natural ecosystems? How do multiple environmental drivers influence the balance of costs and benefits with mutualists? Does a long-lived woody-perennial tree continually change mutualists? A mechanistic understanding of plant–microbe interactions is critical as we attempt to manipulate these interactions to enhance plant growth and health. Furthermore, understanding these relationships in perennial systems is especially important because they may be more likely to have a consequential influence on ecosystem dynamics over time.

LITERATURE CITED

- Abraham, N., Chitampalam, P., Nelson, B. D., Sharma Poudel, R., Chittem, K., Borowicz, P., Brueggeman, R. S., Jain, S., and Leboldus, J. 2019. A microscopic, biochemical, and molecular comparison of moderately resistant and susceptible *Populus* genotypes inoculated with *Sphaerulina musiva*. *Phytopathology* 109:2074–2086.
- Armanhi, J. S. L., de Souza, R. S. C., de Araújo, L. M., Okura, V. K., Mieczkowski, P., Imperial, J., and Arruda, P. 2016. Multiplex amplicon sequencing for microbe identification in community-based culture collections. *Sci. Rep.* 6:29543.
- Aufrecht, J. A., Ryan, J. M., Hasim, S., Allison, D. P., Nebenfuhr, A., Doktycz, M. J., and Retterer, S. T. 2017. Imaging the root hair morphology of *Arabidopsis* seedlings in a two-layer microfluidic platform. *J. Vis. Exp.* 126: e55971.
- Aufrecht, J. A., Timm, C. M., Bible, A., Morrell-Falvey, J. L., Pelletier, D. A., Doktycz, M. J., and Retterer, S. T. 2018. Quantifying the spatiotemporal dynamics of plant root colonization by beneficial bacteria in a microfluidic habitat. *Adv. Biosyst.* 2:1800048.
- Bailey, J. K., Deckert, R., Schweitzer, J. A., Rehill, B. J., Lindroth, R. L., Gehring, C., and Whitham, T. G. 2005. Host plant genetics affect hidden ecological players: Links among *Populus*, condensed tannins, and fungal endophyte infection. *Can. J. Bot.* 83:356–361.
- Barge, E. G., Leopold, D. R., Peay, K. G., Newcombe, G., and Busby, P. E. 2019. Differentiating spatial from environmental effects on foliar fungal communities of *Populus trichocarpa*. *J. Biogeogr.* 46:2001–2011.
- Beckers, B., De Beeck, M. O., Weyens, N., Boerjan, W., and Vangronsveld, J. 2017. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome* 5:25.
- Berch, S. M., Massicotte, H. B., and Tackaberry, L. E. 2005. Re-publication of a translation of ‘The vegetative organs of *Monotropa hypopitys* L.’ published by F. Kamienski in 1882, with an update on *Monotropa mycorrhizas*. *Mycorrhiza* 15:323–332.
- Bewg, W. P., Ci, D., and Tsai, C. J. 2018. Genome editing in trees: From multiple repair pathways to long-term stability. *Front. Plant Sci.* 9:1732.
- Bible, A. N., Fletcher, S. J., Pelletier, D. A., Schadt, C. W., Jawdy, S. S., Weston, D. J., Engle, N. L., Tschaplinski, T., Masyuko, R., Poliseti, S., Bohn, P., Coutinho, T. A., Doktycz, M. J., and Morrell-Falvey, J. L. 2016. A carotenoid-deficient mutant in *Pantoea* sp. YR343, a bacteria isolated from the rhizosphere of *Populus deltoides*, is defective in root colonization. *Front. Microbiol.* 7:491.
- Bitas, V., Kim, H. S., Bennett, J. W., and Kang, S. 2013. Sniffing on microbes: Diverse roles of microbial volatile organic compounds in plant health. *Mol. Plant-Microbe Interact.* 26:835–843.
- Blair, P. M., Land, M. L., Piatek, M. J., Jacobson, D. A., Lu, T. Y. S., Doktycz, M. J., and Pelletier, D. A. 2018. Exploration of the biosynthetic potential of the *Populus* microbiome. *mSystems* 3:e00045–18.
- Blom, D., Fabbri, C., Connor, E. C., Schiestl, F. P., Klauser, D. R., Boller, T., Eberl, L., and Weisskopf, L. 2011. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. *Environ. Microbiol.* 13:3047–3058.
- Bodenhausen, N., Horton, M. W., and Bergelson, J. 2013. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS One* 8: e56329.
- Bokulich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., and Mills, D. A. 2016. Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. *MBio* 7:e00631–16.
- Bonito, G., Hameed, K., Ventura, R., Krishnan, J., Schadt, C. W., and Vilgalys, R. 2016. Isolating a functionally relevant guild of fungi from the root microbiome of *Populus*. *Fungal Ecol.* 22:35–42.
- Bonito, G., Reynolds, H., Robeson, M. S., Nelson, J., Hodkinson, B. P., Tuskan, G., Schadt, C. W., and Vilgalys, R. 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Mol. Ecol.* 23: 3356–3370.
- Brown, S. D., Klingeman, D. M., Lu, T. Y. S., Johnson, C. M., Utturkar, S. M., Land, M. L., Schadt, C. W., Doktycz, M. J., and Pelletier, D. A. 2012a. Draft genome sequence of *Rhizobium* sp. strain PDO1-076, a bacterium isolated from *Populus deltoides*. *J. Bacteriol.* 194:2383–2384.
- Brown, S. D., Utturkar, S. M., Klingeman, D. M., Johnson, C. M., Martin, S. L., Land, M. L., Lu, T. Y. S., Schadt, C. W., Doktycz, M. J., and Pelletier, D. A. 2012b. Twenty-one genome sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of *Populus deltoides*. *J. Bacteriol.* 194:5991–5993.
- Busby, P. E., Peay, K. G., and Newcombe, G. 2016a. Common foliar fungi of *Populus trichocarpa* modify *Melampsora* rust disease severity. *New Phytol.* 209:1681–1692.
- Busby, P. E., Ridout, M., and Newcombe, G. 2016b. Fungal endophytes: Modifiers of plant disease. *Plant Mol. Biol.* 90:645–655.
- Camps, C., Jardinaud, M. F., Rengel, D., Carere, S., Herve, C., Debelle, F., Gamas, P., Bensmihen, S., and Gough, C. 2015. Combined genetic and transcriptomic analysis reveals three major signalling pathways activated by Myc-LCOs in *Medicago truncatula*. *New Phytol.* 208:224–240.
- Carper, D. L., Carrell, A. A., Kueppers, L. M., and Frank, A. C. 2018. Bacterial endophyte communities in *Pinus flexilis* are structured by host age, tissue type, and environmental factors. *Plant Soil* 428:335–352.
- Chamberlain, S. A., Bronstein, J. L., and Rudgers, J. A. 2014. How context dependent are species interactions? *Ecol. Lett.* 17:881–890.
- Chhetri, H. B., Macaya-Sanz, D., Kainer, D., Biswal, A. K., Evans, L. M., Chen, J. G., Collins, C., Hunt, K., Mohanty, S. S., Rosenstiel, T., Ryno, D., Winkler, K., Yang, X. H., Jacobson, D., Mohnen, D., Muchero, W., Strauss, S. H., Tschaplinski, T. J., Tuskan, G. A., and Difazio, S. P. 2019. Multitrait genome-wide association analysis of *Populus trichocarpa* identifies key polymorphisms controlling morphological and physiological traits. *New Phytol.* 223:293–309.
- Chuang, J. S., Rivoire, O., and Leibler, S. 2009. Simpson’s Paradox in a synthetic microbial system. *Science* 323:272–275.
- Chupeau, M. C., Lemoine, M., and Chupeau, Y. 1993. Requirement of thidiazuron for healthy protoplast development to efficient tree regeneration of a hybrid poplar (*Populus tremula* β *p. alba*). *J. Plant Physiol.* 141:601–609.
- Clark, T. J., Friel, C. A., Grman, E., Friesen, M. L., and Shachar-Hill, Y. 2019. Unfair trade underground revealed by integrating data with Nash bargaining models. *New Phytol.* 222:1325–1337.
- Cole, B. J., Feltcher, M. E., Waters, R. J., Wetmore, K. M., Mucyn, T. S., Ryan, E. M., Wang, G. Y., Ul-Hasan, S., McDonald, M., Yoshikuni, Y.,

- Malmstrom, R. R., Deutschbauer, A. M., Dang, J. L., and Visel, A. 2017. Genome-wide identification of bacterial plant colonization genes. *PLoS Biol.* 15:e2002860.
- Cope, K. R., Bascaules, A., Irving, T. B., Venkateshwaran, M., Maeda, J., Garcia, K., Rush, T. A., Ma, C., Labbé, J., Jawdy, S., Steigerwald, E., Setzke, J., Fung, E., Schnell, K. M. G., Wang, Y. Q., Schlieff, N., Bucking, H., Strauss, S. H., Maillet, F., Jargeat, P., Becard, G., Puech-Pages, V., and Ane, J. M. 2019. The ectomycorrhizal fungus *Laccaria bicolor* produces lipochitoooligosaccharides and uses the common symbiosis pathway to colonize *Populus* roots. *Plant Cell* 31:2386-2410.
- Cordovez, V., Dini-Andreote, F., Carrion, V. J., and Raaijmakers, J. M. 2019. Ecology and evolution of plant microbiomes. *Annu. Rev. Microbiol.* 73: 69-88.
- Coutinho, B. G., Mevers, E., Schaefer, A. L., Pelletier, D. A., Harwood, C. S., Clardy, J., and Greenberg, E. P. 2018. A plant-responsive bacterial-signaling system senses an ethanolamine derivative. *Proc. Natl. Acad. Sci. U.S.A.* 115: 9785-9790.
- Cregger, M. A., Veach, A. M., Yang, Z. K., Crouch, M. J., Vilgalys, R., Tuskan, G. A., and Schadt, C. W. 2018. The *Populus* holobiont: Dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* 6:31.
- Currier, W. W., and Strobel, G. A. 1976. Chemotaxis of *Rhizobium* spp. to plant root exudates. *Plant Physiol.* 57:820-823.
- Danhorn, T., and Fuqua, C. 2007. Biofilm formation by plant-associated bacteria. *Annu. Rev. Microbiol.* 61:401-422.
- de Weert, S., Vermeiren, H., Mulders, I. H. M., Kuiper, I., Hendrickx, N., Bloembergen, G. V., Vanderleyden, J., De Mot, R., and Lugtenberg, B. J. J. 2002. Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. *Mol. Plant-Microbe Interact.* 15:1173-1180.
- Edwards, J., Johnson, C., Santos-Medellin, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., and Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. U.S.A.* 112:E911-E920.
- Estenson, K., Hurst, G. B., Standaert, R. F., Bible, A. N., Garcia, D., Chourey, K., Doktycz, M. J., and Morrell-Falvey, J. L. 2018. Characterization of indole-3-acetic acid biosynthesis and the effects of this phytohormone on the proteome of the plant-associated microbe *Pantoea* sp. YR343. *J. Proteome Res.* 17:1361-1374.
- Evans, L. M., Slavov, G. T., Rodgers-Melnick, E., Martin, J., Ranjan, P., Muchero, W., Brunner, A. M., Schackwitz, W., Gunter, L., Chen, J. G., Tuskan, G. A., and Difazio, S. P. 2014. Population genomics of *Populus trichocarpa* identifies signatures of selection and adaptive trait associations. *Nat. Genet.* 46:1089-1096.
- Fahrenkrog, A. M., Neves, L. G., Resende, M. F., Jr., Vazquez, A. I., De Los Campos, G., Dervinis, C., Sykes, R., Davis, M., Davenport, R., Barbazuk, W. B., and Kirst, M. 2017. Genome-wide association study reveals putative regulators of bioenergy traits in *Populus deltoides*. *New Phytol.* 213:799-811.
- Feng, H. C., Zhang, N., Du, W. B., Zhang, H. H., Liu, Y. P., Fu, R. X., Shao, J. H., Zhang, G. S., Shen, Q. R., and Zhang, R. F. 2018. Identification of chemotaxis compounds in root exudates and their sensing chemoreceptors in plant-growth-promoting rhizobacteria *Bacillus amyloliquefaciens* SQR9. *Mol. Plant-Microbe Interact.* 31:995-1005.
- Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., and Johnson, M. T. J. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proc. Natl. Acad. Sci. U.S.A.* 115:E1157-E1165.
- Friel, C. A., and Friesen, M. L. 2019. Legumes modulate allocation to rhizobial nitrogen fixation in response to factorial light and nitrogen manipulation. *Front. Plant Sci.* 10:1316.
- Gaworzewska, E. T., and Carlile, M. J. 1982. Positive chemotaxis of *Rhizobium-Leguminosarum* and other bacteria towards root exudates from legumes and other plants. *J. Gen. Microbiol.* 128:1179-1188.
- González, J. F., and Venturi, V. 2013. A novel widespread interkingdom signaling circuit. *Trends Plant Sci.* 18:167-174.
- Goodrich, J. K., Davenport, E. R., Waters, J. L., Clark, A. G., and Ley, R. E. 2016. Cross-species comparisons of host genetic associations with the microbiome. *Science* 352:532-535.
- Hacquard, S., and Schadt, C. W. 2015. Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome. *New Phytol.* 205: 1424-1430.
- Hansen, R. H., Timm, A. C., Timm, C. M., Bible, A. N., Morrell-Falvey, J. L., Pelletier, D. A., Simpson, M. L., Doktycz, M. J., and Retterer, S. T. 2016. Stochastic assembly of bacteria in microwell arrays reveals the importance of confinement in community development. *PLoS One* 11:e0155080.
- Hartman, K., van der Heijden, M. G. A., Roussely-Provent, V., Walser, J. C., and Schlaeppi, K. 2017. Deciphering composition and function of the root microbiome of a legume plant. *Microbiome* 5:2.
- Hibbing, M. E., Fuqua, C., Parsek, M. R., and Peterson, S. B. 2010. Bacterial competition: Surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* 8:15-25.
- Hirsch, A. M. 2004. Plant-microbe symbioses: A continuum from commensalism to parasitism. *Symbiosis* 37:345-363.
- Jia, Q. D., Chen, X. L., Köllner, T. G., Rinkel, J., Fu, J. Y., Labbé, J., Xiong, W. D., Dickschat, J. S., Gershenzon, J., and Chen, F. 2019. Terpene synthase genes originated from bacteria through horizontal gene transfer contribute to terpenoid diversity in fungi. *Sci. Rep.* 9:9223.
- Johnson, N. C., Graham, J.-H., and Smith, F. A. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol.* 135:575-585.
- Jun, S. R., Wassenaar, T. M., Nookaew, I., Hauser, L., Wanchai, V., Land, M., Timm, C. M., Lu, T. Y. S., Schadt, C. W., Doktycz, M. J., Pelletier, D. A., and Ussery, D. W. 2016. Diversity of *Pseudomonas* genomes, including *Populus*-associated isolates, as revealed by comparative genome analysis. *Appl. Environ. Microbiol.* 82:375-383.
- Jung, H. W., Tschaplinski, T. J., Wang, L., Glazebrook, J., and Greenberg, J. T. 2009. Priming in systemic plant immunity. *Science* 324:89-91.
- Kaschuk, G., Kuypers, T. W., Leffelaar, P. A., Hungria, M., and Giller, K. E. 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol. Biochem.* 41: 1233-1244.
- Kiers, E. T., and Denison, R. F. 2008. Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. *Annu. Rev. Ecol. Evol. Syst.* 39:215-236.
- Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J., and Bucking, H. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333:880-882.
- Kiers, E. T., Rousseau, R. A., West, S. A., and Denison, R. F. 2003. Host sanctions and the legume-rhizobium mutualism. *Nature* 425:78-81.
- Kitano, H. 2002. Systems biology: A brief overview. *Science* 295:1662-1664.
- Klingeman, D. M., Urturkar, S., Lu, T. Y. S., Schadt, C. W., Pelletier, D. A., and Brown, S. D. 2015. Draft genome sequences of four *Streptomyces* isolates from the *Populus trichocarpa* root endosphere and rhizosphere. *Genome Announce.* 3:e01344-15.
- Kumar, S. V., Taylo, G., Hasim, S., Collier, C. P., Farmer, A. T., Campagna, S. R., Bible, A. N., Doktycz, M. J., and Morrell-Falvey, J. 2019. Loss of carotenoids from membranes of *Pantoea* sp. YR343 results in altered lipid composition and changes in membrane biophysical properties. *Biochim. Biophys. Acta Biomembr.* 1861:1338-1345.
- Labbe, J. L., Weston, D. J., Dunkirk, N., Pelletier, D. A., and Tuskan, G. A. 2014. Newly identified helper bacteria stimulate ectomycorrhizal formation in *Populus*. *Front. Plant Sci.* 5:579.
- Lackus, N. D., Lackner, S., Gershenzon, J., Unsicker, S. B., and Köllner, T. G. 2018. The occurrence and formation of monoterpenes in herbivore-damaged poplar roots. *Sci. Rep.* 8:17936.
- Lau, J. A., and Lennon, J. T. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proc. Natl. Acad. Sci. U.S.A.* 109:14058-14062.
- Lerouge, P., Roche, P., Faucher, C., Maillet, F., Truchet, G., Prome, J. C., and Denarie, J. 1990. Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature* 344:781-784.
- Levy, A., Gonzalez, I. S., Mittelviehhaus, M., Clingenpeel, S., Paredes, S. H., Miao, J. M., Wang, K. R., Devescovi, G., Stillman, K., Monteiro, F., Alvarez, B. R., Lundberg, A. D. S., Lu, T. Y., Lebeis, S., Jin, Z., McDonald, M., Klein, A. P., Felcher, M. E., Rio, T. G., Grant, S. R., Doty, S. L., Ley, R. E., Zhao, B. Y., Venturi, V., Pelletier, D. A., Vorholt, J. A., Tringe, S. G., Woyke, T., and Dangel, J. L. 2018. Genomic features of bacterial adaptation to plants. *Nat. Genet.* 50:138-150.
- Li, J. Y., Liu, S. Q., Zhou, L., and Liu, Y. M. 2018. Growth strain in straight and inclined *Populus x euramericana* cv. '74/76' trees, and its relationship with selected wood properties. *Eur. J. Wood Wood Prod* 76:1715-1723.
- Lindroth, R. L., Osier, T. L., Barnhill, H. R. H., and Wood, S. A. 2002. Effects of genotype and nutrient availability on phytochemistry of trembling aspen (*Populus tremuloides* Michx.) during leaf senescence. *Biochem. Syst. Ecol.* 30:297-307.
- Lindroth, R. L., and St. Clair, S. B. 2013. Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. *For. Ecol. Manage.* 299: 14-21.

- Lloyd-Price, J., Mahurkar, A., Ahnavard, G., Crabtree, J. C., Orvis, J., Hall, A. B., Brady, A., Creasy, H. H., McCracken, C., Giglio, M. G., McDonald, D., Franzosa, E. A., Knight, R., White, O., and Huttenhower, C. 2017. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature* 550:61-66.
- Looney, B. P., Meidl, P., Piatek, M. J., Miettinen, O., Martin, F. M., Matheny, P. B., and Labbé, J. L. 2018. Russulaceae: A new genomic dataset to study ecosystem function and evolutionary diversification of ectomycorrhizal fungi with their tree associates. *New Phytol.* 218:54-65.
- Lugtenberg, B. J. J., Dekkers, L., and Bloemberg, G. V. 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu. Rev. Phytopathol.* 39:461-490.
- Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektsen, A., Kunin, V., Del Rio, T. G., Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G., and Dangl, J. L. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488:86-90.
- Maillet, F., Poinot, V., Andre, O., Puech-Pages, V., Haouy, A., Gueunier, M., Cromer, L., Giraudet, D., Formey, D., Niebel, A., Martinez, E. A., Driguez, H., Becard, G., and Denarie, J. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* 469:58-63.
- Martin, F., Tuskan, G. A., Difazio, S. P., Lammers, P., Newcombe, G., and Podila, G. K. 2004. Symbiotic sequencing for the *Populus* mesocosm. *New Phytol.* 161:330-335.
- Martino, E., Morin, E., Grelet, G. A., Kuo, A., Kohler, A., Daghighi, S., Barry, K. W., Cichocki, N., Clum, A., Dockter, R. B., Hainaut, M., Kuo, R. C., Labutti, K., Lindahl, B. D., Lindquist, E. A., Lipzen, A., Khouja, H. R., Magnuson, J., Murat, C., Ohm, R. A., Singer, S. W., Spatafora, J. W., Wang, M., Veneault-Fourrey, C., Henrissat, B., Grigoriev, I. V., Martin, F. M., and Perotto, S. 2018. Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytol.* 217: 1213-1229.
- Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O. H., and Aharoni, A. 2017. Live imaging of root-bacteria interactions in a microfluidics setup. *Proc. Natl. Acad. Sci. U.S.A.* 114:4549-4554.
- McKown, A. D., Guy, R. D., Quamme, L., Klápště, J., La Mantia, J., Constabel, C. P., El-Kassaby, Y. A., Hamelin, R. C., Zifkin, M., and Azam, M. S. 2014. Association genetics, geography and ecophysiology link stomatal patterning in *Populus trichocarpa* with carbon gain and disease resistance trade-offs. *Mol. Ecol.* 23:5771-5790.
- Merkle, S. A., and Nairn, C. J. 2005. Hardwood tree biotechnology. *In Vitro Cell Dev. Biol. Plant* 41:602-619.
- Millet, L. J., Aufrecht, J., Labbé, J., Uehling, J., Vilgalys, R., Estes, M. L., Miquel Guennoc, C., Deveau, A., Olsson, S., Bonito, G., Doktycz, M. J., and Retterer, S. T. 2019. Increasing access to microfluidics for studying fungi and other branched biological structures. *Fungal Biol. Biotechnol.* 6:8.
- Mock, K. E., Rowe, C. A., Hooten, M. B., Dewoody, J., and Hipkins, V. D. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). *Mol. Ecol.* 17:4827-4844.
- Morrell-Falvey, J. L., Alexandre-Jouline, G. M., and Sarles, S. A. 2019. Microscope slide-in chamber. U.S. Patent USD754871S1.
- Muchero, W., Guo, J., Difazio, S. P., Chen, J. G., Ranjan, P., Slavov, G. T., Gunter, L. E., Jawdy, S., Bryan, A. C., Sykes, R., Ziebell, A., Klapste, J., Porth, I., Skyba, O., Unda, F., El-Kassaby, Y. A., Douglas, C. J., Mansfield, S. D., Martin, J., Schackwitz, W., Evans, L. M., Czarnecki, O., and Tuskan, G. A. 2015. High-resolution genetic mapping of allelic variants associated with cell wall chemistry in *Populus*. *BMC Genomics* 16:24.
- Newcombe, G., Muchero, W., and Busby, P. E. 2018. Resistance to an eriophyid mite in an interspecific hybrid pedigree of *Populus*. *PLoS One* 13:e0207839.
- Ottesen, A. R., Pena, A. G., White, J. R., Pettengill, J. B., Li, C., Allard, S., Rideout, S., Allard, M., Hill, T., Evans, P., Strain, E., Musser, S., Knight, R., and Brown, E. 2013. Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). *BMC Microbiol.* 13:114.
- Peay, K. G., Kennedy, P. G., and Talbot, J. M. 2016. Dimensions of biodiversity in the Earth mycobiome. *Nat. Rev. Microbiol.* 14:434-447.
- Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., and Ley, R. E. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci. U.S.A.* 110: 6548-6553.
- Pellegrin, C., Daguerre, Y., Ruytinx, J., Guinet, F., Kemppainen, M., Frey, N. F. D., Puech-Pages, V., Hecker, A., Pardo, A. G., Martin, F. M., and Veneault-Fourrey, C. 2019. *Laccaria bicolor* MiSSP8 is a small-secreted protein decisive for the establishment of the ectomycorrhizal symbiosis. *Environ. Microbiol.* 21:3765-3779.
- Pellegrin, C., Morin, E., Martin, F. M., and Veneault-Fourrey, C. 2015. Comparative analysis of secretomes from ectomycorrhizal fungi with an emphasis on small-secreted proteins. *Front. Microbiol.* 6:1278.
- Pereira, M. de F., Veneault-Fourrey, C., Vion, P., Guinet, F., Morin, E., Barry, K. W., Lipzen, A., Singan, V., Pfister, S., Na, H., Kennedy, M., Egli, S., Grigoriev, I., Martin, F., Kohler, A., and Peter, M. 2018. Secretome analysis from the ectomycorrhizal ascomycete *Cenococcum geophilum*. *Front. Microbiol.* 9:141.
- Pinosio, S., Giacomello, S., Faivre-Rampant, P., Taylor, G., Jorge, V., Le Paslier, M. C., Zaina, G., Bastien, C., Cattonaro, F., Marroni, F., and Morgante, M. 2016. Characterization of the poplar pan-genome by genome-wide identification of structural variation. *Mol. Biol. Evol.* 33:2706-2719.
- Pinto, C., Pinho, D., Sousa, S., Pinheiro, M., Egas, C., and Gomes, A. C. 2014. Unravelling the diversity of grapevine microbiome. *PLoS One* 9:e85622.
- Plett, J. M., Daguerre, Y., Wittulsky, S., Vayssières, A., Deveau, A., Melton, S. J., Kohler, A., Morrell-Falvey, J. L., Brun, A., Veneault-Fourrey, C., and Martin, F. 2014. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proc. Natl. Acad. Sci. U.S.A.* 111:8299-8304.
- Plett, J. M., Kemppainen, M., Kale, S. D., Kohler, A., Legue, V., Brun, A., Tyler, B. M., Pardo, A. G., and Martin, F. 2011. A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Curr. Biol.* 21: 1197-1203.
- Porth, I., Klapste, J., Skyba, O., Hannemann, J., McKown, A. D., Guy, R. D., Difazio, S. P., Muchero, W., Ranjan, P., Tuskan, G. A., Friedmann, M. C., Ehrling, J., Cronk, Q. C. B., El-Kassaby, Y. A., Douglas, C. J., and Mansfield, S. D. 2013. Genome-wide association mapping for wood characteristics in *Populus* identifies an array of candidate single nucleotide polymorphisms. *New Phytol.* 200:710-726.
- Pringle, M. J., Allen, D. E., Orton, T. G., Bishop, T. F. A., Butler, D. W., Henry, B. K., and Dalal, R. C. 2016. Effects of land-use change and management on soil carbon and nitrogen in the Brigalow Belt, Australia: II. Statistical models to unravel the climate-soil-management interaction. *Rangeland J.* 38: 453-466.
- Rasmann, S., and Turlings, T. C. J. 2016. Root signals that mediate mutualistic interactions in the rhizosphere. *Curr. Opin. Plant Biol.* 32:62-68.
- Redford, A. J., Bowers, R. M., Knight, R., Linhart, Y., and Fierer, N. 2010. The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environ. Microbiol.* 12:2885-2893.
- Santiago, T. R., Grabowski, C., Rossato, M., Romeiro, R. S., and Mizubuti, E. S. G. 2015. Biological control of eucalyptus bacterial wilt with rhizobacteria. *Biol. Control* 80:14-22.
- Schaefer, A. L., Lappala, C. R., Morlen, R. P., Pelletier, D. A., Lu, T. Y. S., Lankford, P. K., Harwood, C. S., and Greenberg, E. P. 2013. LuxR- and LuxI-type quorum-sensing circuits are prevalent in members of the *Populus deltoides* microbiome. *Appl. Environ. Microbiol.* 79:5745-5752.
- Schulz, S., and Dickschat, J. S. 2007. Bacterial volatiles: The smell of small organisms. *J. Nat. Prod. Rep.* 24:814-842.
- Schweitzer, J. A., Bailey, J. K., Rehill, B. J., Martinsen, G. D., Hart, S. C., Lindroth, R. L., Keim, P., and Whitham, T. G. 2004. Genetically based trait in a dominant tree affects ecosystem processes. *Ecol. Lett.* 7:127-134.
- Schweitzer, J. A., Fischer, D. G., Rehill, B. J., Wooley, S. C., Woolbright, S. A., Lindroth, R. L., Whitham, T. G., Zak, D. R., and Hart, S. C. 2011. Forest gene diversity is correlated with the composition and function of soil microbial communities. *Popul. Ecol.* 53:35-46.
- Schweitzer, J. A., Madritch, M. D., Bailey, J. K., Leroy, C. J., Fischer, D. G., Rehill, B. J., Lindroth, R. L., Hagerman, A. E., Wooley, S. C., Hart, S. C., and Whitham, T. G. 2008. From genes to ecosystems: The genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. *Ecosystems* (N. Y.) 11:1005-1020.
- Shakya, M., Gittel, N., Castro, H., Yang, Z. M. K., Gunter, L., Labbé, J., Muchero, W., Bonito, G., Vilgalys, R., Tuskan, G., Podar, M., and Schadt, C. W. 2013. A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. *PLoS One* 8: 8e76382.
- Simms, E. L., Taylor, D. L., Povich, J., Shefferson, R. P., Sachs, J. L., Urbina, M., and Tegasczik, Y. 2006. An empirical test of partner choice mechanisms in a wild legume-rhizobium interaction. *Proc. R. Soc. B* 273:77-81.
- Subramoni, S., and Venturi, V. 2009. LuxR-family 'solos': Bachelor sensors/regulators of signalling molecules. *Microbiology* 155:1377-1385.
- Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J., and Van Der Lelie, D. 2009. Genome survey and

- characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl. Environ. Microbiol.* 75: 748-757.
- Timm, A. C., Halsted, M. C., Wilmoth, J. L., and Retterer, S. T. 2017. Assembly and tracking of microbial community development within a microwell array platform. *J. Vis. Exp.* 124:e55701.
- Timm, C. M., Campbell, A. G., Utturkar, S. M., Jun, S. R., Parales, R. E., Tan, W. A., Robeson, M. S., Lu, T. Y. S., Jawdy, S., Brown, S. D., Ussery, D. W., Schadt, C. W., Tuskan, G. A., Doktycz, M. J., Weston, D. J., and Pelletier, D. A. 2015. Metabolic functions of *Pseudomonas fluorescens* strains from *Populus deltoides* depend on rhizosphere or endosphere isolation compartment. *Front. Microbiol.* 6:1118.
- Timm, C. M., Carter, K. R., Carrell, A. A., Jun, S. R., Jawdy, S. S., Velez, J. M., Gunter, L. E., Yang, Z. M., Nookaew, I., Engle, N. L., Lu, T. Y. S., Schadt, C. W., Tschaplinski, T. J., Doktycz, M. J., Tuskan, G. A., Pelletier, D. A., and Weston, D. J. 2018. Abiotic stresses shift belowground *Populus*-associated bacteria toward a core stress microbiome. *mSystems* 3:e00070-17.
- Timm, C. M., Pelletier, D. A., Jawdy, S. S., Gunter, L. E., Henning, J. A., Engle, N., Aufrecht, J., Gee, E., Nookaew, I., Yang, Z. M., Lu, T. Y., Tschaplinski, T. J., Doktycz, M. J., Tuskan, G. A., and Weston, D. J. 2016. Two poplar-associated bacterial isolates induce additive favorable responses in a constructed plant-microbiome system. *Front. Plant Sci.* 7:497.
- Tsai, C.-J., Xu, P., Xue, L.-J., Hu, H., Nyamdari, B., Naran, R., Zhou, X., Goeminne, G., Gao, R., Gjersing, E., Dahlen, J., Pattathil, S., Hahn, M. G., Davis, M. F., Ralph, J., Boerjan, W., and Harding, S. A. 2020. Compensatory guaiacyl lignin biosynthesis at the expense of syringyl lignin in 4CL1-knockout poplar. *Plant Physiol.* 183:123-136.
- Tschaplinski, T. J., Abraham, P. E., Jawdy, S. S., Gunter, L. E., Martin, M. Z., Engle, N. L., Yang, X. H., and Tuskan, G. A. 2019. The nature of the progression of drought stress drives differential metabolomic responses in *Populus deltoides*. *Ann. Bot. (Lond.)* 124:617-626.
- Tsuzuki, S., Handa, Y., Takeda, N., and Kawaguchi, M. 2016. Strigolactone-induced putative secreted protein 1 is required for the establishment of symbiosis by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Mol. Plant-Microbe Interact.* 29:277-286.
- Tuskan, G. A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., Ralph, S., Rombauts, S., Salamov, A., Schein, J., Sterck, L., Aerts, A., Bhaleerao, R. R., Bhaleerao, R. P., Blaudez, D., Boerjan, W., Brun, A., Brunner, A., Busov, V., Campbell, M., Carlson, J., Chalot, M., Chapman, J., Chen, G. L., Cooper, D., Coutinho, P. M., Couturier, J., Covert, S., Cronk, Q., Cunningham, R., Davis, J., Degroove, S., Dejardin, A., Depamphilis, C., Detter, J., Dirks, B., Dubchak, I., Duplessis, S., Ehlting, J., Ellis, B., Gendler, K., Goodstein, D., Gribskov, M., Grimwood, J., Groover, A., Gunter, L., Hamberger, B., Heinze, B., Helariutta, Y., Henrissat, B., Holligan, D., Holt, R., Huang, W., Islam-Faridi, N., Jones, S., Jones-Rhoades, M., Jorgensen, R., Joshi, C., Kangasjarvi, J., Karlsson, J., Kelleher, C., Kirkpatrick, R., Kirst, M., Kohler, A., Kalluri, U., Larimer, F., Leebens-Mack, J., Leple, J. C., Locascio, P., Lou, Y., Lucas, S., Martin, F., Montanini, B., Napoli, C., Nelson, D. R., Nelson, C., Nieminen, K., Nilsson, O., Pereda, V., Peter, G., Philippe, R., Pilate, G., Poliakov, A., Razumovskaya, J., Richardson, P., Rinaldi, C., Ritland, K., Rouze, P., Ryaboy, D., Schmutz, J., Schrader, J., Segerman, B., Shin, H., Siddiqui, A., Sterky, F., Terry, A., Tsai, C. J., Uberbacher, E., Unneberg, P., Vahala, J., Wall, K., Wessler, S., Yang, G., Yin, T., Douglas, C., Marra, M., Sandberg, G., Van De Peer, Y., and Rokhsar, D. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313:1596-1604.
- Tuskan, G. A., Mewalal, R., Gunter, L. E., Palla, K. J., Carter, K., Jacobson, D. A., Jones, P. C., Garcia, B. J., Weighill, D. A., Hyatt, P. D., Yang, Y., Zhang, J., Reis, N., Chen, J. G., and Muchero, W. 2018. Defining the genetic components of callus formation: A GWAS approach. *PLOS One* 13:1-18.
- Uehling, J., Gryganskyi, A., Hameed, K., Tschaplinski, T., Misztal, P. K., Wu, S., Desirò, A., Pol, N. V., Du, Z., Zienkiewicz, A., Zienkiewicz, K., Morin, E., Tisserant, E., Splivallo, R., Hainaut, M., Henrissat, B., Ohm, R., Kuo, A., Yan, J., Lipzen, A., Nolan, M., Labutti, K., Barry, K., Goldstein, A. H., Labbé, J., Schadt, C., Tuskan, G., Grigoriev, I., Martin, F., Vilgalys, R., and Bonito, G. 2017. Comparative genomics of *Mortierella elongata* and its bacterial endosymbiont *Mycoavidus cysteinexigens*. *Environ. Microbiol.* 19: 2964-2983.
- Uehling, J. K., Entler, M. R., Meredith, H. R., Millet, L. J., Timm, C. M., Aufrecht, J. A., Bonito, G. M., Engle, N. L., Labbé, J. L., Doktycz, M. J., Retterer, S. T., Spatafora, J. W., Stajich, J. E., Tschaplinski, T. J., and Vilgalys, R. J. 2019. Microfluidics and metabolomics reveal symbiotic bacterial-fungal interactions between *Mortierella elongata* and *Burkholderia* include metabolite exchange. *Front. Microbiol.* 10:2163.
- Veach, A. M., Morris, R., Yip, D. Z., Yang, Z. K., Engle, N. L., Cregger, M. A., Tschaplinski, T. J., and Schadt, C. W. 2019. Rhizosphere microbiomes diverge among *Populus trichocarpa* plant-host genotypes and chemotypes, but it depends on soil origin. *Microbiome* 7:76.
- Wagner, M. R., Lundberg, D. S., Del Rio, T. G., Tringe, S. G., Dangl, J. L., and Mitchell-Olds, T. 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat. Commun.* 7:12151.
- Ware, I. M., Van Nuland, M. E., Schweitzer, J. A., Yang, Z., Schadt, C. W., Sidak-Lofits, L. C., Stone, N. E., Busch, J. D., Wagner, D. M., and Bailey, J. K. 2019. Climate-driven reduction of genetic variation in plant phenology alters soil communities and nutrient pools. *Glob. Change Biol.* 25:1514-1528.
- Whitham, T. G., Martinsen, G. D., Floate, K. D., Dungey, H. S., Potts, B. M., and Keim, P. 1999. Plant hybrid zones affect biodiversity: Tools for a genetic-based understanding of community structure. *Ecology* 80:416-428.
- Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M., Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S., and Kuske, C. R. 2003. Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology* 84:559-573.
- Wilmoth, J. L., Doak, P. W., Timm, A., Halsted, M., Anderson, J. D., Ginovart, M., Prats, C., Portell, X., Retterer, S. T., and Fuentes-Cabrera, M. 2018. A Microfluidics and agent-based modeling framework for investigating spatial organization in bacterial colonies: The case of *Pseudomonas aeruginosa* and H1-type VI secretion interactions. *Front. Microbiol.* 9:33.
- Wymore, A. S., Keeley, A. T. H., Yturralde, K. M., Schroer, M. L., Propper, C. R., and Whitham, T. G. 2011. Genes to ecosystems: Exploring the frontiers of ecology with one of the smallest biological units. *New Phytol.* 191:19-36.
- Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., Van Der Lelie, D., and Gilbert, J. A. 2015. The soil microbiome influences grapevine-associated microbiota. *MBio* 6:e02527-14.
- Zgadaj, R., Garrido-Oter, R., Jensen, D. B., Koprivova, A., Schulze-Lefert, P., and Radutoiu, S. 2016. Root nodule symbiosis in *Lotus japonicus* drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities. *Proc. Natl. Acad. Sci. U.S.A.* 113:E7996-E8005.
- Zhang, L. L., Jia, Y. T., Wang, L., and Fang, R. X. 2007. A proline iminopeptidase gene upregulated in planta by a LuxR homologue is essential for pathogenicity of *Xanthomonas campestris* pv. *campestris*. *Mol. Microbiol.* 65:121-136.
- Zhang, Q. J., and Gao, L. Z. 2016. The complete chloroplast genome sequence of desert poplar (*Populus euphratica*). *Mitochondrial DNA Part A* 27:721-723.
- Zhang, X. X., Wang, B. Y., and Liu, Z. W. 2019. Impacts of plant secondary metabolites from conifer litter on the decomposition of *Populus purdomii* litter. *J. For. Res.* 30:2237-2245.