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, highlighting 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* species. Further, 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 impact ecosystem sustainability and processes as *Populus* trees function as foundational species in many natural ecosystems and are also deployed in manged ecosystems for various agroforestry applications.

Introduction

A basic premise of systems biology is that biological units (e.g., organisms, cells, 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). While 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). As 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 and/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 et al. 2015). However, the broader goal of manipulating these interactions will require focused genes-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 2003). For example, genetic variation within a keystone species can impact 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 (g) to ecosystem (e) interactions because 1) *Populus* 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, 2) *Populus* is largely undomesticated and its outcrossing nature combined with a propensity for hybridization yields immense genetic and phenotypic diversity, 3) *Populus* are important foundational species in riparian ecosystems, and 4) variation in the *Populus*-microbe system results in altered ecosystem process rates, such as primary productivity.

Generally, all *Populus* species and hybrids can be vegetatively propagated with ease from dormant cuttings (Merkle et al. 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 "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* species and a hybrid poplar (Tuskan et al. 2006; Zhang et al. 2016) [https://phytozome.jgi.doe.gov, https://phytozome.jgi.doe.gov, https://phytozome.jgi.doe.gov, http://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*.

Inter-specific 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 *P. 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 >28M single nucleotide polymorphisms (SNPs) 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 (Pinosio et al. 2016; Chhetri et al. 2019) 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 (Porth et al. 2013; McKown et al. 2014; Muchero et al. 2015; Fahrenkrog et al. 2017; Li et al. 2018), and overall growth and phenological traits (Evans et al. 2014; Chhetri et al. 2019).

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 species *Laccaria bicolor*, the arbuscular mycorrhizal species *Rhizophagus*

116 *irregularis*, the rust pathogen *Melampsora larici-populina*, and several other species (Martin et
117 al. 2004). These resources allowed the development of a "mesocosm" of model species across
118 kingdoms for understanding the genetic basis of plant-microbe interactions and to link specific
119 plant genes to associated microbial genes. Currently, there are over 5,000 isolates in culture that
120 are representative of the *Populus'* microbiome. Members include key bacterial (>3000 isolates)
121 and fungal (>2,000 isolates – Bonito et al. 2016) groups associated with wild and field-grown
122 trees.

123 From these recent microbial collections, over 550 *Populus* bacterial and 65 fungal isolate
124 genomes have been sequenced, largly in collaboration with the Department of Energy - Joint
125 Genome Institute (Taghavi et al. 2009; Brown et al. 2012a; Brown et al. 2012b; Klingeman et al.
126 2015; Bonito et al. 2016; Uehling et al. 2017; Blair et al. 2018; Levy et al. 2018; Looney et al.
127 2018) and the genome sequences in this escalating resource are representative of the current
128 *Populus* microbiome. Further, they are an expansion of the original model fungal symbionts
129 including the ectomycorrhizal fungi (ECM) *Laccaria bicolor*, which has largely served as a
130 research platform for understanding the genetic basis of ECM symbioses (Martin et al. 2004;
131 Plett et al. 2011; Pellegrin et al. 2019). These isolates and genome sequences are now beginning
132 to facilitate broader constructed community experiments (Bible et al. 2016; Timm et al. 2016)
133 and comparative genomic analyses (Schaefer et al. 2013; Timm et al. 2015; Jun et al. 2016; Levy
134 et al. 2018; Martino et al. 2018; Pereira et al. 2018).

135 *Populus* and its microbes are one of the most widely studied perennial plant systems and
136 can therefore facilitate understanding of the genomic underpinnings of other plant-microbe
137 systems in the context of important ecosystem functions. Various *Populus* species serve as
138 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

While 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; Rasmann et al. 2016). LCOs have been studied extensively in arbuscular mycorrhizal (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 (Labbe 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 arbuscular mycorrhizal fungi in Glomales but is indeed 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 fungus *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 (Plett et al. 2011; Pellegrin et al. 2019). 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 to effectively colonize *Populus* roots and form the characteristic symbiotic interface of these interactions, i.e., the Hartig net (Pellegrin et al. 2019). While 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 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 (VOCs) also play important roles in microbial interactions and communications with the host-plant at larger spatial scales than LCOs and SSPs (Schulz et al. 2007; Blom et al. 2011; Bitas et al. 2013). Using *Populus*-derived genome sequences of the above mentioned ecto- and endo-mycorrhizal 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 *Phytophtora 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.

Like 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 et al. 1976; Gaworzewska et al. 1982; de Weert et al. 2002; Feng et al. 2018). 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 et al. 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 bacterial-derived signals (Subramoni et al. 2009; Gonzalez et al. 2013). This inter-kingdom signaling system was first described for the LuxR homologs XccR from *Xanthomonas campestris* pv. *campestris* and OryR from *Xanthomonas 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 (Schaefer et al. 2013; Coutinho et al. 2018). 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 to non-plant 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* has also revealed genes and pathways that are required for efficient plant colonization (Lugtenberg et al. 2001; Cole et al. 2017). 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 impacted 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 *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 to those of other plant hosts (Ottesen et al. 2013; Pinto et al. 2014; Peay et al. 2016). 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 like 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 2019) (Figure 1). These systems have been used to examine the colonization behavior of green fluorescent protein (GFP)-expressing *Pantoea* sp. YR343, as well as other genetically tractable *Populus* microbial isolates such as *Variovorax*, *Methylibium*, and *Caulobacter* (Figure 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; Massalha et al. 2017; Aufrecht et al. 2018), fungal-microbe interactions

253 (Millet et al. 2019; Uehling et al. 2019), and microbial community development (Hansen et al.
254 2016; Timm et al. 2017; Wilmoth et al. 2018).

255 Using these platforms, the colonization behaviors of two *Populus* isolates, *Pantoea* sp.
256 YR343 and *Variovorax* sp. CF313, have been compared and quantified (Aufrecht et al. 2018).
257 The impact of co-colonization was examined, and it was noted that these two bacterial species
258 form spatially distinct associations along developing root tissues. While there did not appear to
259 be any exclusion or co-localization effects, there was a clear impact of the faster growing
260 *Variovorax* sp. CF313 on the ultimate colonization patterns of *Pantoea* sp. YR343 (Aufrecht et
261 al. 2018). Moreover, changes in root phenotype from these bacterial treatments depended both on
262 the initial concentrations and species of the bacterial population. Such distinct colonization
263 patterns suggest that chemical and physical cues, which promote microbial recruitment,
264 attachment, and development, occur at fine scales within the host-symbiont interface.

265 This fine-scale spatial variation within the root microbiome can play an important role in
266 maintaining beneficial plant-microbe interactions. In a phenomenon known as Simpson's
267 Paradox (Chuang et al. 2009), the abundance of mutualistic strains in a system can increase
268 globally, even if mutualists are locally outcompeted in all subcommunities. In a synthetic
269 microbiome, altruism was selected for at the population level as producers generated common
270 goods despite production costs at the individual level (Chuang et al. 2009). This effect requires
271 local heterogeneity in community composition, rewards for community cooperation that
272 accumulate within the local microbial community, and periodic redistribution (but not
273 homogenization) between local communities. Each of these conditions is plausible in the
274 *Populus* rhizosphere, but further research is necessary to examine these hypotheses.
275

Microbiome variability across *Populus* species

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 (Lundberg et al. 2012; Bodenhausen et al. 2013; Zarraonaindia et al. 2015; Wagner et al. 2016). Recently described efforts have thoroughly characterized the microbiomes of numerous plant species including *Populus* (Cregger et al. 2018), *Arabidopsis* (Lundberg et al. 2012; Bodenhausen et al. 2013), rice (Edwards et al. 2015), grape (Zarraonaindia et al. 2015; Bokulich et al. 2016), eucalyptus (Santiago et al. 2015), sugarcane (Armanhi et al. 2016), legumes (Zgadzaj et al. 2016; Hartman et al. 2017), 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* species harbor a great deal of microbial diversity, four bacterial phyla, Proteobacteria, Actinobacteria, Bacteriodetes and Firmicutes, have been shown to represent roughly 40-50%, 15-20%, 5-10% and 1-3%, respectively, of the bacterial community across multiple *Populus* species and genotypes (Shakya et al. 2013; Bonito et al. 2014; Cregger et al. 2018) and 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* selectively recruits fungal and bacterial associates from soil microbiomes that are different from other plant species (*e.g.*, *Quercus* or *Pinus*) when grown with common innocula under greenhouse conditions (Bonito et al. 2014), where oak and pine were dominated by only a few ectomycorrizal taxa and *Populus* contained a more diverse endophytic array of taxa. Furthermore, individual genotypes from closely related species such as *P. trichocarpa, P.*

299 *deltoides,* and their hybrids show significant differences in their microbial composition when
300 grown in common field environments. Interestingly, *P deltoides* had higher fungal diversity
301 relative to hybrid trees (Cregger et al. 2018). These observations of host-dependent differences in
302 microbiome structure seem common across study systems with comparable microbiome
303 observations in animal systems (Goodrich et al. 2016; Lloyd-Price et al. 2017a; Lloyd-Price et al.
304 2017b).

305 Variation in the *Populus* microbiome across *Populus* species and genotypes may be
306 driven by differences in defense-related traits, such as biosynthesis of phenolic glycoside and
307 other plant metabolites (Lindroth et al. 2013). Recent research shows these traits may have some
308 influence on fine tuning microbiome composition in *Populus trichocarpa* (Veach et al. 2019) and
309 fungal endophyte colonization in *P. fremontii*, *P. angustifolia,* and their hybrids (Bailey et al.
310 2005). Within *P. deltoides*, salicylic acid concentrations have been shown to fluctuate in
311 response to abiotic stress (Jung et al. 2009; Tschaplinski et al. 2019). Further, these defense
312 compounds have been shown to fluctuate across *Populus* genotypes with implications for the
313 ability to defend against herbivores (Lindroth et al. 2013). While it is clear changes in plant traits
314 alter microbiome composition, it is unclear how these fine scale processes result in variation in
315 plant-microbe interactions across larger spatial scales.

316 Variation in microbiome composition within *Populus* species is often apparent across
317 landscapes and species native ranges (Shakya et al. 2013; Barge et al. 2019; Ware et al. 2019).
318 Variance partitioning approaches have shown that while the majority of community composition
319 remains unexplained, soil chemical and physical characteristics, geographic distance, climatic
320 gradients, and season of sampling explain greater variance in microbiome composition than tree
321 genotype within *P. deltoides* species (Figure 3) (Shakya et al. 2013; Barge et al. 2019). These

patterns of relatively low intra- vs inter-specific local variation and higher intra-specific variability across landscapes have also been shown in other plant species and ecosystems (Redford et al. 2010). Interestingly, inter-specific variability in microbiome traits is more obvious in controlled greenhouses and common gardens (Busby et al. 2016a; Busby et al. 2016b; 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 the review. While phylogenetic surveys accomplish a critical first step in revealing who is present in the microbiome in natural and controlled, and show that composition can depend on and respond to environmental conditions, host genotype/phenotype, and environmental stressors, a challenge remains in determining how particular microbes are selected and how they collectively function in concert with *Populus*.

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 (Kaschuk 2009; Lau et al. 2012; Timm et al. 2018; Clark et al. 2019; Friel et al. 2019). 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

345 species interactions, such as when photosynthetic limitations force the plant to redirect C from
346 mutualists to other plant C sinks like growth, respiration, and storage (Pringle et al. 2016). Thus,
347 how plants interact with their associated microbial symbionts in a given environment can in part
348 be determined by C fixation potential (i.e., photosynthetic rates) and C allocation patterns. At the
349 same time, the rate of C fixation within a plant can be altered by the microbial constituents living
350 on and within the plant, highlighting the bidirectional nature of this symbiosis.

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

361 Plant genetic control of root exudate profiles and litter chemistry can also have
362 significant impacts on the soil microbial community and on resulting ecosystem functions like
363 decomposition and nutrient cycling. For example, condensed tannin concentrations in *Populus*
364 alter the composition of the soil microbial community and subsequently rates of decomposition
365 (Schweitzer et al. 2004; Bailey et al. 2005; Schweitzer et al. 2008). Similarly, genotypic
366 variation in *Populus* leaf senescence and phytochemistry alter decomposition rates (Lindroth et
367 al. 2002). Often, *Populus* genetic diversity in mixed stands influences soil microbial community

368 composition and exoenzyme activity (Schweitzer et al. 2011). While these studies clearly show
369 higher order consequences of the interactions, fingerprinting methods used at the time did not
370 allow a robust understanding of the phylogenetic microbial community changes associated with
371 them. How these plant level controls scale, through the microbial community, to alter complex
372 ecosystem functions such as nutrient cycles across diverse ecosystems clearly requires futher
373 investigation.
374
375 **Conclusion**
376 The enormous wealth of information available about *Populus* and its microbiome
377 provides an ideal test bed to examine complex plant-microbe interactions from the gene to
378 ecosystem levels. As highlighted, variation in the genes of *Populus* and its symbionts alter which
379 relationships establish and persist, which has consequences that can scale to ecosystem level
380 functions. While *Populus* may be an excellent model to address many of these genes to
381 ecosystems questions, we recognize that there are caveats to keep in mind when using this
382 system. For studies requiring rapid turnaround times, *Populus* may not work well, because
383 growing trees to maturity for experimentation can take years. Furthermore, our understanding of
384 how conclusions drawn from young trees translate as the tree ages is incomplete. Even with the
385 prior research efforts highlighted in this review, there are numerous unanswered questions at
386 each biological scale, and between scales, that will need to be tackled to fully understand how
387 plants and microbes interact within ecosystems and how these interactions alter ecosystem
388 structure and function. Do microorganisms influence plant establishment and persistence in
389 natural ecosystems? How do multiple environmental drivers influence the balance of costs and
390 benefits with mutualists? Does a long-lived woody-perennial tree continually change mutualists?

391 A mechanistic understanding of plant-microbe interactions is critical as we attempt to manipulate
392 these interactions to enhance plant growth and health. Further, understanding these relationships
393 in perennial systems is especially important because they may be more likely to have
394 consequential influences on ecosystem dynamics over time.
395
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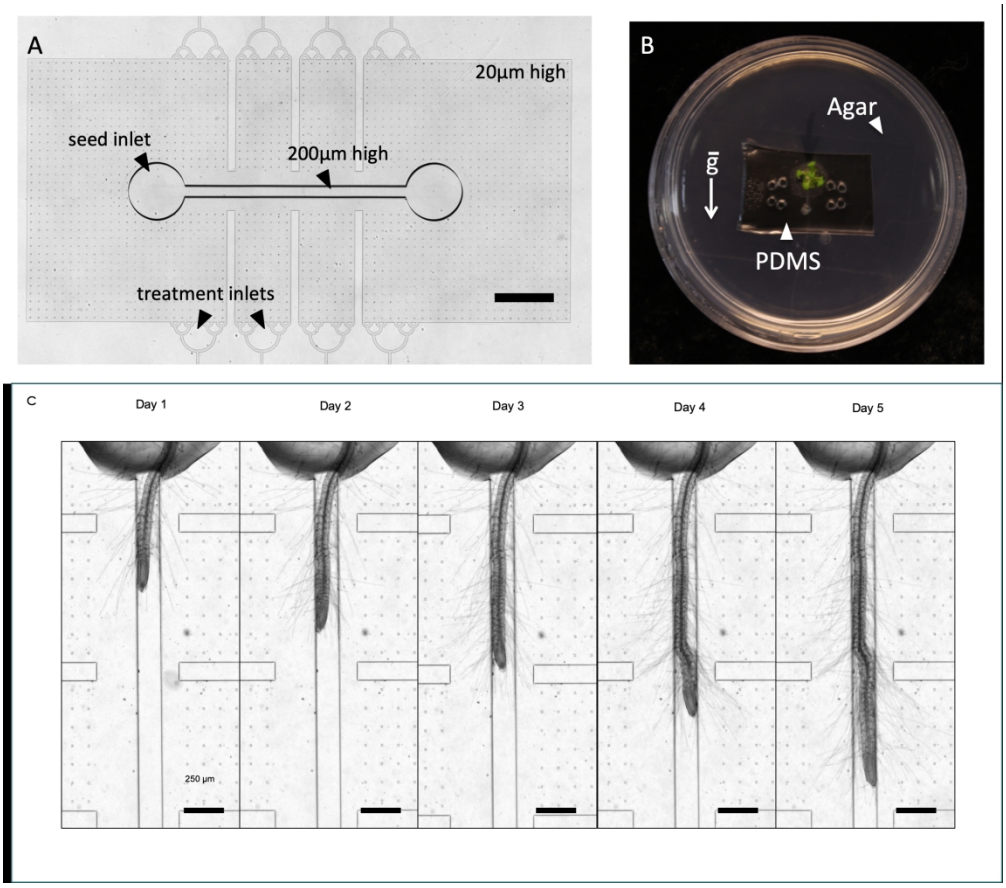
Figure 1: Growth of *Arabidopsis thaliana* in a two-layer microfluidic platform. (A) Device consists of two layers to confine root hairs to the same imaging plane as the main root (scale 1mm). Seedlings may be (B) contained in a gnotobiotic environment, (C) and monitored for growth for the duration of co-culture experiments (scale bar = 250 μm).

Figure 2. Visualizing the dynamics of microbial colonization. (A) *Populus trichocarpa* cutting growing in a 3D printed imaging chamber. (B) *Pantoea* sp. YR343 expressing GFP (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 μm).

Figure 3. Variance partitioning of bacterial (A) and fungal communities (B) 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/spatial distance (orange), host genotype (purple), season of sampling (pink) and beta 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.



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 1mm). Seedlings may be (B) contained in a gnotobiotic environment, (C) and monitored for growth for the duration of co-culture experiments (scale bar = 250 µm).

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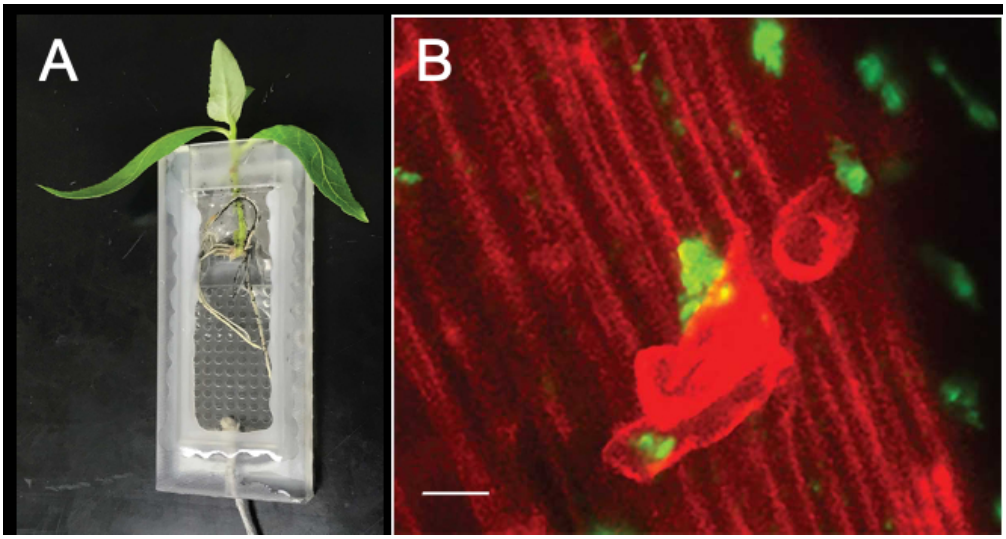

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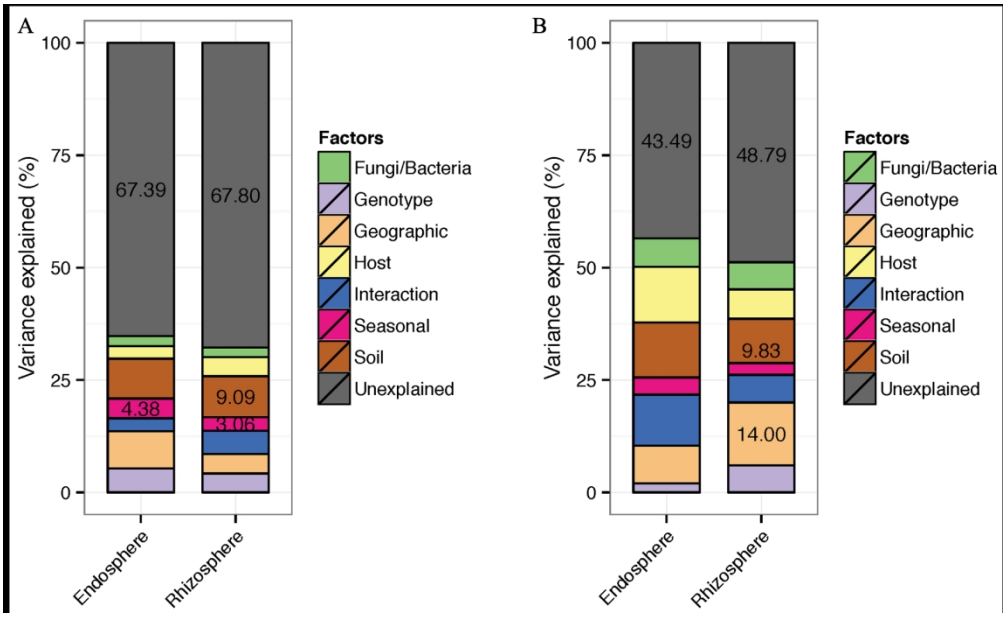

Variance partitioning of bacterial (A) and fungal communities (B) from the root endosphere and soil rhizosphere of Populus deltoides studied in two river systems in Tennessee and North Carolina. Colors represent covariates in the model: soil properties (brown), host properties (yellow), geographic/spatial distance (orange), host genotype (purple), season of sampling (pink) and beta 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.

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