Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome

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Summary:

Interactions between trees and microorganisms are tremendously complex and the multispecies networks resulting from these associations have consequences for plant growth and productivity. However, a more holistic view is needed to better understand trees as ecosystems and superoorganisms; where many interacting species contribute to the overall stability of the system. While much progress has been made on microbial communities associated with individual tree niches and the molecular interactions between model symbiotic partners, there is still a lack of knowledge of the multi-component interactions necessary for holistic ecosystem-level understanding. We review recent studies in *Populus* to emphasize the importance of such holistic efforts across the leaf, stem and rooting zones, and discuss prospects for future research in these important ecosystems.

Keywords: Microbiome, *Populus*, Trees, Mycorrhizas, Endophytes, Bacteria, Fungi

Introduction

Populus trichocarpa was the first tree species genome sequenced **(Tuskan *et al.*, 2006)** and the ability to study genetically tractable *Populus* trees in greenhouses and plantation agroecosytems, as well as in natural ecosystem settings, make *Populus* spp. a powerful system better understanding of plant-microbe relationships. Ectomycorrhizas and arbuscular mycorrhizas both occur within *Populus (***Karlinski *et al.*, 2010)** and *Populus* host genetic variation may influence structure and composition of surrounding plants, soils, and overall ecosystem functions **(**e.g. **Fisher et al. 2007; 2010 & 2014).** Recognizing this importance, a decade ago as the *Populus* genome neared completion, Martin *et al*. (2004) called the community to begin comparable efforts to sequence and study the *Populus* symbiont "mesocosm". They argued for consideration of trees as ecosystems unto themselves and increased understanding of their symbiotic interactions at both holistic levels, and as genome-enabled model systems. In this paper, we discuss the tremendous recent progress and future potential of such efforts across the *Populus* ecosystem (Figure 1).

The root endosphere and rhizosphere microbiome:

Diversity, structure and community level perspectives

A variety of recent studies have examined the root mycorrhizal components of the microbiome in *Populus*. A general focus of many of these studies has been contrasting the communities associated with wild-type and transgenic clones. For example three studies (**Kaldorf *et al.*, 2002; Stefani *et al.*, 2009; Danielson**

***et al*., 2012**) have examined both bulk soil and root fungal populations independently in plantations with different transgenic *Populus* lines. Each of these studies finds no effects of the transgene clones on fungal communities but generally high levels of fungal diversity in association with poplar roots. A few recent whole-microbiome level investigations in natural populations and variants of *P. deltoides* have now included simultaneous examination of both bacteria and fungi in the same sampled environments and experiments, as well as for both the rhizosphere and root endosphere habitats (**Gottel *et al*., 2011; Shakya *et al*., 2013; Bonito *et al*., 2014**). Such studies have done well to begin elucidating how these different plant habitats/niches effect microbial membership, and to begin to disentangle how host, environmental, soil and geographic factors influence each of these *Populus*-associated community types (**Shakya *et al*., 2013; Bonito *et al*., 2014**). Similar results are now being found in a variety of host systems with the widespread application of pyrosequence-based approaches; and patterns of host specificity, host fitness effects, geographic substitution and heritability are now emerging (**Bonito *et al*., 2014; Lundberg *et al*., 2012; Peiffer *et al*., 2013; Talbot *et al*., 2014; Wagner *et al*., 2014**). These studies in both *Populus,* as well as *Arabidopsis* and *Zea* systems have demonstrated that within a host species, habitat (e.g. endosphere vs rhizosphere) and soil type, rather than within species genetic background, have larger effects on overall structure of the microbiome (**Bonito *et al*., 2014; Bulgarelli *et al*., 2012; Lundberg *et al*., 2012; Peiffer *et al*., 2013; Shakya *et al*., 2013**), but the balance of the effects of genetic and soil factors within host habitats on bacteria and fungi is less clear.

Evidence from natural systems, soil inoculum assays, and pairwise colonization assays are suggesting that perhaps due to its often weak ECM nature, root endophytic organisms may be particularly important for *Populus* compared to other ECM trees and result in higher levels of microbiome diversity due to increased niche space *(***Bonito *et al.* 2014; Tschaplinski *et al*. 2014***)***.*

A systematic understanding of how overall rhizosphere communities and their members differ from or complement each other in terms of functioning within the plant and across plant and tree taxa is still lacking. However, meta-analysis and synthesis studies that collectively analyze and compare such communities should now be possible with the widespread adoption of community databasing and standards in microbiome sequence studies (**Yilmaz *et al*., 2011**)

Specific interactions, mechanisms and function

While the basic functions of mycrorrhizas in terms of nutrient and water acquisition are known, the specific detailed signaling mechanisms involved in the formation and functioning of both ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) symbiosis had remained elusive. Genome-enabled studies using the *Laccaria-Populus* system, have led to several insights in this area and suggest mutual signaling mechanisms allow recognition, initiation and reorganization of the symbiotic root organ. Particularly surprising has been the role that small secreted proteins play. Mycorrhizal Induced Small Secreted Protein-(MiSSPs) - MiSSP7 production in *Laccaria,* appears to be induced by

unknown exudates from *Populus* roots (**Plett *et al*., 2011, Plett and Martin, 2012**). MiSSP7 in turn migrates to the plant nuclei and alters the hormonal balance of the plant defense system, allowing mycorrhizal formation to proceed (**Plett *et al*., 2014**). However these detailed patterns of recognition may be species specific even within host *Populus* species. While the above recognition mechanism is effective in *Populus trichocarpa,* in *Populus deltoides* the host defensive system is not effectively suppressed by *Laccaria* and ECM formation does not proceed (**Tschaplinski *et al*., 2014**). Future investigations will need to further explore the phylogenetic distributions of such signaling interactions both with closely related model species and across diverse host-fungal systems, to gain insight into the varying patterns of species specificity and generalist phenomena. The recent completion of the genome sequence of the AM fungus *Rizophagus irregularis* (ex *Glomus*) (**Tisserant *et al*., 2013**) may similarly provide clues necessary to accelerate such research into the functioning of AM systems. Additionally, the use of *Populus* as a host for such studies, with its ability to form both AM and ECM symbioses, should provide insight into the largely unanswered questions of why and under what conditions *Populus* forms both types of symbioses. While there appear to be both genetic and environmental influences on alternation between the two symbiosis modes in *Populus* (**Gehring *et al*., 2006; Karlinski *et al*., 2010; Lodge, 1989**), the detailed mechanisms and *in planta* functioning of such dual symbioses are still unclear.

133 Beyond mycrorrhizal symbionts, *Populus* is also host to a variety of bacterial and
134 fungal rhizosphere partners and root endophytes. Indeed, several studies have
135 shown putative mycorrhizal fungal taxa on and within *Populus* to be outnumbered
136 by other root endopyhtic fungi such as *Atractiella*, *Phialophora, Illyonectria* and
137 *Mortierella* spp. (**Gottel *et al*. 2011; Shakya *et al*. 2013; Bonito *et al*. 2014**).
138 Therefore, elucidating the full potential of microbiome effects on tree growth,
139 health and reproduction also depends on understanding these often neglected
140 plant-microbe interactions. Bacterial endophytes have been shown to have
141 varying functions in altering root branching/allocation patterns through production
142 of plant hormone precursors such as Indole Acetic Acid (IAA) (**Dimpka *et al*.,**
143 **2012; Weyens *et al*., 2012**), transformation and mobilization of nutrients such as
144 nitrogen and phosphorus (**Brown *et al*., 2009**), enhanced mycorrhizal formation
145 (e.g. Mycorrhizal Helper Bacteria) (**Deveau *et al*., 2007**; **Zhao *et al*., 2014**), and
146 aid in pathogen resistance through competitive exclusion or production of
147 antibiotics, (**Lugtenberg *et al*., 2001**) or priming of plant immune responses
148 (**Weston et al., 2012**). None of these effects however seem to be mutually
149 exclusive, as various isolates of even a single genera or species complex such
150 as *Pseudomonas fluorescens,* seem capable of many of these functions, as well
151 as pathogenic effects (**Weston *et al*., 2012**).
152
153 **The phyllosphere and leaf endosphere:**
154 Diversity, structure and community level perspectives

155 The interaction between plants and their associated phyllosphere microbial
156 communities has received growing attention during the last decade (**Vorholt,**
157 **2012**). Microbial diversity and community structure has been described in several
158 woody plant species (**Jumpponen and Jones, 2009; Redford *et al*., 2010**;
159 **Finkel *et al*., 2011**; **Cordier *et al*., 2012; Coince *et al*., 2014**) but our knowledge
160 of the structure of both fungal and bacterial communities associated with poplar
161 leaves remains fragmented. Culture-independent approaches indicate that host
162 genotype is an important factor structuring both fungal and bacterial communities
163 in poplar leaves and suggest that phyllosphere microbial community assemblage
164 is at least partially determined by host genetic variation (**Bálint *et al*., 2013,**
165 **Ulrich *et al*., 2008)**. Consistent with a possible enrichment of infrequent fungal
166 species in the phyllosphere community of trees (**Unterseher *et al*., 2011**), the
167 poplar leaf fungal community was found to be very diverse and is represented by
168 few abundant taxa and numerous rare taxa (**Bálint *et al*., 2013)**. Although the
169 phyllosphere bacterial community of poplar can vary over the growing season
170 (**Redford *et al*., 2009**), the general structure consisting of the dominance of
171 *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* is not strikingly different from
172 the pattern detected for other plant species including angiosperms, grasses and
173 *Arabidopsis*, suggesting an overall conserved structure that is defined by
174 relatively few bacterial phyla (**Ulrich *et al*., 2008; Redford *et al*., 2010**;
175 **Bodenhausen *et al*., 2013; Bulgarelli *et al*., 2013**).
176

177 Integrated approaches are needed to understand processes responsible for
178 determining the structure and assembly rules of phyllosphere communities. One
179 approach recently used various *Arabidopsis* mutants, revealed that cuticular wax
180 and ethylene can significantly affect community composition of phyllosphere
181 bacteria (**Reisberg *et al*., 2013**; **Bodenhausen *et al*., 2014**). In addition, a
182 comprehensive survey of the topographical distribution of fungi and bacteria
183 across various organs of individual tree species is still needed to better
184 understand tissue-type specificity of microbial community assemblages. Finally,
185 recent studies indicate that in addition to the host plant, synergistic, beneficial
186 and antagonistic interactions among microbes may have tremendous impacts on
187 microbial community structure and function in both the phyllosphere and the
188 rhizosphere (**Frey Klett *et al*., 2011**; **Kemen *et al*., 2014**). Therefore,
189 understanding both leaf- and root-associated microbiota structure also rely on the
190 understanding of more complex interactions, where fungal, oomycetes and
191 bacterial communities are not considered as separated entities but as active
192 drivers of microbial community assemblages.
193
194 <u>Specific interactions, mechanisms and function:</u>
195 Although the structure and diversity of bacterial and fungal communities
196 associated with the leaves of woody plants species have been reported, the
197 associated functions remain poorly characterized. It has been recently shown
198 that different fungal endophytes isolated from poplar leaves naturally infected by
199 the poplar rust fungus *Melampsora* can dramatically reduce rust symptoms

severity under laboratory conditions and significantly contribute to quantitative resistance to the foliar rust pathogen (**Raghavendra and Newcombe, 2013**). Interestingly however, some of these same endophytes do not show similar effects against other *Populus* pathogens **(Busby *et al.* 2013).** Strikingly, root-associated microbiota members are also known to induce systemic responses in leaves, resulting in increased resistance to plant pathogens (**Kurth *et al*., 2014; Weston *et al.,* 2012**) and herbivory (**Badri *et al*., 2013**). These selected examples illustrate why a more holistic understanding of plant disease is needed to better understand beneficial interactions across the plant microbiome (**Van der Putten *et al*., 2001**).

The stem and wood microbiome:

While the rhizosphere and phyllosphere have received considerably more attention as microbial habitats, there is increasing evidence that microorganisms inhabiting the heartwood tissues within some woody plants such as *Populus* may have high importance that has been to date unfairly neglected **(Knoth *et al*., 2014**). In *Populus*, many conifers, and other important forest tree species; the heartwood has no living parenchyma cells and only saturated xylem tissues (e.g. wetwood) that can lead to anaerobic conditions favoring fermentation or even methanogenesis (**Zeikus and Ward, 1974**). Prior reports suggested that communities associated with both *Populus trichocarpa* and *P. deltoides* also have the potential to fix nitrogen in these niches as evidenced by acetylene reduction assays **(Schink *et al*., 1981; Kamp, 1986).** Numerous diazotrophic

223 bacteria have been isolated from such habitats. Cross inoculation experiments
224 have shown broad growth promoting effects of these organisms on other plant
225 species, including non-woody plants such as rice and maize (**Govindarajan *et***
226 ***al*., 2008; Knoth *et al*., 2013**) and imply bacterial genera including *Burkholderia,*
227 *Rhizobium, Enterobacter*, and *Paenibacillus* (**Doty *et al*., 2009; Scherling *et al*.,**
228 **2009**) and isolates often show the ability to reduce N_2 in pure cultures outside the
229 host. Isotopic studies from ^{15}N in *P. trichocarpa* inoculated with consortia of
230 bacteria species, show signatures indicative of active fixation and that wetwood
231 may account for up to 65% of the N in leaf tissues **(Knoth *et al*., 2014**).
232 Culturable fungal endophytes have also recently been examined within the
233 woody tissues of branches of *P. angustifolia (***Lamit *et al.,* 2014***).* While
234 functional aspects have not been examined, it is clear from this first work that
235 even the simple communities within woody tissues can be influenced by tree
236 genotype. Additionally, many of the fungal genera identified seem to overlap with
237 those commonly found within leaf and root endophyte habitats.
238
239 Despite indications of the high importance of heartwood habitat, all knowledge to
240 date comes from studies of individual bacterial and fungal isolates, and a few
241 studies of defined consortia. Interestingly there is some indication that these
242 mixed consortia of organisms show differing effects and sometimes more robust
243 growth promotion (**Knoth *et al*., 2014, Knoth *et al*., 2013**) and speculated to be
244 due to increased niche colonization. However microbiome, metagenome, or
245 even Sanger sequencing-based surveys of microbial populations within woody

habitats are lacking. *In planta* localization of N-fixing bacteria has yet to be visualized via FISH or other methods. The use of combinations of advanced microscopy and isotopically resolved mass spectroscopy techniques, such as NanoSIMS, could potentially be very useful (**Pett-Ridge and Weber, 2012**). Given these tantalizing results, and the potential importance of alternative mechanisms of N fixation, microbiome studies of heartwood should be prioritized.

Toward understanding microbiome functions in a community context

Interactions between trees and their associated microbial communities are tremendously complex and the resulting multiorganismal networks have central roles for plant growth and productivity (**Bonfante and Anca, 2009**). A more holistic view of plant health and disease is needed to better understand these "superorganisms", in which interacting species are thought to play a role in the overall stability of the system. Similar to the human microbiota, disruption of the homeostasis between plants and their associated fungal and bacterial communities may alter the stability of the system, with potential impacts on host fitness (**Frey-Klett *et al.*, 2011**). Although culture-independent methods have tremendously contributed to our understanding of tree-associated fungal and bacterial community structures, the study of microbiota functions in a community context remains challenging because of the inherent noise of plant-associated microbial communities seen in nature. One reductionist approach to overcome this limitation is the use of reciprocal transplantation experiments, where plants are moved from one environment to another environment or grown with the same

soil inoculum under controlled conditions. Such an approach has been recently used to decipher the role of soil biota in plant adaptation, revealing that plants are not limited to adapt or migrate, but perhaps utilize microbial consortia to adapt to a novel or disturbed environment (**Lau and Lennon, 2012**; **Gundale *et al*., 2014**). Alternatively, extraction of presumably intact communities from different soil types has also been used to test how distinct environmental microbiomes can alter plant flowering phenology and represents a promising way to search for microbial consortia that alter biological characteristics of interest (**Wagner *et al*., 2014**). Finally, extensive reference culture collections of plant-associated fungal and bacterial stains isolated from model plant species are currently being established and will provide in the near future an inestimable resource for assembling taxonomically defined microbial communities with increasing complexity (**Brown *et al*., 2012**; **Lebeis *et al*., 2012, De Roy *et al*., 2013**). The modularity of synthetic communities has already provided new insights into the structure and the function of plant-associated microbiota (**Rolli *et al*., 2014**; **Bodenhausen *et al*., 2014; Knoth *et al*., 2014**). The assembly of more complex defined microcosms that better mimic environmental microbiomes will aid in 1) understanding the dynamics of host colonization by complex root- and leaf-associated microbial communities, 2) deciphering the contribution of plant-microbe and microbe-microbe interactions in the structuring of microbial consortia and 3) identifying complex microcosms that promote host fitness when exposed to biotic or abiotic stressors. While studies in *Populus* have been informative in their own right, they will become of increasing interest as a

292 comparison for new models such as Eucalyptus, Pine, and others come online
293 now and in the future.

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References

Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM. 2013. Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytologist* **198**: 264-273.

Bálint M, Tiffin P, Hallström B, O'Hara RB, Olson MS, Fankhauser JD, Piepenbring M, Schmitt I. 2013. Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). *PLoS One* **8**: e53987.

Bodenhausen N, Horton MW, Bergelson J. (2013). Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. *PLoS One* **8**: e56329.

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA. 2014. A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genetics* **10**: e1004283.

Bonfante P, Anca IA. 2009. Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annual Review of Microbiology* **63**: 363-383.

Bonito G, Reynolds H, Robeson M, Nelson J, Hodkinson B, Tuskan G, Schadt CW Vilgalys R. 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Molecular Ecology* **23:** 3356-3370

Browne P, Rice O, Miller SH, Burke J, Dowling DN, Morrissey JP, O'Gara F. 2009. Superior inorganic phosphate solubilization is linked to phylogeny within the Pseudomonas fluorescens complex. *Applied soil ecology* **43:** 131-138.

Brown SD, Utturkar SM, Klingeman DM, Johnson CM, Stanton M, Land ML, Lu TY, Schadt CW, Doktycz MJ, Pelletier DA. 2012. Twenty One *Pseudomonas* Genomes and Nineteen Genomes from other Diverse Bacteria Isolated from the Rhizosphere and Endosphere of *Populus deltoides. Journal of Bacteriology* **194:** 5991-5993

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* 488: 91-95.

Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64**: 807-838.

Busby PE, Zimmerman N, Weston DJ, Jawdy SS, Houbraken J, Newcombe G. 2013. Leaf endophytes and Populus genotype affect severity of damage from the necrotrophic leaf pathogen, *Drepanopeziza populi. Ecosphere* **4:** 125

Coince A, Cordier T, Lengellé J, Defossez E, Vacher C, Robin C, Buée M, Marçais B. 2014. Leaf and root-associated fungal assemblages do not follow similar elevational diversity patterns. *PLoS One* **9**: e100668.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. 2012. The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. *New Phytologist* **196**: 510-519.

Danielsen L, Thürmer A, Meinicke P, Buee M, Morin E, Martin F, Pilate G, Daniel R, Polle A, Reich M. 2012. Fungal soil communities in a young transgenic poplar plantation form a rich reservoir for fungal root communities. *Ecology and evolution*, **2**: 1935-1948.

De Roy K, Marzorati M, Van den Abbeele P, Van de Wiele T, Boon N. 2014. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environmental Microbiology* **16**: 1472-1781.

Deveau A, Palin B, Delaruelle C, Peter M, Kohler A, Pierrat JC, Sarniguet A, Garbaye J, Martin F, Frey‐Klett P. 2007. The mycorrhiza helper Pseudomonas fluorescens BBc6R8 has a specific priming effect on the growth, morphology and gene expression of the ectomycorrhizal fungus Laccaria bicolor S238N. *New Phytologist*, **175**:743-755.

Dimkpa, CO, Zeng J, McLean JE, Britt DW, Zhan J, Anderson AJ. 2012. Production of indole-3-acetic acid via the indole-3-acetamide pathway in the plant-beneficial bacterium Pseudomonas chlororaphis O6 is inhibited by ZnO nanoparticles but enhanced by CuO nanoparticles. *Applied and environmental microbiology*, **78**:1404-1410.

Doty SL, Oakley B, Xin G, Kang JW, Singleton G, Khan Z, Vajzovic A, Staley JT. 2009. Diazotrophic endophytes of native black cottonwood and willow. *Symbiosis* **47**: 23-33.

Finkel OM, Burch AY, Lindow SE, Post AF, Belkin S. 2011. Geographical location determines the population structure in phyllosphere microbial communities of a salt-excreting desert tree. *Applied and Environmental Microbiology* **77**: 7647-7655.

Fischer DG, Hart SC, LeRoy CJ, Whitham TG. 2007. Variation in belowground carbon fluxes along a Populus hybridization gradient. *New Phytologist* **176**:415–425.

Fischer DG, Hart S, Schweitzer J, Selmants P, Whitham T. 2010. Soil nitrogen availability varies with plant genetics across diverse river drainages. *Plant and Soil* **331**:391–400

Fischer DG, Chapman SK, Classen AT, Gehring CA, Grady KC, Schweitzer JA Whitham TG. 2014. Plant genetic effects on soils under climate change. *Plant and Soil*, **379:** 1-19.

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A. 2011. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews* **75**: 583-609.

Gehring CA, Mueller C, Whitham TG. 2006. Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. *Oecologia* **149**: 158**–**164

Gottel, NR, Castro HF, Kerley M, Yang ZK, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW. 2011. *Populus deltoides* roots harbor distinct microbial communities within the endosphere and rhizosphere across contrasting soil types. *Applied and Environmental Microbiology* **77:** 5934-5944

Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C. 2008. Effects of the inoculation of Burkholderia vietnamensis and related endophytic diazotrophic bacteria on grain yield of rice. *Microbial Ecology* **55**: 21-37.

Gundale MJ, Kardol P, Nilsson MC, Nilsson U, Lucas RW, Wardle DA. 2014. Interactions with soil biota shift from negative to positive when a tree species is moved outside its native range. *New Phytologist* **202**: 415-421.

Jumpponen A, Jones KL. 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytologist* **184**: 438-448.

Kaldorf M., Fladung M, Muhs HJ, Buscot F. 2002. Mycorrhizal colonization of transgenic aspen in a field trial. *Planta*, **214**: 653-660.

Kamp BJ. 1986. Nitrogen fixation in cottonwood wetwood. *Canadian Journal of Forest Research* **16**: 1118-1120.

Karlinski L, Rudawska M, Kieliszweska-Rokicka B, Leski T. 2010. Relationship between genotype and soil environment during colonization of poplar roots by mycorrhizal and endophytic fungi. *Mycorrhiza* **20**: 315-324.

Kemen E. 2014. Microbe-microbe interactions determine oomycete and fungal host colonization. *Current Opinion in Plant Biology* **20C**:75-81.

Knoth JL, Kim S-H, Ettl GJ, Doty SL. 2013. Effects of cross host species inoculation of nitrogen-fixing endophytes on growth and leaf physiology of maize. *GCB Bioenergy* **5**: 408-418

Knoth JL, Kim SH, Ettl GJ, Doty SL. 2014. Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia. *New Phytologist*, **201**: 599-609.

Kurth F, Mailänder S, Bönn M, Feldhahn L, Herrmann S, Große I, Buscot F, Schrey SD, Tarkka MT. 2014. Streptomyces-induced resistance against oak powdery mildew involves host plant responses in defence, photosynthesis and secondary metabolism pathways. *Molecular Plant-Microbe Interactions*. **27:** 891-900

Lamit LJ, Lau MK, Sthultz M, Wooley SC, Whitham TG, Gehring CA. 2014. Tree genotype and genetically based growth traits structure twig endophyte communities**.** *American Journal of Botany* **101**: 467-478

Lau JA, Lennon JT. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences U.S.A.* **109**: 14058-14062.

Lebeis SL, Rott M, Dangl JL, Schulze-Lefert P. 2012. Culturing a plant microbiome community at the cross-Rhodes. *New Phytologist* **196**: 341-344.

Lodge DJ. 1989. The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in Populus and Salix. *Plant and Soil* **117**: 243**–**253

Lugtenberg BJ, Dekkers L, Bloemberg GV. 2001. Molecular determinants of rhizosphere colonization by Pseudomonas. *Annual review of phytopathology*, **39**: 461-490.

Lundberg, DS, Lebeis SL, Herrera-Paredes S, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrekston A, Kunin V, Glavina del Rio T, Edgar R, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488:** 86-90

Martin F, Tuskan GA, DiFazio SP, Lammers P, Newcombe G, Podila GK. 2004. Symbiotic sequencing for the *Populus* mesocosm. *New Phytologist* **161**: 330-335

Pett-Ridge J, Weber PK. 2012. NanoSIP: NanoSIMS applications for microbial biology. *Methods in Molecular Biology* **881**: 375-408

Peiffer, JA, Spor A, Jin Z, Koren O, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci.* **110**:6548:6553

Plett JM, Kemppainen M, Kale SD, Kohler A, Legué, V, Brun A, Tyler BM, Pardo AG, Martin F. 2011. A secreted effector protein of Laccaria bicolor is required for symbiosis development. *Curr. Biol.* **21**: 1197-1230

Plett JM, Martin F. 2012. Poplar root exudates contain compounds that induce expression of MiSSP7 in Laccaria bicolor. *Plant Signal Behav.* **7**: 12-15.

Raghavendra AK, Newcombe G. 2013. The contribution of foliar endophytes to quantitative resistance to *Melampsora* rust. *New Phytologist* **197**: 909-918.

Redford AJ, Fierer N. 2009. Bacterial succession on the leaf surface: a novel system for studying successional dynamics. *Microbial Ecology* **58**: 189-198.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. 2010. The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology* **12**: 2885-2893.

Reisberg EE, Hildebrandt U, Riederer M, Hentschel U. 2013. Distinct phyllosphere bacterial communities on Arabidopsis wax mutant leaves. *PLoS One* **8**: e78613.

Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, Gandolfi C, Casati E, Previtali F, Gerbino R, Pierotti Cei F, Borin S, Sorlini C, Zocchi G, Daffonchio D. 2014. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environmental Microbiology*. In press. doi: 10.1111/1462-2920.12439.

Scherling C, Ulrich K, Ewald D, Weckwerth W. 2009. A metabolic signature of the beneficial interaction of the endophyte *Paenibacillus* sp isolate and *in vitro*-grown poplar plants revealed by metabolomics. *Molecular Plant–Microbe Interactions* **22***:* 1032-1037*.*

Schink B, Ward JC, Zeikus JG. 1981. Microbiology of wetwood: role of anaerobic bacterial populations in living trees. *Journal of General Microbiology* **123**: 313-322.

Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, Labbe J, Muchero W, Bonito G, Vilgalys R, Tuskan G, Podar M, Schadt CW. 2013. A Multifactor Analysis of Fungal and Bacterial Community Structure in the Root Microbiome of Mature *Populus deltoides* Trees. *PLoS ONE* **8:**e76382

Stefani FO, Moncalvo JM, Séguin A, Bérubé JA, Hamelin RC. 2009. Impact of an 8-year-old transgenic poplar plantation on the ectomycorrhizal fungal community. *Applied and environmental microbiology*, **75:** 7527-7536.

Talbot, JM, Bruns, TD, Taylor JW, Smith DP, Branco S, Glassman, SI, et al. 2014. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences*, **111:** 6341-6346.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, et al. 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences*, **110**: 20117-20122.

Tshaplinski TJ, Plett JM, Engle NL, Deveau A, Cushman KC, Martin MZ, Doktycz MJ, Tuskan GA, Brun A, Kohler A, Martin F. 2014. Populus trichocarpa and Populus deltoides Exhibit Different Metabolomic Responses to Colonization by the Symbiotic fungus Laccaria bicolor. *Molec Plant Microb Int.* **27**: 546-556

Ulrich K, Ulrich A, Ewald D. 2008. Diversity of endophytic bacterial communities in poplar grown under field conditions. *FEMS Microbiology Ecology* **63**: 169-180.

Unterseher M, Jumpponen A, Opik M, Tedersoo L, Moora M, Dormann CF, Schnittler M. 2011. Species abundance distributions and richness estimations in fungal metagenomics--lessons learned from community ecology. Molecular Ecology 20: 275-285.

Van der Putten WH, Vet LEM, Harvey JA, Wäckers FL. 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in ecology and evolution* **16**: 547–554.

Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828-840.

Wagner MR, Lundberg DS, Coleman-Derr D, Tringe SG, Dangl JL, Mitchell-Olds T. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild Arabidopsis relative. *Ecol. Letters.* **17:** 717-726

Weyens N, Boulet J, Adriaensen D, Timmermans JP, Prinsen E, Van Oevelen S, Smeets K, van der Lelie D, Taghavi S, Vangronsveld J. (2012). Contrasting colonization and plant growth promoting capacity between wild type and a gfp-derative of the endophyte Pseudomonas putida W619 in hybrid poplar. *Plant and soil **356*:** 217-230.

Weston DJ, Pelletier DA, Morrell-Falvey JL, Tschaplinski TJ, Jawdy SJ, Lu TY, Allen SM, Karve A, Melton SJ, Martin MZ, Schadt CW, Chen JG, Yang X, Doktycz MJ, Tuskan G. 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant responses in defense networks, primary metabolism, photosynthesis and fitness. *Molecular Plant Microbe Interactions* **25:** 765-778

Xin G, Zhang GY, Kang JW, Staley JT, Doty SL. 2009. A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. *Biology and Fertility of Soils* **45**: 669**–**674.

Yilmaz P, Kottmann R, Field D, Knight R, Cole JR, Amaral-Zettler L, Gilbert JA, *et al*. 2011. Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. *Nat Biotechnol* **29:** 415-420.

Zeikus JG, Henning DL. 1974. Methane formation in living trees: a microbial origin. *Science* **184:** 1181-1183

Zhao L, Wu ZQ, Ye JR, Li H, Li GE. 2014 Isolation and characterization of a mycorrhiza helper bacterium from rhizosphere soils of poplar stands. *Biology and Fertility of Soils,* **50**: 593-561

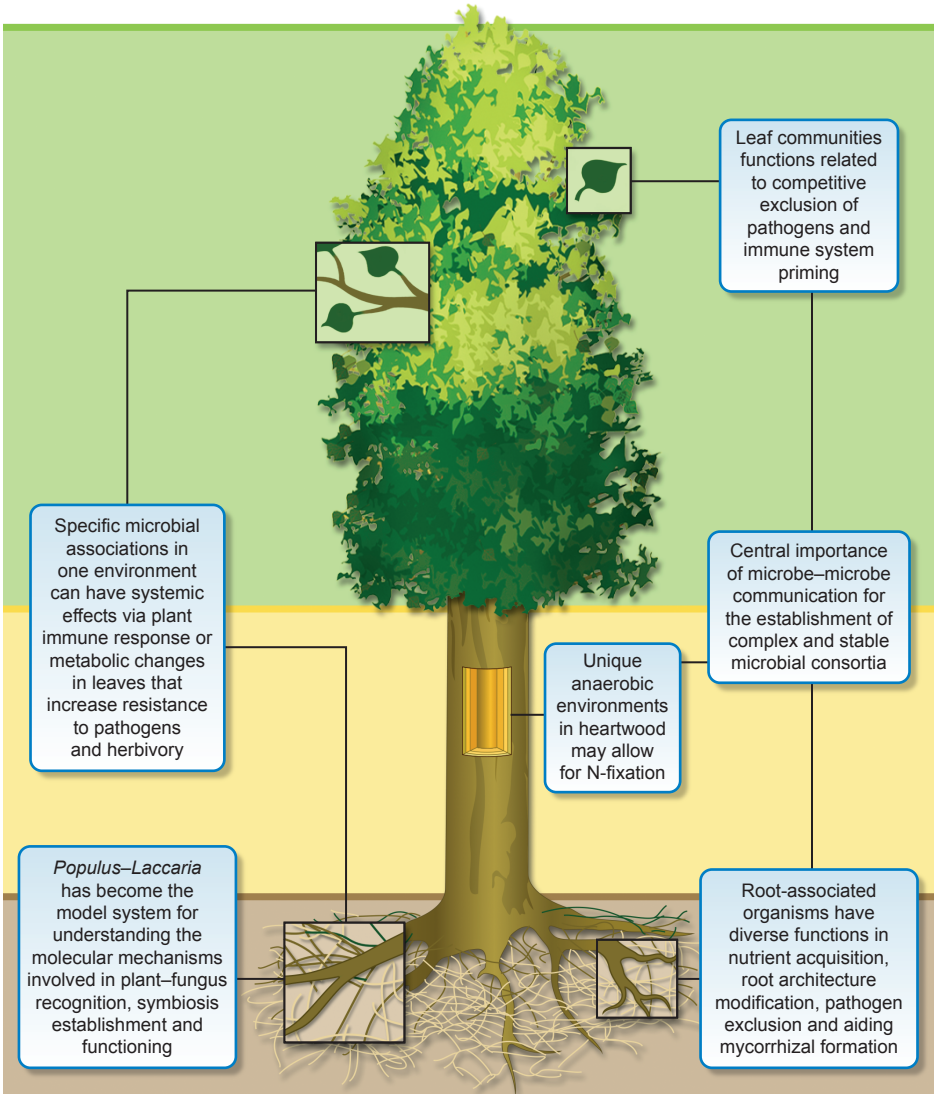

Figure 1
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