Review

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Tansley insight

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 superorganisms, 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.

I. Introduction

Populus trichocarpa was the first tree species genome sequenced (Tuskan *et al.*, 2006), and the ability to study genetically tractable *Populus* trees in glasshouses and plantation agroecosytems, as well as in natural ecosystem settings, make *Populus* spp. powerful systems for obtaining a 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 the structure and composition of surrounding plants, soils, and overall ecosystem functions (Fischer *et al.*, 2007, 2010, 2014). Recognizing its potential as a model system a decade ago, as sequencing of the *Populus* genome neared completion, Martin *et al.* (2004) called for the community to begin comparable efforts to sequence and study the *Populus* symbiont 'mesocosm'. They argued for consideration of trees as ecosystems in

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themselves and for increased understanding of their symbiotic interactions both at a holistic level 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 (Fig. 1).

II. 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; Danielsen *et al.*, 2012) have examined both bulk soil and root fungal populations independently in plantations with different transgenic *Populus* lines. Each of these studies found 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 *Populus 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). In such studies, researchers have done well to begin elucidating how these different plant habitats/niches affect 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 obtained 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 (Lundberg *et al.*, 2012; Peiffer *et al.*, 2013; Bonito *et al.*, 2014; Talbot *et al.*, 2014; Wagner *et al.*, 2014). These studies in *Populus* as well as in *Arabidopsis thaliana* and *Zea mays* systems have demonstrated that, within a host species, habitat (e.g. endosphere vs rhizosphere) and soil type, rather than host genetic background, have larger effects on the overall structure of the microbiome (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Peiffer *et al.*, 2013; Shakya *et al.*, 2013; Bonito *et al.*, 2014), but

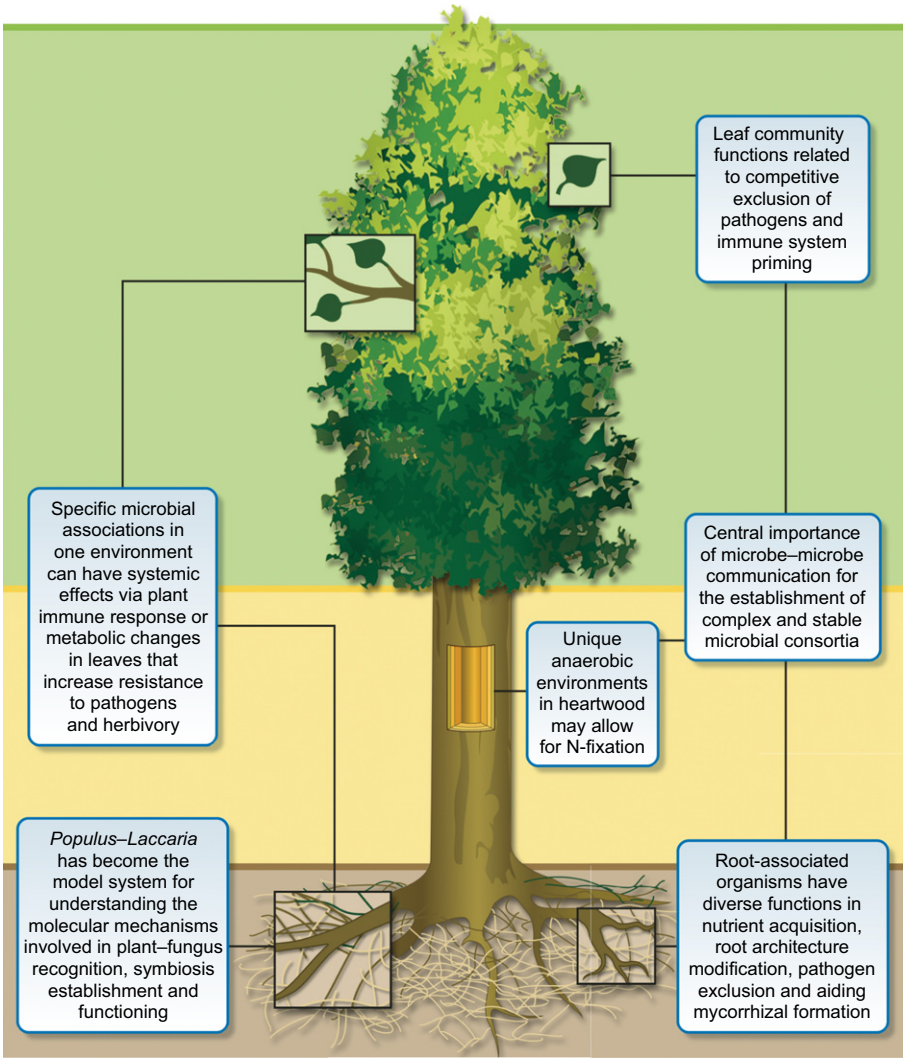

Fig. 1 Understanding of both the diversity of plant-associated microbial habitats and their functioning has greatly increased with sequencing-based analyses. These analyses have provided striking insights into the reductionist functioning of symbiotic partners as well as a more holistic understanding of microbial community interactions.

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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 suggests that, perhaps as a consequence of their often weak ectomycorrhizal (ECM) nature, root endophytic organisms may be particularly important for *Populus* trees compared with other ECM trees and result in higher levels of microbiome diversity due to increased niche space (Bonito *et al.*, 2014; Tshaplinski *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, across the plant and between tree taxa is still lacking. However, meta-analysis and synthesis studies that collectively analyse 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 functions

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 ECM and arbuscular mycorrhizal (AM) symbiosis have remained elusive. Genome-enabled studies using the *Laccaria–Populus* system have led to several insights in this area and suggest that 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 7 (MiSSP7) production in *Laccaria bicolor* appears to be induced by unknown exudates from *Populus* roots (Plett *et al.*, 2011; Plett & 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 *Populus* host species. While the above recognition mechanism is effective in *P. trichocarpa*, in *P. deltoides* the host defensive system is not effectively suppressed by *Laccaria* and ECM formation does not proceed (Tshaplinski *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 *Rhizophagus 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 symbiosis. While there appear to be both genetic and environmental influences on alternation between the two symbiosis modes in *Populus* (Lodge, 1989; Gehring *et al.*, 2006; Karlinski *et al.*, 2010), the detailed mechanisms and *in planta* functioning of such dual symbioses are still unclear.

Beyond mycorrhizal symbionts, *Populus* is also host to a variety of bacterial and fungal rhizosphere partners and root endophytes. Indeed, several studies have shown putative mycorrhizal fungal taxa on and within *Populus* to be outnumbered by other root endopyhtic fungi such as *Atractiella*, *Phialophora, Illyonectria* and *Mortierella* spp. (Gottel *et al.*, 2011; Shakya *et al.*, 2013; Bonito *et al.*, 2014). Therefore, elucidating the full potential of microbiome effects on tree growth, health and reproduction also depends on understanding these often neglected plant–microbe interactions. Bacterial endophytes have been shown to have varying functions in altering root branching/allocation patterns through production of plant hormone precursors such as indole acetic acid (IAA) (Dimkpa *et al.*, 2012; Weyens *et al.*, 2012), transformation and mobilization of nutrients such as nitrogen (N) and phosphorus (Browne *et al.*, 2009), enhanced mycorrhizal formation (e.g. mycorrhizal helper bacteria; Deveau *et al.*, 2007; Zhao *et al.*, 2014), and aiding in pathogen resistance through competitive exclusion or production of antibiotics (Lugtenberg *et al.*, 2001) or priming of plant immune responses (Weston *et al.*, 2012). None of these effects, however, seem to be mutually exclusive, as various isolates of even a single genus or species complex such as *Pseudomonas fluorescens* seem capable of many of these functions, as well as pathogenic effects (Weston *et al.*, 2012).

III. The phyllosphere and leaf endosphere microbiome

Diversity, structure and community-level perspectives

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

Integrated approaches are needed to understand the processes responsible for determining the structure and assembly rules of phyllosphere communities. One approach recently used various

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A. thaliana mutants and revealed that cuticular wax and ethylene can significantly affect the community composition of phyllosphere bacteria (Reisberg *et al.*, 2013; Bodenhausen *et al.*, 2014). In addition, a comprehensive survey of the topographical distribution of fungi and bacteria across various organs of individual tree species is still needed to better understand the tissue-type specificity of microbial community assemblages. Finally, recent studies indicate that, in addition to the host plant, synergistic, beneficial and antagonistic interactions among microbes may have tremendous impacts on microbial community structure and function in both the phyllosphere and the rhizosphere (Frey-Klett *et al.*, 2011; Kemen, 2014). Therefore, understanding of both leaf- and root-associated microbiota structure also relies on the understanding of more complex interactions, where fungal, oomycete and bacterial communities are not considered as separate entities but as active drivers of overall microbial community assemblages.

Specific interactions, mechanisms and functions

Although the structure and diversity of bacterial and fungal communities associated with the leaves of woody plants species have been reported, the associated functions remain poorly characterized. It has been recently shown that different fungal endophytes isolated from poplar leaves naturally infected by the rust fungus *Melampsora* can dramatically reduce rust symptom severity under laboratory conditions and significantly contribute to quantitative resistance to the foliar rust pathogen (Raghavendra & 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 (Weston *et al.*, 2012; Kurth *et al.*, 2014) 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).

IV. 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 great importance that has to date been 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 produce anaerobic conditions favoring fermentation or even methanogenesis (Zeikus & Henning, 1974). Prior reports suggested that communities associated with both *P. trichocarpa* and *P. deltoides* also have the potential to fix N in these niches, as evidenced by acetylene reduction assays (Schink *et al.*, 1981; Kamp, 1986). Numerous diazotrophic bacteria have been isolated from such habitats. Cross-inoculation experiments have shown broad growth-promoting effects of these organisms on other plant species, including nonwoody plants such as rice (*Oryza sativa*) and maize (Govindarajan *et al.*, 2008; Knoth *et al.*, 2013), and isolates of bacterial genera, including *Burkholderia*, *Rhizobium*, *Enterobacter* and *Paenibacillus* (Doty *et al.*, 2009; Scherling *et al.*, 2009) often show the ability to reduce N_2 in pure cultures outside the host. Isotopic studies using ^{15}N in *P. trichocarpa* inoculated with consortia of bacteria species showed signatures indicative of active fixation and that wetwood may account for up to 65% of the N in leaf tissues (Knoth *et al.*, 2014). Culturable fungal endophytes have also recently been examined within the woody tissues of branches of *Populus angustifolia* (Lamit *et al.*, 2014). While functional aspects have not been examined, it is clear from this first work that even the simple communities within woody tissues can be influenced by tree genotype. Additionally, many of the fungal genera identified seem to overlap with those commonly found within leaf and root endophyte habitats.

Despite indications of the great importance of heartwood habitat, all knowledge to date comes from studies of individual bacterial and fungal isolates, and a few studies of defined consortia. Interestingly, there is some indication that these mixed consortia of organisms show differing effects and sometimes more robust growth promotion (Knoth *et al.*, 2013, 2014), and this has been speculated to be attributable to increased niche colonization. However, microbiome, metagenome, or 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 fluorescence *in situ* hybridization (FISH) or other methods. The use of combinations of advanced microscopy and isotopically resolved mass spectroscopy techniques (e.g. NanoSIMS), could potentially be very useful (Pett-Ridge & Weber, 2012). Given these tantalizing results, and the potential importance of alternative mechanisms of N fixation, microbiome studies of heartwood should be prioritized.

V. 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 in plant growth and productivity (Bonfante & 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 contributed tremendously 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,

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revealing that plants are not limited to adapt or migrate, but perhaps utilize microbial consortia to adapt to a novel or disturbed environment (Lau & Lennon, 2012; Gundale *et al.*, 2014). Alternatively, extraction of presumably intact communities from different soil types has also been used to investigate how distinct environmental microbiomes can alter plant flowering phenology, and represents a promising approach in the 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.*, 2014). The modularity of synthetic communities has already provided new insights into the structure and function of plant-associated microbiota (Bodenhausen *et al.*, 2014; Knoth *et al.*, 2014; Rolli *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 comparison with new models such as *Eucalyptus*, *Pinus*, and others being developed now and in the future.

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