Plant-Microbe Interfaces: Dissecting the compatibility and diversity of the mycobiome of *Populus trichocarpa*

Alejandro Rojas*(alejo.rojas@duke.edu), Khalid Hameed[1], Posy Busby[1,5], Gregory Bonito[6], Hui-Ling Liao[7], Brian Looney,[2,4] Jessy Labbé[2], Jessica Vélez[3], Christopher Schadt [2,3], Claire Veneault-Fourrey[4], Aurélie Deveau[4], Annegret Kohler[4], Francis Martin[4], and Rytas Vilgalys[1]

[1]Duke University, Durham, NC; [2]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; [3]Graduate School of Science and Technology, University of Tennessee, Knoxville, TN; [4]INRA and the Université de Lorriaine, Labex ARBRE, UMR 1136, Champenoux, France; [5]Oregon State University, Corvallis, OR, [6]Michigan State University, Plant Soil and Microbial Sciences, East Lansing, MI, and [7]North Florida Research and Education Center, University of Florida, Quincy, FL

Abstract

The *Populus* root microbiome harbors a diverse community of endophytic and ectomycorrhizal fungi that promote nutrient acquisition and plant health. In collaboration with the JGI, we have isolated and sequenced the genomes of over 50 ectomycorrhizal and endophytic fungi isolated from *Populus* roots. These genomes are beings used to interrogate the metagenomes of North American soil fungal communities with other cottonwood species, *P. nigra* collected from France, in order to identify core groups of fungi associated with *Populus*. *Populus* genotypes often vary in their ability to form symbioses with different root-associated fungal taxa, and one aim of our studies is to identify the genetic determinants which underly host-specificity. A diverse collection of root associated fungi are being tested to evaluate their compatibility with different *Populus* genotypes representing different geographical ecotypes of *P. trichocarpa*. Selected species of Populus-associated EMF including *Lactarius, Hebeloma, Cenococcum, Laccaria* and *Paxillus* were inoculated on eight *P. trichocarpa* genotypes to address plant-fungal compatibility and function of the *Populus* mycobiome by in vitro synthesis of ectomycorrhiza and by using split-root systems with stable isotope tracing. The systems are under evaluation and will facilitate the understanding of *Populus*-fungal associations assessing the effects on the plant host using isotope methods, transcriptomics and assessment of plant health and fungal colonization. By using different split-root and in vitro systems in combination with genetic tools and isotopic tracing methods, these studies will provide insight into *Populus*-fungal associations and their development in this model tree genetic system.

Host-specificity: *Hebeloma*

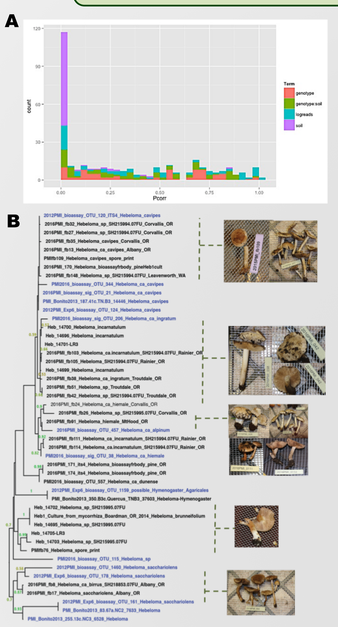


Figure 2. (A) Association between fungal OTUs and *Populus* genotypes; (B) ML phylogenetic tree of *Hebeloma* specimens and OTUs identified from *Populus* bioassays.

P. trichocarpa **GWAS common garden study including 32 *Populus* genotypes and 5 geographically distinct soils suggested an effect of genotype on the mycobiome associated. Different OTUs were identified including EMF and root endophytes, one of the key OTUs was *Hebeloma.***

A follow-up experiment was design to study the genotype-specificity of different pools of spores of *Hebeloma* from Eastern and Western US.

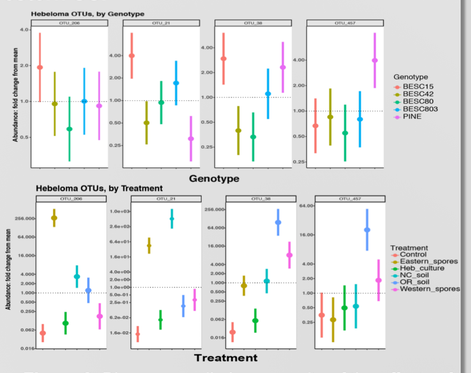


Figure 3. Bioassay preliminary results of the effects of genotype and treatment (natural soil vs spore origin) on the abundance of *Hebeloma* OTUs.

Split-root systems

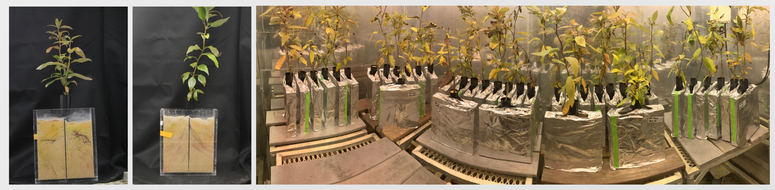


Figure 5. Split-root version 1.0. Eight different genotypes of *Populus trichocarpa* cuttings growing in coarse sand on plastic cuvettes were inoculated with one side of the cuvette. EMF were grown on peat moss saturated with 1/10 MMN liquid media.

EMF treatments

Group	Species
Agaric	*Laccaria 'bicolor'*
Agaric	*Lactarius populinus*
Agaric	*Hebeloma sp*
Agaric	*Paxillus involutus*
Asco	*Cenococcum*

P. trichocarpa **genotypes**

US	CANADA
BESC_80	CYNH_18-3
BESC_42	HOMC_21-3
BESC_184	LILB_26-5
BESC_803	KLNG_20-1

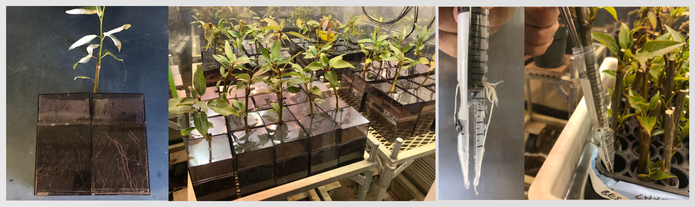
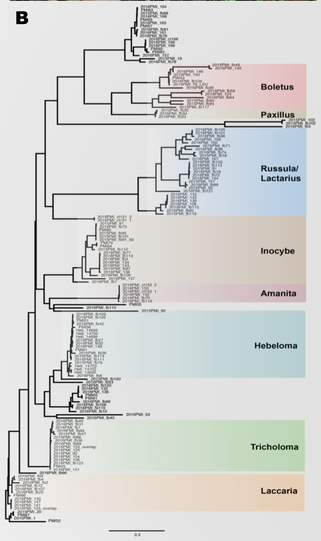


Figure 6. Split-root version 2.0. A low cost approach based on plastic boxes adapted with draining holes. Cuttings were rooted in hydroponic system using a root training devices to facilitate the root-splitting process before transplanting. The close system will reduce contamination by spores present in the environment.

Populus associated EMF



Surveys of macrofungal diversity were undertaken under native *P. trichocarpa* forests in 2015-2016 from five core watersheds in Oregon and Washington. In 2017, a survey was conducted under *P. nigra* in France to identify core groups of fungi associated with *Populus*. The surveys resulted in over 250 collections of EMF including pure cultures, spore prints and bulk soil for use in bioassay studies with different *Populus* genotypes. Taxonomic identification of fungal specimens is aided by multilocus DNA barcodes as well as morphological features. Many of the EMF fungi are uniquely host-specific with *Populus*.

Figure 1. Key groups of ectomycorrhizal fungi collected during surveys in 2015 and 2016 in the *P. trichocarpa* range. (A) Images of some specimens in their native ecosystem; (B) RAxML phylogenetic tree of different fungal species observed based on ITS within some common fungi associated with *Populus.*

P. nigra vs *P. trichocarpa* community

Samples collected at *Populus* sites in Washington and Oregon in the US and in different regions in France. Paired soil samples were collected at each site and processed for amplicon analysis.

Taxa	Ecological guild	Material collected	*Populus trichocarpa* (USA) 2015 sites collected	2015 species collected	2016 sites collected	2016 species collected	*Populus nigra* (France) 2017 sites collected	2017 species collected
Amanitaceae	Ectomycorrhizal	Sporocarp, spore print, frozen, culture	2	2	6	3	2	3
Boletaceae	Ectomycorrhizal	Sporocarp, spore print, culture	3	2	3	3	2	2
Clavulina	Ectomycorrhizal	Sporocarp	1	1	0	0	0	0
Cortinarius	Ectomycorrhizal	Sporocarp, spore print, culture	0	0	5	3	2	7
Hebeloma	Ectomycorrhizal	Sporocarp, spore print, culture	3	3	5	3	3	14
Inocybaceae	Ectomycorrhizal	Sporocarp, spore print	6	6	4	6	3	12
Laccaria	Ectomycorrhizal	Sporocarp, spore print, culture	2	4	3	5	2	3
Paxillus	Ectomycorrhizal	Sporocarp, frozen	1	1	1	1	1	1
Russulaceae	Ectomycorrhizal	Sporocarp, spore print, frozen, culture	3	14	4	11	5	33
Scleroderma	Ectomycorrhizal	Sporocarp	1	1	1	1	0	0
Tricholoma	Ectomycorrhizal	Sporocarp, spore print, frozen, culture	2	2	7	1	1	1

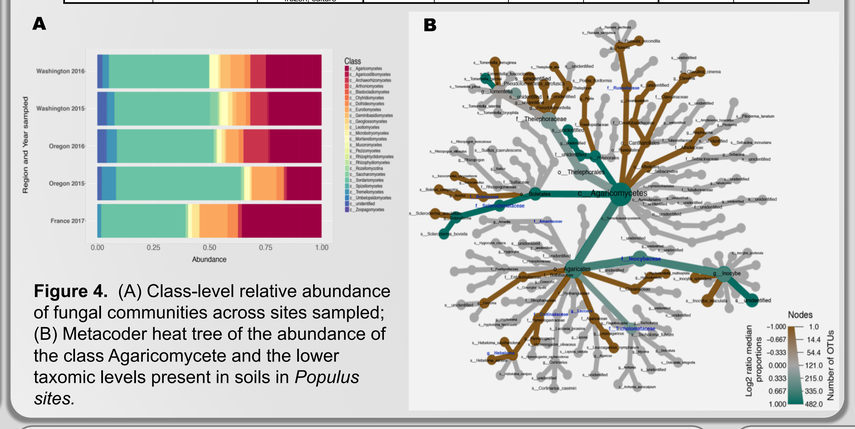


Figure 4. (A) Class-level relative abundance of fungal communities across sites sampled; (B) Metacoder heat tree of the abundance of the class Agaricomycete and the lower taxomic levels present in soils in *Populus sites.*

EMF host specificity

Figure 7. Host specificity bioassay across 8 *P. trichocarpa* genotypes using spore print inoculation of *Hebeloma, Laccaria, Boletus, Tricholoma, Lactarius* and *Paxillus.*

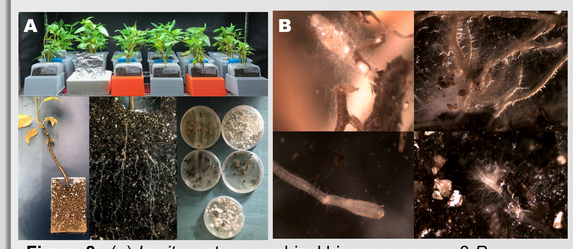


Figure 8. (a) *In vitro* ectomycorrhizal bioassay across 8 *P. trichocarpa* genotypes to develop emergent models of EMF, including established models as *Laccaria bicolor* as control, in comparison with *Lactarius populinus, Hebeloma sp., Paxillus sp.* and *Cenococcum sp.* (b) *Hebeloma m*ycorrhizal root tips on *Populus.*

Spore inoculations of specimens collected during surveys were used to inoculate distinct *Populus* genotypes in order to address host specificity and the underlying regulation of the host-symbiont interaction. Different scales will allow the characterization of the EMF system on *Populus*.

References:

Bonito, G., Hameed, K., Ventura, R. & Krishnan, J. Isolating a functionally relevant guild of fungi from the root microbiome of Populus. Fungal Ecology (2016).

Bonito, G. et al. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Mol. Ecol. 23, 3356–3370 (2014).

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