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Title: Fungal endophytes of *Populus trichocarpa* alter host phenotype, gene expression and rhizobiome composition

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Abstract:

Mortierella and *Ilyonectria* include common species of soil fungi which are frequently detected as root endophytes in many plants including *Populus* spp. However, the ecological roles of these and other endophytic fungi with respect to plant growth and function are still not well understood. The functional ecology of two key taxa from the *Populus* rhizobiome, *Mortierella elongata* PMI93 and *Ilyonectria europaea* PMI82, was studied by coupling forest soil bioassays with environmental metatranscriptomics. Using soil bioassay experiments amended with fungal inoculants, *M. elongata* was observed to promote the growth of *Populus*. This response was cultivar independent. In contrast, *I. europaea* had no visible effect on *Populus* growth. Metatranscriptomic studies reveal that these fungi impact rhizophytic and endophytic activities in *Populus* and induce shifts in soil and root microbial communities. Differential expression of core genes in *P. trichocarpa* roots was observed in response to both fungal species. Expression of *Populus* genes for lipid signaling and nutrient uptake were up-regulated and expression of genes associated with gibberellin signaling were altered in plants inoculated with *M elongata*, but not *I. europaea*. Up-regulation of genes for growth promotion, down-regulation of genes for several LRR-receptors/kinases, and alteration of expression of genes associated with plant defense responses (e.g., JA/ET/SA pathways) also suggest that *M. elongata* manipulates plant defenses while promoting plant growth.

Introduction

Soils of *Populus* and forest trees harbor a high diversity of rhizospheric fungi with diverse ecological functions, including mycorrhizal fungi, endophytes, saprophytes and pathogens (Bonito, et al. 2016). In particular, *Populus* species associate with a high diversity of root endophytes which play key roles in rhizosphere function and plant fitness (Shakya et al. 2013; Cregger et al. 2018). While several *Populus*-ectomycorrhizal interactions have been reported (Lodge 1989; Podila et al. 2009; Baum and Makeschin 2000; Bois et al. 2005; Gottel et al. 2011; Guevara et al. 2013; Martin et al. 2016), the mechanisms involved in *Populus*-endophytes interactions that affect plant growth and fitness remain unexplored.

Recent studies have identified *Mortierella* spp. as part of the core *Populus* microbiome (Gottel et al. 2011; Shakya et al., 2013; Bonito et al. 2014; Uehling et al. 2017). *Mortierella* belongs to Mucoromycota, an early diverging phylum of fungi, which is comprised of Glomeromycotina (arbuscular mycorrhizal fungi), Mortierellomycotina and Mucoromycotina (Bidartondo et al. 2011; Spatafora et al. 2016; Strullu-Derrien et al. 2018). Most *Mortierella* spp. are considered to be soil saprophytes, however they are also frequently isolated as endophytes from surface sterilized healthy root tissue of *Populus* and other plant species (Bonito et al. 2016). Beneficial interactions between *Mortierella* and plants are known to exist, but functional and mechanistic studies on plant-*Mortierella* interactions are few.

A recent study showed that *Mortierella hyalina* enhanced aboveground biomass of *Arabidopsis* and activated host Ca^{2+} signaling to suppress immune responses (Johnson et al. 2018). Another study showed the ability of endophytic *Mortierella alpina* to enhance the stress tolerance in host plants as a root endophyte via biosynthesis of the tetraterpenoid-derived phytohormones *in planta*, including apocarotenoid (Wani et al. 2017). Genome analysis and carbon utilization assays suggest that *M. elongata* metabolism is largely based on simple carbon utilization (e.g., D-glucose, D-trehalose and D-mannose) and that its metabolism is enriched in lipids and polyunsaturated fatty acids (PUFAs) anabolism (Uehling et al. 2017). Based on their enzyme profile, *Mortierella* spp. can acquire organic nitrogen through chitinolytic activities (Uehling et al. 2017; Vadivelan and Venkateswaran 2014) utilizing the chitin monomer N-acetyl glucosamine as a nitrogen and carbon source.

Ilyonectria spp. are another common group of ubiquitous rhizosphere fungi whose function as endophytes is poorly known. *Ilyonectria* belongs to the family of Nectriaceae (Hypocreales, Sordariomyceta, Ascomycota), however, the taxonomy status of *Ilyonectria* and other related genera is still in flux. For instance, molecular systematic studies revealed a high amount of cryptic speciation within the *Ilyonectria* complex (Cabral et al. 2012). *Ilyonectria* spp. are commonly isolated from rhizosphere soils and as endophytes from surface sterilized healthy roots from a wide range of woody and herbaceous plants including *Populus* (Cui et al. 2015; Kwaśna et al.

2016). *Ilyonectria* species are generally assumed to be commensals or weak plant pathogens, since some species are associated with disease of certain plant hosts, including root rot in grapes (Cabral et al. 2012; Hersh et al. 2012) and ginseng (Farh et al. 2018). However, as with *Mortierella*, the ecological function of most *Ilyonectria* species is unknown.

We hypothesize signaling between *Populus* and other non-mycorrhizal fungal root endophytes can occur in a community context and is bidirectional, such that each symbiont impacts the transcriptional regulation of its partner. However, we predict that *P. trichocarpa* uses different strategies to interact with *Mortierella* compared to *Ilyonectria*, given these fungal taxa are separated by a large phylogenetic distance and are assumed to represent opposite ends of the 'pathogen-mutualist' ecological spectrum. We carried out bioassay experiments with *P. trichocarpa* to test our hypotheses that *M. elongata* (PMI93) and *I. europaea* (PMI82) elicit different molecular responses from their native host *Populus trichocarpa*. We used RNA-Seq to investigate the functional activities of these fungal generalists (PMI93 and PMI82) in the rhizosphere and to identify the key plant genes responsive to fungal inoculation. This study highlights how different functional groups of endophytic fungi interact with a single host plant, *P. trichocarpa* and provides new insights into the relationships between species coexistence, plant fitness and ecosystem functions.

Results

125 Mortierella elongata *(PMI93) promotes plant growth*

126 To test if endophytic fungal taxa belonging to the core *Populus*
127 rhizobiome contribute to plant fitness, we used bioassay experiments to
128 examine the response of *Populus* to inoculation with two fungi isolated from
129 *Populus* roots, *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82)
130 (Bonito *et al.*, 2016). *Populus* cuttings were grown in a background of soils
131 collected from *Populus* sites in North Carolina, USA and inoculated with *M.*
132 *elongata* (PMI93) or *I. europaea* (PMI82), respectively. *M. elongata* (PMI93)
133 enhanced whole plant dry weight (30%, $p \leq 0.05$), and leaf expansion
134 ($p \leq 0.05$). Additionally we observed an increased amount of chloroplasts in
135 *Populus* (Fig. 1; Supplementary Fig. 1). Particularly, *M. elongata* (PMI93)
136 enhanced the dry weight in *Populus* roots more than it did in aboveground
137 organs (Fig. 1). While *Populus* growing in both soil and sand, *M. elongata*
138 (PMI93)-triggered-growth of aboveground organs was not plant genotype-
139 dependent (Supplementary Fig. 1). In contrast, *I. europaea* (PMI82) promoted
140 *Populus* growth in sterilized sand, but otherwise had no effect on plant
141 growth (Fig. 1B). The response of *Populus* to *I. europaea* was investigated
142 only on a single genotype, BESC4 (Fig. 1B), we cannot exclude a positive or
143 negative response with other genotypes.

144 *Fungi as biotic factors that influence the composition of fungal*
145 *communities in* Populus *roots and soils*

RNA-Seq data indicate that there were populations of *Mortierella* and *Ilyonectria* in the soils used for these bioassays (Supplementary Dataset 1; Fig. 2), however, inoculation resulted in a larger population of the target taxa and a higher relative abundance of target mRNA recovered (Fig. 2A). The inoculation with *M. elongata* (PMI93) increases the target fungus only in soil samples, whereas that with *I. europaea* (PMI82) in both root and soil samples (Fig. 2A). *M. elongata* (PMI93) inoculation resulted in an increase of relative abundance of *Ilyonectria* mRNA in the root and soil (Fig. 2B). *I. europaea* (PMI82) inoculation resulted in an increase of relative abundance of *Mortierella* mRNA in the soil (Fig. 2B).

To explore the interactive effects of *M. elongata* (PMI93) and *I. europaea* (PMI82) on the fungal community in *Populus* roots and soils, fungal LSU rRNA reads (corresponding to divergent domains D1 and D2) were extracted from the RNA-Seq data and used to identify fungal community composition in soil and roots as described by Liao *et al.* (2014). Species-rich communities of root-associated fungi were detected in individual *Populus* roots and rhizosphere soils (Fig. 3). A high diversity of fungi across different ecological guilds, from mutualists to pathogens, were present in all samples. A block effect was observed (Fig. 3), whereby the microbial community structure of uninoculated *M. elongata* (PMI93) samples were more similar to inoculated *M. elongata* (PMI93) samples and the microbial community structure of uninoculated *I. europaea* (PMI82) samples were more similar to *I. europaea* (PMI82) inoculated samples, likely explained by the fact that two

different soils (harboring different microbial communities) were used for the two separate experiments. Therefore, the two experiments were analyzed independently. Further, higher variability of soil and root fungal community structure was found in the *I. europaea* (PMI82) experimental samples compared to *M. elongata* (PMI93) experimental samples, regardless of the addition of fungal inoculum (Supplementary Fig. 3; Supplementary Table 2). Over 90% of the detected fungi are root associated fungi, including arbuscular (AMF) and ectomycorrhiza fungi (EMF), endomycorrhizal fungi and fungal endophytes. It is not known if physiological conditions of *Populus* cuttings used for the individual replicates contributed to the variability of fungal microbiomes. The physiological conditions of plants were not examined other than the measurement of plant biomass (Fig. 1). In addition, inoculation with either *M. elongata* (PMI93) or *I. europaea* (PMI82) resulted in a shift in the composition of the fungal soil community compared to uninoculated plants grown in the same soils ($p \leq 0.05$) (Supplementary Fig. 3A; Supplementary Table 2A). AMF transcriptome activity changed significantly in the roots and soil inoculated with *I. europaea* (PMI82), but not with *M. elongata* (PMI93) ($p \leq 0.05$; Supplementary Fig. 3B1). *M. elongata* (PMI93) inoculations reduced some AMF taxa in root tissues, including those of Glomeromycota, *Septoglomus* and *Scutellospora* (Fig. 3; Supplementary Table 2C). Soil inoculation with *M. elongata* (PMI93) resulted in increased transcriptome activity by EMF (Supplementary Fig. 3B2 and 3B3), an effect that has also been observed for other fungi in the *Nectriaceae* (Swett and

9

Gordon 2016). The transcriptome composition of endophytes in the soils was shifted in response to both *M. elongata* (PMI93) and *I. europaea* (PMI82) inoculation (Supplementary Fig. 3B4 and 3B5). Inoculation with *M. elongata* (PMI93) resulted in enrichment of certain fungal endophytes (*Gibberella, Bionectria, Neonectria, Neocosmospora, Nectria* and *Trichoderma*) in both root and soil systems (Fig. 3). *I. europaea* (PMI82) inoculation resulted in the enrichment for the fungal pathogens, *Leptosphaerulina* and *Didymella* (Fig. 3; Supplementary Fig. 3B6; Supplementary Table 2C). Finally, inoculation with *M. elongata* (PMI93) and *I. europaea* (PMI82) resulted in a shift in the composition of transcribed rRNA of saprotrophs (Supplementary Fig. 3B7).

Shared and unique responses of Populus *responses to* Mortierella elongata *(PMI93) and* Ilyonectria europaea *(PMI82) inoculations*

We compared the net transcriptomic activity of *Populus* roots with and without fungal inoculation. We recovered ~34 million reads from individual pools of fine roots (around 1 mg; Supplementary Dataset 1). The average read proportion of expressed genes was 72%:0.1%:28 %, (plant: inoculated fungi: other species not mapped to plant hosts or inoculated fungal genomes) for individual samples regardless of whether the samples were inoculated with fungi (Fig. 2; Supplementary Dataset 1). Additional details of computational pipeline used for data assemblies can be found in Supplementary Fig. 2.

Inoculation with *M. elongata* (PMI93) and *I. europaea* (PMI82) resulted in a strong molecular plant response (Fig. 4; Supplementary Dataset 2). Over 4,497 individual genes of *Populus* were significantly altered in response to *M. elongata* (PMI93) inoculation (FDR≤0.05; ≥2-fold changes; n=4) (Fig. 4A). In contrast, only 380 genes of *Populus* changed their expression patterns in response to inoculation with *I. europaea* (PMI82) (Fig. 4A). Further, replicate samples of *I. europaea* (PMI82) appear to be much more variable than for *M. elongata* (PMI93) (Fig. 3 and 5). Likely, the higher variability in gene expression across replicates is the cause of fewer differentially expressed *Populus* genes in response to *I. europaea* (PMI82) compared to *M. elongata* (PMI93) inoculation.

Expression patterns of 260 genes changed in *Populus* in response to both *M. elongata* (PMI93) and *I. europaea* (PMI82) (shared genes) (Fig. 5; Supplementary Dataset 3), indicative of molecular commonalities in how *Populus* responds to different species of rhizosphere fungi. Pathways mediating this shared response include carbohydrate metabolism, plant cell wall development, fatty acid/lipid biosynthesis and metabolisms, IAA signaling, heat shock, stress response and transport and intracellular signaling/transcriptional regulation. As part of this common response, a few functional groups, including several plant defense related genes (18 genes), are significantly up- and down- regulated (FDR≤0.05; ≥2-fold changes; n=4). These include JA/ET/ABA biosynthesis and signaling and salicylic acid (SA)-LRR-mediated signaling. For example, one gene for JA signaling (12-

oxophytodienoate reductase) was up-regulated (Gene ID: Potri.013G102700) and two genes encoding lipoxygenase were down-regulated (Potri.005G032700; Potri.005G032400) (Supplementary Table 1; Supplementary Dataset 3). The NDR1/NIH1-like gene (Potri.017G154000), which has been reported to respond to SA-mediated biotic stress, was also up-regulated in response to fungal inoculation (Wu et al. 2012).

Aside from shared genes, 4,237 and 120 *Populus* genes were predicted to respond to *M. elongata* (PMI93) and *I. europaea* (PMI82), respectively. *Populus* responded more strongly to *M. elongata* (PMI93) compared to *I. europaea* (PMI82) at physiological and molecular levels. Thus, further analyses focused specifically on *Populus* responses to *M. elongata* (PMI93). The majority of functional groups of these unshared genes are involved within transmembrane functions (32% of total unshared genes), extracellular functions (7%) and transcriptional regulators (8%) regardless of soil batch or fungal species was used as inoculum (Supplementary Fig. 4). Of 356 plant genes involved in extracellular activities during *Populus-M. elongata* (PMI93) interaction, 147 genes (41% of extracellular proteins) were considered to be plant small secreted proteins (pSSPs), comprising up to 8% total pSSPs (1,680 pSSPs) from *P. trichocarpa* genome (Tuskan et al. 2006; Yang et al. 2011). Of 147 *Populus* pSSPs, 94 were up-regulated and 53 genes were down-regulated in response to *M. elongata* (PMI93) (Fig. 6; Supplementary Dataset 4). Genes encoding pSSPs involved in plant lipid-transfer proteins (pLTPs) and cell wall loosening (expansin) were up-regulated, while genes

encoding pSSPs related to cell adhesion and plant defense response were down-regulated (Gene IDs were shown in column A and the predicted functions were shown in column CG of Supplementary Dataset S4A).

Of 85 plant genes involved in extracellular activities during *Populus- I. europaea* (PMI82) interaction, 15 genes (18%) were predicted to be pSSPs (Supplementary Dataset 4B). One gene encoding an pSSPs for pLTPs (Potri.013G131500), two genes for protein app1 and three genes for cell wall protein gp1-like were up-regulated. Other genes encoding pSSPs, including serine protease inhibitor (Kazal-type), clavata3 and plant natriuretic peptide A, were down-regulated. None of those 15 genes encode for cell adhesion and plant defense response. The protein structure analysis were furthered applied to study the structural architectures of those pSSP groups (Supplementary Fig. 5).

Two distinct pLTPs families (Family 1 LTPs; Family 2 LTPs) have been biochemically characterized (Yeats and Rose 2008). All 12 *Populus* pLTPs identified in response to *M. elongata* (PMI93) share a structural architecture of a hydrophobic cavity enclosed by four alpha-helices that are folded using four disulfide bounds (Supplementary Fig. 5A). The conserved eight-cysteine motif contributes to these four disulfide bounds. The presence of Tyrosine-16 and the small hydrophobic amino acid (Ile, Val, Leu, Ala) direct *Populus* pLTPs as the Family 1 pLTPs. These *Populus* pLTPs vary in amino acid identity (between 1%-90% identity).

Populus pSSPs associated with defense responses were predominantly down-regulated in response to *M. elongata* (PMI93) including three groups: dirigent-like protein, germin-like protein and PR-thaumatin associated protein (Fig. 6; Supplementary Fig. 5B; Supplementary Dataset 4). Ligand and enzyme activity prediction analysis showed different modes of the plant immune systems, including JA/ET/SA/ABA mediated pathways, were suppressed in association with *M. elongata* (PMI93) inoculation. Along this line, although one gene for JA signaling (12-oxophytodienoate, Potri.013G102700) was up-regulated in *Populus* in response to both *M. elongata* and *I. europaea* inoculation, other 12-oxophytodienoate reductases (Potri.003G004600 and Potri.003G004200) were conversely downregulated in the root inoculated by *M. elongata* (PMI93). All detected genes for lipoxygenases (Potri.005G032400, Potri.005G032700, Potri.005G032600, Potri.013G022100, Potri.009G022400) (Supplementary dataset 1) were also downregulated in *M. elongata* (PMI93) inoculated roots. Expression of 23 *Populus* genes for gibberellin signaling was altered in plants inoculated with *M elongata*, but not *I. europaea* (Supplementary Table 1B).

We further categorized these genes through different annotation methods, including KEGG mapper (Supplementary Fig. 6), KOG gene groups (JGI annotation) (Supplementary Fig. 7) and ClueGO gene enrichment analysis (Bindea et al. 2009;2013) (Supplementary Fig. 8). Results of these analyses indicate that *M. elongata* (PMI93) inoculation contributed to an up-regulation in *Populus* pathways involved in fatty acid/glycerolipid

biosynthetic processes and metabolism, and oxidative phosphorylation. Conversely, there was a down-regulation of genes involved in carotenoid biosynthesis and ET/JA/SA signaling ($p \leq 0.05$; ≥2-fold changes; n=4). The most abundant differentially expressed genes appear to be mostly involved in signaling (Fig. 5; Fig. 6; Supplementary Dataset 2A), including receptor kinases and transcription factors. A majority of receptor kinases were down-regulated in response to *M. elongata* (PMI93) including 61 genes for LRR-receptor kinases (LRR-RKs) (Supplementary Dataset 2A). ClueGo gene enrichment analysis also shows that inoculation with *M. elongata* (PMI93) enhances the activities of fatty acid biosynthesis, thiolester hydrolase, response to inorganic substrates, cytokinin metabolism and disaccharide biosynthesis (Supplementary Fig. 8B).

Of 120 genes in *Populus* that responded to *I. europaea* (PMI82) inoculation, but not *M. elongata* (PMI93) inoculation, two genes were predicted to be pLRRs (Potri.005G043700; Potri.019G110800) and were down-regulated (Supplementary Dataset S2B).

Fungal genes up-regulated in response to Populus

Our initial attempts to profile the expression pattern of *M. elongata* (PMI93) and *I. europaea* (PMI82) genes in roots and soils was hampered by a low abundance of fungal endophyte reads (Fig. 2). For example, only 56K paired-reads of *M. elongata* (from over 28M qualified reads) were detected from individual soil samples (Supplementary Dataset 1). This may be a

general feature of many endophytes which are characterized by lower activity and abundance especially within plant tissues. Because *Mortierella* expressed higher numbers of mRNA transcripts than to *Ilyonectria* for the soil and root samples without inoculation (Fig. 2), the molecular activities of *M. elongata* (PMI93) in the bioassay were further investigated. The higher abundance of *M. elongata* (PMI93) mRNA transcripts in rhizosphere soil (Fig. 2) permitted us to compare relative expression comparisons of fungal genes (% reads of *M. elongata* = 5%, around 1.4 M reads per sand sample) (Supplementary Dataset 1). In total, 7,950 genes of *M. elongata* (PMI93) were detected across all (4) biological replicates of sand samples inoculated with M. elongata (PMI93). To study the functional categories active in *M. elongata* (PMI93), we investigated the number of transcribed genes and their functional proportions of *M. elongata* (PMI93) detected in sand and *in vitro* (Supplementary Fig. 9; Supplementary Dataset 6). In general, a similar pattern and proportion of *M. elongata* (PMI93) functional genes was detected across sand and culture conditions. However, fewer *Mortierella* genes encoding secreted proteins (fSSPs), leucine-rich receptors and WD40 were detected in *Populus*-sand bioassay treatments inoculated by *M. elongata* (PMI93), compared to when *M. elongata* (PMI93) was grown in pure culture axenically ($p \leq 0.05$) (Supplementary Dataset 6). Of 87 fSSPs detected in the sand with *Populus* grown nearby, only 3 fSSPs were not detected in the culture (Supplementary Dataset 7). Comparative metatranscriptomics also show genes for RNA modification, translation, signal transduction, lipid

transport and metabolism and chitinase were significantly up-regulated in *M. elongata* (PMI93) when *M. elongata* (PMI93) grew with *Populus* compared to pure culture (Supplementary Fig. 9; Supplementary Dataset 7).

Discussion

Some fungal endophytes are known as beneficial symbiotic microbes able to promote plant growth and induce plant defense (Vaarma, et al., 1999; Lee et al., 2011; Zuccaro, et al., 2014; Greletet al., 2017). In this study, soil inoculation with the ubiquitous fungal endophyte *M. elongata* (PMI93) promoted plant growth, while the effects of inoculation with another common endophyte *I. europaea* (PMI82) were neutral in phenotype (Fig. 1). RNA-Seq data demonstrate that *M. elongata* (PMI93) and *I. europaea* (PMI82) both have a dual lifestyle: each can grow as a root endophyte or as soil saprotroph (Fig. 2). The mycelium of *M. elongata* (PMI93) forms a biofilm on plant roots indicating that *M. elongata* (PMI93) can directly interact with plant roots (Supplementary Fig. 10). The differentiation of an individual fungal mycelial network between two life strategies also implies that a fungal isolate itself may utilize multiple resources, while interacting with plant host(s). Fungal endophytes have been classified into four classes according to their life histories (Rodriguez et al. 2009). *Mortierella* and other Class 4 fungal endophytes live within their host plant roots for at least a part of their life cycle without apparent symptoms (Wilson 1995; Rodriguez and Redman

1997; Rodriguez et al. 2009). In contrast, saprotrophic fungi live off dead organic matter in soils and dead plant tissues. The present of fungal transcriptomes in root and soil suggests that *M. elongata* (PMI93) and *I. europaea* (PMI82) participate in a combination of endophytic and saprotrophic activities (Fig. 2). However, the low proportion of *Mortierella* and *Ilyonectria* transcripts in the soil and root RNA probably reflects the low biomass of this fungus in these two niches. This fact prevented the authors from carrying out a detailed analysis of the *Mortierella* transcriptome in response to a host plant.

Results from this study also demonstrated how enrichment of a single fungal taxon can shift the whole community of root and soil-associated microbes and thus altering the ecological functions of associated plants and diverse soil taxa (Fig. 3). *Mortierella elongata* (PMI93) may promote plant growth indirectly by manipulating the community and functioning of other rhizosphere microbes (Fig. 3), by altering the nutrient composition of soil to facilitate resource acquisition (e.g., nitrogen, lipid) or by modulating plant phytohormones (e.g., IAA, GA) (Supplementary Fig. 6, 7 and 8). The common surveillance genes in *Populus* are activated in response to inoculation of both fungal species (Fig. 5), even though the two fungal species play different roles in associated with *Populus* (Fig. 1). This indicates that *Populus* may react to different biotic conditions through a common set of signaling pathways. Since soils collected destructively over a time interval were used to study the effect of *M. elongata* (PMI93) and *I. europaea* (PMI82)

respectively, we cannot exclude the possibility that the different responses of *Populus* to these two endophytes may be influenced by interactions between the inoculated endophytes and soil microorganisms.

Unlike pathogenic or mycorrhizal fungi that utilize a battery fungal effectors to modulate plant defenses, the *M. elongata* genome has reduced amount of fSSPs (417 fSSPs genes in Morel1 genome, Supplementary Dataset 8) in comparison with fungal specialists or obligate biotrophs (around 700-1,400 fSSPs) (Kim et al. 2016). We observed similar pattern and proportion of *M. elongata* (PMI93) fSSPs were present in soil, as compared to when growing in pure in culture (Supplementary Fig. 9). Thus, direct interactions between fungal effectors and plant receptors likely play a lesser role in *Populus-Mortierella* interactions.

Populus spp. form functional symbioses with both AMF and EMF using effector-receptor communication (Plett et al. 2011; Martin et al. 2016). During ectomycorrhizal interaction between *P. trichocarpa* and *Laccaria bicolor*, a fungal effector fSSP (MiSSP7) is taken up by *Populus* and imported into plant nuclei where it suppresses JA-mediated plant defense response (Plett et al. 2011; Plett et al. 2014). In the present study, inoculation of *P. trichocarpa* with *M. elongata* (PMI93) was found to alter plant JA signaling, possibly affecting the suppression of JA-derived pathway. Thus, many beneficial microorganisms such as *Mortierella* and *Ilyonectria* may use different strategies to interact with their *Populus* host.

Enrichment of genes for plant lipid pathway in response to PMI93

Given that root-endophytes do not generally grow within plant cells, interactions between *Populus* and its endophytes must occur within extracellular spaces. The proportion of genes encoding predicted small-secreted proteins (pSSPs) for *Populus* in response to *M. elongata* (PMI93) is high (~40%; 147 genes) compared to other genes for extracellular activities (Supplementary Datasets 2 and 4). RNA-Seq data indicates that the plant lipid-transfer proteins (pLTPs) are the primary pSSPs produced by *Populus* in response to *M. elongata* (PMI93) inoculation (Fig. 6). Only 15 genes for pSSPs were differentially expressed in *Populus* in response to *I. europaea* (PMI82) (Supplementary Dataset 4B). In addition, genes for fatty acid/lipid biosynthetic processes represent the primary set of *Populus* genes enriched in response to *M. elongata* (PMI93) (Supplementary Fig. 8B), suggesting the involvement of lipids in *Populus-Mortierella* interactions. As mentioned above, *Populus* genes for pLTPs were enriched in response to *M. elongata* (PMI93). These pLTPs contain signal peptides that direct their secretion into the extracellular matrix. In addition, the affinity of pLTPs for lipids is presumably fundamental to their function. In other plants, pLTPs have been shown to be responsible for translocating phospholipids and other fatty acid groups across cell membranes (Kader 1996). They may also bind to ligands that contain acyl groups. Ligand prediction analysis indicates these *Populus* pLTPs can bind to saturated fatty acid (e.g., stearic acid and palmitic acid) (Supplementary Dataset 4, Supplementary Fig. 5A). The biological roles of

pLTPs are still unclear, however several studies suggest their involvement in antimicrobial activity, defensive signaling, cuticle deposition and cell wall loosening (Yeats and Rose 2008). Growing evidence also supports another function of family 1 pLTP to promote plant cell walls extension (Nieuwland et al. 2005). Up-regulation of gene groups for pLTPs and six expansin-like proteins and 10 cell wall loosening associated enzymes of *Populus* (Fig. 6; Supplementary Dataset 4) implicates pLTPs as potential modulators of non-enzymatic and enzymatic cell wall loosening (Marowa et al. 2016). Similar to other family 1 pLTPs (Pagnussat et al. 2012), *Populus* pLTPs may function as the extracellular lipid transfer protein and may be re-localized intracellularly in order to facilitate fatty acid and lipid-associated pathways.

In a separate study, *M. elongata* (PMI93) was observed to produce polyunsaturated fatty acids under normal growth conditions (Uehling et al., 2017, Supplementary Fig. 11). The higher number of genes and higher expression rates of lipid transport and metabolism genes in *M. elongata* (PMI93) detected in sand-grown *Populus* cuttings compared to cultured isolates suggests higher induction of *M. elongata* (PMI93) lipid metabolic activities in the plant-soil system (Supplementary Fig. 9; Supplementary Dataset 6 and 7). We hypothesize that lipids produced by *Mortierella* may serve as ligands for pLTPs.

PMI93 manipulates Populus *SSP-(pSSPs) and LRR-(pLRR) genes invloved in defense responses*

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462 In *Populus*, pSSPs for defense responses (Fig. 6) and pLRR-RKs
463 (Supplementary Dataset 2A; S4), were predominantly down-regulated in
464 response to *M. elongata* (PMI93). Three groups of these pSSPs were
465 identified: (a) dirigent-like protein; (b) germin-like protein (c) PR-thaumatin
466 associated protein (Supplementary Dataset 4). Ligand prediction analysis
467 showed that these pSSPs are able to bind diverse ligands, implying that
468 different parts of plant immunity system were suppressed, including JA, SA,
469 hypersensitive response and LRR-RK associated defense response (see below
470 explanation).

471 Enzyme active site prediction showed that dirigent-like pSSPs contain
472 the activity of allene oxide cyclase (AOC) (Supplementary Fig. 5B). These
473 dirigent-like pSSPs contain an eight-stranded antiparallel beta-barrel with a
474 central hydrophobic ligand binding site. The predicted ligands of dirigent-like
475 pSSPs include reaction intermediates required for allene oxide synthase
476 (AOS) activities (i.e. enoic acid, vernolic acid, Supplementary Fig. 5B)
477 (Wasternack and Kombrink 2010). The essential function of AOC and AOS in
478 JA biosynthesis has been reported in other studies (Wasternack 2007; von
479 Malek et al. 2002; Park et al. 2002; Ishiga et al. 2003), implicating the
480 manipulation by *M. elongata* (PMI93) in the suppression of JA-mediated plant
481 defense. Enzyme active site prediction suggests that germin-like pSSPs have
482 oxalate oxidase (OXO) activity, which can catalyze the conversion between
483 oxalate and CO_2 + H_2O_2. The OXOs are involved in hypersensitive plant cell
484 death (Lane 2002) and increase their activity under biotic stress (Zhou et al.

1998; Hurkman and Tanaka 1996). Together these results suggest the possibility that *M. elongata* (PMI93) can down-regulate the hypersensitive response of its *Populus* host. Down regulation of several PR-thaumatin associated proteins with beta-1,3-glucanases activity shows that *M. elongata* (PMI93) may suppress multiple routes of plant immunity, including the salicylic acid mediated pathway (Liu et al. 2010) and fungal cell wall degradation (Lusso and Kuć 1996; Kuc 1995).

Plant membrane-localized receptor kinases play important roles in sensing and responding to environmental signals (Osakabe et al. 2013). These receptors perceive the extracellular ligands in order to phosphorylate intracellular kinase domains to activate downstream pathways. Within the *Populus* genome, pLRR-RKs account for the largest group of membrane-localized receptor kinases. pLRR-RKs can exhibit diverse biological functions and most have been shown to play a role in plant defense (McHale et al. 2006). Down regulation of *Populus* pLRR-RKs raises the possibility that *M. elongata* (PMI93) can balance pLRR-RKs signaling and growth tradeoffs to optimize plant fitness.

Conclusions

In conclusion, our findings show differential responses of *Populus* to two functionally different fungal endophytes, *M. elongata* (PMI93) and *I. europaea* (PMI82). These two fungal endophytes have endophytic and saprotrophic activities. The enrichment of a single fungal taxon (PMI93 or PMI82) can shift

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the whole community of root and soil-associated microbes and can alter the gene expression of their host plant. RNA-Seq results suggest that *M. elongata* (PMI93) can modulate *Populus* defense responses, nutrient uptake, and photosynthetic-associated energy production through direct or indirect interactions with its host. Particularly, the presence of *M. elongata* (PMI93) leads to downregulation of genes involved in plant immune response and hormones signaling (e.g., JA/SA/ET signaling, HR response and fungal cell wall degradation), and alters expression of genes involved in gibberellin and lipid associated pathways which may result in the observed growth enhancement. Future studies may consider targeting lipid-derived communication and metabolism between *Mortierella* and *Populus* roots to better understand the interactions of these symbionts.

Materials and Methods

Inoculum preparation

Sterile millet seeds were used as the medium to sustain viability and growth of fungal isolates. To prepare sterile millet seeds for fungal inoculum, millet seeds were soaked overnight in sterile distilled water. The excess water was drained, and the wet millet seeds were transferred to mushroom spawn bags with micropore patch. All bags were tightly closed and autoclaved at 120C, 15 psi for 45 minutes for two cycles and cooled between cycles.

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Prior to inoculating spawn bags, fungal mycelium [(*M. elongata* (PMI93) and *I. europaea* (PMI82)] was grown on pure modified Melin Norkrans (MMN) 1% agar media at 25 °C (Rossi and Oliveira 2011). After 3-days of culturing, the fungal biomass with solid media was finely chopped into small cubes (around 3x3x3 mm^3) and transferred to sterile culture bags containing sterile millet seeds. After one month of incubation at 25 °C, the fungi had completely colonized the millet seeds which were used as inoculum in soil bioassay experiments.

Soil bioassay and sample collection for metatranscriptomics

The fresh cuttings of *Populus trichocarpa* were collected from Washington and Oregon in spring of 2015, including four genotypes: BESC86, GW7974, BESC4 and BESC320. The 30-cm long vegetative cuttings of *Populus trichocarpa* were submerged in tap water for three days, with daily water change. Prior to further use cutting were surface sterilized by soaking in 5-7% bleach solution with 0.01% Tween 20 for 15-20 min and then rinsed with sterile distilled water. Cuttings were planted into sterile sand and allowed to root under fluorescent lighting and regular watering. After one month of growth, the plants were transferred on the same day to 1) soil collected from forest sites and diluted with sterile sand - soil:sand; 30:70 (w/w), and to 2) sterile sand only – to serve as “no soil control (sand bioassay)”. Because fungal inocula grew at different rates, two different fresh soil samples from

the same field site were used for inoculation with *M. elongata* (PMI93) and *I. europaea* (PMI82), respectively. Potted plants were inoculated in replicate with *M. elongata* (PMI93) and *I. europaea* (PMI82), respectively (soil/millet inoculum at 99:1 w:w ratio). Negative control treatments used sterile millet mixed with each soil. Inoculated plants were placed in a growth chamber at 25 °C, 80% humidity and fluorescent light at 200 µmol $m^{-2}s^{-1}$ for 16 hours per day. After 2-3 months, some replicates of *P. trichocarpa* growing in the sand only without *M. elongata* (PMI93) inoculation did not survive and could not be used for the further studies (Fig.1; Supplementary Fig. 1). After 4 months growth, roots and soils (four biological replicates each) collected from *P. trichocarpa* BESC4 soil bioassay, sand bioassay (with PMI93 inoculum) were harvested for metatranscriptomic analysis. Supplementary Dataset S1 summarized root and soil samples used for metatranscriptomic analysis. Physiological measurements for each plant are presented in Fig. 1 and Supplementary Fig. 1. Additional methods used for soil bioassay are described in Supplementary Fig. 1.

RNA preparation, cDNA library construction and Illumina sequencing

Total RNA from roots and soils were extracted following a CTAB/chloroform extraction and LiCl precipitation protocol previously described (Liao et al. 2014). The mRNA and cDNA for RNA-Seq analysis were purified with a TruSeq RNA sample preparation kit (Illumina, San Diego, CA). cDNA pools

were sequenced on the Illumina HiSeq 2000 instruments (Illumina, San Diego, CA) in the Duke Center for Genomic and Computational Biology (GCB). Twelve samples were sequenced in an individual lane to generate a total of ~40Gb of data. In total, 32 samples (root and soils) in soil bioassay, 4 samples in sand bioassay and 3 fungal culture samples were sequenced for this study. RNA-Seq data have been deposited at NCBI Short Read Archive (SRP057033).

Sequence assembly and annotation

Genome sequences produced by the Joint Genome Institute for *P. trichocarpa* v3.1 (Tuskan et al. 2006), *Mortierella elongata* AG77 v. 1 (Morel 1) (Uehling et al. 2017) and *Ilyonectria* (*Ilyonectria europaea* v1.0) were used as references for RNA-Seq filtered read mapping using Tophat/Cufflink packages (Trapnell et al. 2010, 2009). The genome and transcriptome of *I. europaea* were sequenced using Illumina platform. The pipelines applied for the assembly of *Ilyonectria* genome were described in the Supplementary Text. Ribosomal rRNA mapping method was employed to sort reads for all other fungal rRNA as well. Computational workflows for the sequence assembly are described in Supplementary Fig. 2 (Liao et al., 2014; Liao et al., 2016). Recovered rRNA reads containing D1/D2 regions were used to calculate relative abundance of fungal communities (Supplementary Fig. 2). Recent studies indicate that rRNA reads recovered from metatranscriptome (poly-A

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enrich strategy) and RNA-based amplicon sequencing detected similar trends of microbial diversity and community (Liao et al., 2014; Chen et al., 2018). Nonmetric multidimensional scaling (NMDS) was performed on both euclidean and bray-curtist dissimilarity matrices, and results from multiple different dimensions were examined. Results from different configurations demonstrated similar classification, and results from two dimensions are presented in Supplementary Figure 3. Differences in community composition among the treatments (w/o fungal inoculation) were tested using permutational multivariate analysis of variance (PERMANOVA). Results for PERMANOVA were corrected for multiple comparison using false discovery rate (FDR). P-value were calculated based on pseudo-F statistics, and results with $P \leq 0.05$ were considered as statistically significant (Results shown in Supplementary Table 2). Both NMDS and PERMANOVA were performed using vegan package version 2.5.3 in R (3.5.1). They are performed using metaMDS and adonis functions respectively. Comparative metatranscriptomics using Cuffdiff and Cuffcompare packages were applied to identify key plant genes that differentially respond to fungal inoculation (Trapnell et al. 2010). A false discovery rate (FDR) of 5% was used to identify highly expressed transcripts with at least 2-fold change for the genes. A combination of GO (Ashburner et al. 2000), KEGG (Kanehisa et al. 2012) and KOG (Tatusov et al. 2003) packages was used for gene annotation for *P. trichocarpa* v.3.1. Gene enrichment analysis was applied using the ClueGO plateform (Bindea et al. 2009;2013). Parameters and statistical analysis for

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gene enrichment analysis were shown in the legend of Supplementary Fig. 8. It is important to note that only around 18% of the *Populus* genes in the *P. trichocarpa* genomes were assigned to the KEGG and/or GO category. For example, of 60,000 *P. trichocarpa* genes, 10,876 genes were assigned to 7068 KO numbers. In addition to gene enrichment analysis, several other software packages were used to better annotate domains to identify the subcellular locations of genes with an unknown function or predicted with the extracellular enzymes. For domain analysis, EMBL-EBI, Phobius (Käll et al. 2004), Signal-3L (Zhang and Shen 2017; Shen and Chou 2007) , Signal P v4.1 (Kihara 2017) and TMHMM v2.0 (Krogh et al. 2001) were used for the prediction of signal-peptides and transmembrane helix domains. ER retention signal ScanPrositeTool (de Castro et al. 2006) and Euk-mPLoc 2.0 (Chou and Shen 2010) were used to identify the subcellular localization of contigs. Small secreted proteins were defined as having 1) a size smaller than 300 amino acids (detected manually), 2) signal peptide predicted at the N-end (Signal-P v4.1), 3) extracellular location (Euk-mPLoc 2.0), 4) no transmembrane domains (Euk-mPLoc 2.0, TMHMM v 2.0, EMBL-EBI and Signal-3L) and 5) no ER retention motifs (ER retention signal_ScanPrositeTool). The tertiary structures, ligand binding sites and enzyme activation sites of the individual SSPs were predicted using I-TASSER v3.0 (Bateman et al. 2002; Zhang 2008; Roy et al. 2010; Yang et al. 2015). With this approach, we were able to assign the majority of contigs with unknown function to either small-secreted proteins or transmembrane

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proteins. Plots (Fig. 4) and heatmaps (Fig. 5 and 6) were generated with statistical packages in R (R Development Core Team 2003). Given that different batches of soil were used for *M. elongata* (PMI93) and *I. europaea* (PMI82) inoculations, we did not attempt to compare the differential responses of plant between two fungal species. Only the expression of *Populus* genes that were manipulated by both fungal species are presented (Fig. 5). The greater number of plant genes responsive to *M. elongata* (PMI93) were reported in Fig. 6.

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Figure legends

Figure 1. Plant dry weight (*P. trichocarpa* BESC4) in response to *Mortierella elongata* (PMI93) (A), and *Ilyonectria europaea* (PMI82) (B) inoculation. Two soil treatments, sterile sand and 30% natural soil (w/ 70% of sterile sand w/w) collected from *Populus* site (NC1, USA), were used. The data were collected 1-year post inoculation. Error bars indicate the standard deviation of data from above-ground tissues (error bars above) and root tissues (error bar below), n=4. Tukey test was used to test significance of the whole plant biomass across the combinations ($p \leq 0.05$, n=4). Means marked by the same letters were not significantly different.

Figure 2. *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) are transcriptomically active in the forest soil and *Populus* roots and enriched by inoculation. Bars show the percentage of total RNA-Seq reads of the root and soil samples (+/- =w/o inoculums) mapped to the genome databases of *M. elongata* and *I. europaea* respectively. Fig. 2A indicates the individual sample set was mapped to the genome databases of their inoculum, while Fig. 2B indicates the individual sample set was mapped to the genome databases of *I. europaea* or *M. elongata*, but not inoculum. The error bars indicate the standard deviation of the data, n=4. Tukey test was used to test significance

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of the percentage reads across the combinations in a bar graph ($P \leq 0.05$, n=4). Means marked by the same letters were not significantly different.

Figure 3. Taxonomic composition of fungal communities in uninoculated (-) root and soil samples and those inoculated (+) with *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82). Individual bars show the normalized abundance of ribosomal RNA sequences (LSU D1D2). Biological replicates (n=4) show a consistent taxonomic representation of reads recovered from root and soil samples across treatments. Percentages indicate the relative values of paired reads. The relative values below 1 appear as zero (Over 80% of the relative values <1 were singletons representatives, data not shown). Taxa are ordered and coded in color based upon the ecological function of the fungal taxa according to FunGuild (Nguyen et al. 2016). PERMANOVA were applied to identify the taxa with significant differential abundance in associated with *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) inoculation. In the right side of the boxes, the abundance of normalized rRNA reads (using Deseq2) that were significantly increased (blue boxes) or reduced (red boxes) (p-value ≤ 0.05), or not significantly changed (grey boxes) (p-value>0.05). The detailed statistical results were also shown in Supplementary Table 2 and Supplementary Fig. 3.

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Figure 4. Volcano plots elucidate the counts and expression rate of *P. trichocarpa* genes up- (green dots) and down- (blue dots) regulated in response to *Mortierella elongata* (PMI93) (A) and *Ilyonectria europaea* (PMI82) (B) inoculation. Black dots represent the expression of genes with no significant difference across the comparisons. Data were generated according to the normalized expression rates using Cufflink packages (see workflow Supplementary Fig. 2). Data of the loading gene factors were generated using the coordinate scales on the left (log10 of expression rate) and the bottom (mean of log2-fold changes). Cross-comparative expression of the genes was analyzed using t-text to compare *P. trichocarpa* with fungal inoculation versus without fungal inoculation (n=4; P≤0.01; FDR ≤0.05; fold changes>=2). The total counts of genes detected and the counts of genes significant changes in response to the inoculation were listed in Supplementary Dataset 2.

Figure 5. *Populus* genes whose expression changes in response to both *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) inoculation. Significant changes in individual expression between inoculated root (+) v.s. non-inoculated root samples (-) were clustered according to their biological function (>2-fold changes; FDR ≤0.05, Benjamini-Hochberg test). The color key represents RPKM normalized log2 transformed counts of the genes. Wilcoxon signed-rank test (Bauer, 1972) was applied to filter the data. The

genes for other catalytic activities and unknown function were not included in heatmap (Supplementary Dataset 3). Each *Populus* gene manipulated by the fungal species is given the expression profiles across the four biological replicates per treatment.

Figure 6. Significant changes of *Populus* pSSP genes in response to *Mortierella elongata* (PMI93) inoculation (>2-fold changes; FDR $\leq$ 0.05). The method for data analysis see Fig. 5 legend.

Captions for Supplementary Figures

Supplementary Figure 1. (A) Growth enhancement of *Populus trichocarpa* BESC86 in response to inoculation with *Mortierella elongata* PMI93. Without *M. elongata* (PMI93) inoculation, *P. trichocarpa* (BESC86) cuttings did not survive sterile sand conditions. (B) The enhancement of physiological traits (shoot growth, leaf expansion) across 4 different *Populus* genotypes (BESC86, GW7974, BESC4, BESC320) in response to PMI93 inoculation. Soil bioassay (30% soil means 30% soil: 70% sterile sand /w:w) was conducted using natural soil collected from *Populus* field site NC1 (Bonito et al., 2014). (C) Seedling growth and changes in leaf color of *P. trichocarpa* BESC4 in response to PMI93 inoculation. Leaves of inoculated plants were darker green compared to the un-inoculated plants (similar to effect of adding N fertilizer).

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Supplementary Figure 2. Computational flowchart used to sort the RNA-Seq reads of *Populus* root and soil samples. See Dataset S1 for the reads, %reads sorted out from each root and soil samples.

Supplementary Figure 3. NMDS plots showing the taxonomic composition of fungal communities in uninoculated (-) root and soil samples versus those inoculated (+) with *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82). PERMANOVA were applied to identify the differential abundance of ribosomal RNA sequences (LSU D1D2) in associated with Mortierella elongata (PMI93) and Ilyonectria europaea (PMI82) inoculation. In particular, the pairwise comparisons using PERMANOVA were applied to compare the uninoculated samples (Group A in green) vs. inoculated samples (Group B in orange) across (A) the whole communities and (B) a predicted ecological functional group. The statistical outcomes were also shown in Supplementary Table 2.

Supplementary Figure 4. The combination of sub cellular localization analysis and functional annotation show the differential expression of *Populus trichocarpa* genes in response to *M. elongata* (PMI93) (A), and *I. europaea* (PMI82) (B).

Supplementary Figure 5. The primary and tertiary (ribbon model) protein structures and the predicted ligands of pSSP, include **(A)** lipid transferring (pLTPs) and **(B)** defense responsive proteins.

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Supplementary Figure 6. Key functional categories (KEGG) of P. *trichocarpa* genes up- (green) and down- (blue) regulated in response to PMI 93.

Supplementary Figure 7. Of 1,055 KOG categorized gene groups detected in populous root in response to PMI93 inoculation, top 32 gene groups were listed (>2-fold changes, FDR ≤0.05 for each selected gene; n=4).

Supplementary Figure 8. The ClueGO pie charts show the enrichment of populus genes (% Go terms per group) without (A) versus with PMI93 inoculation (B) (p≤0.05).

Supplementary Figure 9. (A) Functional proportions of fungal [*M. elongata* (PMI93)] genes detected in sand, in culture and presented in *M. elongata* genome. (B) Number of *M. elongata* (PMI93) genes significantly expressed (>2-fold changes; FDR ≤0.05) in sand versus in culture system.

Supplementary Figure 10. The image observing the mycelium of *M. elongata* (PMI93) forming the biofilm in associated with corn root (**A**) and extending out from the corn root (**B**).

Supplementary Figure 11. *Mortierella elongata* (PMI93) growing on MMN media producing lipid globules at lipid producing centers (**A** and **B**). Lipid release (examples shown by arrow) when the culture is injured with sharp object (**C**).

Captions for Supplementary Table and Text

Supplementary Table 1. The list of *Populus* genes for phytohormone biosynthesis/signaling that were responsive to both *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) vs. either one treatment.

Supplementary Table 2. Taxonomic composition of fungal communities in uninoculated (-) root and soil samples vs. those inoculated (+) with *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82). PERMANOVA were applied to identify the differential abundance of ribosomal RNA sequences (LSU D1D2) in associated with *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) inoculation. In particular, the pairwise comparisons using PERMANOVA were applied to compare the uninoculated samples vs. inoculated samples across **(A)** the whole communities, **(B)** a predicted ecological functional group, or **(C)** each listed fungal taxa.

Supplementary Text The pipeline applied for the genome sequencing and genome assembly of *Ilyonectria europaea* v1.0

Captions for Supplementary Dataset

Supplementary Dataset S1. Reads and %reads of fungal D1D2 LSU rRNA, fungal genes and plant genes sorted from root and soil samples using computational workflow (Supplementary Fig. 5)

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Supplementary Dataset S2. *Populus* gene significantly changes in response to *M. elongata* (PMI93) and *I. europaea* (PMI82) inoculation (FDR≤0.05; >2-fold; n=4) (S10_9to12 versus S11_1to4). The data of Supplementary Dataset S2 was used to generate Volcano plots in Fig 4.

Supplementary Dataset S3. The list of shared genes of *P. trichocarpa* in response to *M. elongata* (PMI93) and *I. europaea* (PMI82). The data of Supplementary Dataset S3 was used to generate heatmap for Fig 5.

Supplementary Dataset S4. Differential expression of pSSP-like genes of *P. trichocarpa* in response to PMI93 (Dataset S4A, 147 genes) and PMI82 (Dataset S4B, 15 genes) respectively. The data of Supplementary Dataset S4 was used to generate heatmap for Fig 6.

Supplementary Dataset S5. Gene products (7950 transcripts) of *M. elongata* (PMI93) detected in sand with *P. trichocarpa* BESC4 grown nearby (n=4).

Supplementary Dataset S6. Gene counts and % of gene counts of *M. elongata* (PMI93) detected in sand with *P. trichocarpa* BESC4 grown nearby (n=4), culture condition and presented in Morel1 genome. The data of Supplementary Dataset S7 was used to generate bar graph of Fig. S8A.

Supplementary Dataset S7. Comparative transcriptomic data showing the differential expression of *M. elongata* (PMI93) genes in sand condition (with

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P. trichocarpa BESC4 grown nearby) verse culture conditions. The data of Supplementary Dataset S7 was used to generate bar graph of Fig. S8B.

Supplementary Dataset S8. The list of fungal small secreted protein (fSSP) predicted in *Mortierella elongata* (Morel1) genome.

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