Root endophytic fungal communities associated with pitch pine, switchgrass and rosette grass in the pine barrens ecosystem

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ABSTRACT

Almost all plants in nature harbor fungi in their roots but the knowledge on distribution and the underlying principles of assemblage is still poorly developed for the root-associated fungi. In this study we analyzed the root endophytic fungal communities associated with switchgrass, rosette grass, and pitch pine in the acidic, oligotrophic pine barrens ecosystem. A total of 434 fungal isolates were obtained from 600 root segments of 60 plant samples. DNA barcoding and morphological analyses identified 92 fungal species, which belong to 39 genera in 6 classes. Compared to other ecosystems, the pine barrens has a higher proportion of *Leotiomycetes*. The fungal community associated with pitch pine was significantly different from those associated with the grasses, while less difference was found between those associated with the two grasses. Our results suggest that edaphic factors and host specificity play a role in shaping root endophytic fungal community. This study also corroborates our previous finding that plant roots in the pine barrens are a rich reservoir of novel fungi.

Keywords:

Endophytes

Fungal ecology

Host specificity

Pine barrens

Root-associated fungi

Switchgrass

1 Introduction

Fungal endophytes refer to fungi that colonize living plant tissue without causing any immediate, overt negative effects to the hosts (Hirsch and Braun 1992). Root fungal endophytes reside within plant roots for all or part of their life cycle (Sieber 2002). The concept of root endophytes used in this paper is in the broad sense that includes all symbiotic fungi from surface sterilized roots of apparently healthy plants. In practice, certain mycorrhizal and pathogenic species may also be isolated.

Fungal root endophytes are ubiquitous and have been reported from a wide host range including ferns, lycophytes, conifers and angiosperms (Jumpponen and Trappe 1998; Rodriguez et al. 2009; Zubek et al. 2010). Some root endophytes were reported to play a role in deterring pathogens, decreasing herbivory, and enhancing host plant's tolerance to biotic and abiotic stresses. However, the functions of most root endophytes remain elusive due to the inconsistent results or simply the lack of study (Mandyam and Jumpponen 2005; Rodriguez et al. 2009).

Fungal root endophytes are taxonomically diverse. Various *Ascomycota*, *Basidiomycota* and *Mucoromycotina* fungi have been isolated from plant roots, among which *Ascomycota* fungi are usually predominant. For example, 96% fungal root endophytes isolated from three boreal tree species belonged to *Ascomycota* (Kernaghan and Patriquin 2011). High proportion of species belonging to *Ascomycota* was also reported from *Arabidopsis thaliana* (89%) (Keim et al. 2014), *Microthlaspi perfoliatum* (100%) (Keim et al. 2014), *Holcus lanatus* (97%) (Sánchez Márquez et al. 2010), *Dactylis glomerata* (98%) (Sánchez Márquez et al. 2007), *Oryzae granulate* (98%) (Yuan et al. 2010), and *Pseudotsuga menziesii* and *Pinus ponderosa* (52%) (Hoff et al. 2004). Within *Ascomycota*, *Sordariomycetes* and *Dothideomycetes* were reported as the predominant classes in most studies (Keim et al. 2014; Khidir et al. 2010; Sánchez Márquez et al. 2007, 2010; Yuan et al. 2010). However, recent studies using a culture-independent metabarcoding method revealed that *Basidiomycota* fungi and other unculturable or slow growing fungi account for a large proportion of the root fungal community (Mundra et al. 2015).

Many fungal pathogens and endophytes of leaves or stems are host-specific (Butin 1986; Fisher et al. 1995; Newton and Haigh 1998; Wood and Graniti 1976; Zhou and Hyde 2001). Root endophytes, however, were generally believed to be less host-specific and have broad host ranges (Jumpponen and Trappe 1998; Rodriguez et al. 2009). For example, *Phialocephala fortinii*, a wide spread root endophyte was found to be associated with more than 20 plant species (Jumpponen and Trappe 1998). However, more recent molecular phylogenetic studies indicated that *P. fortinii* is a species complex, within which each cryptic species may be associated with a narrower host range (Grünig et al. 2004, 2008a, 2008b). Several other recent studies also demonstrated that different host plant species harbor different root endophyte communities (Keim et al. 2014; Kernaghan and Patriquin 2011). However, the data on the root endophyte distribution in various host plants and ecosystems are still very limited, and our knowledge on the underlying principles of root

endophyte community assemblage is poorly developed (Davison et al. 2015; Tedersoo et al. 2014).

New Jersey Pine Barrens is a 1.4 million acre (57,000 km²) coastal plain located in southern New Jersey in the United States. It has a temperate climate with average monthly temperatures from 0.3 to 24.3 °C and average annual precipitation of 116.5 cm (1981–2010, NJ State Climatologist). There are more than 850 plant species inhabiting this ecosystem, where pines and oaks are the dominant trees, and the understory is composed of grasses (*Poaceae*), sedges (*Cyperaceae*), blueberries and other heath family members (*Ericaceae*) (Forman 1998, <http://nj.gov/pinelands/reserve/plants/>). The soils in the pine barrens are highly acidic (pH 4–5), sandy, dry (low moisture holding capacity) and nutrient poor (low in P, K, N) (Forman 1998; Joffe and Watson 1933; Tedrow 1952; Turner et al. 1985). The New Jersey Pine Barrens represents one of a series of barrens ecosystems in the eastern United States and one of a series of similar ecosystems around the world. Only few studies have assessed the fungal diversity in the pine barrens, and much remains unknown about root endophytes in this ecosystem (Forman 1998; Tuininga and Dighton 2004). Our recent survey indicated that plant roots in this acidic, oligotrophic ecosystem harbor diverse fungal endophytes, many of which are undescribed species (Luo et al. 2014a).

In this study, root endophytic fungi associated with one pine and two grass species that are commonly found in the New Jersey Pine Barrens were examined. The objectives were to (1) identify the endophytic fungi inhabiting the pine and grass roots in the pine barrens, and (2) identify the factors that shape the root endophytic fungal communities in this ecosystem.

2 Materials and methods

2.1 Sample collection

In June 2014, a total of 60 root samples were collected from three common native plant species, i.e., rosette grass (*Dichanthelium acuminatum*), switchgrass (*Panicum virgatum*) and pitch pine (*Pinus rigida*) from Collier's Mills (N40°04', W74°26', Alt. 42m) and Wharton State Forest (N39°46', W74°40', Alt. 40m) in the New Jersey Pine Barrens. There is a distance of 64 km between the two sampling locations. They are both typical sand plains with similar vegetation. Both sites are low density forests composed mostly of pitch pines. The scattered trees or groves are interspersed with openings in which isolated tussocks of rosette grass and switchgrass are prominent. Healthy plants of similar size were chosen and their root samples were collected by digging up the plant root system approximately 50 cm deep in soil. At each location, 10 apparently healthy root samples including coarse roots, fine roots and root tips were randomly collected for each plant species, at least 5 m apart to avoid clonal ramets and maximize diversity (Kleczewski et al. 2012). Samples were kept on ice and transported to the laboratory for fungal isolation within 24 h.

2.2 Fungal isolation

The collected roots were rinsed in tap water to remove soil particles and cut into 10–20 mm long segments. The segments were surface sterilized with 95% ethanol for 30 s, followed by 2 min in 0.6% sodium hypochlorite and 70% ethanol for 2 min, then two final rinses in sterile distilled water. Samples from the same host species and same location were pooled and further cut into 3 mm long small segments. For each of the six pooled samples, 100 surface sterilized 3 mm long root segments were air dried and placed on 2% malt extract agar (MEA, BD Difco, Sparks, Maryland) with 0.07% lactic acid. Lactic acid was used to limit bacterial growth during isolation (Bills and Foster 2004). Plates were incubated at room temperature and observed daily in the first two weeks, then twice a week afterwards for six months. Fungal cultures were isolated and purified by subculturing from emergent hyphal tips. Imprints of root segments were made on MEA plates to confirm the effectiveness of the surface disinfection protocol. No fungal growth was observed from these imprint plates during 4 weeks of incubation. Spore morphology, if present, and colony characteristics were examined and recorded as morphological data for identification. Fungal cultures are preserved at -80 °C in glycerol and 4 °C on agar in the Zhang lab at Rutgers University, USA. Fungal DNA sequences from this study were deposited in GenBank (Table S1).

2.3 Molecular identification and phylogenetic analysis

The DNA sequences of the internal transcribed spacer (ITS) of the ribosomal RNA genes were obtained for all the isolated fungal cultures. The PowerSoil DNA Isolation Kit (MoBio, California) was used for DNA isolation, and the primers ITS1 and ITS4 (White et al. 1990) were used for PCR and sequencing. DNA extraction, PCR amplification and sequencing of ITS followed the protocols described in Luo and Zhang (2013). With Usearch 7 (Edgar 2010), the ITS sequences were clustered into operational taxonomic units (OTUs) using 97% sequence similarity according to O'Brien et al. (2005) and 99% similarity suggested by Gazis et al. (2011), respectively. The OTUs were then identified based on the search results with Blastn in UNITE (<https://unite.ut.ee/>), GenBank (<http://www.ncbi.nlm.nih.gov/>) and AFTOL (<http://aftol.org/>) databases, as well as the morphological data, which contributed to the identification of the sporulating taxa such as *Acidomelania*, *Barrenia*, *Curvularia*, *Fusarium*, *Magnaporthiopsis*, *Penicillium*, *Pseudophialophora*, and *Umbelopsis*. Uncultured environmental samples in the databases were excluded. The close matching sequences were included in our phylogenetic analyses to infer taxonomic assignments (Tables S1, Figs S1–S3). Because the two clustering criteria led to similar conclusions regarding the fungal community structure, only the results from the 97% similarity clustering were presented in the paper, while the results from the 99% similarity were provided in the supplementary files (Tables S3–S4, Figs S4–S10). Another reason to prefer the 97% similarity is that this criterion applied well to the predominant *Leotiomyces* and *Sordariomyces* species from the pine barrens plant roots (Luo et al. 2014a, 2015b; Walsh et al. 2014, 2015). A species was categorized as undescribed when it was diagnosably distinct from any reference taxon in phylogeny, had

<97% ITS sequence similarity to any known taxon in GenBank, and morphologically, there was no match with available fungal descriptions in the literature.

Phylogenetic analyses that include reference sequences were performed to further confirm the fungal identification. Because fungal ITS sequences are highly variable at higher taxonomic levels (Nilsson et al. 2008), three separate phylogenetic analyses were performed: *Sordariomycetes* and *Leotiomycetes*, *Dothideomycetes* and *Eurotiomycetes*, and *Basidiomycota* and *Mucoromycotina*. All sequences of representative phylotypes were aligned in Clustal X V.1.8 (Thompson et al. 1997) and slightly refined manually in BioEdit 7.0.5 (Hall 1999). MrModeltest (Nylander 2004) was used to specify the best-fit nucleotide substitution model under hierarchical likelihood ratio tests (hLRT) and Akaike information criterion (AIC). The general time reversible with a proportion of invariable sites and gamma distributed rate variation among sites (GTR+I+G) was the selected model for the *Sordariomycetes* and *Leotiomycetes*, as well as the *Dothideomycetes* and *Eurotiomycetes*. The Hasegawa-Kishino-Yano model with a proportion of invariable sites and gamma distributed rate variation among sites (HKY+I+G) was used for the third group including *Basidiomycota* and *Mucoromycotina*. Maximum likelihood (ML) analyses with the selected models were carried out in PhyML 3.0 (Guindon et al. 2010). Branch support was assessed with 1000 bootstraps replicates.

2.4 Community analysis

Fungi isolated from rosette grass (R), switchgrass (S) and pitch pine (P) from Collier's Mills (C) and Wharton State Forest (W) in New Jersey Pine Barrens were abbreviated as CR, CS, CP, WR, WS and WP, respectively. Species rarefaction curves, species richness analyses, and diversity indices were calculated using 50 randomizations of sample order in EstimateS 9.1 (Colwell et al. 2012). Chao, Jackknife and Bootstrap values were applied for species richness estimates, and only Chao1 index was provided in Table 1. Fungal diversity was measured using Shannon-Wiener's and Fisher's alpha indices.

The composition of fungal communities was compared among hosts and locations using two similarity indices: Sørensen's (SI) and Jaccard's (JI) indices. Additionally, the relationships among the fungal communities were visualized using nonmetric multidimensional scaling (NMDS) analysis according to Bray Curtis dissimilarities in R package vegan (Oksanen 2015). The statistical significances of fungal assemblages were evaluated with analysis of similarities (ANOSIM) using distance matrices.

3 Results

3.1 Fungal isolation and identification

A total of 434 fungal isolates were obtained from 600 root segments of 60 plant samples. Based on ITS sequences and morphology, they were identified to belong to 92 species in 39 genera, 25 families, 18 orders and 6 classes (Fig 1), among which 47 (51%) species were likely undescribed. The majority (85.9%) of these fungal species are in the phylum *Ascomycota*, including 32 *Leotiomycetes* species (209 isolates), 25 *Sordariomycetes* (149

isolates), 12 *Dothideomycetes* (28 isolates) and 10 *Eurotiomycetes* (27 isolates). Only 8 *Mucoromycotina* (15 isolates) and 5 *Basidiomycota* species (6 isolates) were uncovered.

There were 96 isolates uncovered from CR, 86 from WR, 76 from WP, 71 from WS, 70 from CP, and 35 from CS (Table 1). The highest fungal species richness was found from CP (33 species), followed by WP (25 species), WR (20 species), CR (19 species), WS (19 species) and CS (13 species). All species rarefaction curves failed to reach asymptote, indicating that more species will likely be found with additional sampling (Fig 2). The species diversity and the estimated richness are listed in Table 1. Based on the calculated Chao1 index, CP had the highest fungal species richness, followed by WS, CR, WP, CS and WR. Additionally, the highest percentage of species captured based on the Chao1 index was found from WR (95.2%), followed by WP (73.5), CP (61.1%), CR (54.3%), WS (51.4%), and CS (43.3%), which indicated that more than 50% species were successfully obtained from most sampling locations except CS. The Shannon's diversity index ranged from 2.01 to 3.11, while Fisher's alpha index was from 7.1 to 24.38 (Table 1).

Distribution of fungal classes found among hosts and locations in the New Jersey Pine Barrens was illustrated in Fig 3. *Sordariomycetes* and *Leotiomyces* were the most frequently isolated fungi in CR, WR, WS and WP, *Sordariomycetes* and *Dothideomycetes* were predominant in CS, while *Leotiomyces* and others were predominant in CP. At the species level, the most frequently isolated species were listed in Table 1.

Pseudophialophora eragrostis was shared by CR and WR; *Acephala* sp4 was shared by CR, CR and WS, and *Mollisia* sp. was shared by CS and WS. Among the 92 identified fungal species, 24 occurred in two or more communities. There were 19 fungal species exclusive to CP, 15 to WP, 12 to WR, 9 to CR, 9 to WS, and 5 to CS.

3.2 Difference among hosts and locations

The pairwise similarity of six samples ranged from 0.438 to 0.044 for the Sørensen's index, and 0.28 to 0.023 for the Jaccard's index (Table 2). The highest similarity was found between CS and CR (SI = 0.438, JI = 0.28), followed by WS and WR (SI = 0.41, JI = 0.258), CP and WP (SI = 0.31, JI = 0.184).

The NMDS identified two major groups (Fig 4). WS, WR, CS and CR constituted one group, while WP and CP formed the other group. They were distantly related with significant difference (ANOSIM $R = 1$, $P = 0.04$). The root fungal communities from the rosette grass and switchgrass were closely related, and were distinct from those from the pine host. CR and CS from Collier's Mills clustered as a subgroup and separated from another subgroup including WR and WS from Wharton State Forest (Fig 4). However, the ANOSIM test suggested that the difference between these two subgroups is not significant (ANOSIM $R = 0.75$, $P = 0.33$).

4 Discussion

4.1 *Leotiomyces* and edaphic factors

In the acidic, oligotrophic pine barrens ecosystem, we found that *Leotiomyces* fungi

account for a relatively high proportion of the root endophyte communities. Thirty-two species in eight genera were identified to belong to *Leotiomyces*, including *Rutstroemia*, *Lachnum*, *Meliomyces*, *Acephala*, *Phialocephala*, *Mollisia*, *Acidomelania*, and *Barrenia* (Fig 1). In other ecosystems, *Leotiomyces* usually constitute less than 15% of the root endophytic fungi according to a number of published papers on poaceous plants, conifers and brassicaceous herbs (Keim et al. 2014; Sánchez Márquez et al. 2007, 2010; Yuan et al. 2010). In our preliminary study (Luo et al. 2014a), the root fungal community in *Poaceae* grasses in the New Jersey Pine Barrens was compared with that of the Yunnan sub/tropical rain forest. The results showed that 22% of fungal isolates from the former belonged to *Leotiomyces*, while the latter counterpart had only 1.8% *Leotiomyces*. In this study, we focused on three common native plant species in the pine barrens and sampled more extensively. The percentage of *Leotiomyces* ranged from 29% (rosette grass in the Wharton State Forest) to 75% (switchgrass in the Wharton State Forest) in the six communities analyzed here, which is significantly higher than those reported in other ecosystems.

Leotiomyces are also frequently found from coniferous and ericaceous roots in the nutrient-limiting boreal and arctic area (Mundra et al. 2015; Sieber and Grünig 2006; Vrålstad et al. 2002). For instance, a total of 6.65% of the reads (11.54% OTUs) were represented from *Bistorta vivipara* root in the Arctic according to a pyrosequencing study (Mundra et al. 2015). The results from a recent global survey using pyrosequencing showed that *Leotiomyces* formed 7.1% of the total soil fungal community, within which the Arctic had the highest (about 25%) and the tropical savannas and tropical forests had the least *Leotiomyces* (1–2%). Although the soil and root are different habitats, the similar trend also was observed in our grass root fungal survey (Luo et al. 2014a), indicating that edaphic factors play a key role in determining the distribution of soil and root-associated *Leotiomyces*.

Geographic locality also was suggested as a factor determining fungal communities (Herrera et al. 2010; Sánchez Márquez et al. 2008). The two sampling locations in this study are only 64 km apart and have similar soil properties (Table S2). As expected, location did not show significant impact on root fungal endophytes from this study (Fig 4, Table 2). Similar results were also reported from other studies (Loro et al. 2012; Macia-Vicente et al. 2008), in which fungal root endophytic communities were more influenced by the edaphic factors rather than location.

Despite the ubiquity of plant root endophytic fungi, their ecological functions in nature remain elusive. Many researchers in this field speculated that certain endophytes function as mycorrhizae that increase host plant stress tolerance (Mandyam and Jumpponen 2005; Knapp et al. 2012; Sieber and Grünig 2013). However, the specific sources of stress have not been identified and the host-fungus interaction experiments reported to date often produced variable or inconsistent results, ranging from pathogenic to mutually beneficial interactions (Mandyam and Jumpponen 2005). The ecological functions of the root-colonizing *Leotiomyces* from the pine barrens also are unclear but some evidence

indicated that some of them likely play a role in increasing the plant hosts' fitness in the poor nutrient environment, or protection against root pathogenic fungi (Terhonen et al. 2014). Our plant-fungal interaction experiments showed that *Acidomelania panicicola* and *Barrenia panicia*, two newly described *Leotiomyces* fungi we isolated from the New Jersey Pine Barrens wild switchgrass root promoted switchgrass root hair growth under low nutrient conditions (Walsh et al. 2014, 2015). In addition, the ITS sequence of *A. panicicola* and *B. panicia* each had over 50 matches in GenBank (>97% identities) from a broad geographic range (Africa, Asia, Europe, Central, North and South America). Despite the wide distribution, the matched samples were all from the roots of plants in acidic and infertile soils, similar to the pine barrens (Walsh et al. 2015). Further studies are needed to better understand the ecological functions of these extensively colonizing *Leotiomyces* in the pine barrens.

4.2 Host specificity of root fungal endophytes

Another finding from this study is that host plants likely play a role in shaping root endophytic fungal communities. For example, *Pseudophialophora*, a grass root associated genus we recently described (Luo et al. 2014b, 2015b) was not found from any pine samples in this study. Fungi with host specificity for pine were also revealed in this study, e.g., *Phialocephala fortinii* and *Meliniomyces* species. Another example is *Barrenia*, within which *B. panicia* was only from grass while *B. taeda* was only from pine (Walsh et al. 2015). Pitch pine is a woody gymnosperm plant obligatorily bearing ectomycorrhiza, while the two *Poaceae* grasses are herbaceous monocots generally forming arbuscular mycorrhiza (Smith and Read 2008). The genus *Meliniomyces* was reported to include ectomycorrhizal species (Hambleton and Sigler 2005). In this study, we identified four *Meliniomyces* species from the pine roots and whether they also are ectomycorrhizal symbionts needs further studies to verify.

There were 42 fungal species found exclusively from pitch pine root, 21 from rosette grass and 14 from switchgrass (Fig 5). However, only four fungal species were found from all the three hosts, i.e., *Chaetomium aureum*, *Mollisia* sp., *Acephala* sp4., and *Phoma herbarum* (Figs 1 and 5). More species (12 species) were shared by the two grasses than those between pine and switchgrass grass (6 species), or rosette grass (5 species). In addition to the four common fungal species listed above, *Codinaeopsis* sp., *Pseudophialophora* sp4, *Pseudophialophora panicorum*, *Lachlum virgineum*, *Lachnum* sp., *Acephala* sp3, *Leotiomyces* sp., and *Phoma* sp. were found from both of the grasses; *Fusarium verticillioides* and *Umbelopsis dimorpha* were from the pitch pine and switchgrass; *Umbelopsis isabellina* was shared by the pine and rosette grass (Figs 1 and 5). These results suggested host specificity of certain fungal species.

The difference between the grass and pine fungal communities was significant with only 8% shared fungal species, while the host specificity was not well supported between the two grasses based on the ANOSIM test. Similar results were reported in a number of published papers (Hoff et al. 2004; Kernaghan and Patriquin 2011; Khidir et al. 2010;

Macia-Vicente et al. 2008). Khidir et al. (2010) reported a general suite of root fungal endophytes from two *Poaceae* species, which was different from the *Agavaceae* weed. Hoff et al. (2004) and Kernaghan and Patriquin (2011) found that the root endophytes showed little host specificity within the *Pinaceae* family. No host specificity was observed when *Helotiales* fungi were investigated from *Ericaceae* species (Walker et al. 2011). Our data corroborate other published results that host specificity of root endophytes is evident at the family level of the plants (Hoff et al. 2004; Kernaghan and Patriquin 2011; Walker et al. 2011).

4.3 Pine barrens plant roots—a rich reservoir of undescribed fungi

Our previous survey on grass root associated fungi from the pine barrens and Yunnan sub/tropical rain forest showed that pine barrens plant roots were a rich reservoir of novel fungi with about 47% being undescribed species (Luo et al. 2014a). This study added more data and corroborated this finding. To date, a total of 136 fungal species have been identified from 1,011 cultures isolated from pine barrens plant roots, of which 62 species (46%) are likely undescribed (Luo et al. 2014a, 2014b, 2015b; Walsh et al. 2014, 2015, unpublished data). It is possible that some species may be described but have not been sequenced. However, it was estimated that over 90% of fungal species have not been discovered and the unmatched sequences more likely are new taxa (Hibbett and Taylor 2013). Furthermore, we also check morphology to confirm their novelty. We are aware that ITS sequences are highly variable at family or higher levels and therefore are not appropriate to infer phylogenetic relations for all fungal taxa collected in this study. Fig 1 is shown only to demonstrate overall fungal diversity. Our species identification was based on a number of separate phylogenetic analyses for each individual OTU (not shown due to limited space), rather than only based on BLAST or the ITS tree. Six-locus (SSU, LSU, ITS, *EF-1alpha*, *MCM7* and *RPB1*) phylogenetic analysis was done with the new *Pseudophialophora* and *Magnaporthiopsis* species (Luo et al. 2014b, 2015b), and three-locus phylogeny was generated when proposed the new *Acidomelania* and *Barrenia* species (Walsh et al. 2014, 2015). In addition, our phylogenomic analysis based on over 200 genes further supported recognition of the new *Pseudophialophora* *Magnaporthiopsis* species (Luo et al. 2015a).

The New Jersey Pine Barrens has about 850 plant species (State of New Jersey Pinelands Commission, 2012) but only a handful of them have been studied for their root endophytes (Luo et al. 2014a, 2014b, 2015b; Walsh et al. 2014, 2015). Based on the host association data (Fig 5) and our species accumulation curves (Fig 2), further sampling in the pine barrens will likely result in uncovering more novel fungal species. These discoveries will fill in the missing lineages of the fungal tree of life, shed light on their functions in the ecosystems, and contribute to finding the principles of fungal distribution and community assemblage.

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Table and Figure legends:

Table 1 – Comparison of fungal communities associated with plant roots from different locations and hosts.

Table 2 – Similarity of fungal communities from grass roots between different locations and hosts. Sørensen's index is showed above the diagonal, while Jaccard's index is below the diagonal.

Fig 1 – Maximum likelihood tree inferred from ITS sequences of 92 OTUs obtained in this study. Branch values ($\geq 50\%$) of ML bootstrap proportions are noted above internodes. Bars show the number of isolates from corresponding plant hosts.

Fig 2 – Species accumulation curves of root associated fungi from rosette grass (R), switchgrass (S) and pitch pine (P) from Collier's Mills (C) and Wharton State Forest (W) in New Jersey Pine Barrens.

Fig 3 – Relative abundance and composition of root associated fungal classes found in rosette grass (R), switchgrass (S) and pitch pine (P) from Collier's Mills (C) and Wharton State Forest (W) in New Jersey Pine Barrens.

Fig 4 – Non-metric multidimensional scaling (NMDS) of root associated fungi from rosette grass (R), switchgrass (S) and pitch pine (P) from Collier's Mills (C) and Wharton State Forest (W) in the New Jersey Pine Barrens.

Fig 5 – Venn diagram of shared root fungal species across the three host plants.

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Figure1

Figure2

Figure3

Figure4

Figure5

Table 1 – Comparison of fungal communities associated with plant roots from different locations and hosts.

	Number of root samples	Number of fungal isolates	Number of fungal species	Chao1	Fisher's alpha	Shannon's index	Most frequently isolated fungal species
Colliers Mills _Rosette grass (CR)	10	96	19	35	7.1	2.05	<i>Pseudophialophora eragrostis</i> (28.1%), <i>Acephala</i> sp4. (27.1%), <i>Chaetomium aureum</i> (15.6%)
Colliers Mills _Switchgrass (CS)	10	35	13	30	7.49	2.01	<i>Phoma herbarum</i> (31.4 %), <i>Acephala</i> sp4. (28.6%), <i>Mollisia</i> sp.
Colliers Mills _Pitch pine (CP)	10	70	33	54	24.38	3.11	<i>Phialocephala</i> sp2. (21.4%), <i>Acephala</i> <i>macrosclerotium</i> (8.6%), <i>Umbelopsis</i> <i>dimorpha</i> (5.7%)
Wharton State Forest _Rosette grass (WR)	10	86	20	21	8.19	2.56	<i>Pseudophialophora</i> sp4. (25.6 %), <i>Pseudophialophora</i> sp2. (10.5%), <i>Mollisia</i> sp. (9.3%), <i>Pseudophialophora eragrostis</i> (9.3%)

Wharton State Forest _Switchgrass (WS)	10	71	19	37	8.5	2.3	<i>Mollisia</i> sp. (26.8%), <i>Lachnum</i> sp. (21.1%), <i>Acephala</i> sp4. (14.1%)
Wharton State Forest _Pitch pine (WP)	10	76	25	34	12.99	2.63	<i>Trichoderma strigosum</i> (30.3%), <i>Meliniomyces</i> sp1. (13.2%), <i>Aspergillus</i> <i>cerrinus</i> (6.6%)

Table 2 – Similarity of fungal communities from grass roots between different locations and hosts. Sørensen's index is showed above the diagonal, while Jaccard's index is below the diagonal.

	Colliers Mills _Rosette grass (CR)	Colliers Mills _Switchgrass (CS)	Colliers Mills _Pitch pine (CP)	Wharton State Forest_Rosette grass (WR)	Wharton State Forest_Switchgra ss (WS)	Wharton State Forest_Pitch pine (WP)
Colliers Mills _Rosette grass (CR)		0.44	0.19	0.26	0.21	0.05
Colliers Mills _Switchgrass (CS)	0.28		0.17	0.18	0.25	0.05
Colliers Mills _Pitch pine (CP)	0.11	0.10		0.08	0.12	0.31
Wharton State Forest_Rosette grass (WR)	0.15	0.10	0.04		0.41	0.04
Wharton State Forest_Switchgrass (WS)	0.12	0.14	0.06	0.26		0.09

Wharton State

0.02

0.03

0.18

0.02

0.05

Forest_Pitch pine

(WP)