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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 harbour 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 analysed 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 six 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.

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Introduction

Fungal endophytes refer to fungi that colonize living plant tissue without causing any immediate, overt negative effects to the hosts (Hirsch & 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,

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conifers, and angiosperms (Jumpponen & 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 & 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 & 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 (Sánchez Márquez *et al.* 2007, 2010; Khidir *et al.* 2010; Yuan *et al.* 2010; Keim *et al.* 2014). 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 (Wood & Graniti 1976; Butin 1986; Fisher *et al.* 1995; Newton & Haigh 1998; Zhou & Hyde 2001). Root endophytes, however, were generally believed to be less host-specific and have broad host ranges (Jumpponen & 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 & 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 harbour different root endophyte communities (Kernaghan & Patriquin 2011; Keim *et al.* 2014). 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 (Tedersoo *et al.* 2014; Davison *et al.* 2015).

New Jersey Pine Barrens is a 1.4 million acre (57 000 km^2) 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) (Joffe & Watson 1933; Tedrow 1952; Turner *et al.* 1985; Forman 1998). 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 & Dighton 2004). Our recent survey indicated that plant roots in this acidic, oligotrophic ecosystem harbour 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.

Materials and methods

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. 42 m) and Wharton State Forest (N39°46′, W74°40′, Alt. 40 m) 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.

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 & Foster 2004). Plates were incubated at room temperature and observed daily in the first two weeks, then twice a week afterwards for 6 m. Fungal cultures were isolated and purified by subculturing from emergent hyphal tips. Imprints of root segments were made on MEA plates to confirm

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