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Comparisons of Ectomycorrhizal Fungi and Fine Roots of *Pinus virginiana* Hosts from Two Soil Sources at the Grassy Hill Natural Area Preserve, Franklin County, Virginia

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ABSTRACT

Roots of Virginia Pine (*Pinus virginiana* Mill.) trees from soils of Basic Oak-Hickory Forest (BOHF) and Mountain/Piedmont Acidic Woodland (MPAW) ecological communities at the Grassy Hill Natural Area Preserve (Franklin County, Virginia) differing in soil pH and moisture were compared for ectomycorrhizal (ECM) fungal properties and fine root length. ECM colonization, community composition, morphotype/species richness, and fine root length were assessed from eight BOHF and nine MPAW trees. While soil cores from these trees represented a relatively low sample size, colonization was found to not differ, but ECM fungal composition varied as richness and the respective numbers of dominant and less abundant morphotypes differed from each soil source. Total richness was greater, and mean richness per meter fine root was significantly greater in the more acidic xeric MPAW soil, while fine root length was significantly greater in the less acidic sub-mesic BOHF soil. Our results are the first to characterize ECM properties and fine root growth from *P. virginiana* trees growing in these two soil sources.

Key words: ectomycorrhizae, fine roots, Grassy Hill, pH, *Pinus virginiana*, soil moisture.

INTRODUCTION

Ectomycorrhizal (ECM) fungi are key components of temperate forests, benefiting hosts by facilitating their nutrient and water uptake, and increasing their tolerance of stressful soil conditions (Smith & Read, 2008). Many trees in Virginia's Blue Ridge Mountains, including Virginia Pine (*Pinus virginiana* Mill.), Sourwood (*Oxydendrum arboreum* [L.] DC.), and Chestnut Oak (*Quercus prinus* L.), grow in acidic and xeric soils (Virginia Department of Conservation and Recreation, 2012), partly due to ECM facilitation (McQuilkin, 1990). This is not surprising, given that both conventional morphotyping and more contemporary DNA-based methods have found that ECM fungi tolerate a range of soil conditions, including moisture levels and pH values (Slankis, 1974; Gehring et al., 1998; Peter et al., 2001; Jany et al., 2003; Abler, 2004; Buée et al., 2005). To better understand the influence of variable soils on ECM fungi and their hosts, we compared ECM fungal and fine root properties of *P. virginiana* trees growing in Basic Oak-Hickory Forests (BOHF) and Mountain/Piedmont Acidic Woodlands (MPAW) communities, whose soils differ in moisture levels and pH.

MPAW communities are rare in the southeastern U.S., but occur in Virginia mountains as barrens characterized by shallow, highly xeric soils. In contrast, BOHF communities are more common across the state, and have deeper, more mesic soils (Virginia Department of Conservation and Recreation, 2012). Both are coniferous or coniferous-deciduous, often being dominated by *Pinus* and *Quercus* species that associate with numerous ECM fungal taxa, many of

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which tolerate acidic soils (Brundrett, 2003). In fact, most ECM fungi grow well between pH values of 4.5 and 5.5 (which include the values of our soils), while others do so under lower values (McAfee & Fortin, 1987; Lehto, 1994).

Ultimately, the success of temperate trees growing in acidic soils depends on ECM fungi. Tree growth and survival are positively correlated with ECM colonization in acidic soils (Erland & Söderström, 1990), due to increased nutrient access. In addition, ECM fungi increase host water access in xeric soils (Gehring & Whitham, 1994). Although studies have examined ECM communities in soils defined by a range of moisture levels and pH values as single variables, fewer have done so in soils with two variables, and none to our knowledge has examined ECM communities on *P. virginiana* hosts in BOHF and MPAW communities. In this study, we examined *in situ* ECM properties and root growth on *P. virginiana* trees growing in these two community types at the Grassy Hill Natural Area Preserve in Franklin County, Virginia. We predicted that there would be differences in ECM colonization, community composition, and diversity between BOHF and MPAW soils based on studies finding differences in these variables in similarly contrasting soils (Gehring & Whitham, 1994; Gehring et al., 1998). However, given the lack of studies reporting differences in fine root length from ECM hosts from similarly contrasting soil types, no prediction was made regarding fine root length.

MATERIALS AND METHODS

Study Sites and Host Species

We conducted our study at the Grassy Hill Natural Area Preserve, located at the northwest edge of Rocky Mount, Virginia (36° 59′ 60″ N, 79° 53′ 23″ W). The Virginia Department of Conservation and Recreation's Division of Natural Heritage manages the Preserve to conserve biodiversity and ecological communities. It lies in the Piedmont physiographic province (Roberts & Bailey, 2000) and southern oak/pine forest zone (Yahner, 2000). It is composed primarily of *Carya* and *Quercus* stands, interspersed with *P. virginiana*, that are fairly undisturbed except for a few roads and power lines (Turner & Demkó, 2007). The terrain is described by magnesium-rich bedrock overlain with heavy clay soils (Virginia Department of Conservation and Recreation, 2013), with rocky slopes reaching 535 m ASL (United States Geological Survey and Virginia Division of Mineral Resources, 1985). Average monthly precipitation ranges from 7.7 to 12.8 cm and temperatures range from –3.4 to 30.2 C (National Weather Service, 2011; values derived from data collected at the Rocky Mount station from 1981 to 2010).

Basic Oak-Hickory Forests (BOHF) and Mountain/Piedmont Acidic Woodlands (MPAW) communities were compared because their soil moisture levels and pH differ (Table 1; M. Leahy, unpubl. data). Although tree composition was similar in each community, there were differences: *Quercus*, *Carya*, and *Acer* species were the dominant trees in the BOHF, whereas *Oxydendrum arboreum*, *Pinus*, and *Quercus* species were dominant in the MPAW communities. *Pinus virginiana* served as our host species because it associates with many ECM fungal taxa (e.g., *Cenococcum*, *Russula*, and *Tomentella*; Hepting, 1971; Abler, 2004) and is found in both communities. The species has shallow roots, grows well in xeric to sub-mesic soils (Carter & Snow, 1990) and tolerates pH values of 4.2 to 7.9 (Miller & Cumming, 2000) – values in which ECM fungi enable its survival (Thiet & Boerner, 2007). Thus ECM fungi were expected to associate with this host in both soil sources.

Field Sampling

In May 2006, we identified *P. virginiana* trees in each of three BOHF and MPAW plots designated within sites previously surveyed for abiotic and vegetative profiles (M. Leahy, unpubl. data). Only two sites of each community were used because only two BOHF sites had a sufficient number of trees to sample. Plots were located more than 500 m apart, and in each, three *P. virginiana* trees with DBH >10 cm were randomly selected, except in one BOHF site where only two suitable host trees occurred. Trees were farther than 5 m from one another, given that ECM fungi less than 3 m apart may be from the same mycelium (Turner et al., 2009). Root extractions were timed to coincide with spring ECM flush (Walker et al., 2008). Blocks of 500 cm^3 (i.e., soil blocks 5 x 10 x 10 cm deep) were cut and extracted 1-3 m from each tree base (i.e., 2 plots × 3 trees × 3 blocks + 1 plot × 2 trees × 3 blocks = 24 BOHF blocks; 3 plots × 3 trees × 3 blocks = 27 MPAW

Table 1. Soil properties from Basic Oak-Hickory Forest (BOHF) and Mountain/Piedmont Acidic Woodland (MPAW) communities.

	Ecological Community	
	BOHF	MPAW
Soil pH range	4.9-5.0	4.3-4.5
Mean % organic matter	4.2	4.3
Soil moisture regime	Sub-mesic	Xeric
Mean soil depth (cm)	7.8	6.6

blocks) by use of a soil spade immersion-sterilized in a 9:1 mixture of bleach and water, followed by rinsing before each extraction. Blocks were then wrapped in new aluminum foil and taken to Ferrum College for analysis.

Fungal Morphotyping, Quantification, and Statistics

We exposed roots in each sample block by soaking and gently rinsing them with tap water over sieves to remove adhered pebbles, soil, and dead organic matter. Any remaining pebbles or organic matter was then removed from each sample manually, using tweezers and root snips. We randomly selected a subsample of all of the cleaned fine roots (i.e., any root <1 mm in diameter), representing approximately 50% of all fine roots per sample. Species were identified, and morphotypes were described, using macroscopic morphotyping methods (i.e., Ingleby et al., 1990) based upon root tip branching pattern and shape, mantle color and texture, and presence and abundance of hyphae and rhizomorphs (Table 2, Fig. 1), using an Olympus SZ61 stereoscope. All but one type was not identifiable to species using these procedures, and so were named based on the order in which they were described and on their predominant color. Colonization was expressed as the total numbers of colonized tips per meter fine root. Tips at least partially covered by fungal tissue were considered colonized. We characterized community composition by determining the percent contribution of each morphotype/species. Our assessments of ECM diversity relied upon morphotype richness and evenness. Richness was measured as the number of ECM types per meter fine root length, while evenness was determined by comparing the ranked proportional contributions of each morphotype per soil source. We quantified fine root length using Tennant's (1975) root intercept method for all fine roots.

Our study was intended to test for differences in ECM and host properties between BOHF and MPAW soils. However, our design was limited by a lack of resources, thus we examined the cumulative effects of BOHF and MPAW soil parameters on these properties. In addition, given the variability in the number of fine roots, the amount of dead organic matter, and the number of viable ECM roots tips found in each root sample block, blocks from each tree were consolidated to yield a total of nine MPAW and eight BOHF samples to analyze. After performing tests for normality (i.e., histograms, skewness and kurtosis, and homogeneity of variance), we analyzed colonization data with t-tests, while richness and fine root length were analyzed with

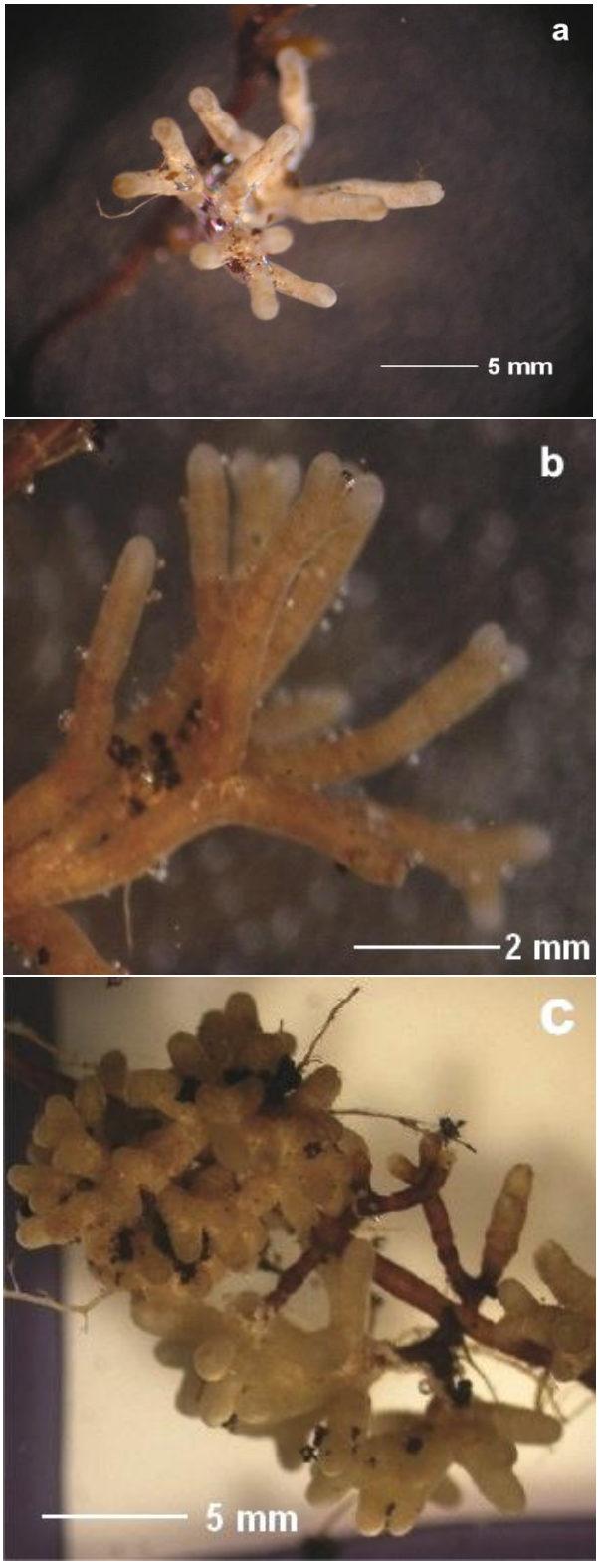

Fig. 1. (a) Irregularly pinnate copper morphotype, (b) dichotomous rust morphotype, and (c) irregularly coralloid white morphotype intermingled with charcoal black *Cenococcum geophilum*.

Table 2. Descriptions and proportional percentage colonization of ectomycorrhizal (ECM) fungal morphotypes in relation to all root tips colonized by all types pooled in Basic Oak-Hickory Forests (BOHF) and Mountain/Piedmont Acidic Woodlands (MPAW) soils.

ECM type	Branching pattern; tip shape; mantle color and texture; presence and abundance of hyphae; presence of rhizomorphs.	% Colonization	
		BOHF	MPAW
Cenococcum geophilum	Unbranched; straight; charcoal black, grainy; common; not present	24.2	31.6
E1br	Unbranched; straight; brown, grainy; not present; not present		0.5
E2co	Irregularly pinnate; slightly bent; copper, grainy; sparse; sparse		0.1
E3cr	Monopodial pinnate; slightly bent; cream, grainy; not present; not present		0.1
E4og *Tomentella*-like	Monopodial pyramidal; straight to slightly bent; olive green, grainy to smooth; rare; not present	1.3	
E5rw	Irregularly pinnate; slightly bent; reddish white, smooth; not present; not present		16.3
E6ru	Dichotomous; slightly bent; rust, smooth; not present; not present	1.5	5.7
E7si *Boletus*-like	Irregularly pinnate; straight to slightly bent; silver, felty; common; not present	0.4	1.2
E8w	Irregularly coralloid; straight; white, smooth; not present; not present	61.8	31.3
E9y	Irregular; bent; yellow tan, smooth; not present; not present	10.8	13.2

Mann-Whitney U tests (SPSS version 16.0, Chicago, IL). Differences in the percentage of root tips colonized by morphotypes between soil sources were analyzed with G-tests. We used Mann-Whitney and G-tests because the data for each violated the assumptions of t-tests and Chi-squared tests, respectively. Differences for all tests were considered significant if $P < 0.05$.

RESULTS

ECM colonization did not differ between *P. virginiana* roots from the two communities ($F = 1.101$, $P = 0.415$). Mean colonized root tips per meter fine root were 67.7 ± 8.8 (SE) and 80.9 ± 12.5 in BOHF and MPAW soils, respectively. Nine distinct morphotypes and the ubiquitous Ascomycete *Cenococcum geophilum* Fr. were described or identified in both soil sources (Table 2). One and four types were exclusive to BOHF and MPAW soils, respectively, whereas five occurred in both soils. E8w and *C. geophilum* were abundant in both soils, representing 62 and 24% of colonized tips, respectively, in BOHF soils, and approximately 1/3 each in MPAW soils. E9y was relatively abundant in BOHF soils, as were E5rw and E9y in MPAW soils. Collectively, E8w, *C. geophilum*, and E9y accounted for ca. 97% of colonization in BOHF soils, while *C. geophilum*, E8w, E5rw, and E9y accounted for ca. 92% in MPAW soils. Two infrequent types, E6ru and E4og, and the rare type E7si accounted for just over 3% of colonization in BOHF soils, while two infrequent types, E6ru and E7si, and rare types E1br, E2co, and E3cr accounted for 7.6% in MPAW soils. Overall, ECM community composition differed between soil sources; BOHF soils were dominated by one type and had less diversity whereas MPAW soils had no dominant type and higher diversity. Furthermore, while all but E8w was more abundant in MPAW soils, G-tests found that E6ru and E7si were significantly more abundant in MPAW than BOHF soils. *Cenococcum geophilum*, E8w, and E9y did not differ between soils.

Mean morphotype richness was significantly different ($U = 7.595$, $P = 0.007$), being three times greater per meter fine root in MPAW as compared to BOHF soils (i.e., 0.57 ± 0.13 versus 0.19 ± 0.05), while evenness was qualitatively similar in MPAW and BOHF soils (i.e., fewer dominant types and more spread; Fig. 2). Similarly, host fine root length was significantly different ($U = 13.000$, $P = 0.027$), being more than twice as long in BOHF than MPAW soils (28.8 ± 4.55 vs. 10.7 ± 1.6 cm).

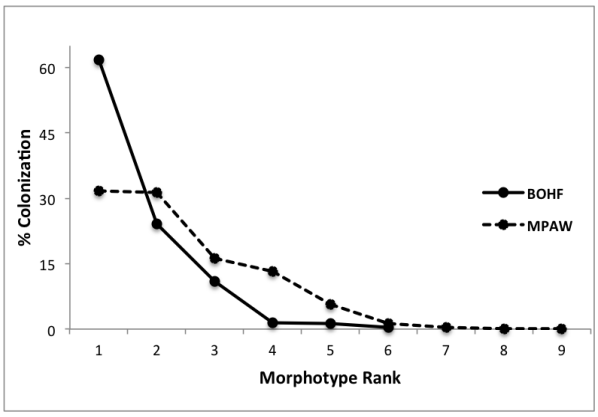

Fig. 2. Rank abundance patterns for ECM morphotypes from *Pinus virginiana* host trees growing in Basic Oak-Hickory Forests (BOHF) and Mountain/Piedmont Acidic Woodlands (MPAW) soils.

DISCUSSION

ECM colonization did not differ between soil sources, which is not surprising given that other relevant studies report similar findings. For example, Edwards & Kelly (1992) found no colonization differences on Loblolly Pine (*P. taeda* L.) from soils with pH values of 3.8 and 5.2, though they assessed seedlings, rather than trees, exposed to ozone and magnesium in open air chambers. A study of Pinyon Pine (*P. edulis* Engelm.) from xeric and less xeric soils in an Arizona forest found that there were no differences in colonization (Gehring et al., 1998), and, like our results, that only one or a few morphotypes dominated ECM composition. However, it is important to note that we had more limited sampling, our types were based on conventional morphotyping, and that most current analogous studies use DNA identification methods (e.g., PCR analyses and sequencing), often finding greater sample species richness and more complex composition from various hosts and systems (Dahlberg, 2001; Jany et al., 2003; Tedersoo et al., 2003; Smith & Read, 2008).

Regardless, we found that composition varied, given that total richness and the numbers of dominant and rare types differed between soil sources. Differences in colonization shown by individual types may reflect responses to factors unique to each soil. For example, three types (i.e., E6ru, E7si, and E9y) were more abundant in MPAW soils (E6ru and E7si significantly so), suggesting that these types may be more acid-tolerant than others, as Erland & Söderström (1990) and Lehto (1994) found for *Pisolithus* and *Suillus* species associated with *Abies* and *Picea* hosts. We also found that *C. geophilum* and E9w colonization were similar in both soils, suggesting that these fungi tolerate a wide range of pH values, as Rao et al. (1997) observed for *P. kesiya*-associated *C. geophilum* in soils with variable pH values. However, while colonization by some morphotypes in our study may at least partly reflect responses to pH, these same types, and others, may also have responded to differences in soil moisture, as Gehring et al. (1998) observed. E5rw and E8w, for example, may have affinities for xeric and sub-mesic soils, respectively. By contrast, *C. geophilum*, with roughly equal abundances in both soil sources, likely tolerates a greater range of moisture levels, as Worley & Hacskaylo (1959) observed for it colonizing *P. virginiana* seedlings grown in Maryland forest soils in the greenhouse.

Richness differed significantly between soil sources, with three times more ECM morphotypes per meter fine root in MPAW than BOHF soils. Greater MPAW richness may reflect the ability of more types to tolerate lower pH and xeric soils, as Gehring & Whitham (1994) found for *P. edulis* types, and some types that may be acidophilic (e.g., E1br and E5rw). Another factor that may have influenced differences in richness is fine root length, with which it has been positively correlated on *Picea* and *Quercus* hosts (Korkama et al., 2006; Turner et al., 2009). However, our results differ from these patterns, because we found that fine root length was significantly lower in the more morphotype-rich MPAW soils. In addition, ECM fungi were less evenly structured in BOHF than MPAW soils (Fig. 2) as evidenced by the steeper slope representing the BOHF community (i.e., 62% proportional colonization by E8w), and the occurrence of fewer dominant and more rare types in MPAW soils. Considering evenness with richness, our results suggest that ECM communities may be more diverse on *P. virginiana* hosts from MPAW than BOHF soils.

Fine root length was significantly greater in BOHF than MPAW soils. Organic matter and soil depth did not differ greatly between soils (Table 1). Although these factors can affect root growth (Gehring et al., 1998; Hertel et al., 2003), it is unlikely they did so in our study. Soil pH also affects root growth, though no clear patterns have emerged from the literature. For example, Lehto (1994) reports negative effects while Brunner et al. (2002) found weak or no effects. In contrast, soil moisture may have been influential because it is known to be positively correlated with fine root growth (López et al., 1998; Wilcox et al., 2004; Olesinski et al., 2011). *Pinus virginiana* may operate similarly, growing longer fine roots in the moister BOHF than the xeric MPAW soils.

In summary, ECM composition and richness on *P. virginiana* hosts differed between BOHF and MPAW soils at the Grassy Hill Natural Area Preserve.

Morphotype richness was greater in MPAW soils and, like composition, may have been affected by differences in the response of individual morphotypes to moisture levels and pH. Greater *P. virginiana* fine root length in BOHF soils likely reflects the host's ability to grow longer fine roots in moister soils. Our findings corroborate some studies reporting differences in ECM fungi in response to variable soil moisture levels or pH, respectively. However, as stated earlier, our explanations were based on cumulative plot-level differences in key soil parameters and relied on small, consolidated samples. Thus, more research, including bioassays, outplantings, and local-scale soil parameter manipulations would go far in helping us to better understand how ECM fungi and fine roots respond to differences in key soil parameters. In addition, future studies might also consider that factors like soil moisture and pH, root length, and vegetative composition may be covariates for ECM colonization.

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