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Topographic and Host Effects on Arbuscular Mycorrhizal and Ectomycorrhizal Fungal Communities in a Forested Watershed

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Abstract

In a forested watershed, identity of tree species and topographical position could be important driving factors shaping mycorrhizal fungal communities. Here we aimed to disentangle the contributions of these two factors to mycorrhizal fungal community structure. We collected tree roots colonized by either arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi in a small, temperate, forested watershed of the Susquehanna-Shale Hills Critical Zone Observatory. Relative abundances of fungal OTUs were assessed using high-throughput DNA sequencing. The structures of fungal communities, both AM and EM, were compared between different host species at the same slope position, and within the same host species at different slope positions that vary in soil moisture, nutrient content and belowground biomass. We found that structures of AM fungal communities were significantly affected by host species but not by slope position. Although the structures of EM fungal communities were not significantly affected by either host identity or slope position, there were three core EM fungal OTUs (occurrence ≥ 50%) for which their relative abundances were significantly affected by slope position and three for which their relative abundances were significantly affected by host species. In our system, the effects of host identity and slope position were only moderately strong and varied between mycorrhizal types. Our findings provide guidance to those attempting to link the fine-scale distribution of mycorrhizal fungi and mycorrhizal-mediated ecosystem functions to both host species and topographic position.

Key words: critical zone observatory; host specificity; Illumina sequencing; temperate forest; trees; slope position.

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Author Contributions: WC conceived the idea. WC, RTK and DME designed the study. WC performed the research and analyzed the data. WC wrote the paper, with input from RTK and DME.

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Highlights

- Effects of host identity varied between mycorrhizal fungal types

- Arbuscular mycorrhizal fungi were more strongly driven by host than by topography
- Topography affected the relative abundance of a few individual fungal taxa

Introduction

Mycorrhizal fungi exchange resources with plant hosts while simultaneously interacting with the physical environment (Smith and Read 2008). The distribution of mycorrhizal fungi, therefore, can be constrained by two potential factors: location of preferred hosts and variation in the abiotic environment (Johnson and others 1992, 2017; Helgason and others 2002; Tedersoo and others 2008; Hazard and others 2013; Bonfim and others 2016; van der Linde and others 2018). Understanding the relative contributions of these factors in structuring mycorrhizal fungal communities may provide a practical way to map mycorrhizal fungal-mediated ecosystem functions at both local and regional scales (Agerer 2001; Clemmensen and others 2015; Treseder and others 2018).

In many forested watersheds of a particular lithology, the physical and chemical soil properties vary across slope position, partly because of differences in erosional and depositional processes (Ovalles 1986; Ludwig and others 2005) and partly because of differences in host species composition and vegetation productivity (Smith and others 2016). Variation in soil factors may shift the distribution of certain mycorrhizal fungal taxa (Toljander and others 2006; Branco and others 2013; Bonito and others 2014; Erlandson and others 2016), resulting in a gradient of mycorrhizal fungal taxon abundance across hillslopes of different scales and environmental conditions (Day and others 1987; Gibson and Hetrick 1988; Yao and others 2013).

Host tree species, independent of slope position, select for specific mycorrhizal fungal taxa from tens to hundreds of candidates at their habitats (Lang and others 2011; Roy and others 2013; Toju and others 2013; Martínez-García and others 2015). However, the degree of host specificity varies (Peay and others 2015; Hempel 2018) and could be confounded by other factors such as slope position. If the structure of mycorrhizal fungal communities is predominately driven by host identity, irrespective of slope position, we can simply track spatial patterns of fungal communities via the host species distribution across the watershed. Otherwise, we need to consider how, independent of host identity, slope position influences fungal communities and mediates host–fungal interactions. Systematic analyses of both factors are needed to better understand the drivers on the abundance of individual fungal taxa or the species composition of the fungal community.

In this contribution, we selected common tree species from different slope positions across a temperate forested watershed. These tree species form associations with either arbuscular mycorrhizal (AM) fungi or ectomycorrhizal (EM) fungi. Soil microenvironments vary along slope positions. Valley floor soils may be thick and poorly drained, whereas mid-slope soils are often thinner and prone to drought. We hypothesized that the relative abundance of individual AM and EM fungal OTUs, as well as the overall structure of AM and EM fungal communities (1) vary between slope positions for a given host and vary among host species of the same slope position; and (2) respond more strongly to slope position than to host identity.

Materials and Methods

Site Description and Root Collection

The study watershed is located in a natural forest within the Shale Hills catchment of the Susquehanna-Shale Hills Critical Zone Observatory in central Pennsylvania, USA (40° 39′ N, 77° 54′ W). A detailed description of this site can be found in Smith and others (2016). In early August 2014, we sampled roots of *Acer* and *Liriodendron* species on the valley floor, both of which are associated with AM fungi, and we also sampled roots of *Acer* species in the mid-slope position (Figure 1). Similarly, we sampled roots of EM tree species *Tsuga* and *Quercus* on the valley floor, with additional root samples of *Quercus* from the mid-slope positions (Figure 1). Although AM colonization has been observed in *Quercus* roots, we consider it to be an EM plant genus in this study. We previously obtained evidence that AM fungi colonizing the roots of *Quercus rubra*, one of the dominant tree species at our site, do not improve plant nutrient uptake (Dickie and others 2001). Moreover, the nutrient foraging strategies by mycorrhizal roots of *Q. rubra*, as well as *Quercus alba* (also at our site), are more similar to other EM tree species than AM tree species (Chen and others 2016). The number of individual trees sampled within a slope position was approximately proportional to the tree species' natural abundance, and the sampling points were designed to encompass the majority of the valley floor and mid-slope position in this small watershed (Figure 1). Some

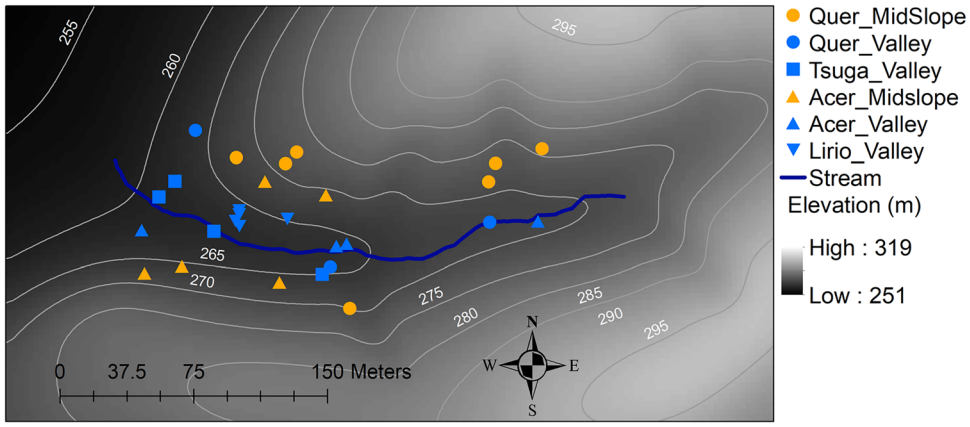

Figure 1. Sampling locations of mycorrhizal roots at the Shale Hills watershed. Symbol color represents host tree species identity, and symbol shape represents slope position. Quer = *Quercus* spp. (*Q. alba*, *Q. prinus*, and *Q. rubra*); Tsuga = *Tsuga canadensis*; Acer = *Acer* spp. *(A. saccharum and A. rubrum)*; Lirio = *Liriodendron tulipifera*. The blue line indicates the valley intermittent stream (Color figure online).

environmental variables of the two topographic positions are given in Table 1.

For each tree individual, four root branches (15–20 cm long) were harvested in the 0–10 cm depth from 4 random locations (1 branch per location) within a 2 m radius of the trunk. Soil particles adhering to root surfaces were carefully removed, and all root samples were stored at − 80°C for subsequent DNA analysis. The first two orders of the root branches were selected for fungal molecular studies because they often contain the majority of the mycorrhizal fungal tissue (Guo and others 2008). Twenty root segments (from the first two orders, usually < 1 cm in length) were dissected from each sampled root branch. The 80 root segments from a given individual tree were combined for DNA extraction.

Molecular Methods

We used MoBio Power Soil DNA extraction kits (MoBio Laboratories, Carlsbad CA) to extract DNA from all samples, following the manufacturer's recommendations. The PowerSoil kits were selected because they handle PCR inhibitors, such as polyphenolic compounds, better than the other MoBio kits, and polyphenolic compounds occur in high concentrations in *Quercus* and *Pinus* roots. Before the molecular studies on mycorrhizal fungi, we first determined tree species identity of the root samples. The plant chloroplast *rbcL* was amplified using the primers rbcLaF-rbcLajf634R (ATGTCACCACAAACAGAGACTAAAGC/GAAACGGTCTCTCCAACGCAT, Fazekas and others 2008). Successful PCR products were Sanger sequenced (Genomics Core Facility, The Pennsylvania State University, USA). Sequences were BLASTed and the results with the highest scores were chosen. In this process, samples were relabeled with the DNA-identified hosts if differed from targeted hosts. Because of the limitations of plant sequence resolution, root samples were categorized at the genus level.

Amplification of fungal DNA was conducted with DNA samples identified for each of the plant species. For the AM samples, we used primers of NS31-AML2 (TTGGAGGGCAAGTCTGGTGCC/GAACC-

Table 1. Abiotic and Biotic Variables (mean ± S.E.) of the Two Slope Positions at the Shale Hills Watershed

Variable	Unit	Mid-slope	Valley floor
Soil temperature	°C	20.1 ± 0.1	19.8 ± 0.1
Soil water content	%	17.3 ± 0.3	19.7 ± 0.8
NH_4 (10 cm depth)	mg kg^{-1}	0.05 ± 0.01	0.34 ± 0.11
NO_3 (10 cm depth)	mg kg^{-1}	0.02 ± 0.01	0.05 ± 0.01
DOC (10 cm depth)	mg kg^{-1}	3.51 ± 0.45	8.26 ± 1.32
RLD (0–10 cm depth)	cm cm^{-3}	3.67 ± 0.66	2.41 ± 0.39

Soil temperate and soil water content are averaged from June 1 to August 31.
DOC dissolved organic carbon, RLD root length density.

CAAACACTTTGGTTTCC, Liu and others 2011) to amplify the partial 18S rRNA gene sequences. For EM samples, we used primers of ITS1F and ITS2 (CTTGGTCATTTAGAGGAAGTAA/GCGTTCTTCATC GATGC, White and others 1990) to amplify the ITS1 region. Successful PCR products were sent to the DNA Sequencing Facility at the University of Wisconsin-Madison for the remaining procedures for Illumina sequencing. There, a library was prepared by adding a unique barcoding sequence to each sample in a second PCR step. All amplicons were sequenced on the MiSeq platform with 2 × 300 bp pair-end reading.

We used PANDAseq assembler (Masella and others 2012) to merge the pair-end reads. Full-length amplicons were quality-filtered and processed using the QIIME v.1.8.0 (Caporaso and others 2012). In the bioinformatics pipeline, sequences were clustered into operational taxonomic units (OTUs) using an open reference-based (that is, reference-based + de novo) approach with the UCLUST algorithm (Edgar 2010) and a 97% similarity threshold. The relative abundance of a fungal OTU within a sample was represented by its percentage of sequencing read numbers.

The UNITE (Abarenkov and others 2010) and MaarjAM (Öpik and others 2010) databases, respectively, were used as reference assignment databases for EM fungi and AM fungi. Taxonomy was assigned via the RDP classifier (Wang and others 2007) with the aforementioned databases using a 0.7 confidence threshold. In particular, because primers of ITS1F-ITS2 amplified the ITS1 region of all fungal DNA, we checked the trophic status (EM, non-EM and unknown) of the assigned genus. We included only EM OTUs in subsequent analyses by referencing online databases such as UNITE (http://unite.ut.ee/) and DEEMY (http://www.deemy.de/) and other literature (Tedersoo and Smith 2013; Trocha and others 2016). We assumed that the trophic status of EM fungi is conserved within a genus.

Statistical Analyses

For the AM and EM OTU tables, we performed normalization using *DESeq2* to remove the influence of variation in sequencing depth among samples. Using all OTUs, we performed PerMANOVAs (Hellinger-transformed data) to test for the effects of host identity and slope position on fungal community structure. We also performed PerMANOVAs on binary-transformed OTU data (presence = 1, absence = 0) on fungal community structure to determine how much of the variation in community structure is driven by the OTU relative abundance versus occurrence. Corresponding ordinations (NMDS) were performed for data visualization. Both PerMANOVAs were performed in R (R Core Team 2019). We also calculated the dissimilarities in community structure using *vegdist* in R with the ''horn'' distance. We calculated fungal community dissimilarities (ranging from 0 to 1) between AM tree species at the same slope position, between slope positions for a given AM tree species, between EM tree species at the same slope position, and between slope positions for a given EM tree species. Finally, we compared between slope positions and between host species the relative abundances of each AM and EM core OTUs, those that were detected in 50% or more of AM or EM root samples.

Results

Using *DESeq2*-normalized OTU read counts, we found that the dissimilarity in AM fungal community structure between different AM hosts on the valley floor averaged 0.89 for all possible sample-pairs, which was significantly larger than the dissimilarity of AM fungal communities associated with the same host (*Acer*) at different slope positions (mean = 0.80 for all possible sample-pairs, $P = 0.02$, Figure 2a). For the AM fungal communities, host species explained 11.7% of the variation in community structure ($F = 1.727$, $P = 0.037$), whereas the contribution of slope position was smaller, explaining 6.9%, and not statistically significant ($F = 1.012$, $P = 0.42$) (Figure 3a). We found similar results using binary (presence/absence) data. Dissimilarity was significantly greater between the different AM hosts on the valley floor (mean = 0.91) than for the same host (*Acer*) at different slope positions (mean = 0.84, $P < 0.001$) (Figure 2b). Host species significantly explained 9.9% of the variation in the overall structure of AM OTU presence/absence ($F = 1.424$, $P = 0.020$), but the contribution of slope position was insignificant ($F = 1.016$, $r^2 = 7.0\%$, $P = 0.39$) (Figure 3c).

The dissimilarity of EM fungal community structure between the different host species on the valley floor did not significantly differ from the dissimilarity for the same species at different slope positions (mean = 0.41 vs. 0.38, $P = 0.15$, Figure 2a). Among the EM fungal communities, neither host species nor slope position explained significant variation in community structure ($F = 1.115$, $r^2 = 8.4\%$, $P = 0.17$ for slope position and $F = 1.018$, $r^2 = 7.8\%$, $P = 0.43$ for host species,

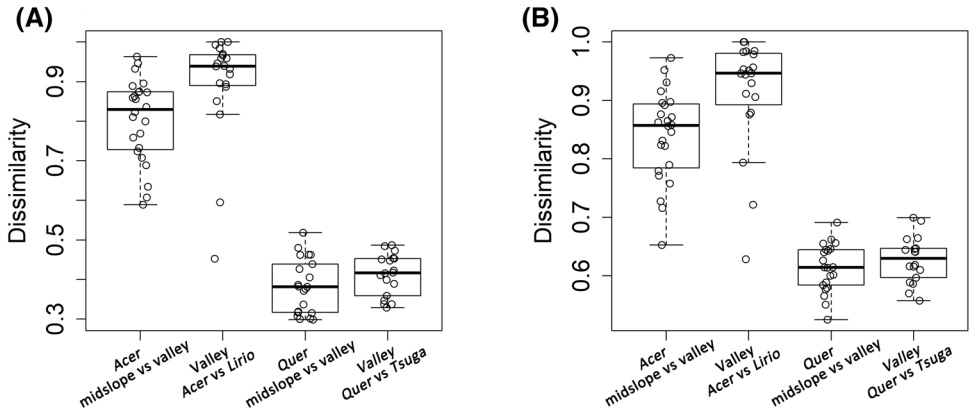

Figure 2. Dissimilarities of fungal communities between any two arbuscular mycorrhizal or ectomycorrhizal samples of the same host species at two slope positions, and of different host species at the same slope position. Calculations of dissimilarities were based on DESeq2-normalized OTU counts (**A**) or OTU presence/absence (**B**).

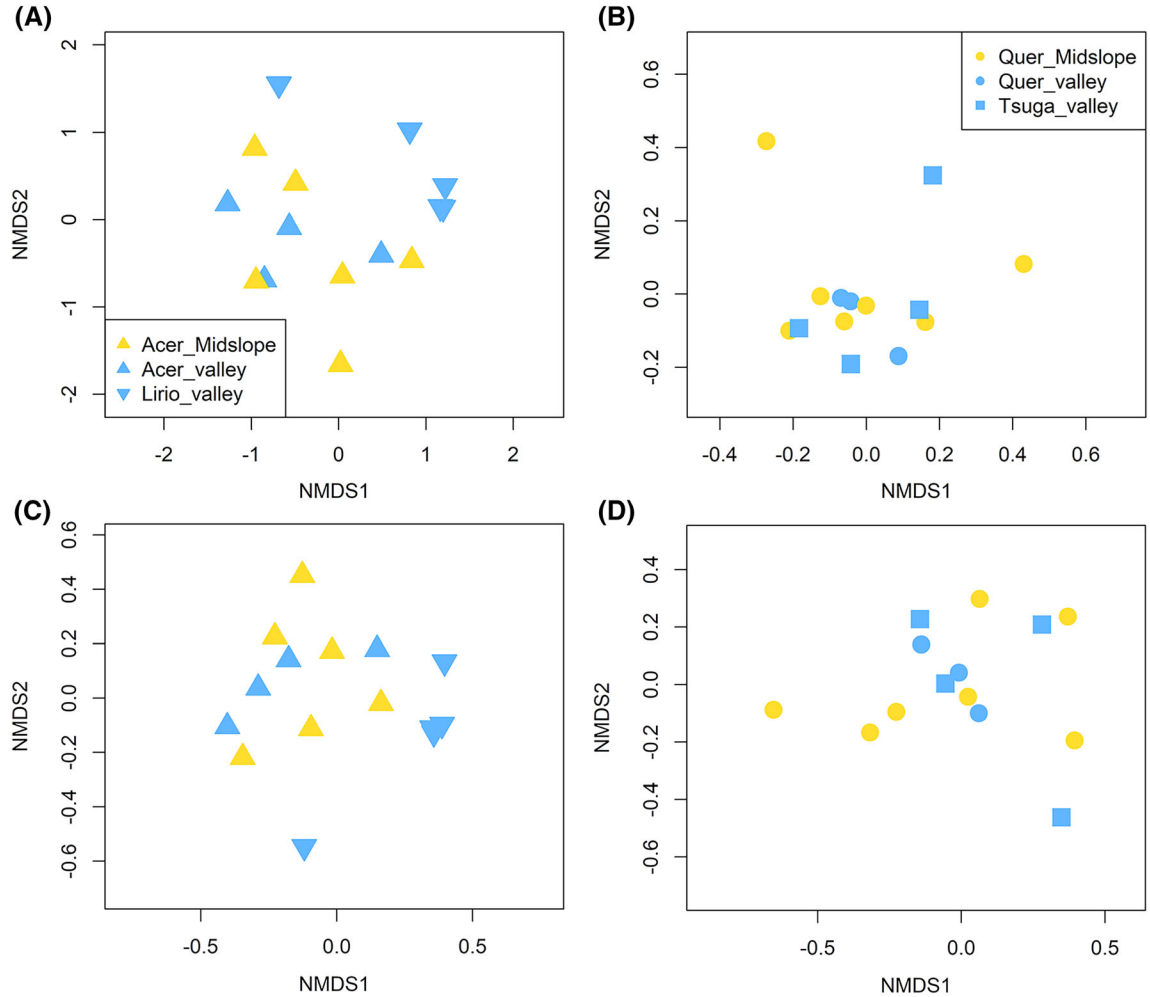

Figure 3. Non-metric multidimensional scaling (NMDS) plot ordination of arbuscular mycorrhizal (AM, **A**, **C**) and ectomycorrhizal (EM, **B**, **D**) fungal communities among different host tree species (indicated by symbol shape, as abbreviated in Figure 1) and slope positions (indicated by symbol color). Ordinations were based on DESeq2-normalized OTU counts (**A**, **B**) or OTU presence/absence (**C**, **D**) (Color figure online).

Figure 3b). Again, we found similar results using the binary (presence/absence) data. Dissimilarity was 0.61 for the same host (*Quercus*) at different slope positions, similar to the dissimilarity of different host on the valley floor (mean = 0.63, $P = 0.27$) (Figure 2b). And neither host species nor slope position were significant with respect to overall EM fungal community structure ($F = 1.044$, $r^2 = 8.0\%$, $P = 0.27$ for slope position and $F = 1.018$, $r^2 = 7.8\%$, $P = 0.37$ for host species, Figure 3d).

Discussion

Influence of Slope Position

Within the study watershed, soil physical and chemical conditions exhibited considerable variation due to slope position (Table 1). The soil of the valley floor is moister and more fertile than the soil at mid-slope (Table 1). These abiotic environmental variations were expected to influence the structure of mycorrhizal fungal communities (Toljander and others 2006; Branco and others 2013; Bonito and others 2014; Erlandson and others 2016; Williams and others 2017). In addition, we observed differences in root length density along the slope (Table 1) that are also expected to influence the structure of EM fungal communities (Peay and others 2011). However, we did not find strong evidence of variation in the relative abundance of individual mycorrhizal fungal taxa or in the structure of whole fungal communities due to slope position in our study watershed. Our findings suggest the possibility that environmental filtering due to slope position was negligible for the majority of AM and EM fungi in this small watershed.

It is possible that our sampling effort was insufficient to detect a slope position effect. However, our sampling locations were well dispersed across the majority of the valley floor and mid-slope area of this small watershed. Previous studies revealing significant topographic effects on mycorrhizal fungi were conducted at larger geographic scales for which topography and spatial dispersal may have been somewhat confounded (Shakya and others 2013; Goldmann and others 2016). For instance, some studies included an elevation gradient of nearly 1 km (Li and others 2014; Bonfim and others 2016), while our study had an elevation gradient of only about 20 m (altitude 261–280 m.a.s.l.). Therefore, the lack of strong variation in the relative abundance of individual mycorrhizal fungal species or in the structure of whole communities due to slope position in our study may be consistent with the lack of AM and EM fungal propagule dispersal limitation within the small (8 ha) watershed as has been suggested in other systems (Lekberg and others 2007; Peay and others 2012; Vályi and others 2016). Nevertheless, our methods were capable of detecting significant variation in mycorrhizal fungal communities if it had existed. Indeed, we identified distinct mycorrhizal fungal communities at our Shale Hills site and a site about 7 km away, even when the fungal communities were associated with the same host species between sites (Chen 2017).

We did detect three EM fungal individuals associated with *Quercus* trees that were significantly influenced by slope position within this relatively small watershed (Table 2). Two *Tomentella* OTUs were relatively more abundant in the valley floor, whereas one *Hymenogaster* OTU was relatively more abundant at mid-slope (Table 2). It is likely that *Tomentella* species, which are often wood-rotting fungi, prefer moister habitats, such as the valley floor (Přívětivý and others 2016). The *Hymenogaster* species form truffle-like fruitbodies that can insulate fungal spores from drying (Thiers 1984), allowing them to be adapted to drier habitats such as at mid-slope positions (Table 1). Previous studies have also shown variation in the distribution of individual fungal taxa along a slope. For example, in an alpine region the EM fungi *Sebacinales*, *Cortinarius* and *Meliniomyces* showed distinct affinities either to ridge-top or to valley floor that varied largely in soil content of carbon, nitrogen, and phosphorus (Yao and others 2013). In a pasture where the valley floor had sandier surface textures than other positions of the slope, the AM fungal genera *Gigaspora* were more favored than *Acaulospora* and *Glomus* in terms of fungal spore abundance (Day and others1987). In a tallgrass prairie, *Glomus etunicatum* decreased but *Glomus geosporum* increased in abundance from the top to the bottom of a slope (Gibson and Hetrick 1988). The full reason for these spatial patterns of AM and EM fungi is not very clear and may be highly context-dependent. However, these patterns suggest that topography cannot be overlooked with respect to the spatial distributions of various fungal species.

Influence of Host Identity

Increasing numbers of high-throughput sequencing studies of mycorrhizal fungal community structures have suggested a scale-dependent host identity effect (Hempel 2018). Neuenkamp and others (2018) found a strong host effect on the species composition of AM fungal communities

Table 2. The Relative Abundance of Core OTUs (occurrence ≥ 50%) that Were Significantly Influenced by Host Identity or Slope Position ($P < 0.05$)

Taxa	Occurrence (%)	Average relative abundance (%)	Significant factor	Effect on relative abundance
AM OTUs				
Glomeraceae spp	53	1.7	Host identity	*Acer* > *Liriodendron*
EM OTUs				
Amanita fulva	93	0.8	Host identity	*Quercus* < *Tsuga*
Tricholoma columbetta	93	0.6	Host identity	*Quercus* < *Tsuga*
Tomentella spp 1	93	0.6	Slope position	Valley > Mid-slope
Tomentella spp 2	57	0.4	Slope position	Valley > Mid-slope
Hymenogaster spp 2	57	0.3	Host identity	*Quercus* > *Tsuga*
Hymenogaster spp 1	50	0.3	Slope position	Valley < Mid-slope

Occurrence of an OTU was defined as the percentage of AM or EM root samples that host this fungal OTU.
AM arbuscular mycorrhizas, EM ectomycorrhizas.

within an area of 2 km^2. Our study confirmed the significant host effect for AM fungal communities in an even smaller area (< 0.1 km^2). We also found that in this topographically heterogeneous environment, the effect of host identity on AM fungi was stronger than the effect of slope position (Figure 2). The two selected AM hosts, *Acer* and *Liriodendron*, exhibit large differences in root morphology and nutrient foraging behavior (Chen and others 2016), suggesting that host specificity of AM fungi would likely occur when hosts employ different strategies for soil nutrient acquisition. It appears that variation in fungal OTU presence/absence is the main contributor to the variation in AM fungal community structure between *Acer* and *Liriodendron* because quantitative and qualitative (binary) OTU data resulted in similar results (Figures 2, 3).

Although EM tree species with variation in root morphology, such as root tip diameter, are also expected to associate with EM fungi of different hyphal exploration distances (Chen and others 2018), the degree of difference in root tip diameter between *Quercus* and *Tsuga* (0.4 vs. 0.5 mm, Comas and Eissenstat 2009) may not be strong enough to cause detectable shifts of hyphal exploration distance of the associated EM fungal communities. Previous studies have shown that shifts in forest composition between AM-dominated and EM-dominated trees may cause fundamental shifts in ecosystem function including carbon and nitrogen cycling (Phillips and others 2013), and our study additionally suggests that shifts in tree species composition within the AM or EM functional groups could change the relative abundance of individual fungal OTUs or the structure of fungal communities, resulting in altered mycorrhiza-mediated ecosystem processes.

Because of the limited nature of our study, it is dangerous to extrapolate our findings to other tree species or other forested watersheds. But we do suggest that examining the effect of host and slope position for more tree species in other systems will be critical in attempts to link the existing high-resolution documentation of tree species at various slope positions across broad ecological regions, such as the Database of Forest Inventory and Analysis (FIA) program within the United States Forest Service and the National 1-meter Digital Elevation Models within the United States Geological Survey, to the understudied fine-scale distributions of mycorrhizal fungal communities.

Summary

In this study, we collected AM and EM fungi from four different host tree species at two slope position in a small, temperate, forested watershed to disentangle the influence of host identity and slope position on the distribution of mycorrhizal fungal species and mycorrhizal fungal community structure. We found that the AM fungal communities of *Acer* and *Liriodendron* trees were mainly affected by host identity. Variation of slope position only affected a few core EM OTUs (occurrence > 50%) associated with *Pinus* and *Quercus* trees. These findings suggest a way to link host species/topographic position to fine-scale distribution of mycorrhizal fungi to better understand the impact of mycorrhizal fungi on important ecosystem functions.

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DATA AVAILABILITY

Data of host species identity, slope position and OTU counts are available at Open Science Framework, with direct URL https://osf.io/kmd96/.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

REFERENCES

Abarenkov K, Henrik Nilsson R, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R. 2010. The UNITE database for molecular identification of fungi–recent updates and future perspectives. New Phytol 186:281–5.

Agerer R. 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11:107–14.

Bonfim JA, Vasconcellos RLF, Gumiere T, Mescolotti DDLC, Oehl F, Cardoso EJBN. 2016. Diversity of arbuscular mycorrhizal fungi in a Brazilian Atlantic forest toposequence. Microb Ecol 71:164–77.

Bonito G, Reynolds H, Robeson MS, Nelson J, Hodkinson BP, Tuskan G, Schadt CW, Vilgalys R. 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Mol Ecol 23:3356–70.

Branco S, Bruns TD, Singleton I. 2013. Fungi at a small scale: spatial zonation of fungal assemblages around single trees. PLoS ONE 8:e78295.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–4.

Chen W. 2017. Mycorrhizal-mediated nutrient foraging strategies of temperate tree species. Doctoral dissertation, Pennsylvania State University.

Chen W, Koide RT, Adams TS, Deforest JL, Cheng L, Eissenstat DM. 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. Proc Nat Acad Sci USA 113:8741–6.

Chen W, Eissenstat DM, Koide RT. 2018. Root diameter predicts the extramatrical hyphal exploration distance of the ectomycorrhizal fungal community. Ecosphere 9:e02202.

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol 205:1525–36.

Comas LH, Eissenstat DM. 2009. Patterns in root trait variation among 25 co-existing North American forest species. New Phytol 182:919–28.

Day LD, Sylvia DM, Collins ME. 1987. Inter-actions among vesicular-arbuscular mycorrhizae, soil and landscape position. Soil Sci Soc Am J 51:635–9.

Dickie IA, Koide RT, Fayish AC. 2001. Vesicular–arbuscular mycorrhizal infection of *Quercus rubra* seedlings. New Phytol 151:257–64.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–1.

Erlandson SR, Savage JA, Cavender-Bares JM, Peay KG. 2016. Soil moisture and chemistry influence diversity of ectomycorrhizal fungal communities associating with willow along an hydrologic gradient. FEMS Microbiol Ecol 92:fiv.148.

Fazekas AJ, Burgess KS, Kesanakurti PR, Graham SW, Newmaster SG, Husband BC, Percy DM, Hajibabaei M, Barrett SC. 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS ONE 3:e2802.

Gibson D, Hetrick BAD. 1988. Topographic and fire effects on the composition and abundance of VA-mycorrhizal fungi in a tallgrass prairie. Mycologia 80:433–41.

Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z. 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. New Phytol 180:673–83.

Goldmann K, Schröter K, Pena R, Schöning I, Schrumpf M, Buscot F, Polle A, Wubet T. 2016. Divergent habitat filtering of root and soil fungal communities in temperate beech forests. Sci Rep 6:31439.

Hazard C, Gosling P, Van Der Gast CJ, Mitchell DT, Doohan FM, Bending GD. 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. ISME J 7:498–508.

Hempel S. 2018. Passengers and drivers of arbuscular mycorrhizal fungal communities at different scales. New Phytol 220:952–3.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J Ecol 90:371–84.

Johnson NC, Tilman D, Wedin D. 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034–42.

Johnson NC, Miller RM, Wilson GW. 2017. Mycorrhizal interactions with climate, soil parent material, and topography. In: Johnson NC, Gehring C, Jansa J, Eds. Mycorrhizal mediation of soil: fertility, structure, and carbon storage. Amsterdam: Elsevier Inc. p 47–66.

Lang C, Seven J, Polle A. 2011. Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza 21:297–308.

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J Ecol 95:95–105.

Li XL, Gai JP, Cai XB, Christie P, Zhang FS, Zhang JL. 2014. Molecular diversity of arbuscular mycorrhizal fungi associated with two co-occurring perennial plant species on a Tibetan altitudinal gradient. Mycorrhiza 24:95–107.

Liu Y, He J, Shi G, An L, Öpik M, Feng H. 2011. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. FEMS Microbiol Ecol 78:355–65.

Ludwig JA, Wilcox BP, Breshears DD, Tongway DJ, Imeson AC. 2005. Vegetation patches and runoff-erosion as interacting ecohydrological processes in semiarid landscapes. Ecology 86:288–97.

Martínez-García LB, Richardson SJ, Tylianakis JM, Peltzer DA, Dickie IA. 2015. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. New Phytol 205:1565–76.

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. 2012. PANDAseq: paired-end assembler for Illumina sequences. BMC Bioinform 13:31.

Neuenkamp L, Moora M, Öpik M, Davison J, Gerz M, Männistö M, Jairus T, Vasar M, Zobel M. 2018. The role of plant mycorrhizal type and status in modulating the relationship between plant and arbuscular mycorrhizal fungal communities. New Phytol 220:1236–47.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–41.

Ovalles FA. 1986. Soil landscape relationships and soil variability in north central Florida. Soil Sci Soc Am J 50:401–8.

Peay KG, Kennedy PG, Bruns TD. 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecol 4:233–40.

Peay KG, Schubert MG, Nguyen NH, Bruns TD. 2012. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. Mol Ecol 21:4122–36.

Peay KG, Russo SE, McGuire KL, Lim Z, Chan JP, Tan S, Davies SJ. 2015. Lack of host specificity leads to independent assortment of dipterocarps and ectomycorrhizal fungi across a soil fertility gradient. Ecol Lett 18:807–16.

Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. New Phytol 199:41–51.

Přívětivý T, Janík D, Unar P, Adam D, Král K, Vrška T. 2016. How do environmental conditions affect the deadwood decomposition of European beech (*Fagus sylvatica* L.)? For Ecol Manag 381:177–87.

R Core Team. 2019. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/.

Roy M, Rochet J, Manzi S, Jargeat P, Gryta H, Moreau PA, Gardes M. 2013. What determines Alnus-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. New Phytol 198:1228–38.

Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, Labbé J, Muchero W, Bonito G, Vilgalys R, Tuskan G, Podar M. 2013. A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature Populus deltoids trees. PLoS ONE 8:e76382.

Smith LA, Eissenstat DM, Kaye MW. 2016. Variability in aboveground carbon driven by slope aspect and curvature in an eastern deciduous forest, USA. Can J For Res 47:149–58.

Smith SE, Read DJ, Eds. 2008. Mycorrhizal symbiosis. Cambridge: Academic Press.

Thiers HD. 1984. The secotioid syndrome. Mycologia 76:1–8.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U. 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–90.

Tedersoo L, Smith ME. 2013. Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27:83–99.

Toju H, Sato H, Yamamoto S, Kadowaki K, Tanabe AS, Yazawa S, Nishimura O, Agata K. 2013. How are plant and fungal communities linked to each other in belowground ecosystems? A massively parallel pyrosequencing analysis of the association specificity of root-associated fungi and their host plants. Ecol Evol 3:3112–24.

Toljander JF, Eberhardt U, Toljander YK, Paul LR, Taylor AF. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytol 170:873–83.

Treseder KK, Allen EB, Egerton-Warburton LM, Hart MM, Klironomos JN, Maherali H, Tedersoo L. 2018. Arbuscular mycorrhizal fungi as mediators of ecosystem responses to nitrogen deposition: a trait-based predictive framework. J Ecol 106:480–9.

Trocha LK, Rudy E, Chen W, Dabert M, Eissenstat DM. 2016. Linking the respiration of fungal sporocarps with their nitrogen concentration: variation among species, tissues and guilds. Funct Ecol 30:1756–68.

Vályi K, Mardhiah U, Rillig MC, Hempel S. 2016. Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. ISME J 10:2341–51.

Van Der Linde S, Suz LM, Orme CD, Cox F, Andreae H, Asi E, Atkinson B, Benham S, Carroll C, Cools N, De Vos B. 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 558:243–8.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–7.

White TJ, Bruns T, Lee SJ, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, Eds. PCR protocols—a guide to methods and applications. New York: Academic Press. p 315–22.

Williams A, Manoharan L, Rosenstock NP, Olsson PA, Hedlund K. 2017. Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (*Hordeum vulgare*) mycorrhizal carbon and phosphorus exchange. New Phytol 213:874–85.

Yao F, Vik U, Brysting AK, Carlsen T, Halvorsen R, Kauserud H. 2013. Substantial compositional turnover of fungal communities in an alpine ridge-to-snowbed gradient. Mol Ecol 22:5040–52.