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Influences of host plant identity and disturbance on spatial structure and community composition of ectomycorrhizal fungi in a northern Mississippi uplands ecosystem

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ABSTRACT

We sought to characterize how abiotic and biotic factors, including identities of tree hosts, influence ectomycorrhizal (ECM) fungal composition on tree roots in mixed upland forests of northern Mississippi, where sites have been subject to restoration treatments through burning, manual and natural (tornado) thinning. We identified both plant and fungal components of root tips and collected data on abiotic factors that potentially drive variation in ECM fungal community composition. We found that plant host identity and measured abiotic factors explained less than 8% of variation in ECM fungal community composition. ECM fungal community composition did not differ significantly between control and burned/thinned plots; however, it did differ substantially at the tornado-damaged plot, which also exhibited significant spatial structure. These results suggest that much variation in ECM communities is unexplained by commonly measured biotic and abiotic variables and natural disturbance may play a role in both community and spatial structure of ECM fungi.

1. Introduction

Mycorrhizal fungi are ubiquitous symbionts on plant roots and are prolific in most major biomes and plant communities (Brundrett, 2009). In many temperate forests, the dominant mycorrhizal fungi are ectomycorrhizal (ECM), which colonize the roots of many different tree species, including those of economically important *Quercus* and *Pinus* species (Brundrett, 2009). Mycorrhizas play key roles in influencing soil structure (Perry et al., 1989), plant community dynamics (van der Heijden et al., 1998; Koide and Dickie, 2002), and nutrient cycling (Treseder and Allen, 2000; Treseder, 2004), as well as directly affecting plant growth (Smith and Read, 2008). Molecular techniques developed in the last 20 y, including sequencing and the use of the internal transcribed spacer (ITS) region of the nuclear DNA for identifying fungi to species level (Schoch et al., 2012), have provided a window into the composition of ECM fungal communities, but much still remains to be discovered about the factors driving variation in this composition (Peay et al., 2008). A recent analysis of global patterns in ECM fungal communities indicated that temperature and precipitation are the most important factors driving variation in species richness at large geographic scales, while host plant family was most significant in determining fungal phylogenetic community composition (Tedersoo et al., 2012). Despite these overall patterns, local processes and habitat heterogeneity account for wide variation in fungal structure, indicating that fungal communities can be very context specific (Peay et al., 2008; Hoeksema et al., 2010).

Disturbance can play a very important role in structuring ECM communities. Initially after a disturbance, ECM communities generally consist largely of 'early stage' successional species that can survive in adverse conditions present during or after disturbances, or quickly colonize following disturbance. As the community recovers from disturbance, more species of fungi are introduced by either mycelia that survived the disturbance or by spores from surrounding undisturbed habitat. For example, Visser (1995) found distinctly different ECM fungi in jack pine (*Pinus banksiana*) stands disturbed by fire at time intervals of 6, 41, 65 and 122 y. *Russula* and *Tricholoma* species dominated the root

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community in the 6 and 41-y-old community, while species diversity was much higher in the older stands. Similarly, ECM fungi in the genera *Tuber* and *Rhizopogon* dominated pine seedlings in the first few years following stand-replacing wildfires in bishop pine (*Pinus muricata*) forests, although some taxa (e.g., *Tomentella sublilacina*) are relatively common regardless of time since disturbance (Taylor and Bruns, 1999). The frequency and intensity of the disturbance impacts community structure, as single low intensity disturbances only have minor impacts on ECM communities compared to undisturbed stands (Hart et al., 2005), while more frequent fires have much longer term impacts on species diversity and composition (Tuininga and Dighton, 2004). In general, different species of ECM fungi may respond differently to the changes in biotic and abiotic factors caused by disturbances (such as soil properties, litter depth, ambient light levels, and host plant composition), resulting in shifts in the ECM fungal community (Bruns, 1995).

We investigated what factors may structure the ECM fungal community in an upland forest site in Mississippi, USA, where a diversity of primarily hardwood tree species are hosts for ECM fungi. In this region, attempts are underway to restore portions of the historical open oak woodland habitat from the dense, closed-canopy hardwood forest that has resulted from fire suppression practices of the past century (Brewer and Menzel, 2009). We compared ECM fungal community composition on the roots of trees among plots that were not recently disturbed and plots that were subject to recent disturbances geared towards open oak woodland restoration, including different combinations of burning, anthropogenic thinning, or natural thinning by wind damage from a tornado. In addition, data on specific abiotic and biotic factors were collected, such as soil properties, canopy openness, and the identities of roots of tree hosts colonized by each ECM fungus.

This project considered the following questions and hypotheses:

Question 1: Are ECM fungal communities more variable (in their composition and diversity) among sites or in response to forest disturbances within sites?

We hypothesized that ECM fungal community composition would differ between recently disturbed and control (less disturbed) plots due to significant changes in abiotic conditions expected with reduced canopy cover in disturbance plots.

Question 2: Which abiotic and biotic factors, including plant host identity, most influence ECM fungal community composition?

We hypothesized that abiotic factors of light, soil density, litter depth, and recent burn history, and biotic factors of plant host identity, would explain a significant amount of variation in the species composition of ECM fungi, since those factors are known to change in response to burning and thinning disturbances.

Question 3: Does spatial proximity explain any variation in the ECM fungal community structure?

We hypothesized that there would be no detectable spatial autocorrelation at the 10 m spatial scale at which we sampled between cores because previous studies have primarily found spatial autocorrelation at smaller scales (Lilleskov et al., 2004; Bahram et al., 2011; Pickles et al., 2012).

2. Methods

2.1. Study sites

Two of the three study sites were located at Strawberry Plains Audubon Center (SPAC), a 1052 acre wildlife sanctuary located in Marshall County, Mississippi, USA (34.833° N,−89.470° W). The study area was characterized by gently rolling hills, 10–50 m in elevation from ridge to hollow (Surrette et al., 2008). The trees that dominate this area include mostly second growth stands of oaks such as *Quercus velutina*, *Quercus marilandica*, *Quercus rubra* and *Quercus stellata* in the upland areas, with *Liquidambar styraciflua*, *Acer rubrum*, *Quercus alba*, and *Nyssa sylvatica* commonly occurring in the floodplain regions. The soil at this site is characterized as Providence-Cahaba with a loamy silt texture (Brewer, 2001; Surrette et al., 2008). The two SPAC sites were located approximately 1.6 km from each other. The third study site was located in the Little Tallahatchie Experimental Forest (LTEF), in Holly Springs National Forest, Lafayette County, Mississippi, USA (34.505° N,−89.440° W), approximately 36 km from the SPAC sites. The LTEF site consists of a mixed upland forest with similar composition to the SPAC sites, but with a larger population of *Pinus echinata* (shortleaf pine) and *Pinus taeda* (loblolly pine). The soil at this site is designated as Loring silt loam, and is heavier in density than at SPAC, with sandier composition on the slopes (J.S. Brewer pers. obs.). At each of the three study sites, we established paired Disturbance and Control (less disturbed) plots measuring 70 × 75 m (Table 1). Table 1 summarizes the characteristics and locations of the three study sites and the paired plots at each site. See below for additional details on history of disturbances in each plot.

2.2. History of disturbances in plots

Disturbance plots at SPAC received anthropogenic thinning and burning treatments, including four recent fires: September 2004 (Front Strawberry only), April 2005 (Front Strawberry only), October 2006 (Front Strawberry only) and July 2008 (both sites). The spring fire in 2005 burned the entire Front Strawberry Disturbance plot, while the other three fires were patchier and only affected areas near the edges of the plots. Thinning at the SPAC sites consisted mostly of mechanical tree removal via girdling along with chemical application of 8% Triclopyr (an herbicide), especially focusing on *L. styraciflua,* which historically has been relegated to flood plains, but due to fire suppression has extended its range into upland forests (Brewer, 2001; Surrette et al., 2008; Brewer and Menzel, 2009). The mechanical thinning at Front Strawberry and Back Strawberry disturbance plots has been ongoing since 2005 and 2007, respectively. Thinning in the Disturbance plot at the

Table 1
Site locations and characteristics at the time of sampling.

Site	Plot	Latitude/Longitude	Elevation	Disturbance history
Front Strawberry	FSC: Control FSD: Disturbance	34°49′51.70″N 89°28′33.64″W	143.26 m	FSD burned Sept. 2004, April 2005, Oct. 2006 and July 2008. Mechanical thinning at FSD since 2005.
Back Strawberry	BSC: Control BSD: Disturbance	34°50′14.67″N 89°28′15.17″W	137.12 m	Both BSD plots showed evidence of burn ~10 years prior or more. BSD was also burned July 2008. Mechanical thinning at BSD since 2007.
Tallahatchie	TC: Control TD: Disturbance	34°30′17.52″N 89°26′10.40″W	121.92 m	Both plots were burned during the 1980s. TD was hit by a tornado in Feb. 2008 and burned March of 2005.

Tallahatchie site occurred naturally from a tornado in February 2008, with canopy coverage thinned to approximately 30% of the original canopy. The Tallahatchie Disturbance plot was also burned in March of 2005. The entire experimental forest area had previously been burned at various intervals during the 1980s, and Control plots at Strawberry Plains may have burned previously, but none of the Control plots were subject to burning or thinning in at least the 20 y prior to our study, and likely longer.

2.3. Sample collection and processing

In May 2009, within each of the six plots (paired Disturbance and Control plots at each of the three sites), 36 root cores 15 cm deep and 3 cm in diameter were collected. In each plot, a systematic grid sampling design consisting of 6 parallel transects 10 m apart was utilized, collecting cores every 10 m along each transect (6 cores per transect) for a total of 36 cores per plot. We chose this sampling design to maximize sampling of ECM diversity while minimizing potential spatial autocorrelation between samples, which has typically been found to occur among samples of ECM fungal communities at smaller scales (Lilleskov et al., 2004). Soil cores were kept on ice in coolers until they were returned to the laboratory, where they were then refrigerated at 4 °C until processing. Roots in each core were washed carefully over a 2 mm sieve, and 10 individual ECM root tips were randomly sampled from each core for molecular identification using the aid of a dissecting microscope. Only clearly ectomycorrhizal root tips were selected for sampling, based on swelling, lack of root hairs, and presence of a hyphal mantle. ECM root tips were sorted into morphotypes within each sample based on color and texture of ectomycorrhizas, the number of root tips belonging to each morphotype in each sample were counted, and three representative root tips from each morphotype in each sample were saved for molecular identification of fungi and plants. We also collected leaves from dominant trees at the field sites, for use in molecular identification of unknown roots.

Abiotic variables were measured for each of the soil core samples in the field, including litter depth (from top of mineral soil layer), soil density (at 7.5 cm and 15 cm depths using a penetrometer), evidence of recent fire disturbance (i.e., scorched tree trunks and coarse woody debris), and canopy cover. Canopy photos were taken with a Nikon Coolpix 990 digital camera fit with a fisheye lens and then analyzed using Gap Light Analyzer software (version 2, Frazer et al., 1999) to produce estimates of % canopy openness and total light penetration. Subsamples of soil from each soil core were analyzed for texture (percent sand, silt, and clay) using a LaMotte soil texture kit (Chestertown, MD, USA).

2.4. Molecular identification of ECM fungi and host plant roots

Plant and fungal DNA were extracted from fresh root tip and tree leaf samples using the Sigma Extract-N-Amp kit (Sigma-Aldrich, St. Louis, MO), as follows: 10 μl of the Sigma Extraction Buffer was added to each root tip, each sample was heated at 65 °C for 10 min and 95 °C for 10 min in a thermocycler, and then 30 μl of the Sigma Neutralization Solution was added to each sample. Polymerase chain reaction (PCR) for fungal identification was performed using the fungal-specific primers ITS1-F and ITS4 (Gardes et al., 1991). Plant DNA was amplified using the universal chloroplast primers ucp-e and ucp-f, which amplify the trnL-trnF intergenic spacer (Taberlet et al., 1991). Each 8 μl PCR reaction contained 0.4 μl (10 μM stock concentrations) of each primer, 2.7 μl of sterile PCR-grade water, 4 μl of Sigma Extract-N-Amp PCR Reaction mix, and 0.5 μl of DNA extract. Thermocycling for PCR included the following conditions: initial denaturation at 93 °C for 3 min followed by 35 cycles of denaturation for 1 min at 93 °C, annealing for 55 s at 53 °C (for fungal PCR) or 55 °C (for plant PCR), and extension for 35 s with +5 s per cycle at 72 °C, followed by a final extension of 10 min at 72 °C. The PCR products were checked for amplification on a 1% agarose gel with SYBR® Safe DNA gel stain (Molecular Probes, Eugene, OR, USA). Excess primer and unincorporated nucleotides were removed enzymatically using ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA), with the following protocol: 1 μl of ExoSAP-IT and 4 μl of PCR-grade water were combined with 5 μl of PCR product, and each sample was heated to 37 °C for 45 min, 80 °C for 15 min and 4 °C for 5 min. Sanger sequencing was performed using only the forward ITS1 primer (for fungal sequencing) or the forward ucp-e primer (for plant sequencing) and the ABI Big Dye Terminator Sequencing Kit (v3.1). Each Big Dye reaction for fungal sequencing contained 1 μl Big Dye Reaction Pre-Mix, 1.5 μl Big Dye 5X sequencing buffer, 0.5 μl of the primer (10 μM stock concentration), 6 μl of sterile PCR-grade water, and 1 μl of the cleaned PCR product. Each Big Dye reaction for plant sequencing contained 0.25 μl Big Dye Reaction Pre-Mix, 1.875 μl Big Dye 5X sequencing buffer, 0.5 μl of the primer (10 μM stock concentration), 6.55 μl of sterile PCR-grade water, and 1 μl of the cleaned PCR product. Thermocycling for sequencing included the following conditions: initial denaturation at 96 °C for 1 min followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 20 s at 50 °C (for fungal sequencing) or 53 °C (for plant sequencing), and extension at 60 °C for 4 min. Reactions were then dried and mailed overnight to the DNA Lab in the School of Life Science at Arizona State University (Tempe, AZ, USA), where sequencing reactions were purified and read on a capillary genetic analyzer.

Raw sequences were imported into CodonCode Aligner software (version 1.6.3; CodonCode Corporation) where sequence ends were trimmed and sequences with fewer than 200 bases were removed from the dataset. In addition, sequences with >6% ambiguous bases (defined as bases with Phred-Phrap quality scores of less than 15) were not used for further analysis. Sequences passing these initial screening criteria were then assembled into operational taxonomic units (OTUs) with the CAP3 software package (Huang and Madan, 1999) running on the University of Alaska, Fairbanks (UAF) Life Science Informatics computing cluster, using default parameters with the exception of the following changes: h = 60 (max. % overhang length), m = 6 (match score factor), p = 97 (overlap % identity cutoff), y = 6 (clipping range). This analysis sorted the sequences into contigs (OTUs appearing more than once) and singlets (OTUs appearing only once). After OTU assembly, singlets with >3% ambiguous bases were removed. Because we only sequenced in the forward direction, it is likely that a number of fungal sequences were discarded due to sequence ambiguities caused by ITS length polymorphisms, potentially biasing our data set against taxa with such polymorphisms.

A merged file containing the filtered singlets and consensus sequences for the contigs was submitted for BLAST comparisons with GenBank using the BLASTALL utility on the UAF Life Science Informatics computing cluster. Database hits with overlap of less than 150 bases were not used. OTU sequences of fungi were also compared with matches from the UNITE database (Kessy et al., 2010) as well as our in-house database generated from identified ectomycorrhizal mushroom samples collected in northern Mississippi. Plant sequences were also compared with sequences from leaves collected from dominant trees at the sites. Top hits from these comparisons and database queries were used to assign likely taxonomic identities to plants and fungi based on the degree of matching, with hits matching at 99% or greater identity assigned to matching species. Sequences with 95–98% similarity were assigned to genus level resolution, designated with a number based on the order with which they were determined (e.g., *Lactarius* 1). OTU matches at the 90–94% identity level were assigned family level

resolution, with a number denoting the order with which they were assigned (e.g., Russulaceae 1). All queries found to be <90% or matching non-ectomycorrhizal fungal species were excluded from the final analyses. Representative plant and fungal sequences were uploaded to GenBank (accession numbers KX619482–KX619567).

2.5. Data analysis

Question 1: Are ECM fungal communities more variable (in their composition and diversity) among sites or in response to forest disturbances within sites?

Permutational analysis of variance (PermANOVA) was used to test the influences of disturbance and site on multivariate fungal community structure, with site and disturbance as fixed-effect predictor variables in the model. We tried preliminary versions of this analysis with several different subsets of the data, including all of the fungal OTUs occurring more than once (i.e., all of the contigs), only the 17 OTUs occurring in more than 5 cores, and only the five dominant OTUs occurring in at least 4% (15) of cores. The results from these three analyses were very similar, so we only present results from the analysis of the latter data set. This analysis was conducted in PRIMER 6 (version 6.1.11) plus PERMANOVA (version 1.0.1) software package (Clarke and Gorley, 2006).

OTU richness and diversity for each plot, using all OTUs including those occurring only once, were estimated with EstimateS (version 8.2.0) software (Colwell, 2009), including Chao 2, an incidence-based estimator of richness for sample-based incidence data, and the Shannon and Simpson diversity indices. PC-ORD (version 5.0, McCune and Mefford, 2006) was used to generate sample based rarefaction species accumulation curves for each plot. ANOVA (in SAS version 9.2, Proc GLM) was then used to test the influences of disturbance and site on plot-level estimated fungal species richness and diversity, with site and disturbance as fixed-effect predictor variables in the model.

Question 2: Which abiotic and biotic factors most influence ECM fungal community composition?

Distance-based linear modeling (DBLM; Legendre and Anderson, 1999) was used to test which measured environmental factors, including abiotic variables (canopy cover, soil density, litter depth, and burn history) and host plant root identity, had the strongest influence on multivariate ECM fungal community structure. Disturbance from treatment (thinning, prescribed burning or tornado damage) was excluded as a factor in these analyses, after disturbance was deemed insignificant in structuring the fungal community (see Question 1), and we desired to elucidate which specific environmental factors were most predictive of ECM fungal community composition. Canopy openness was also omitted from the analysis, as we lacked canopy openness data for some samples, and it was found to be a highly insignificant predictor in preliminary analyses. Site was included as a candidate predictor variable, so that we could test whether significant differences in ECM community composition among sites were still detected when the measured biotic and abiotic factors were included in the models. Only the main effects of predictors were examined, to avoid overfitting of models, and because we did not have any strong hypotheses about how interactions would affect ECM community composition. Significance of candidate predictors was assessed in the full model with all predictors using *p*-values, but model selection based on Akaike's information criterion corrected for small sample sizes (AICc) was also conducted to assess which predictor variables were most important in explaining the multivariate community data. The proportion of variation in ECM community composition explained by the best model was assessed by summing the proportion of variation explained by each of the first four multivariate DBLM axes. This statistical analysis used only the dominant OTUs occurring in at least 4% of cores, and was conducted in the PRIMER 6 (version 6.1.11) software package (Clarke and Gorley, 2006).

Question 3: Does spatial proximity explain any variation in the ECM fungal community structure?

A statistical approach to measure spatial autocorrelation at the plot level was used, with cores as the sample units (Legendre and Fortin, 1989). Specifically, a Mantel test was conducted separately for each plot to test for a correlation between two distance matrices, a species distance matrix (consisting of Sorensen distances generated from species relative abundance data using all fungal OTUs occurring more than once), and a physical distance matrix (consisting of Euclidean physical distances among cores generated from X–Y coordinates). Monte Carlo permutation (in PC-ORD version 5.0, McCune and Mefford, 2006) was used to generate *p*-values for the significance of this test of spatial autocorrelation. When significant spatial autocorrelation was found in a plot, the scale of spatial autocorrelation was explored using a Mantel correlogram, consisting of normalized Mantel correlation coefficients for multiple distance classes of soil cores (as described by Legendre and Fortin (1989)).

3. Results

3.1. Overall patterns in community composition

A total of 484 ECM fungal sequences were generated from the 216 root cores, after low quality and non-mycorrhizal sequences were discarded. The fungal community was highly diverse, with 69 operational taxon units (OTUs) occurring more than once, and 104 singlet OTUs (i.e., OTUs occurring only once) across all sites. The most abundant species included taxa from the Cantharellaceae, Thelephoraceae, Russulaceae, and Sebacinaceae families (Fig. 1, see also Table S1). Observed OTU richness in the six plots ranged from 25 (in the Back Strawberry Disturbed plot) to 59 (in the Front Strawberry Disturbed plot) (Table S2). Sample based rarefaction OTU accumulation curves did not reach an asymptote when all OTUs were included, but began to asymptote when singlets (OTUs occurring only once in the data) were excluded, indicating that our sampling effort did not reach saturation for rare taxa (Supplemental Fig. S1).

For plant roots identified with molecular techniques, a total of 368 root sequences, excluding those that failed to meet the screening criteria, were generated from 136 root cores. The molecular data generated did not have the resolution to discern plant root identity at the species level in all cases, but it did distinguish among plant genera and, in the case of the oaks (*Quercus* spp.), among major sub-generic clades (red versus white oak clades). Across all six plots, the community composition of plant host roots comprised 7 different taxa: red oak clade (*Quercus* subgenus *Erythrobalanus*, 44% of all ECM roots), white oak clade (*Quercus* subgenus *Quercus*, 31%), hickory (*Carya* spp., 18%), pine (*Pinus* spp., 3%), winged elm (*Ulmus alata,* 2%), red maple (*A. rubrum,* 2%), and black cherry (*Prunus serotina, <1%*).

Question 1: Are ECM fungal communities more variable (in their composition and diversity) among sites or in response to forest disturbances within sites?

ECM fungal community composition differed significantly

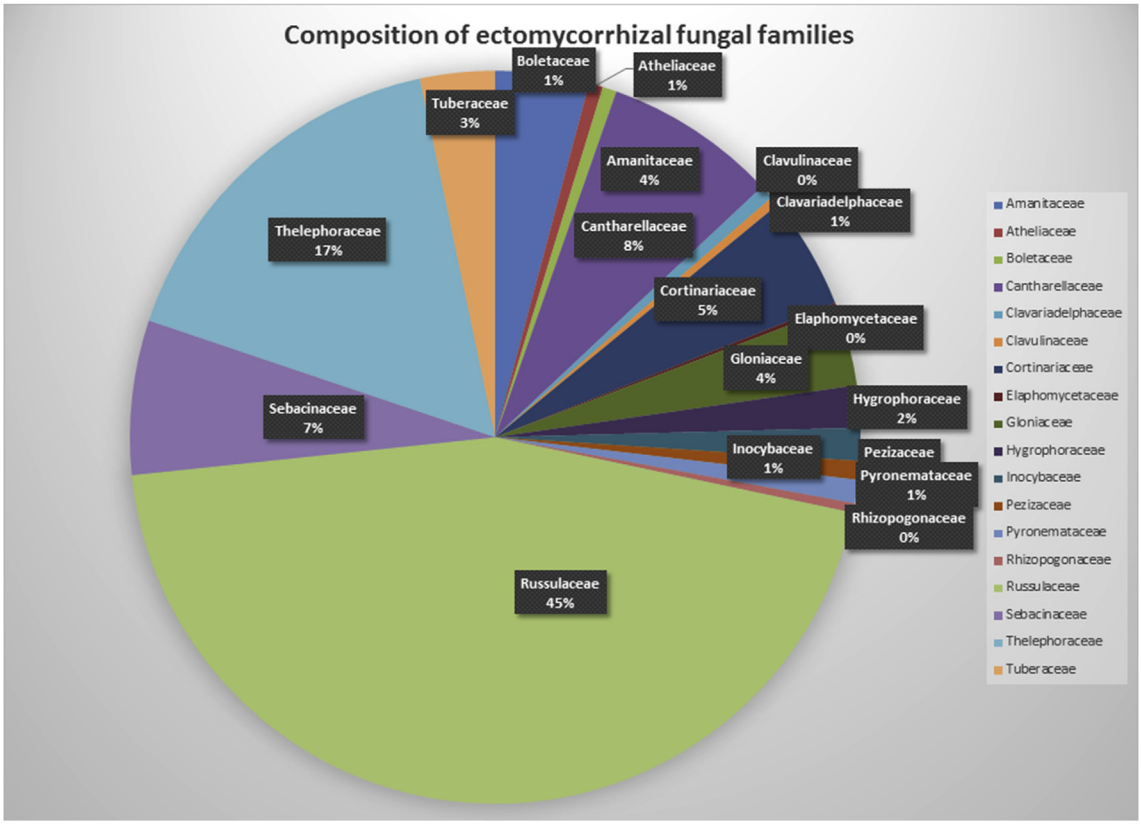

Fig. 1. Percent composition of ectomycorrhizal fungal operational taxonomic units (OTUs) at the family level across all study sites.

among sites, (df = 2, Pseudo-F = 2.28, and $p = 0.001$), but not with disturbance. The Back and Front Strawberry sites were not different from one another, but differed significantly from the Tallahatchie site. Richness and diversity estimators did not differ significantly by site or disturbance. The most abundant OTUs (making up 6% or more of the total species community) made up 67% of the total samples and included OTUs from the families Sebacinaceae, Thelephoraceae, and Russulaceae. OTUs in the family Russulaceae alone accounted for 45% of the total OTUs for all plots (Fig. 1, see also Table S1).

At the higher taxonomic levels of family and genus, the fungal communities were similar across all three sites; however, numerous taxa at the species level occurred in only 1 plot or were specific to a site (see Table S1). Russulaceae and Thelephoraceae consistently made up the largest proportion of the ectomycorrhizal fungal community; however, the next most abundant taxa differed between the Strawberry sites and the Tallahatchie sites (see Table S1).

Question 2: Which abiotic and biotic factors most influence ECM fungal community composition?

Distance-based linear modeling (DBLM) was employed to relate measured abiotic and biotic (plant root identity) data to ECM fungal community composition data (Table 2). Significant predictors of ECM fungal community composition included site, recent burn evidence, and the presence of red oak (Table 2). Soil density at 17 cm depth and presence of hickory roots were marginally significant ($p < 0.1$). Conclusions from model selection (not shown) were not qualitatively different from those using significance of predictors, and the AICc-best model (i.e., the model with the lowest AICc score), which contained the three significant factors (site, recent burn evidence, and red oak), explained only 7% of variation in ECM fungal community composition.

Question 3: Do spatial patterns explain any variation in the ECM fungal community structure?

One plot had significant spatial autocorrelation of fungal community composition, i.e., a correlation between spatial proximity and species composition similarity among cores: Tallahatchie Disturbance (TD), where tornado damage resulted in significant thinning of the canopy. This result indicates similar species composition among nearby cores at distances of 10 m or greater at this plot, whereas for the other 5 plots, communities were uncorrelated at those spatial scales. A distance class correlogram showed that the spatial autocorrelation was highest at the 10–15 m scale

Table 2
DBLM results for analysis of variation in ectomycorrhizal fungal community composition associated with abiotic and biotic environmental factors.

Predictor variable	Sum of squares	Pseudo-F	*p*-value
Site	7025.4	4.0692	0.013
Soil density at 10 cm depth	2633.1	1.4929	0.184
Soil density at 17 cm depth	3790.7	2.1613	0.07
Litter depth	3165.4	1.7993	0.132
Recent burn evidence	5255.5	3.0178	0.019
White oak subgenus	1343.8	0.75722	0.5
Red oak subgenus	4389.4	2.5099	0.046
Hickory (*Carya* spp.)	3997.2	2.2813	0.06
Pine (*Pinus* spp.)	1335.5	0.75252	0.486
Black cherry (*Prunus serotina*)	383.82	0.21529	0.972
Winged elm (*Ulmus alata*)	1541.8	0.8696	0.394
Red maple (*Acer rubrum*)	886.99	0.49873	0.637

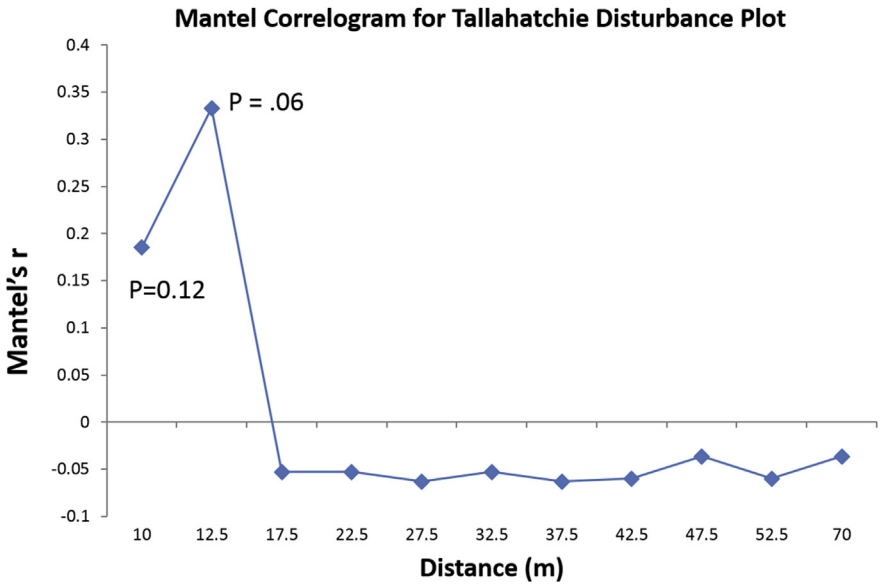

Fig. 2. Correlogram indicating spatial autocorrelation among pairs of cores in different distance classes in the Tallahatchie Disturbance plot after 999 permutations. *p*-values for spatial autocorrelation at distances 17.5 m or greater were highly non-significant ($p > 0.6$). The X-intercept was 16.8 m.

for the TD plot (Fig. 2).

4. Discussion

4.1. Overall patterns of ECM fungal community composition

Results from this study indicate that the ECM fungi found in our mixed hardwood forest sites in northern Mississippi are very diverse, a pattern repeatedly confirmed in other ectomycorrhizal community surveys of belowground diversity (see reviews by Horton and Bruns, 2001; Smith and Read, 2008). Other studies that have surveyed ECM fungal belowground diversity in oak forests, or forests containing oak species, have found an impressive amount of diversity, generating species area curves that do not asymptote for typical sampling intensities, indicating an even greater diversity than sampled (Avis et al., 2003; Walker et al., 2005, 2008; Morris et al., 2008; Walker et al., 2008). The ecological significance of such high diversity on root tip communities is a question still debated among mycorrhizal ecologists, though multiple hypotheses, including micro-niche partitioning (Bruns, 1995) and regional temperature and rainfall (Tedersoo et al., 2012) have been proposed to explain the hyper diversity of these cryptic communities at some locations. To our knowledge, no molecular studies of the ECM fungi of upland mixed hardwood/conifer forests have previously been conducted in this region, and knowledge of ECM fungal communities in forests of the southeastern United States is generally sparse.

4.2. Variation in ECM fungal community composition among sites, and the influence of abiotic and biotic factors

The ECM fungal community had significant site fidelity, with composition varying significantly in the Tallahatchie site compared to the two SPAC sites; however the best model of abiotic and biotic factors only explained 7% of variation in ECM composition. This result suggests that stochastic variation in community assembly may contribute to differentiation in ECM fungal community composition at the scale of 36 km that separates the Tallahatchie site from the two SPAC sites. In addition, unmeasured variables such as soil chemical factors and legacies of early land history may vary among sites and influence ECM fungal composition. The measured abiotic factor found to have the strongest relationship with fungal community composition was recent burn evidence, suggesting that unmeasured aspects of soil chemistry and microclimate of the edaphic environment related to fire have a short-term influence on which fungal taxa will occur in a certain area. Overall, it is striking how little variation in this ECM fungal community is explained by the variables included in our analyses.

In comparison to other forest types in the literature, the hardwood dominated upland forests of Northern Mississippi are similarly diverse in belowground ECM fungal community composition. Our molecular survey of belowground diversity found a total of 69 reoccurring OTUs and 106 single species. In a *Eucalyptus* forest in Australia, a molecular survey of ectomycorrhizal fungi found a total of 123 species occurring on root tips (Tedersoo et al., 2008). A survey of fungal diversity in a Mediterranean forest dominated by *Quercus ilex* recovered around 140 different ectomycorrhizal species (Richard et al., 2005). Using 454 pyrosequencing, a study on ectomycorrhizal fungi in urban versus rural sites in forests in central Kansas found 1077 unique OTUs (Jumpponen et al., 2010). A study of ECM diversity in an *Quercus* forest in Japan found 345 distinct taxa, with the greatest species abundance in the genera of *Russula, Lactarius, Cortinarius, Tomentella, Amanita, Boletus, and Cenococcum* (Toju et al., 2013), similar to the same abundant genera we found in our survey. It is likely that as methods for characterizing belowground fungal communities improve, further surveys of ectomycorrhizal fungal community will continue to reveal even greater diversity than previously reported.

The presence of red oak was also found to influence ECM community composition, and hickory was marginally significant. These trees comprise a large proportion of the aboveground plant host community, and their significance as predictors of ECM fungal community composition suggests some degree of specificity in compatibility or preference among tree species within the ECM fungal community. Oaks have been shown to host very diverse ectomycorrhizal fungal communities in other surveys that looked at belowground community diversity, often finding over a hundred fungal species on root tip communities (Smith et al., 2007; Avis et al., 2008). Some ECM fungal species have relatively narrow host ranges and are specific to a host genus or family, such as the

ECM fungal genus of *Suillus* and its specific associations with *Pinus* (Bruns et al., 2002b). Many of the fungi found in this survey including *Russula, Amanita,* and *Cenococcum,* are reported to have broader host associations, and likely there are ECM fungal species that will associate with both oaks and other tree host species (Dickie et al., 2004). While plant host identities did not account for a large proportion of variation in ECM community structure, previous studies have found that plant host does influence fungal diversity and what species will occur in certain areas (Molina et al., 1992; Richard et al., 2005; Ishida et al., 2007; Tedersoo et al., 2008, 2012).

It is likely that unmeasured factors, including soil physiochemical properties such as organic matter content and pH, play important roles in structuring ECM fungal communities in these habitats. Soil organic matter content, for example, is likely significantly lower in the sandier soils of the LTEF site, compared to the two SPAC sites. In a meta-analysis using data from papers on ECM fungal community structure from all over the world, Tedersoo et al. (2012) found that temperature and precipitation had the strongest effect on fungal community structure. For this study, we did not include factors related to precipitation, including soil moisture, as accurate and meaningful measures of this variable are difficult to obtain. However, given the lack of explanatory power from the factors we measured, such efforts may be important if we are to make progress in explaining local variation in ECM fungal communities, and specifically in accounting for the large amount of variation unexplained in this study.

Potentially relevant to site-specificity of the ECM fungal community structure are the life history and dispersal patterns of the different fungal taxa. For instance, fungi in the Amanitaceae were prevalent at both the Strawberry Plains sites, but were not found in the Tallahatchie sites. *Amanita* species have often been observed as late-successional taxa that colonize by growing from one plant root to another, and often will not occur in young recently-disturbed plant assemblages (Bruns et al., 2002a). Overall, the Strawberry sites were less disturbed than the Tallahatchie, potentially permitting more favorable conditions for the occurrence of *Amanita*.

While some taxa were specific to particular sites or plots, fungal OTUs in the Russulaceae family prevailed throughout all 3 sites in the greatest abundance, a pattern that has been reported in numerous ECM fungal community studies (Taylor and Bruns, 1999; Richard et al., 2005; Smith et al., 2007; Avis et al., 2008). Russulaceae are globally disturbed and have been reported in both tropical and temperate ecosystems, making them one of the most widely occurring ectomycorrhizal families (Mueller et al., 2007).

4.3. Spatial autocorrelation of ECM fungal communities

Perhaps the most surprising finding of this study was discerning spatial autocorrelation at a relatively large spatial scale (between 10 and 15 m) in the Tallahatchie Disturbance plot. Most previous studies have shown spatial autocorrelation in ECM fungal communities only at smaller scales (Lilleskov et al., 2004; Bahram et al., 2011) typically less than 3 m. To our knowledge, spatial autocorrelation in ECM fungal communities at this large scale using similar analyses has never been previously reported, and suggests an interesting topic for further research into factors structuring ECM fungal communities on a local scale. We hypothesize that the substantial disturbance provided by the tornado generated homogeneity in biotic or abiotic factors at a relatively large spatial scale, which drove corresponding homogeneity in the ECM fungal community at that scale.

This result may also indicate an important role for ECM fungal networks in highly disturbed sites. ECM fungi form extensive mycelial networks through the soil (Simard and Durall, 2004), and *Quercus* species have been shown to facilitate congeneric seedlings through these networks (Dickie et al., 2002). If the oaks in historical landscapes maintained by fire were at greater distances apart from congeneric neighbors, as may result from contemporary thinning and burning efforts towards oak woodland restoration, perhaps their fungal linkages were shared at greater spatial scales as well. Additionally, fungal dispersal and autocorrelations in environmental variables caused by soil disturbance may also contribute to the patterns we discerned in this particular plot. Using data from co-occurrence analysis, Pickles et al. (2012) found that spatial structure of mycorrhizal communities was driven by competitive interactions between species and that community structure was more similar at distances equal to or less than 3.41 m. Spatial analysis of belowground fungal community structure may be a fruitful topic for research that has the potential to elucidate a more holistic concept of what the historical conditions for these ecosystems might have been.

5. Conclusions

Ectomycorrhizal fungi comprise the dominant symbioses of many economically and ecologically important trees throughout the world, and many of these species of trees depend on these fungi to establish and persist in the environment (Smith and Read, 2008). However, our ability to identify fungal taxa in the soil through molecular methods far exceeds our ability to explain the function of these taxa or factors that structure these cryptic communities, though recent work has suggested that structure and function of soil communities is linked strongly to climate and dispersal limitations at larger regional scales (Talbot et al., 2014). We found that our measured abiotic and biotic variables only accounted for 7% of total variance in species composition, and that spatial autocorrelation in fungal community composition may exist at greater spatial scales than previously reported in systems where there is significant disturbance from a natural event. To make progress in better understanding the dynamics that structure fungal communities, we need more studies that measure not only composition of these belowground communities, but also aspects of function, while seeking to understand the key factors that drive composition. Further investigation into spatial dynamics in belowground fungal connections can provide greater understanding of how these networks function and interact, as well as the role disturbance may place in structuring these networks. Knowledge of the interplay between the aboveground and belowground will provide a more complete understanding of our environment and how these unseen fungal constituents influence the visible aboveground ecology of temperate ecosystems.

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Supplementary data

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