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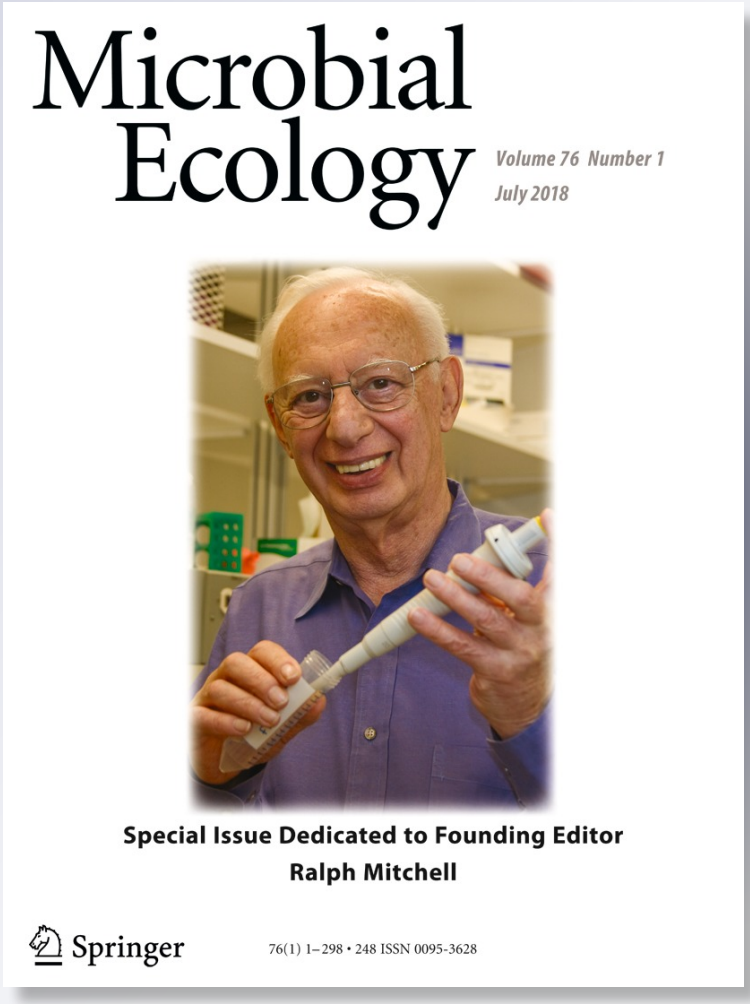

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Fungal Communities and Functional Guilds Shift Along an Elevational Gradient in the Southern Appalachian Mountains

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Abstract

Nitrogen deposition alters forest ecosystems particularly in high elevation, montane habitats where nitrogen deposition is greatest and continues to increase. We collected soils across an elevational (788–1940 m) gradient, encompassing both abiotic (soil chemistry) and biotic (vegetation community) gradients, at eight locations in the southern Appalachian Mountains of southwestern North Carolina and eastern Tennessee. We measured soil chemistry (total N, C, extractable PO_4, soil pH, cation exchange capacity [ECEC], percent base saturation [% BS]) and dissected soil fungal communities using ITS2 metabarcode Illumina MiSeq sequencing. Total soil N, C, PO_4, % BS, and pH increased with elevation and plateaued at approximately 1400 m, whereas ECEC linearly increased and C/N decreased with elevation. Fungal communities differed among locations and were correlated with all chemical variables, except PO_4, whereas OTU richness increased with total N. Several ecological guilds (i.e., ectomycorrhizae, saprotrophs, plant pathogens) differed in abundance among locations; specifically, saprotroph abundance, primarily attributable to genus *Mortierella*, was positively correlated with elevation. Ectomycorrhizae declined with total N and soil pH and increased with total C and PO_4 where plant pathogens increased with total N and decreased with total C. Our results demonstrate significant turnover in taxonomic and functional fungal groups across elevational gradients which facilitate future predictions on forest ecosystem change in the southern Appalachians as nitrogen deposition rates increase and regional temperature and precipitation regimes shift.

Keywords ITS2 gene sequencing · Fungal ecology · Coweeta hydrologic laboratory · Great Smoky Mountains · Soil chemistry

Introduction

Complex fungal assemblages of symbionts, parasites, and saprophytes occur through large geographical and climatic regions and can be highly diverse [1]. Across landscapes, such as the southern Appalachian Mountains, knowledge of fungal community dynamics is limited [2–4] because of tedious and often inconsistent methods for field sampling and taxon identification. However, metabarcode sequencing [5, 6] has allowed for the identification of fungal groups at a high taxonomic resolution facilitating a more holistic view of fungal community composition within and across landscapes.

Environmental factors such as available soil nutrients [7, 8] or soil moisture [9] can drive fungal community composition. For example, Avis [10] observed that ectomycorrhizal (ECM) sporocarp richness and abundance decreased by more than 50% in nitrogen fertilized plots. Trudell and Edmonds [11] determined that both total soil nitrogen and soil moisture strongly influenced sporocarp production across a nitrogen

Allison M. Veach and Richard Baird contributed equally to this work.

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availability gradient and that changes in production were due to functional differentiation between species. It has been documented that ECM sporocarp abundance associated with Scots Pine have substantially declined in Europe partially due to N deposition [12, 13]. Fungal diversity, as determined by rRNA gene sequencing, may also decrease due to changes in plant-derived carbon resulting from N fertilization [9]. Similarly, carbon quality can define phylogenetically distinct fungal communities across land use [8]. In sum, fungal communities respond strongly to many edaphic variables, but particularly to the quantity and quality of nitrogen and carbon pools.

Passage of the Clean Air Act of 1970 and the Clean Air Act Amendment of 1990 have resulted in reduced levels of sulfur oxides in the atmosphere across the eastern USA [14]. Yet, levels of nitrous oxides have remained stable and nitrogen deposition continues [15, 16], mainly because of anthropogenic factors such as urbanization [17]. Nitrogen deposition across landscapes is related to rainfall amount and proximity to urbanized areas. Air currents move atmospheric nitrogen from urban areas and greater nitrogen deposition occurs in areas of high rainfall, such as high elevation montane habitats. Nitrogen deposition increases with elevation in the southern Appalachian Mountains: lower elevations (780 m) receive 9.5 kg ha^{-1} $year^{-1}$, whereas higher elevations (1380 m) receive 12.4 kg ha^{-1} $year^{-1}$ [16]. Not only are higher elevation forests subject to greater N deposition, but also to greater litterfall N and stream N export [16]. Prolonged nitrogen deposition can result in N saturation (N availability is greater than plant and microbial uptake) and reductions in plant growth and primary production [18, 19]. However, the impact of N deposition on soil fungal communities in montane forests, particularly in the Appalachian Mountains, remains largely unknown.

Incremental long-term nitrogen increases have measurable effects on ECM composition and abundance. For example, sporocarp production may be an effective bioindicator of nitrogen deposition [11]. One such mycorrhizal group, the stipitate hydnate fungi, is abundant in forested ecosystems, but has dramatically decreased in the southern USA as documented in several monographic investigations [4, 20]. The decline of ECM sporocarps in Europe has been linked to eutrophication [12, 13, 21], resulting in increased abundances of fungal saprobes [4]. The evolution of new sequencing methods to evaluate fungal community dynamics bears the promise of yielding information to answer questions about commonly occurring fungi at sites with previous records of sporocarp production. Furthermore, assessing changes in soil chemical variables and concurrent fungal community shifts is critical for identifying nitrophilic and nitrophobic indicator fungi and subsequently understanding changes in forest health.

Our goal was to improve current understanding on how abiotic (soil chemistry) and biotic (vegetation community) gradients may alter the fungal communities in southern Appalachian forests. Specifically, we aimed to determine (1) the differences in soil chemistry across an elevation gradient and (2) the correlation between fungal diversity, ecological guild abundances (e.g., ECM, plant pathogens, saprotrophs), and community composition with soil chemical variables measured within this ecosystem. These data provide necessary baseline information on the relationship between fungal community dynamics and environmental gradients and may thus aid future work in how anthropogenic factors influence forest health.

Methods

Site Description

The study was conducted in the southern Appalachian Mountains of North Carolina and eastern Tennessee. The North Carolina sites are in the Coweeta Hydrologic Laboratory (CWT), a USDA Forest Service Experimental Forest and National Science Foundation Long-Term Ecological Research (LTER) site in southwestern North Carolina, whereas Tennessee sites are in the Great Smoky Mountain National Park (GRSM). In CWT, five study locations were established in 1991 representing a gradient in elevation (788–1389 m) and across the major vegetation community types within the Coweeta basin [22–24]. These vegetation types include dry mixed-oak pine (OP), cove hardwoods (CH), low elevation mesic mixed-oak (LO), high elevation mesic mixed-oak (HO), and northern hardwoods (NH; Table 1). In 2013, three study locations from GRSM were added to extend the elevation gradient (1539–1940 m) and increase the diversity of plant communities including high elevation northern hardwood (HNH), mixed northern hardwood spruce fir (NHS), and spruce fir (SF; Table 1).

Soil Sampling

In December 2013, we sampled for soil chemistry and fungal communities from all 8 study locations (Table 1) following previously established sampling protocols [22]. In brief, we randomly selected 8 x, y coordinates within each 80 × 80 m location for soil sampling; all soil samples were collected using a 2.54-cm diameter soil probe. Fungal community samples represented the top 5 cm of mineral soil plus the Oa horizon which consisted of a mor type of forest floor humus with well-decomposed organic matter of an unrecognizable origin. At each x, y location, we collected 6–10 subsamples and pooled them into one composite (8 composited samples × 8 study locations, $N = 64$). Each composite sample represents an experimental unit ($N = 64$). Soil samples for all soil chemistry analyses were collected similarly. Soils were placed on

Table 1 Site characteristics of Coweeta Hydrological Laboratory LTER (CWT) and Great Smoky Mountain (GRSM) plots which primarily vary by elevation, vegetation, and soil type. All locations have a mesic moisture regime. fl denotes fine-loamy soils, whereas cl denotes coarse-loamy soils

Site	GPS Coordinates	Elevation (m)	Aspect (degrees)	Slope (degrees)	Dominant species	Soil subgroup
CWT						
OP (mixed oak/pine)	35° 3′ 3.69″ N, 83° 26′ 3.012″ W	788	180	34	*Pinus rigida, Quercus coccinea, Q. prinus, Carya sp., Kalmia latifolia*	fl, parasesquic, mesic Typic Hapludults cl, micaceous, mesic Typic Dystrudepts cl, mixed, active, mesic Typic Dystrudepts
CH (cove hardwood)	35° 2′ 56.4252″ N, 83° 26′ 2.4468″ W	801	340	21	*Liriodendron tulipifera, Q. prinus, Carya sp.*	fl, mixed, superactive, mesic Humic Hapludults fl, isotic, mesic Typic Humudepts
LO (mixed oak-low)	35° 2′ 51.4176″ N, 83° 26′ 1.8564″ W	860	15	34	*Q. prinus, Carya sp., Q. rubra, Rhododendron maximum*	fl, mixed, active, mesic Humic Hapludults
HO (mixed oak-high)	35° 2′ 17.538″ N, 83° 27′ 33.7752″ W	1094	75	33	*Q. prinus, Q. rubra, Carya sp., R. maximum*	cl, micaceous, mesic Typic Dystrudepts
NH (northern hardwood)	35° 2′ 38.796″ N, 83° 27′ 25.812″ W	1389	20	33	*Betula allegheniensis, Q. rubra, Betula lenta, Tilia heterophylla*	fl, isotic, mesic Typic Humudepts
GRSM						
HNH (High Elevation NH)	35° 35′ 16.296″ N, 83° 4′ 50.736″ W	1539	190	30	*Q. rubra, Q. alba, Prunus serotina, Acer saccharum*	fl, isotic, frigid Humic Dystrudepts
NHS (mixed NH spruce fir)	35° 33′ 52.272″ N, 83° 28′ 35.436″ W	1737	205	17	*Picea rubens, B. alleghanensis, Fagus grandifolia, Abies fraseri*	fl, isotic, frigid Humic Dystrudepts
SF (spruce fir)	35° 33′ 54.972″ N, 83° 29′ 41.568″ W	1940	125	24	*P. rubens, A. fraseri*	fl, isotic, frigid Humic Dystrudepts

ice and returned to the laboratory. Upon arrival, soils for all measured nutrients, described below, were refrigerated at 4 °C for up to 72 h prior to processing whereas fungal community soils had large roots and litter fragments removed and then stored at − 80 °C until DNA extractions.

Soil Chemical Analyses

Samples for chemical analyses were air dried and sieved through a 2 mm sieve. All soil chemical data are presented on an air-dry weight basis. Soil pH was determined in a 1:1 soil to 0.01 M $CaCl_2$ slurry using a Thermoscientific Orion 3-star pH bench-top meter with a Thermo Scientific Orion pH probe. We extracted 5.0 g of soil with 50-ml 1.0 M NH_4Cl on a mechanical vacuum extractor (SampleTek, Science Hill, KY, USA) for determination of exchangeable cation concentrations followed by determination of effective cation exchange capacity (ECEC) by rinsing the soil with 95% ethanol to extract remaining NH_4 with 1 M KCl. Cation concentrations in NH_4Cl solution were determined using an inductively coupled plasma spectrometer (ICP) (Thermo Fisher iCAP 6300, Thermo Fisher Scientific, Madison, WI, USA); NH_4 concentration in KCl extraction solution was determined colorimetrically using the alkaline phenol method [25] and used to calculate ECEC (meq 100 kg $soil^{-1}$). Percent base saturation (% BS) was calculated: %BS = ((Ca + Mg + Na + K) / ECEC) * 100. Extractable phosphate (PO_4) was determined by extracting 5.0 g of soil in 20-ml dilute double acid (0.05 M HCl + 0.0125 M H_2SO_4) followed by centrifugation; PO_4 in solution was determined by ICP as described above [26, 27]. Subsamples (~ 5 g) of each soil sample were ground to a fine powder prior to total C and N analysis by combustion using a Flash EA 1112 series CN analyzer (CE Elantech, Lakewood, NJ, USA).

DNA Extraction and Illumina MiSeq Library Preparation

Total DNA was extracted from three replicate 0.5 g subsamples of each composite soil sample ($n = 192$) using a

PowerSoil DNA extraction kit (MoBio Laboratory, Carlsbad, CA, USA). Subsamples were homogenized in lysis solution for 30 s using a Biospec Mini-beadbeater (Biospec, Bartlesville, OK, USA). The extractions from the three technical replicate subsamples were combined for each experimental unit ($N = 64$). We chose to extract from three technical replicates to have a better representation of fungal communities in the heterogeneous soils collected. DNA was quantified with a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). A two-step PCR approach was used to avoid 3′-end amplification bias during PCR DNA barcoded tagging [28]. Primary PCRs were composed of three technical replicates per experimental unit in 50-μl reaction volumes with the following: 10-μl 5× Phusion Green Buffer, 1-μl 10 mM dNTPs, 1-μL forward primer ITS1F (5′-CTTG GTCATTTAGAGGAAGTAA-3) and reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [29], 5-μl genomic DNA template, 0.5-μl Phusion Hot Start II DNA polymerase, and 31.5 μl molecular grade water. PCR conditions included initial denaturation of 30 s at 98 °C, followed by 30 cycles of denaturing, annealing, and extension at 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s. Final extension was at 72 °C for 10 min. For secondary PCRs, 10-μl aliquots of each of 3 PCR technical replicates were included as template and amplified using primers gITS7 (5′-GTGAATCATCGARTCTTTG-3′) and ITS4 with a ligated unique 12 bp barcode (ITS4-MID; Supplementary Table 1), using the same PCR conditions except PCRs were run for 5 cycles.

Technical PCR replicates from each experimental unit were pooled and cleaned with a magnetic bead system (AmPure SPRI; Beckman Coulter, Inc., Brea, CA, USA) using a ratio of 1:1 AMPure bead solution to PCR volume to remove short PCR amplicons and residual primers. The cleaned amplicons (one per experimental unit) were quantified and pooled equally (100 ng per sample). The amplicon library was paired-end sequenced at the Integrated Genomics Facility at Kansas State University using Illumina MiSeq (v. 3; 2 × 300 cycles).

Bioinformatic Analyses

Sequence data (.fastq) were processed using the mothur pipeline (version 1.33.3) [30]. Sequences were joined (22,365,729 reads after joining) and reads with any ambiguous bases, any mismatches to primers or MIDs, homopolymers longer than 8 bp, or less than 300 bp in length were removed. Sequences were truncated to 300 bp and preclustered to reduce potential sequencing biases [31] resulting in 3,049,142 sequences. Subsequently, sequences were screened for chimeras using the uchime algorithm [32]. After removal of chimeras (78,029 chimeric sequences detected), a pairwise distance matrix was calculated and sequences clustered into operational taxonomic units (OTUs) at 97% sequence similarity. OTUs were assigned to taxonomic affinities using the Naïve Bayesian Classifier [33] with the UNITE taxonomy reference database (http://unite.ut.ee/respository.php). No contaminant OTUs (unclassified to Kingdom Fungi, plant) were detected (2,971,113 high-quality sequences). Experimental units were subsampled to 5400 sequences, and singletons and doubletons were removed as these are likely sequencing artifacts [34, 35], yielding a total of 2412 OTUs and 286,200 sequences in the final dataset. Good's coverage ($G = 1\text{-}(n_1/N)$ where n_1 = the number of OTUs sampled once and N = the total number of individuals in the sample), observed OTU richness (S_{obs}), Chao1 richness estimator ($S_{obs} + F1^2/2F2$) where F1 = number of OTUs with one occurrence, F2 = number of OTUs with two occurrences, and the complement of Simpson's Diversity ($1\text{-}D: 1\text{-}\sum p_i^2$), and Simpson's Evenness ($E_D: 1/\sum p_i^2/S_{obs}$) with p^i representing the frequency of each OTU within a sample, were iteratively calculated in mothur (version 1.33.3) [30]. Richness, diversity, and evenness are collectively referred to as diversity estimates throughout the remainder of the text. Paired sequence data (.fastq files) are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject number PRJNA381709 and Study Accession SRP103512.

Statistical Analysis

One of our goals was to determine if soil chemistry (response variables: total N, total C, C/N, PO_4, soil pH, % BS, and ECEC) and fungal diversity estimates (response variables: richness [S_{obs}, Chao1], diversity [complement of Simpsons Diversity], and evenness [Simpson's Evenness]) differed among study locations (categorical explanatory variable) or elevation (continuous explanatory variable). As a result, we used two separate statistical models to evaluate study location and elevation effects—Kruskal-Wallis rank sum tests to test for location effects and linear regressions with a normal error distribution to determine elevational differences in soil chemistry. We also used Spearman's correlation coefficients to identify which soil chemical variables significantly correlated with each other (function *rcorr* in the Hmisc package) [36].

Because some response variables were not normally distributed, a nonparametric Kruskal-Wallis rank sum test was used to determine differences among study locations (function *kruskal.test* in PMCMR package) [37]. Dunn's post hoc correction for multiple pairwise comparisons test of mean rank sums was subsequently performed for each Kruskal-Wallis test that was statistically significant (*posthoc.kruskal.dunn.test* in PMCMR package) [37]. A linear regression model was used to discern elevation differences (function *lm* in the stats package). Some response variables exhibited data curvature across elevation (i.e., plateaued at intermediate elevations); we elected to also run quadratic regression models to account for this. An ANOVA for linear model fits was then used to determine whether a linear or quadratic regression significantly

reduced model SSE (function *anova* in stats package) [38]. This approach identifies if there is a significant reduction in residual SSE if the quadratic term is added to the model. If the quadratic term did not significantly reduce model SSE, then the linear model is reported. Otherwise, the model with quadratic term included is reported. For regression models, total C, ECEC, and % BS were $\log_{10}$-transformed prior to analyses.

We also used a multiple, linear regression model with a stepwise selection and Akaikie's information criterion (AIC) minimization procedure (function *stepAIC* in MASS package) [39, 40] to determine if soil chemistry (total N, total C, PO_4, soil pH, and % BS) and elevation explains variation in fungal diversity estimates. C/N and ECEC were multicollinear across multiple variables and were thus excluded from these analyses. Further, study location SF had only 1 viable set of technical replicates extracted from the 8 sample collection locations; therefore, data from this location were not included in statistical analyses, but were included in study location characteristics and beta diversity for comparison purposes. Simpson's diversity was arc-sin transformed prior to multiple regression analyses.

Bray-Curtis dissimilarity matrices were calculated from fungal OTU abundances and used in non-metric dimensional scaling (NMDS) ordinations (function *metaMDS* in vegan package) [41] to visualize fungal community compositional differences. We set the number of NMDS dimensions to 3 dimensions and maximum number of random starts to 20. This configuration provided an acceptable stress value of 0.16. Bray-Curtis dissimilarity was used as input for a permutational multivariate ANOVA model (perMANOVA, 999 permutations, function *adonis* in vegan package) [41, 42] to determine how much variation in community composition was represented by study location. We then conducted further pairwise comparisons to determine which locations differed in fungal community composition (function *pairwise.perm.manova* in RVAideMemoire package) [43]. Environmental vectors were also fit with the NMDS ordination (function *envfit* in vegan) [41] to determine which variables (elevation, total N, total C, soil pH, PO_4, % BS) were most strongly correlated with fungal community composition across locations. We provide only those environmental vectors which significantly correlated with community composition ($p < 0.05$).

We classified each OTU into an ecological guild using FUNGuild [44], to determine if specific functional groups of fungi differed among study locations and across the measured environmental gradients. Only those OTUs that were identified with a confidence level designated as "highly probable" or "probable" [44] were included in this analysis. OTUs which remained unclassified or were classified to a guild as "possible" were considered "unclassified" and were excluded from further analyses. In addition, OTUs which were placed in more than 1 guild, with confidence, were placed in the "> 1 guild" category (e.g., *Cladophialophora* spp. could be an animal pathogen or an undefined saprotroph). OTUs which were confidently classified were further annotated individually as some genera were either incorrectly classified into a guild (e.g., *Pyrenochaeta* as a saptrotroph) or belong in multiple guilds but originally classified into one guild (e.g., *Ceratobasidium* can be a saprotroph or pathogen). Undefined pathogens refer to pathogens not specific to fungi, animals, or plants whereas undefined saprotrophs refer to saprotrophs not specific to wood or litter-soil. Further, we exclude endophyte guilds as these are not relevant for understanding how soil fungi shifted in this study. The relative abundance of each guild was calculated across samples, and differences among locations were determined using non-parametric Kruskal-Wallis rank sum tests. Further, stepwise regression models, with an AIC minimization procedure, were used to determine if variation in functional guild abundances were explained by any of the measured soil chemical variables and elevation. Lastly, the most abundant genera confidently classified to an ecological guild (the 18 most abundant genera comprising 50% of sequences) were also analyzed for study location differences (Kruskal-Wallis rank sum tests) and variation explained by soil chemical variables and elevation (stepwise regression models). All statistical analyses were carried out in R (version 3.1.1) [45] using the type 1 error rate of $\alpha = 0.05$ after post hoc statistical corrections when appropriate.

Results

Soil Chemical Variables Differ Across Elevation

All soil chemical variables differed among study locations (Table 2), across elevation (Fig. 1), and most chemical variables significantly correlated with each other (Supplementary Table 2). For example, total N (within location means: 1.4–7.1 g kg soil^{-1}) concentrations varied among locations ($\chi^2 = 43.21$, $p < 0.001$); NH at CWT (highest elevation at CWT) had significantly higher N than other, lower elevation locations (Table 2). Both total N and total C concentrations increased with elevation and plateaued at ~1400 m elevation at the NH study location (Adj. $R^2 = 0.57$, $p < 0.001$; Fig. 1). PO_4 increased in concentration until ~1400 m in elevation, then declined (Adj. $R^2 = 0.30$, $p < 0.001$). Soil pH showed a similar trend (Adj. $R^2 = 0.51$, $p < 0.001$; Fig. 1). Percent BS increased until ~1400 m in elevation, then declined (Adj. $R^2 = 0.35$, $p < 0.001$). ECEC linearly increased with increasing elevation (Adj. $R^2 = 0.77$, $p < 0.001$; Fig. 1), whereas soil C/N decreased with increasing elevation (Adj. $R^2 = 0.23$, $p < 0.001$; Fig. 1).

Table 2 Summary statistics (mean ± 1 standard deviation) of fungal diversity estimates and chemical variables measured across all CWT and GRSM locations. Letters denote Dunn's post hoc corrections for multiple comparisons of mean ranks (each chemical variable = response variable, location = explanatory variable) after Kruskal-Wallis rank sum tests were deemed statistically significant ($p \leq 0.002$). Any response variable which differed among study location is italicized (note that only chemical variables differed among locations). SF had one sample for fungal communities so is not included in this analysis, but means are reported for this location

Response variable	OP	CH	LO	HO	NH	HNH	NHS	SF
OTU richness	280 (26.8)	315 (34.4)	333 (31.1)	329 (26.9)	344 (23.6)	302 (32.4)	323 (55.0)	252
Chao1	471 (55.1)	516 (55.2)	538 (56.5)	557 (79.0)	544 (62.2)	503 (61.1)	503 (87.7)	562
Simpsons diversity	0.88 (0.07)	0.88 (0.13)	0.93 (0.04)	0.89 (0.09)	0.94 (0.04)	0.93 (0.03)	0.89 (0.06)	0.35
Simpsons evenness	0.04 (0.02)	0.04 (0.02)	0.06 (0.03)	0.05 (0.03)	0.07 (0.03)	0.05 (0.01)	0.04 (0.03)	0.006
total N (g kg soil^{-1})	1.4 (0.4)^{A}	2.7 (1.2)^{AC}	2.2 (0.5)^{AC}	2.3 (0.4)^{AC}	7.1 (1.2)^{B}	4.9 (1.3)^{BC}	4.5 (1.1)^{BC}	4.9
total C (g kg soil^{-1})	36.5 (7.7)^{A}	49.6 (17.4)^{AC}	45.9 (12.3)^{AC}	55.2 (13.2)^{ABC}	106.5 (22.7)^{B}	90.2 (26.5)^{BC}	81.8 (20.7)^{BC}	78.8
C:N	26.3 (4.9)^{A}	19.0 (2.6)^{ABC}	20.6 (2.1)^{AC}	24.0 (1.9)^{AC}	15.1 (2.1)^{BC}	18.4 (1.8)^{ABC}	18.1 (0.9)^{C}	16
PO_4 (mg kg soil^{-1})	4.3 (1.5)^{A}	4.3 (0.8)^{A}	4.7 (1.1)^{A}	5.7 (1.2)^{AB}	7.6 (1.4)^{B}	7.1 (3.6)^{AB}	4.7 (1.6)^{AB}	7.5
Soil pH	4.1 (0.2)^{AB}	4.2 (0.1)^{A}	4.0 (0.2)^{AB}	3.9 (0.2)^{AB}	4.0 (0.3)^{AB}	4.1 (0.1)^{B}	3.3 (0.2)^{A}	3.2
ECEC (meq 100 g soil^{-1})	3.8 (0.7)^{A}	5.4 (0.9)^{AB}	5.3 (0.9)^{AB}	5.7 (0.7)^{ABC}	9.4 (1.4)^{B}	11.3 (2.9)^{BC}	13.7 (4.1)^{BC}	12.2
% BS	19.2 (14.4)^{AB}	29.2 (17.2)^{A}	21.7 (8.8)^{A}	16.9 (3.8)^{AB}	28.4 (13.8)^{A}	20.2 (11.8)^{AB}	7.1 (3.2)^{B}	10.2

Fungal Diversity and Community Composition

Good's coverage was high on average (mean = 0.97) indicating that fungal communities were adequately sampled. The observed OTU richness was greater at NH (mean ± standard deviation: 344 ± 23.6), the highest elevation location at CWT, compared to OP (280 ± 26.8) at CWT, the lowest elevation location ($\chi^2 = 13.10$, $p = 0.04$) and increased with total N (Adj. $R^2 = 0.12$, $p = 0.02$). However, no other edaphic variable predicted OTU richness. Fungal diversity and evenness did not differ among sites ($\chi^2 \leq 9.31$, $p \geq 0.16$) or across soil chemical variables and elevation ($p \geq 0.09$).

Soil fungal communities were dominated by Basidiomycota with 189,043 sequences (66.1%), Ascomycota with 56,587 sequences (19.8%), and basal fungi (former Zygomycota) with 29,566 sequences (10.3%). Other phyla were infrequent (Glomeromycota—248 sequences, 0.09%; Chytridiomycota—143 sequences, 0.05%). The most abundant families were Russulaceae (12.6%), Mortierellaceae (10.0%), Herpotrichiellaceae (9.3%), Atheliaceae (6.1%), and Hygrophoraceae (5.2%). The most abundant genera were *Mortierella* (9.8%) and the ectomycorrhizal mushroom *Lactarius* (7.6%). Fungal communities differed compositionally among locations—30% of the observed variation in community composition was explained by this effect (perMANOVA: $R^2 = 0.30$, $p = 0.001$). Specifically, the three highest elevation sites—NH, HNH, and NHS—differed compositionally at the OTU level from other sites and each other at both CWT and GRSM ($p \leq 0.02$; Supplementary Table 3). Correlation between elevation and OTU community composition was greater than any measured soil chemistry variable ($R^2 = 0.67$, $p = 0.001$). Total N ($R^2 = 0.58$) and pH ($R^2 = 0.58$) had greater correlation coefficients with community composition ($p = 0.001$) than other chemical variables. Total C ($R^2 = 0.43$, $p = 0.001$) and % BS ($R^2 = 0.24$, $p = 0.004$) were also significantly correlated with community composition (Fig. 2), whereas extractable PO_4 was not ($R^2 = 0.10$, $p = 0.08$). Total N and C vectors indicated that higher elevation fungal communities had greater total N whereas pH and % BS vectors indicated that mid to low elevation communities had greater pH and base saturation (Fig. 2).

The abundance of three functional guilds (ectomycorrhizae, plant pathogens, and undefined saprotrophs) differed among study locations (Table 3). Ectomycorrhizae were similar across locations, except the second lowest elevation, CH, was lower than all other locations ($\chi^2 = 18.58$, $p = 0.005$; Table 3). Both undefined saprotrophs and plant pathogens had greater abundance either at NH at CWT (plant pathogens: $\chi^2 = 16.59$, $p = 0.01$) or HNH at GRSM (saprotrophs: $\chi^2 = 22.89$, $p < 0.001$) compared to at least one low elevation location (Table 3). There were several significant correlations between fungal guild abundances and soil chemical variables. Animal, plant, and undefined pathogens, and wood saprotrophs increased with total N ($p \leq 0.02$). Mycoparasites ($p < 0.001$) and arbuscular mycorrhizae ($p = 0.01$) increased with total C, whereas plant pathogens ($p < 0.001$) and wood saprotrophs ($p < 0.001$) decreased with total C. Ectomycorrhizae ($p = 0.03$) increased with PO_4 ($p = 0.03$), whereas arbuscular mycorrhizae ($p = 0.04$), animal pathogens ($p = 0.04$), and undefined saprotrophs ($p = 0.05$) decreased with PO_4. Ectomycorrhizae ($p = 0.01$), orchid mycorrhizae ($p = 0.003$), and wood saprotrophs ($p = 0.004$) decreased with soil pH, whereas undefined saprotrophs ($p < 0.001$) and arbuscular mycorrhizae ($p = 0.02$) increased with soil pH. Notably, undefined saprotroph abundance increased with elevation ($p < 0.001$) whereas other guilds did not (Supplementary

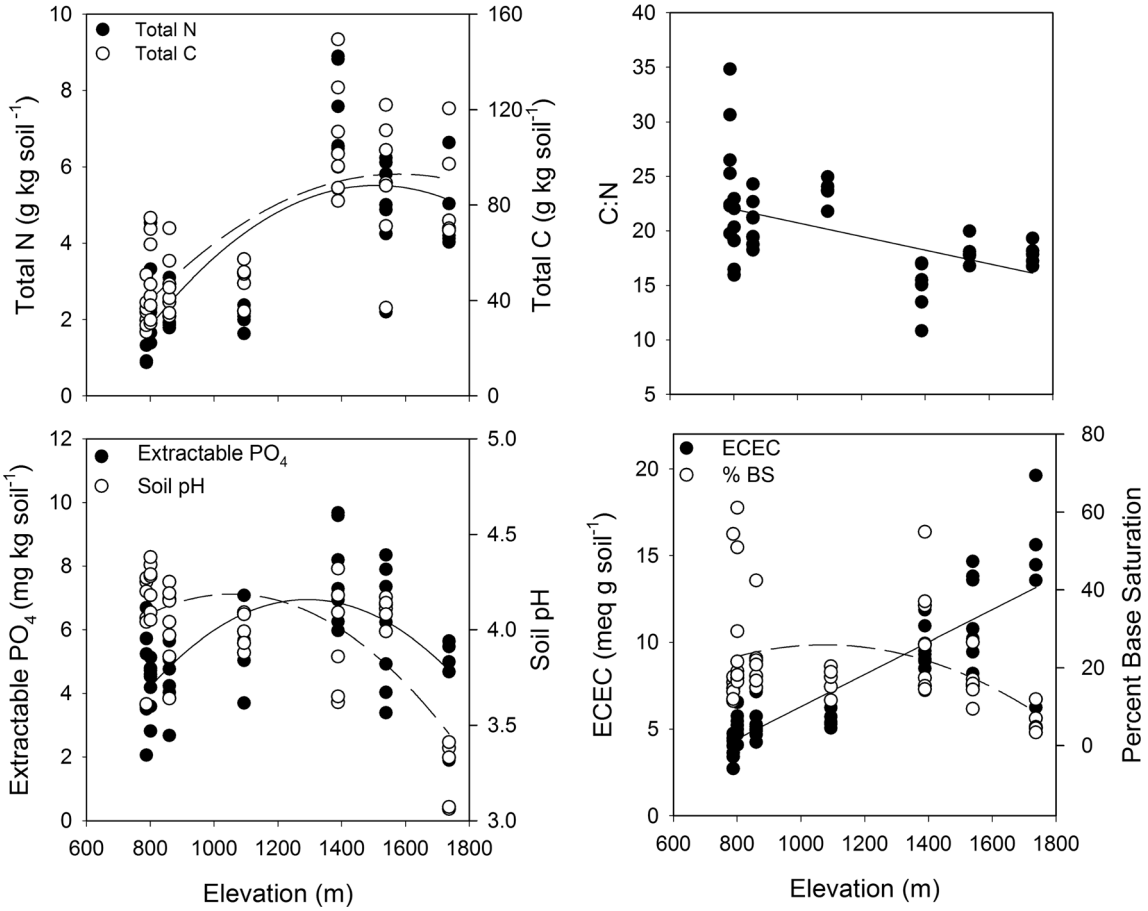

Fig. 1 All soil chemical variables differed across elevation ($p \leq 0.001$). The carbon to nitrogen ratio (C/N) decreased and effective cation exchange capacity (ECEC) linearly increased with elevation, whereas all nutrients, pH, and percent base saturation exhibited significant curvature in data distribution; therefore, quadratic regressions provided the best fit. Closed circles correspond with bolded regression lines and open circles correspond with dashed regression lines in each panel

Table 4). Ericoid mycorrhizae and litter-soil saprotrophs (Table 3) did not differ across elevation or any chemical variable.

Several abundant genera (which combined made up 50% of all sequences) confidently classified to a functional guild differed in relative abundance among study locations (Table 4) and varied with chemical variables or elevation (Supplementary Table 5). Among these, the ectomycorrhizal *Amanita* ($p < 0.001$) and saprotrophic *Mortierella* ($p < 0.001$) abundance increased with elevation. The ectomycorrhizal *Lactifluus* ($p = 0.003$) and *Tomentella* ($p = 0.001$) decreased with elevation. The mycorrhizal *Clavulina* ($p = 0.01$), *Thelephora* ($p < 0.001$), and mycoparasite *Mycena* ($p = 0.001$) decreased with soil pH, whereas *Cryptococcus* ($p < 0.001$), saprotrophic *Geminibasidium* ($p < 0.001$), *Mortierella* ($p = 0.02$), and mycorrhizal *Russula* ($p = 0.002$) increased with soil pH. In addition, *Cryptococcus* ($p < 0.001$), *Inocybe* ($p = 0.03$), and *Tomentella* ($p = 0.006$) increased with total N, whereas *Amanita* ($p < 0.001$) decreased with total N. Lastly, *Russula* increased with total C ($p = 0.001$) and *Hygrocybe* increased with % BS ($p = 0.03$).

Discussion

In this study, higher elevation locations in the southern Appalachian Mountains were greater in soil N and C whereas lower elevation locations were greater in soil pH and base saturation—thus soil chemistry varied spatially. Furthermore, N increased at a greater rate compared to C along elevation as demonstrated by reductions in soil C/N with increasing elevation. Total nitrogen was the only measured predictor of fungal richness and the soil chemical variable with strongest correlation with fungal community composition, although other chemical factors (e.g., total C, soil pH) were also important correlates (Fig. 2). Fungal saprotrophs, composed of primarily *Mortierella* abundance, increased with elevation whereas other guilds, such as ectomycorrhizae and plant pathogens, did vary among locations although no clear patterns along elevation were discernible. These results suggest that although soil chemical variables likely partly explain some variability in fungal community structure, other spatial effects drive community dynamics in this ecosystem. Pinpointing

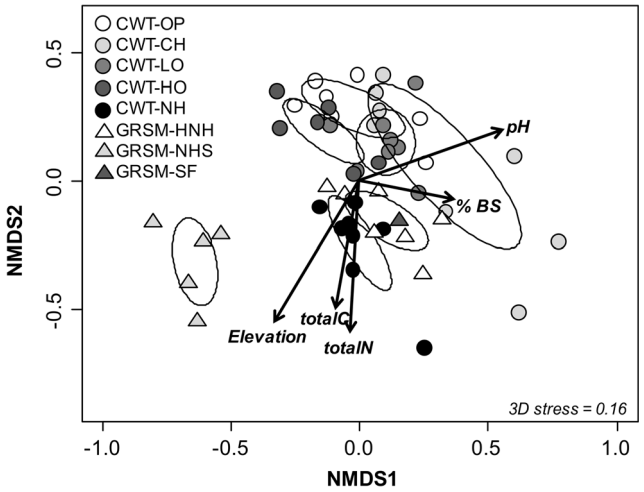

Fig. 2 Non-metric multidimensional scaling (NMDS) ordination of fungal communities across CWT (circle symbols) and GRSM (triangle symbols) locations. Based on perMANOVA, fungal communities differ among the locations ($R^2 = 0.30$, $p = 0.001$). Elevation, total N, total C, pH, and percent base saturation (% BS) significantly correlated with composition ($p \leq 0.001$) as shown by fitted vectors. Study location SF was included in NMDS ordination for comparison purposes, but not included in perMANOVA and environmental vector fitting analyses. 95% confidence interval centroid ellipses are given per study location

specific mechanisms for fungal community turnover across this gradient is not plausible—further experimentation will reveal the importance of local (abiotic and biotic) and regional-scale factors that influence fungal distributions in highly-diverse temperate forests, such as the southern Appalachian Mountains.

Fungal Diversity and Community Composition

A large proportion of variation in fungal community composition was explained by location ($R^2 = 0.30$, Fig. 1). These effects were largely due to the highest elevation locations—NH at CWT, HNH and NHS at GRSM—differing in community composition from other, low elevation locations (Fig. 1, Supplementary Table 3). In addition, functional guilds such as plant pathogens, saprotrophs, and ectomycorrhizae exhibited turnover either among locations and/or across elevation (Table 3). Understanding elevation effects on diversity and community turnover is informative for understanding how future environmental scenarios (e.g., N deposition, vegetation) will select for either functional changes within fungal communities (turnover from an ECM-dominating community to a pathogenic one) or less biodiverse fungal assemblages. Global distribution analyses have indicated that overall fungal diversity is impacted by climatic factors primarily and chemical and spatial effects secondarily [46]. However, other studies suggest that spatial effects are as important as abiotic conditions, at least for AM fungi [47]. Regardless of specific

Table 3 Ecological guilds relative abundance across study locations. Based on Kruskal-Wallis rank sum tests, guilds which differed among study locations are italicized. Plant pathogens, undefined saprotrophs, and ectomycorrhizae guilds differed among locations ($p \leq 0.05$). Letters denote Dunn's post hoc correction for multiple comparisons of mean ranks after Kruskal-Wallis rank sum tests were deemed statistically significant

	Mean (SD) relative abundance							
Functional guild	OP	CH	LO	HO	NH	HNH	NHS	SF[a]
Animal pathogen	0.10 (0.11)	0.11 (0.13)	0.17 (0.15)	0.06 (0.04)	0.27 (0.19)	0.44 (0.80)	0.17 (0.22)	0.0
Plant pathogen	0.31 (0.18)[a]	0.75 (0.52)[ab]	0.37 (0.16)[ab]	0.34 (0.13)[ab]	1.23 (0.77)[b]	0.48 (0.32)[ab]	0.51 (0.52)[ab]	0.5
Undefined pathogen	0.003 (0.01)	0.002 (0.01)	0.007 (0.01)	0.01 (0.01)	0.03 (0.04)	0.01 (0.01)	0.02 (0.03)	0.0
Mycoparasite	0.04 (0.05)	0.08 (0.10)	0.09 (0.05)	0.08 (0.08)	0.61 (0.62)	0.27 (0.26)	0.12 (0.09)	0.0
Foliar epiphyte	0.0	0.02 (0.04)	0.01 (0.02)	0.03 (0.05)	0.0	0.05 (0.01)	0.01 (0.02)	0.0
Undefined endophyte	0.03 (0.03)	0.16 (0.39)	0.45 (1.02)	0.09 (0.15)	0.04 (0.04)	0.17 (0.21)	0.19 (0.08)	0.0
Litter—soil saprotroph	0.003 (0.01)	0.005 (0.01)	0.005 (0.01)	0.0	0.0	0.0	0.0	0.0
Wood saprotroph	0.32 (0.16)	0.36 (0.10)	0.81 (0.44)	1.0 (1.51)	0.78 (0.40)	0.42 (0.26)	4.88 (8.71)	0.2
Undefined saprotroph	9.59 (3.30)[a]	17.04 (9.25)[ab]	14.31 (5.93)[ab]	8.90 (3.26)[a]	14.54 (3.36)[ab]	29.81 (8.25)[b]	16.43 (12.14)[ab]	2.4
Ectomycorrhizal	39.19 (7.98)[a]	18.63 (6.89)[b]	24.61 (10.34)[ab]	23.45 (17.32)[ab]	37.46 (13.54)[ab]	25.22 (6.92)[ab]	41.22 (21.73)[ab]	5.7
Arbuscular mycorrhizal	0.04 (0.06)	0.13 (0.15)	0.05 (0.07)	0.05 (0.05)	0.03 (0.04)	0.22 (0.21)	0.08 (0.17)	0.1
Ericoid mycorrhizal	0.0	0.002 (0.01)	0.0	0.0	0.0	0.0	0.0	0.0
Orchid mycorrhizal	0.0	0.0	0.03 (0.05)	0.03 (0.07)	0.01 (0.02)	0.01 (0.03)	0.06 (0.07)	0.0
> 1 guild designation[b]	3.25 (1.15)	23.69 (20.13)	4.77 (2.72)	7.97 (6.46)	7.90 (4.19)	11.66 (2.93)	3.84 (2.54)	82.2
Low confidence guilds	1.75 (1.31)	3.94 (3.75)	4.27 (5.69)	2.50 (1.44)	3.96 (4.94)	3.84 (3.53)	7.94 (10.73)	0.9
Unclassified	45.38 (7.16)	35.07 (13.52)	50.05 (9.64)	55.48 (13.34)	33.15 (9.56)	27.46 (7.57)	24.55 (13.92)	7.9

[a] One replicate was recovered for fungal community composition therefore, this sample data is reported with no standard deviation

[b] OTUs which were classified into greater than 1 guild had abundances summed and means calculated per location

mechanisms for fungal community assembly and selection of hyper-diverse assemblages among local patches, the multitude of both spatial and chemical predictors in explaining community composition and functional guild abundances suggest that habitat filtering is at least partially driving soil fungal community turnover in this ecosystem.

Although fungal diversity estimates did not differ among study locations, fungal richness increased with increasing soil total N concentrations and total N was most correlated with community composition, second only to elevation. These results contrast with others that indicate that fungal diversity declines with nitrogen fertilizer application [9, 48] or nitrogen deposition [49] due to the loss of diverse functional fungal groups, such as ectomycorrhizae [50]. In this study, we did see a decline in ectomycorrhizae with total N confirming previous findings [50] and an increase in pathogens (animal, plant, and undefined) and wood saprotrophs with total N. Two mutually non-exclusive reasons may be responsible for these results. Ecosystem N availability may change competitive pressure between fungal groups, allowing a greater number of copiotrophic fungi and increased species richness [48] while total N promotes fungi with pathogenic traits [51]. Although we cannot necessarily discern the life history strategies among fungi in this study, such as *r*-selected traits (i.e., copiotrophs), the prevalence of multiple groups of pathogens with increasing total N is in agreement with previous work that concludes selection of pathogens under high N scenarios [51]. These data indicate that future increases in N deposition [52] may select for a greater load of fungal pathogens potentially impacting other animal or plant biota. However, it is noteworthy that no pathogenic group was abundant and ranged between 0.003% abundance (undefined pathogens) to 1.23% (plant pathogens) whereas more common soil groups such as ectomycorrhizae (18–41%) and undefined saprotrophs (9–29%; Table 3) were most abundant. High throughput sequencing may not provide an accurate representation of all possible fungal groups as sequence data does not mirror organismal abundances [53]; thus, these data require further validation and experimentation to confirm such conclusions.

Several variables we examined co-vary with elevation, including climatic variables and vegetation turnover not included in these analyses. For example, sites with greater total soil N concentrations also have higher rates of long-term N deposition, lower mean annual temperatures, and greater annual precipitation [16, 23]. Other studies indicate that climatic factors best predict fungal richness globally followed by chemical variables [46]. Therefore, the positive relationship between OTU richness and total soil N may be attributable to regional variation in climate or environmental perturbation, such as long-term atmospheric N deposition, or increased soil water availability due to greater precipitation [54]. Plant communities may also play a role in controlling microbial community structure as a result of belowground symbioses (e.g., mycorrhizal abundances), or selection of specific fungal functional groups [55, 56]. Plant species may select for specific fungal taxa in rhizosphere soils [57]. Many locations across the elevation gradient shared dominant plant species, such as the Chestnut Oak (*Quercus prinus*), whereas the NHS site was spruce-dominated (Red spruce; *Picea rubens*) mixed with American beech (*Fagus grandifolia*) but lacked oak species. Changes in litter chemistry or plant community turnover may strongly influence overall fungal community composition. For example, Uroz et al. [58] demonstrated that soil fungal community composition differentiates more so between tree species stands (Norway spruce and European beech stands) than between soil types (rhizosphere versus bulk soils) due to differences in litter chemistry or other plant-mediated edaphic conditions. Given the complexity in environmental covariates (temperature, precipitation, nutrients) across elevation [59], we cannot conclude specific mechanisms to the relative contribution of spatial and chemical effects on fungal diversity and community composition.

Spatial and Environmental Variation Associated with Functional Guild Abundance

Ectomycorrhizal fungus abundance varied among study locations, but did not differ in abundance across elevation (Table 3, Supplementary Table 3). Yet, different genera dominated along the elevational gradient: *Clavulina*, *Inocybe*, and *Thelephora* were more abundant at high elevation areas, whereas *Lactifluus* and *Tomentella* were more abundant at lower elevations and declined overall with increasing elevation (Table 4). Ectomycorrhizal groups can differ in host specificity and they can respond differentially to soil conditions [54, 60] which may contribute to the lack of elevational differences in ectomycorrhizal abundance. The dominant ectomycorrhizal family in this study was the Russulaceae, which includes *Lactarius* and *Lactifluus* (known as milkcaps) [61], and *Russula* (Table 4). *Russula* species have been abundant in previous studies in this ecosystem, whereas *Lactarius* species have not been detected at spruce-fir NHS or SF locations previously [54, 60–64]. In similar northern hardwood forested ecosystems, *Clavulina*, *Lactifluus*, and *Russula* species are common [63, 65, 66], but other commonly observed genera, such as *Laccaria*, were not detected in this study. Further, the ectomycorrhizal fungus *Piloderma* has been reported in previous studies on leaf litter surface in hardwood forest ecosystems in CWT and GRSM [63, 65]. It was rarely observed here, possibly because of our choice of omitting leaf litter material during soil sample collection. Other common ectomycorrhizal fungi were members of genus *Tomentella* which are observed in forest habitats primarily as resupinate basidiomata on wood appearing to be saprotrophs,

Table 4 The 18 most abundant fungal genera across the study confidently designated with a guild. Relative abundance represents means across all samples. Genera which differed in abundance across study locations are italicized. Letters denote Dunn's post hoc correction for multiple comparisons of mean ranks after Kruskal-Wallis rank sum tests were deemed statistically significant. SF had one sample for fungal communities; therefore, only that sample data is given and no mean or standard deviations are included

				Mean (SD) relative abundance							
Genus	Functional guild	Relative abundance	Number of OTUs	OP	CH	LO	HO	NH	HNH	NHS	SF
Amanita	Ectomycorrhizal	2.36	26	1.32 (0.99)	1.83 (2.87)	0.57 (0.36)	1.16 (2.03)	0.68 (0.59)	4.67 (8.59)	7.58 (13.32)	0.33
Cladophialophora	Animal pathogen, Undefined saprotroph	0.59	45	0.56 (0.46)	0.96 (0.66)	0.53 (0.34)	0.37 (0.23)	0.33 (0.14)	0.67 (0.65)	0.80 (0.92)	0.13
Clavulina	Ectomycorrhizal	1.68	4	0.18 (0.03)^{a}	1.25 (2.92)^{ab}	1.23 (2.58)^{ab}	0.46 (0.21)^{bc}	0.68 (1.02)^{ab}	0.21 (0.09)^{ac}	9.56 (11.62)^{b}	0.37
Clavulinopsis	Undefined saprotroph	0.76	10	0.22 (0.27)	3.37 (4.57)	0.33 (0.34)	0.21 (0.16)	0.11 (0.06)	0.71 (0.75)	0.12 (0.04)	0.11
Cortinarius	Ectomycorrhizal	2.27	71	3.43 (7.34)	1.81 (2.73)	3.87 (3.90)	3.45 (5.09)	0.98 (0.64)	2.09 (2.59)	0.29 (0.09)	0.26
Cryptococcus	Undefined Saroptroph	3.71	25	1.67 (0.75)^{ac}	6.44 (3.39)^{ab}	2.06 (1.23)^{ac}	3.53 (3.02)^{abc}	5.38 (3.21)^{ab}	7.38 (3.47)^{b}	0.85 (0.32)^{c}	1.07
Elaphomyces	Ectomycorrhizal	1.04	8	0.35 (0.56)	1.10 (2.19)	0.82 (1.47)	0.78 (1.56)	2.10 (2.29)	0.62 (0.88)	1.62 (3.39)	0.09
Geminibasidium	Undefined saprotroph	0.68	1	0.42 (0.38)^{ab}	0.97 (1.41)^{ab}	0.98 (0.78)^{a}	0.98 (0.76)^{a}	0.49 (0.35)^{ab}	0.75 (0.34)^{a}	0.10 (0.03)^{b}	0.15
Geoglossum	Undefined saprotroph	0.68	6	0.10 (0.05)	0.32 (0.66)	2.02 (5.50)	0.074 (0.04)	0.30 (0.61)	1.59 (2.76)	0.12 (0.10)	0.04
Hygrocybe	Undefined saprotroph, Symbiotroph	4.68	13	0.43 (0.11)^{a}	15.41 (22.24)^{b}	1.18 (1.68)^{ab}	0.62 (0.19)^{ab}	0.79 (0.79)^{ab}	2.19 (2.95)^{ab}	0.57 (0.23)^{ab}	80.94
Inocybe	Ectomycorrhizal	3.22	37	0.44 (0.35)^{a}	0.91 (1.18)^{ab}	1.89 (3.95)^{ab}	0.41 (0.10)^{a}	10.32 (12.43)^{b}	2.92 (5.06)^{ab}	5.94 (10.32)^{ab}	0.52
Lactarius	Ectomycorrhizal	6.80	25	17.88 (17.51)	4.24 (4.04)	2.70 (2.19)	9.61 (17.34)	8.04 (3.36)	3.88 (5.14)	2.63 (1.33)	1.07
Lactifluus	Ectomycorrhizal	0.90	7	2.68 (3.04)^{a}	1.44 (2.85)^{ab}	0.23 (0.28)^{ab}	1.43 (2.43)^{a}	0.45 (0.29)^{a}	0.11 (0.07)^{b}	0.14 (0.05)^{ab}	0.26
Mortierella	Undefined saprotroph	9.78	75	6.15 (2.95)^{a}	8.43 (6.12)^{a}	7.26 (2.72)^{a}	4.96 (1.57)^{a}	9.70 (2.70)^{ab}	22.11 (8.34)^{b}	9.83 (7.61)^{ab}	1.72
Mycena	Mycoparasite	0.95	31	0.26 (0.22)^{a}	0.47 (0.77)^{a}	1.32 (1.51)^{ab}	1.06 (1.19)^{ab}	0.37 (0.25)^{ab}	0.88 (1.15)^{ab}	2.79 (2.51)^{b}	0.06
Russula	Ectomycorrhizal	4.35	59	4.67 (4.27)	1.85 (1.38)	3.82 (2.86)	1.76 (1.37)	6.58 (3.53)	8.17 (6.16)	3.42 (6.98)	1.78
Thelephora	Ectomycorrhizal	0.88	2	0.02 (0.01)^{a}	0.03 (0.03)^{a}	0.04 (0.03)^{ab}	0.03 (0.02)^{a}	0.18 (0.16)^{ab}	0.09 (0.23)^{a}	7.24 (10.14)^{b}	0.02
Tomentella	Ectomycorrhizal	2.90	64	3.84 (5.60)^{ab}	2.51 (2.08)^{ab}	6.19 (7.81)^{a}	1.56 (0.82)^{ab}	3.91 (1.40)^{a}	1.50 (0.68)^{ab}	0.42 (0.12)^{b}	0.48

but members of the genus have also been reported as root symbionts at the soil surface and on roots embedded in decaying wood [67]. The lower abundance of *Tomentella* in the spruce-fir zone may be due to its nutritional requirements or lack of available arthropod and other animal vectors that play an important role in the dispersal of basidiospores [68].

Saprotrophs were the second most abundant guild which increased in abundance with increasing elevation (Table 3). This group had significantly greater abundance at HNH compared to OP (lowest elevation) and HO at CWT (Table 3), a result primarily driven by changes in *Mortierella* abundance (Table 4). *Mortierella* species have been reported to be saprobes on soil and roots in forest ecosystems [69, 70] or acting as plant root endophytes [71]. Although *Mortierella* abundance did not correlate with soil N or C concentrations (Supplementary Table 5), it may either be tracking changes in nutrient quality instead of quantity or partially be driven by vegetation turnover due to its potentially endophytic trophic lifestyle. However, its overall ecology within soils and association with plant roots requires further testing. Both *Cryptococcus*, a dominant genus in this study (3.7% relative abundance; Table 4), and *Mortierella* were absent from similar habitats in CWT that were studied using cloning and sequencing of soils from Eastern hemlock and rhododendron study areas in this ecosystem [62, 64]. The mushroom forming saprotrophic genus *Hygrocybe*, also dominant (4.7% relative abundance, Table 4), had the second highest abundance of saprophytic fungi and showed greatest abundance at low elevation. *Hygrocybe* species are more common in young forest stands in lower elevations [60] and dependent on soil C and N fruiting more abundantly when nutrients are low [72]. Despite this, *Hygrocybe* constituted 80.9% sequence abundance of all genera in the SF community ($N = 1$; Table 4) so they may be dominant, and potentially frequently dormant, soil fungi in high elevation sites, although not detected previously.

In conclusion, fungal community dynamics were influenced by both spatial and soil chemistry gradients in this ecosystem. Saprotrophic fungi increased in abundance with elevation whereas fungal richness and pathogen abundance increased with total N. Although the decay process can be slow within high elevation habitats due to low soil and litter pH, a cooler climate, and shorter growing season, greater abundances in saprotrophs may have consequences for decomposition in these locations. Over time, as N deposition and air temperature increase due to urbanization and climate change, spruce-fir sites will potentially see increases in soil pathogens. Spatial changes in fungal communities can serve as a baseline for understanding the underlying processes impacting fungal functional turnover and forest health. Our contribution establishes a foundation for future forest monitoring of critical taxa that may be impacted by anthropogenically driven environmental change within the southern Appalachian Mountains.

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References

1. Cannon PF, Sutton BC (2004) Microfungi on wood and plant debris. In: Foster MS, Bills GF, Mueller GM (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam, pp 217–239
2. Baird RE, Watson CE, Woolfolk S (2007) Microfungi from bark of healthy and damaged American beech, Frasier fir, and eastern hemlock trees during an all taxa biodiversity inventory in forests of the great Smoky Mountains National Park. Southeast. Nat. 6:67–82
3. Baird RE, Woolfolk S, Watson CE (2009) Microfungi of forest litter from healthy American beech, Fraser fir, and eastern hemlock stands in great Smoky Mountains National Park. Southeast. Nat. 8:609–630
4. Baird RE, Wallace L, Baker G, Scruggs M (2013) Stipitate hydnums of the temperate southeastern united state. Fungal Divers 62:41–114
5. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci U S A 109:6241–6246
6. Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjoller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, Kauserud H (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol. 199:288–299
7. Toljander JF, Eberhardt U, Toljander YK, Paul LR, Yalor AF (2006) Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytol. 170:873–883
8. Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40:2407–2415
9. Allison SD, Hanson CA, Treseder KK (2007) Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biol. Biochem. 39:1878–1887
10. Avis PG (2003) The effects of long-term nitrogen fertilization on the ectomycorrhizal communities of a temperate deciduous ecosystem. Dissertation, University of Minnesota
11. Trudell SA, Edmonds RL (2004) Macrofungus communities correlate with moisture and nitrogen abundance in two old-growth conifer forests, Olympic National Park, Washington, USA. Can. J. Bot. 82:781–883
12. Arnold EEF (1991) Decline of ectomycorrhizal fungi in Europe. Agric. Ecosyst. Environ. 35:209–244
13. Arnold AE, Lamit LJ, Gehring CA, Bidartondo MI, Callahan H (2010) Interwoven branches of the plant and fungal trees of life. New Phytol. 185:874–878

14. Driscoll CT, Whitall D, Aber J, Boyer E, Castro M, Cronan C, Goodale CL, Groffman P, Hopkinson C, Lambert K, Lawrence G, Ollinger S (2003) Nitrogen pollution in the northeastern United States: sources, effects, and management options. Bioscience 53: 357–374
15. Baumgardner R, Lavery TF, Rogers CM, Isil SS (2002) Estimates of the atmospheric deposition of sulfur and nitrogen species: clean air status and trends network, 1990-2000. Environ Sci Technol 36: 2614–2629
16. Knoepp JD, Vose JM, Swank WT (2008) Nitrogen deposition and cycling across an elevation and vegetation gradients in southern Appalachian forests. Int. J. Environ. Stud. 65:391–410
17. IPCC (2014) Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Core Writing Team, Pachauri RK, Meyer LA (eds). IPCC, Geneva. IPCC ARS Synthesis Report website: http://ar5-syr.ipcc.ch
18. Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7:737–750
19. Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in temperate forest ecosystems. Bioscience 48: 921–934
20. Coker WC, Beers AH (1951) The stipitate hydnums of the eastern United States. Chapel Hill
21. Newton AC, Holden E, Davy LM, Ward SD, Fleming LV, Watling R (2002) Status and distribution of stipitate hydnoid fungi in Scottish coniferous forests. Biol. Conserv. 107:181–192
22. Knoepp JD, Swank WT (1998) Rates of nitrogen mineralization across an elevation and vegetation gradients in the southern Appalachians. Plant Soil 204:235–241
23. Knoepp JD, Coleman DD, Crossley DA, Clark JS (2000) Biological indices of soil quality: an ecosystem case study of their use. For Ecol Manag 138:357–368
24. Elliott KJ, Vose JM (2011) The contribution of Coweeta hydrologic laboratory to developing an understanding of long-term (1934-2008) changes in managed and unmanaged forests. For Ecol Manag 261:900–910
25. USEPA (1983) Methods for chemical analysis of water and waste. Determination of nitrogen as ammonia. Method 350.1, environmental monitoring and support laboratory, Office of Research and Development, USEPA, Cincinnati
26. Kuo S (1996) Phosphorus. In: Sparks DL (ed) Methods of soil analysis, part 3 – chemical methods, Madison, pp 869–920
27. Miniat CF, Brown CL, Harper C, Gregory S, Welch B (2014) Procedures for chemical analysis at the Coweeta hydrologic laboratory. Coweeta files. Coweeta Hydrologic Laboratory, Otto, p 206
28. Berry D, Mahfoudh KB, Wagner M, Loy A (2011) Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. Appl Environ Microbiol 77:7846–7849
29. Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–138
30. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
31. Huse SM, Dethlefsen L, Huber JA, Welch DM, Relman DA, Sogin ML (2008) Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. PLoS Genet 4:1–10
32. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200
33. Wang Q, Garrity GM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267
34. Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I, Bahram M, Bechem E, Chuyong G, Kõljalg U (2010) 454 pyrosequencing and sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. New Phytol 188:291–301
35. Brown SP, Veach AM, Rigdon-Huss AR, Grond K, Licktieg SK, Lothamer K, Oliver AK, Jumpponen A (2015) Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Fungal Ecol 13:221–225
36. Harrell F, Dupont C (2017) Hmisc: Harrell Miscellaneous. R package. http://biostat.mc.vanderbilt.edu/Hmisc
37. Pohlert T (2016) PMCMR: calculate pairwise multiple comparisons of mean rank sums. R package. https://CRAN.R-project.org/package=PMCMR
38. Chambers JM (1992) Linear models. In: Chambers JM, Hastie TJ (eds) Statistical models in S. Chapman & Hall/CRC, Boca Raton, pp 95–138
39. Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodal interference and model averaging in behavioural ecology using Akaike's information criterion. Behav Ecol Sociobiol 65:13–21
40. Venables WN, Ripley BD (2002) Modern applied statistics. Springer, New York
41. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2017) Package vegan. R package. https://CRAN.R-project.org/package=vegan
42. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46
43. Hervé M (2017) RVAideMemoire: diverse basic statistical and graphical functions. R package. https://CRAN.R-project.org/package=RVAideMemoire
44. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke L, Schilling JS, Kennedy PG (2015) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248
45. R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
46. Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Põldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Pärtel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson KH, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo L, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, Kesel AD, Dan T, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K (2014) Global diversity and geography of soil fungi. Science 346:1078
47. Kivlin SN, Hawkes CV, Treseder KK (2011) Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biol Biochem 43: 2294–2303
48. Weber CF, Vilgalys R, Kuske CR (2013) Changes in fungal community composition in response to elevated atmospheric CO_2 and nitrogen fertilization varies with soil horizon. Front Microbiol 4:1–14
49. Lilleskov EA, Fahey TJ, Horton TR, Lovett GM (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83:104–115

50. Teseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347–355
51. Paungfoo-Lonhienne C, Yeoh YK, Kasinadhuni NRP, Lonhienne TGA, Robinson N, Hugenholtz P, Ragan MA, Schmidt S (2015) Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Sci Rep 5:8678. https://doi.org/10.1038/srep08678
52. Li Y, Schichtel BA, Walker JT, Schwede DB, Chen X, Lehmann CN, Puchalski MA, Gay DA, Collett JL (2016) Increasing importance of deposition of reduced nitrogen in the United States. Proc Natl Acad Sci U S A 113:5474–5879
53. Kircher M, Kelso J (2010) High-throughput DNA sequencing—concepts and limitations. BioEssays 32:524–536
54. Walker JF, Miller Jr OK, Horton JL (2005) Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Mol Ecol 14:829–838
55. 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 3:3112–3124
56. Hui N, Jumpponen A, Francini G, Kotze DJ, Liu X, Romantschuk ML, Strömmer RH, Setälä HM (2017) Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environ Microbiol 19:1281–1295
57. Bezemer TM, Lawson CS, Hedlund K, Edwards AR, Brook AJ, Igual JM, Mortimer SR, Van der Putten WH (2006) Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. J Ecol 94:893–904
58. Uroz S, Oger P, Tisserand E, Cébron A, Turpault MP, Buée M, De Boer W, Leveau JHJ, Frey- Klett P (2016) Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. Sci Rep 6:27756. https://doi.org/10.1038/srep27756
59. Sundqvist MK, Sander NJ, Wardle DA (2013) Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. Annu Rev Ecol 44:261–280
60. Baird R, Stokes CE, Wood-Jones A, Alexander M, Watson C, Taylor G, Johnson K, Diehl S (2014) Fleshy ectomycorrhizal fungal community associated with healthy and declining eastern hemlock stands in great Smoky Mountains National Park. Southeast Nat 13:192–218
61. Verbeken A, Nuytinck J (2013) Not every milkcap is a *Lactarius*. Scripta Botan Belg 51:162–168
62. Baird R, Stokes CE, Wood-Jones A, Alexander M, Watson C, Taylor G, Johnson K, Threadgill P, Diehl S (2014) A molecular clone and culture inventory of the root fungal community associated with eastern hemlock in great Smoky Mountains national park. Southeast Nat 13:219–237
63. Baird R, Stokes CE, Frampton J, Smith B, Watson C, Pilgram C, Scruggs M (2014) Density and diversity of ectomycorrhizal fungal community present in high elevation Fraser fir of great Smoky Mountains National Park. North Am Fungi 9:1–21
64. Baird R, Wood-Jones CA, Varco J, Watson C, Starrett W, Taylor G, Johnson K (2014) Rhododendron decline in great Smoky Mountains and surrounding areas: intensive site study of biotic and abiotic parameters associated with the decline. Southeast Nat 13:1–25
65. Bills GF, Holtzman GI, Miller OK (1986) Comparison of ectomycorrhizal-basidiomycete communities in red spruce versus northern hardwood forests of West Virginia. Can J Bot 64:760–768
66. Bird C, McCleneghan C (2005) Morphological and functional diversity of EM fungi on Roan Mountain (NC/TN). Southeast Nat 4:121–132
67. Suvi T, Tedersoo L, Abarenkov K, Beaver K, Gerlach J, Kõljalg U (2010) Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. Mycologia 102:522–533
68. Lilleskov EA, Bruns TD (2005) Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia 97:762–769
69. Watanabe T (1994) Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC Press LLC, Boca Raton
70. Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi10th edn. Cromwell Press, Trowbridge
71. Leite DP, Amadio JVRS, Martins ER, Simões SAA, Yamamoto ACA, Leal-Santos FA, Takahara DT, Hahn RC (2012) *Cryptococcus spp* isolated from dust microhabitat in Brazilian libraries. J Ocul Med Toxicol 7:11
72. Reverchon F, Ortega-Larrocea MP, Pérez-Moreno J (2010) Saprophytic fungal communities change in diversity and species composition across a volcanic soil chronosequence at sierra del Chichinautzin, Mexico. Ann Microbiol 60:217–226