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Organic matter removal associated with forest harvest leads to decade scale alterations in soil fungal communities and functional guilds

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ABSTRACT

Intensive organic matter removal (OMR) associated with timber harvest has the potential to impart long-term alterations to soil biota and associated properties and processes; however, there is a lack of data to say if this trend persists at depth. This study investigated how OMR influences long-term stability of soil fungi to a depth of 1 m using a replicated experimental pine forest in the Gulf Coastal Plain, USA. Treatments included unharvested control stands as well as low- and high-OMR stands. Intensive OMR led to significant differences in community structure and the abundance of functional guilds in surficial soil. Saprophytic taxa increased while ectomycorrhizal (ECM) taxa decreased with intensive-OMR, which correlated strongly with increased surface temperature and reduced soil nitrogen. Ericoid mycorrhizae (ERM) also increased in intensive-OMR stands, which may indicate that following disturbance, ERM could outcompete ECM for colonization of subsequent seedlings. Overall, no differences were observed below 30 cm, except for alpha diversity, which was a function of high inter-replicate variability. Our results illustrate a distinct long-term structural and functional response of soil fungi to intensive-OMR in the upper portions of the soil profile, which could lead to altered stand productivity and ecosystem services.

1. Introduction

Over the past century, coniferous forests in the southern USA have been extensively managed for timber-related products, such as merchantable and industrial timber as well as pulpwood. This region represents roughly 31% of the total US forest area (Oswalt et al., 2014), approximately half of which is utilized for plantation forestry (Fox et al., 2007). In addition, there is growing interest in the removal of non-merchantable woody debris, including slash, sawdust, and forest litter, to be used as substitute feedstocks in industrial processes, for bioenergy production, and as merchantable mulch (Janowiak and Webster, 2010; Dickens et al. 2012). These intensive organic matter removal (OMR) techniques, such as whole-tree harvest + forest floor removal (WT + FF), result in forest soil disturbance that is of higher magnitude than commonly used harvest techniques such as bole-only harvest (BO). The removal of the forest floor can have profound effects on microclimate conditions such as surface temperature and water infiltration as well as soil biotic and abiotic properties. Specifically, intensive OMR-related disturbances have been shown to negatively affect concentrations of soil organic carbon (Johnson and Curtis, 2001; Nave et al., 2010; Achat et al., 2015a, 2015b), total and inorganic nitrogen (Thiffault et al., 2011; Zummo and Friedland, 2011; Kellman et al., 2014; Prest et al., 2014; Foote et al., 2015; Mushinski et al., 2017a, 2017b), and soil phosphorus (Mushinski et al., 2018). Additionally, intensive OMR can lead to reductions in soil microbial biomass (Busse et al., 2006; Holden and Treseder, 2013; Foote et al., 2015; Mushinski et al., 2017a), alterations in microbial community composition (Hartmann et al., 2009; Hartman et al., 2012), and decreased abundance of specific microbial functional groups such as lignocellulolytic taxa (Cardenas et al., 2015; Leung et al., 2016) as well as nitrifiers and denitrifiers (Mushinski et al., 2017b). Biogeochemical processes such as cellulolysis and nitrification have also been shown to be altered in stands subjected to intensive OMR (Mushinski et al., 2017b; Wilhelm et al., 2017a).

Fungi are a major component of the soil biota, and contribute to the carbon sink strength of forest soil (Clemmensen et al., 2013). Fungal community structure is fundamentally linked to vegetation composition (Wardle et al., 2004; van der Heijden et al., 2008) as well as abiotic and edaphic factors including elevation (Meng et al., 2013; Thébault et al., 2014), precipitation regimes (Sorensen et al., 2013), fires (Sun et al.,

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2011; Brown et al., 2013; Buscardo et al., 2015; Oliver et al., 2015), substrate composition (Bååth and Anderson, 2003; Zinger et al., 2009; Anderson et al., 2016; Martínez-Garcia et al., 2015; Mayor et al., 2015), and soil pH (Rousk et al., 2010; Wang et al., 2015). Previous studies have shown that soil disturbance associated with timber harvest can negatively influence overall fungal biomass (Holden and Treseder, 2013) and richness (Bader et al., 1995), which has been attributed to changes in soil organic matter (SOM) pool sizes as well as an alteration in plant host abundance. Specific fungal taxa, as well as functional fungal guilds, have variable responses to intensive OMR, which can vary across different forest ecosystems (Wilhelm et al., 2017b). Some have shown that the removal of forest floor material leads to no long-term observable change in some groups of saprophytic fungi (Allmér et al., 2009), while other studies have shown increased abundance of similar groups (Wilhelm et al., 2017a). Some studies have observed increases in ectomycorrhizal (ECM) dominance and diversity with increased disturbance (Twieg et al., 2007) while others show reductions in ECM diversity (Simard, 2009; Wilhelm et al., 2017b). It has also been observed that the conversion to a plantation system can lead to a virtual disappearance of ECM (McGuire et al., 2015). Furthermore, arbuscular mycorrhizal taxa (AM) have been shown to increase in intensive OMR sites, which has been attributed to increases in early successional symbionts following OMR (Wilhelm et al., 2017b).

As previously noted, there have been a multitude of studies investigating not only the effect of disturbance on soil fungi, but also the specific long-term effect of intensive OMR on fungi present in the forest litter and surface mineral soil. However, there has been only one other study that investigated how intensive OMR specifically affects fungal communities below 20 cm (i.e., Hartman et al., 2009), which did show significant OMR-induced fungal differences up to 30 cm in a Canadian coniferous forest. The lack of data on deeper soils is most likely due to multiple factors including sampling difficulty and the generalization that organic matter inputs, nutrient stores, and microbial activity are greatest in the uppermost portion of the soil profile (Fierer et al., 2003; Eilers et al., 2012; Stone, 2015). While this generalization is true, a large portion of forest soil organic matter (SOM) is stored below 30 cm with the majority of this SOM originating from deep tree roots where symbiotic taxa are likely to be observed (Jobbágy and Jackson, 2000; Hartmann et al., 2009; Schutz et al., 2010; Laclau et al., 2013). Mushinski et al. (2018) recently showed that the relative abundance of bacterial N-cycle genes were significantly altered at deeper depths (i.e., 60–100 cm) in response to intensive OMR. This not only illustrates the potential for intensive OMR to significantly affect functional microbial groups at deeper depths, but the potential changes in N-cycle processes could have profound effects on certain mycorrhizal groups that rely on oxidized forms of N to maintain successful symbiotic relations with plant roots.

In this study, we explored the decade-scale response of soil fungal community composition and functional characteristics to intensive OMR throughout the upper 1 m of the soil profile. We hypothesized that: (a) increasing forest OMR will lead to reduced fungal diversity, altered community structure, and alteration in the relative abundance of functional fungal guilds due to reductions in organic matter availability, and (b) changes in community metrics, structure, or the relative abundance of functional groups such as saprophytic and mycorrhizal fungi would be most evident in surficial soil due to higher abundance of substrate and roots at these depths.

2. Materials & methods

2.1. Study site and experimental design

Research was conducted in Davy Crockett National Forest near the town of Groveton, TX, USA (31° 06′ 32.48″ N, 95° 09′ 59.15″ W). The study site is part of the Long-Term Soil Productivity (LTSP) network aimed at understanding the effects of forest management on soil properties and processes (Powers et al., 2005; Powers, 2006). Soils across the study area are uniform (fine-loamy siliceous, thermic Oxyaquic Glossudalf in the Kurth series), and topography is relatively flat. The experimental design included unharvested control stands dominated by *Pinus taeda* (tree age = 60–80 yrs), and two harvest treatments differing in the extent of organic matter removal. Harvest treatments consisted of a low intensity treatment, bole-only (BO) harvest (40–70% of aboveground organic matter removed), and a high-intensity treatment, whole-tree harvest + forest floor removal (WT + FF) (100% of aboveground organic matter removed). During harvest, trees were hand-felled and lifted off of the plots with a loader to reduce soil compaction. Controls and both harvest treatments were replicated 3X and each replicate was 0.2 ha. All plots are located within a 1.5 km radius. Plots were harvested in 1996 and then replanted in 1997 with containerized *P. taeda* (loblolly pine) seedlings of 10-half sib families from the US Forest Service seed orchards using 2.5-m x 2.5-m spacing. At the time of this study, bole width and height of *P. taeda* as well as forest floor biomass and root biomass were all significantly lower in the WT + FF stands; however, total understory cover, which was dominated by *Ilex vomitoria* (yaupon holly), was statistically similar between treatments (Table S1).

2.2. Soil sampling and physiochemical properties

Field sampling took place in late April 2015. Vertical soil cores (0–100 cm) were extracted using a JMC Environmentalist's Sub-Soil Probe PLUS (Clements Associates Inc., Newton, IA, USA) (2.8 cm diameter x 120 cm length). For control and treatment plots, soil cores were taken at 1.8 m from the base of a randomly selected *P. taeda* individual with a diameter at breast height (DBH) between 18 and 24 cm. A 7.5 m buffer from the outside of the 0.2 ha plots was not sampled to avoid edge effects. In some of the WT + FF stands, the forest floor had not yet redeveloped; because of this, the organic soil horizon in all other plots (approximate thickness: < 3 cm) was removed prior to coring in order to investigate mineral soil horizons exclusively. Soil sampling followed a stratified random sampling design in which four cores were taken from each plot and homogeneously pooled by depth (i.e., 0–10, 10–30, 30–60, 60–100 cm) to increase sample mass and reduce error introduced by environmental heterogeneity. This resulted in 1 composited core per plot, separated into 4 depth increments, and replicated 3X per treatment. Soil depth increment selection was based on previous LTSP studies. On the day in which soil cores were taken from the ground, samples were transported at 4 °C from the field to the lab, aseptically homogenized by hand, and 5-g subsamples (3 per sample) were immediately stored at −80 °C for future DNA extraction and subsequent analysis. The remaining soil was stored at 4 °C for physicochemical analysis. Soil chemical properties have been previously reported (Mushinski et al., 2017a, 2017b, and 2018), and are summarized in Table S2.

2.3. DNA extraction, PCR amplification, DNA library construction, and sequencing

Because of the difficulty in extracting environmental DNA from soil with the potential for high humic co-extractant (He et al., 2005) as well as an overall low microbial biomass, DNA extraction followed the modified version of the International Standard for the extraction of DNA from soil as described by Terrat et al. (2014). Further modification was made to extract DNA from 3 g of soil rather than the prescribed 1 g. DNA was extracted from 3 analytical replicates per sample and then pooled to increase mass and reduce environmental heterogeneity. DNA library preparation and sequencing of soil fungal communities was done by Molecular Research DNA Laboratory (www.mrdna.com, Shallowater, TX, USA) through target-based amplification of the entire fungal internal transcribed spacer (ITS) region (ITS1, 5.8s, ITS2) with primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3'; White et al., 1990) and

ITS4 (5′-TCCTCCGCTTATTGATATGC-3'; White et al., 1990). These primers (ITS1 and ITS4) were selected because of sequence variability and representation in public data repositories and reference databases (Nilsson et al., 2009). PCR amplification was accomplished by utilizing the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA) under the following conditions: an initial denaturation step at 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. After amplification, PCR products were verified via gel electrophoresis (2% agarose gel), barcoded, pooled together in equal proportions based on their molecular weight and DNA concentrations, and purified with calibrated AMPure XP beads (Agencourt Biosciences Co., Brea, CA, USA). Synthesis-based sequencing on an Illumina MiSeq followed the manufacturer's guidelines and resulted in single-end ITS reads of 343 ± 67.5 (mean ± standard deviation) bp.

2.4. Bioinformatic analysis

Raw Illumina reads were reoriented so that all amplicons were oriented 5′-3'. Resulting .fasta and .qual files were demultiplexed, quality filtered, and analyzed using the QIIME 1.9.1 pipeline (Caporaso et al., 2010). Illumina sequences with < 200 and > 900 bp, barcode or primer sequence errors, and those with homopolymers or ambiguous base calls that exceed six nucleotides were discarded. Sequences were trimmed to contain only the ITS1 region using the ITSx 1.0.11 software (Nilsson et al., 2010; Bengtsson-Palme et al., 2013). The trimming of the ITS1 region allowed for the extraction of a defined gene segment that was better suited for community comparisons and also provides further quality control by confirming the authenticity of the target. Raw sequences were deposited in NCBI's sequence read archives under the accession number SRR5202284. Operational taxonomic units (OTUs) were defined by clustering at 97% sequence identity (Nilsson et al., 2008; Tedersoo et al., 2010) using the QIIME implementation of UCLUST (Edgar, 2010). Subsequent taxonomic annotations, refer to assignments based on > 97% sequence identity for species, 95–97% sequence identity for genera and 77–80% for phyla using the UNITE fungal database (Lemos et al., 2011). Chimera checking was performed on remaining sequences using the QIIME-based ChimeraSlayer (Haas et al., 2011). For functional analysis, fungal OTUs were transformed into text formatting and then uploaded to FUNGuild: Taxonomic Function (http://www.stbates.org/guilds/app.php) and analyzed according to Nguyen et al. (2016).

2.5. Statistical analysis

All statistical analyses on fungal communities were carried out using the sequence count within each OTU as an abundance value for the fungal community. Similarly, OTU counts within functional guilds were used as an abundance value within each respective grouping. All datasets were tested for normality using Shapiro-Wilk's test. When OTU data were not of normal distribution, non-parametric statistical tests or log transformations were applied. Community metric calculations and functional analyses were analyzed using normalized OTU data set to 11 620 reads per sample. OTU data generated in QIIME were used to calculate alpha diversity metrics including Simpson's diversity index, Shannon's diversity index, and OTU evenness. Fungal OTU abundance values, community metric estimates, and functional abundances were statistically analyzed for OMR and soil depth responses using a linear mixed model ANOVA. Because of the inherent autocorrelation between differing soil depths, a repeated measures experimental design was employed with OMR as the fixed main plot and soil depth as the repeated measure. Replicated plots were nested within harvest treatment and considered a random effect. When differences were significant, Tukey's honest significant difference (HSD) test was performed to assess post hoc contrasts with significance inferred at $p \leq 0.05$. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix was performed on normalized fungal ITS data. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using the Bray-Curtis matrix listed above was employed to characterize differences in the soil fungal community based on OMR treatment and soil depth. PERMANOVAs were run using 999 permutations. Environmental factors were fitted onto NMDS ordinations using the function envfit of the vegan package in R (R Development Core Team, 2011). Significance of the associations was determined by 1000 random permutations. Spearman's correlations were calculated from all data points using SAS (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. OTU counts and site diversity

Of the initial 1,098,145 sequences, a total of 724,669 high-quality sequences were retained following filtering. Using normalized OTU data, sequences corresponded to 936 ± 306 (mean ± SD) unique OTUs per sample, which did not vary by OMR treatment, but decreased significantly with increasing soil depth (Table S3). Measures of alpha diversity illustrated large variability between samples as well as some significant OMR and soil depth differences. Shannon index values (0–100 cm) ranged from 0.99 to 7.75 (mean = 5.68, SD = 1.59) and were significantly reduced with increasing OMR and soil depth. Simpson index values (0–100 cm) ranged from 0.16 to 0.98 (mean = 0.86, SD = 0.21), which did not vary with OMR but did decrease significantly with depth (Table S3). Evenness (0–100 cm) ranged from 0.05 to 0.99 (mean = 0.71, SD = 0.22) and was significantly higher in the control relative to the OMR treatments. OMR-based differences in Shannon's index and evenness were largely driven by difference between the control and two OMR treatments at 60–100 cm where the control was higher (Table S3).

3.2. Taxonomic distribution

We were able to successfully classify 73% of all OTUs to the genus level. Excluding unassigned OTUs, phylum-level characterization indicated that communities were dominated by Basidiomycota (64.6 ± 11.6%) and Ascomycota (31.6 ± 10.1%) across all treatments and soil depths (Fig. 1). To a lesser extent, libraries were composed of Zygomycota (3.3 ± 1.9%) (Fig. 1). The remaining 0.6 ± 0.4% of sequences were annotated to either Blastocladiomycota, Chytridiomycota, Cryptomycota, Entomophthoromycota, Glomeromycota, Monoblepharidomycota or Neocallimastigomycota (Fig. 1). The five most abundant fungal orders included Eurotiales and Helotiales (Ascomycota) as well as Agaricales, Boletales, and Russulales (Basidiomycota) (Fig. 1). Neither OMR treatment nor soil depth statistically impacted the abundance of OTUs annotated to Basidiomycota, Ascomycota, or Zygomycota (Table 1); however, noteworthy trends did emerge. The number of sequences annotated to Ascomycota increased by 20% while those annotated to Basidiomycota decreased by 17% in the WT + FF treatment compared to the unharvested control. The relative abundance of OTU sequences similar to Ascomycota was positively correlated to concentrations of SOC and Mehlich-III extractable P, while sequences annotated to Basidiomycota were positively correlated to concentrations of $NO_2^- + NO_3^-$ and negatively correlated to soil pH (Table S4).

The three most abundant genera, *Russula*, *Inocybe*, and *Rhizopogon* comprised over 43% of all OTUs (Table 1) and belong to Basidiomycota. The fourth and fifth most abundant genera, *Cladophialophora* and *Penicillium*, were associated with Ascomycota and accounted for 8% of all annotated OTUs. *Russula* (> 23% of all OTUs) was significantly reduced with increasing OMR intensity across all sample depths; however, none of the other 20 most abundant genera were significantly influenced by OMR. The relative abundance of these most abundant genera were significantly correlated to multiple soil properties. For example, *Russula*

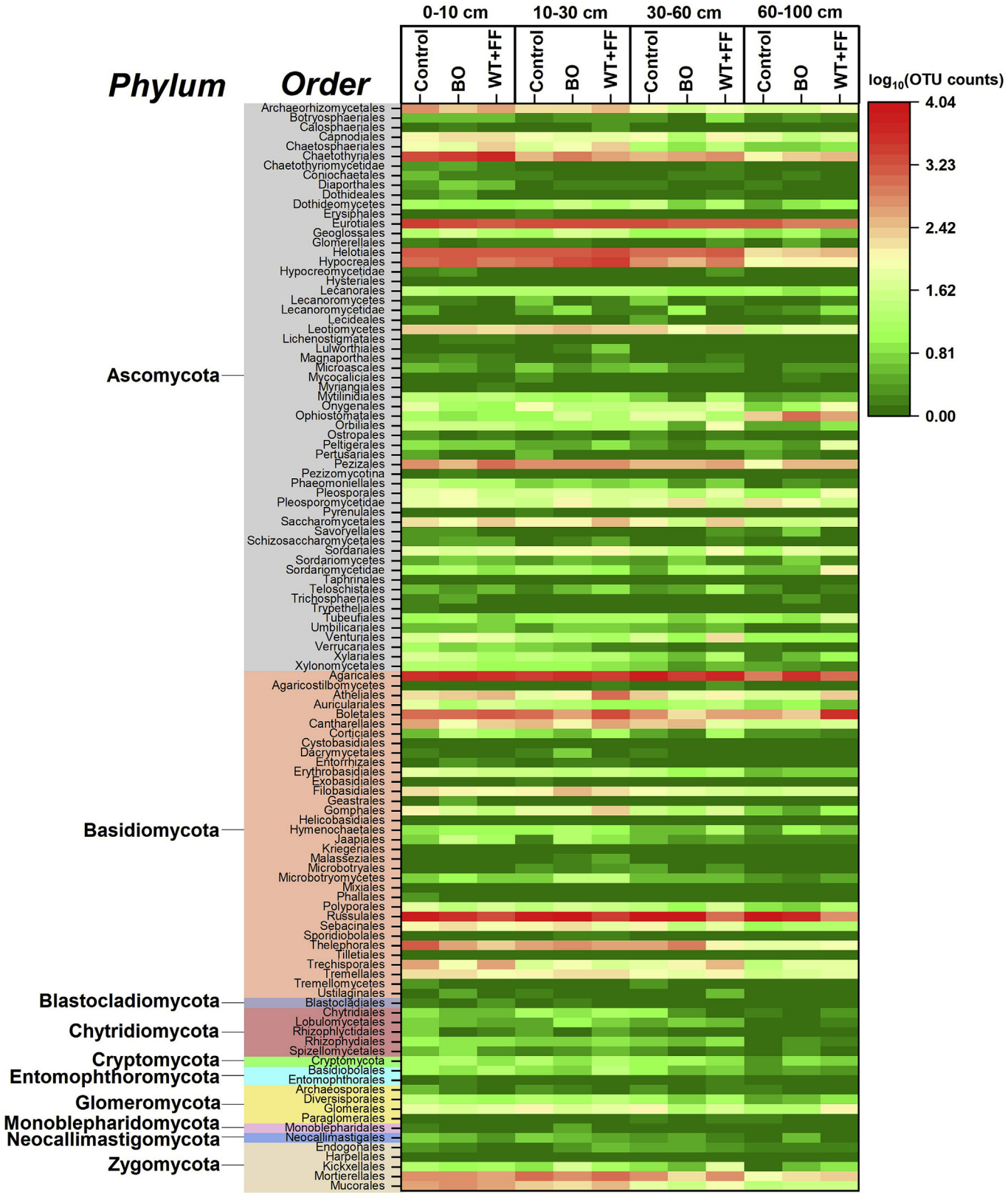


Fig. 1. Heatmap of phylum and order level abundance for each organic matter removal treatment nested within each soil depth. The scale is set as the $\log_{10}$ value of normalized abundance values.

was positively correlated to concentrations of NH_4^+, NO_2^- + NO_3^-, and magnesium (Mg) and negatively correlated to soil pH and Mehlich-III P. Of the less abundant genera, the relative abundance of 5 were influenced by OMR (i.e., *Chaetosphaeria, Craterellus, Meliniomyces, Pycnidiophora, and Rhizoscyphus*) (Table S5). Numerous genera showed significant variation with respect to soil depth. Of note, the abundance of saprophytic genera such as *Penicillium* and *Aspergillus* significantly decreased with depth.

Table 1
Relative abundance of major phyla and genera in soil samples as well as results from a mixed model ANOVA. *$p < 0.05$,**$p < 0.01$,***$p < 0.001$. All significant changes were due to a reduction in the relative abundance of fungal genera with increasing harvest intensity or soil depth.

Phylum	Genus	Relative Abundance (%)	OMR	Soil Depth (SD)	OMR x SD
			F-Ratio		
Ascomycota		31.6	0.54	2.98	0.49
	Aspergillus	1.4	1.99	5.69**	1.47
	Beauveria	1.0	0.99	3.09*	0.69
	Cladophialophora	3.8	3.35	17.06***	1.73
	Elaphomyces	0.9	3.17	0.97	0.95
	Leptodontidium	2.9	0.3	4.58**	0.29
	Penicillium	3.8	0.04	4.75*	0.48
	Raffaelea	2.0	0.6	4.33*	0.59
	Talaromyces	1.1	1.51	1.22	0.81
	Trichoderma	1.4	0.19	1.76	1.28
	Other Ascomycete Genera	13.3	2.24	1.64	0.74
Basidiomycota		64.6	0.36	0.6	0.37
	Amanita	3.1	2.95	2.87	0.54
	Amphinema	1.4	0.84	0.26	1.25
	Cryptococcus	1.0	0.29	4.34*	0.59
	Inocybe	14.1	0.05	2.12	1.01
	Laccaria	3.2	0.34	0.84	0.51
	Lactarius	0.9	1.25	1.31	1.11
	Rhizopogon	6.0	2.86	0.77	0.99
	Russula	23.1	11.42**	2.25	0.65
	Tomentella	1.2	3.19	3.05*	2.08
	Trechispora	2.1	0.02	1.09	0.92
	Other Basidiomycete Genera	8.5	2.24	4.28*	0.68
Zygomycota		3.3	1.36	5.25**	0.77
	Mortierella	2.2	1.22	3.83*	0.71
	Other Zygomycete Genera	1.1	1.63	10.99***	1.97
Other Fungal Phyla		0.6	2.36	0.7	0.94

3.3. Fungal community structure

Non-metric multidimensional scaling (NMDS) plots, based on Bray-Curtis distance matrices, of OTUs showed distinct separation of the three OMR treatments, across multiple depth increments (Fig. 2); furthermore, when soil depth was analyzed without regard for OMR treatments, the shallowest depth increment (i.e., 0–10 cm) was more separated from the other depths, with lower standard deviation, while the 10–30, 30–60 and 60–100 cm depth increments were more tightly clustered with each other. Based on PERMANOVA, fungal community composition was statistically altered by OMR across 0–10 cm and 10–30 cm, but not 30–60 cm and 60–100 cm (Table S6). Root biomass, SOC, TN, and soil pH were significantly linked to fungal community structure and positively correlated with the more surficial soil depths (Fig. 2).

3.4. Guild classification

Using the normalized OTU dataset, 27 ± 5% of all OTUs were functionally classified into fungal guilds. Control (29 ± 4%) and BO (30 ± 6%) stands possessed a higher percentage of classifiable OTUs, relative to WT + FF stands (23 ± 6%); however, these differences were not significant. Excluding unassigned OTUs, ectomycorrhizal fungi (ECM) dominated all functionally classified OTUs (62 ± 3%) followed by undefined saprotrophs (7 ± 1%) and ericoid mycorrhizal fungi (ERM) (1 ± 0.1%) (Fig. 3). ERM were represented primarily by

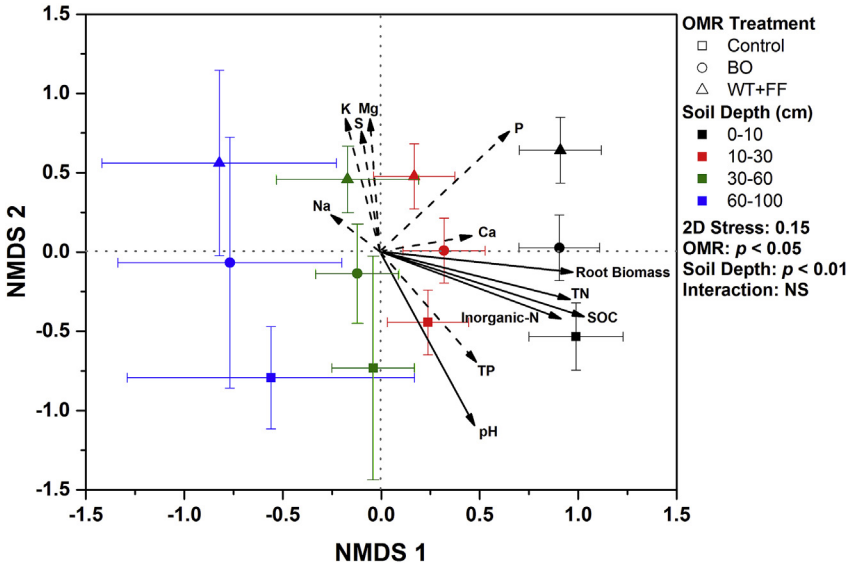
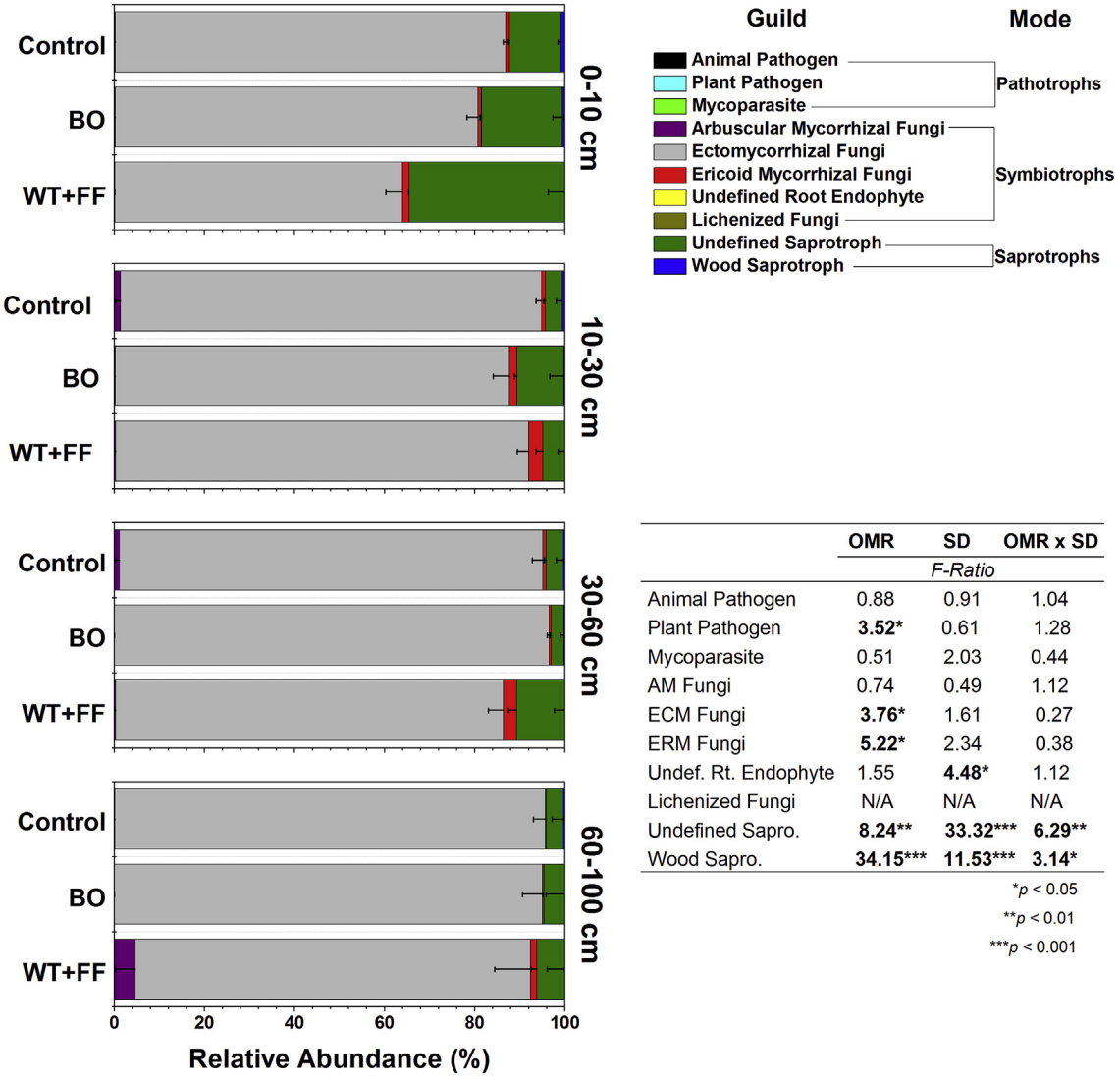


Fig. 2. Nonmetric multidimensional scaling (NMDS) ordinations of soil fungal communities based upon their OTU composition derived from Bray-Curtis distances matrices. For each point, N = 3. Error bars indicate standard deviation. Vectors represent the strength/direction of the weight of environmental variables on bacterial communities. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; Mehlich III extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and sulfur (S); Inorganic-N: combined concentration of NH_4^+ and $NO_2^- + NO_3^-$. Significant factors are shown by black solid lines, while the non-significant factors are indicated using dotted lines. Control = unharvested control; BO = bole-only harvest; WT + FF = whole-tree harvest + forest floor removal.



	OMR	SD	OMR x SD
	F-Ratio		
Animal Pathogen	0.88	0.91	1.04
Plant Pathogen	**3.52***	0.61	1.28
Mycoparasite	0.51	2.03	0.44
AM Fungi	0.74	0.49	1.12
ECM Fungi	**3.76***	1.61	0.27
ERM Fungi	**5.22***	2.34	0.38
Undef. Rt. Endophyte	1.55	**4.48***	1.12
Lichenized Fungi	N/A	N/A	N/A
Undefined Sapro.	**8.24****	**33.32*****	**6.29****
Wood Sapro.	**34.15*****	**11.53*****	**3.14***

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

Fig. 3. Distribution and relative abundance of fungal functional guilds for each organic matter removal treatment (Control = unharvested control; BO = bole-only harvest; WT + FF = whole-tree harvest + forest floor removal) nested within soil depth increment. Error bars indicate the standard deviation of 3 biological replicates. Results of mixed model ANOVA are shown in below legend.

Table 2
Results from a one-way ANOVA investigating the influence of organic matter removal on the abundance of fungal functional guilds at each depth increment. *p < 0.05,**p < 0.01,***p < 0.001.

	0–10 cm	10–30 cm	30–60 cm	60–100 cm
	F-Ratio			
Animal Pathogen	1.01	0.89	N/A	N/A
Plant Pathogen	2.06	1.22	1.41	0.89
Mycoparasite	N/A	0.57	N/A	N/A
Arbuscular Mycorrhizal Fungi	0.85	1.11	0.99	1.02
Ectomycorrhizal Fungi	10.44*	0.54	2.23	0.28
Ericoid Mycorrhizal Fungi	3.19	1.89	1.57	0.79
Undefined Root Endophyte	1.23	N/A	N/A	N/A
Lichenized Fungi	N/A	N/A	N/A	N/A
Undefined Saprotroph	14.11**	4.41	4.06	0.01
Wood Saprotroph	43.46***	16.89**	1.88	2.92

Rhizoscyphus ericae, which significantly increased with OMR. Increasing OMR lead to significant increases in the relative abundance of undefined saprotrophs and ERM as well as significant decreases in ECM, wood saprotrophs, and plant pathogens. Increasing depth significantly decreased the relative abundance of undefined saprotrophs, wood saprotrophs, and root endophytes, while no guild significantly increased with depth. When we analyze the effect of OMR for each depth increment, we note that significant differences were only observed in the 0–10 cm increment for wood saprotrophs, undefined saprotrophs, and ECM as well as in the 10–30 cm increment for wood saprotrophs (Table 2). Fungal guilds did not correlate well with soil properties (Table S7).

4. Discussion

Compared to similar soil fungal sequencing studies, measures of fungal diversity at 0–10 cm were comparable to southeastern coniferous forests in Georgia (USA) (Brown et al., 2013) and slightly higher than reported for eastern coniferous forests in North Carolina (USA) (O'Brien et al. 2005) and boreal forests in Alaska (Allison et al., 2007). Previous studies at this LTSP site have shown no significant differences in fungal diversity metrics for mineral soil following OMR (Wilhelm et al., 2017b); however, when we analyze OMR independent of depth, we observed the highest levels of diversity in control stands, for which Shannon's diversity index was statistically significant. Additionally, evenness metrics illustrated that OTU abundance was significantly

more uniform in control stands relative to both BO and WT + FF treatments. This indicates that the overall number of unique taxa throughout the profile (0–100 cm) may be somewhat susceptible to perturbation over the long term, with intensive OMR resulting in environmental conditions that are better suited to a smaller number of taxa who are able to persist with reduced substrate and altered microclimate conditions. However, *post hoc* analyses illustrated that OMR-based differences at 60–100 cm largely contributed to overall differences observed when OMR is analyzed without regard for depth, and differences at shallower depths are non-significant. Considering all other OMR-based differences in community structure as well as functional distribution are primarily observed in surficial soil, it is likely that these differences in diversity (0–100 cm) are a function of heterogeneity where fewer taxa persist in deeper depths and are different in each biological replicate.

In agreement with our hypothesis as well as previous fungal work at this and other LTSP sites, we found that community differences at the phylum level were evident, and mainly constrained to surface mineral soil. As postulated by Wilhelm et al. (2017b), the decline in Basidiomycota, which dominate ECM, and concurrent increase in Ascomycota could simply be due to heat-intolerance of ECM relative to thermophilic saprophytic taxa commonly found in Ascomycota. This argument is in agreement with the largest variation in fungal guilds being observed in the most surficial depth increment (0–10 cm) where ECM decreased 23% between the control and WT + FF stands while undefined saprotrophs increased to the same degree. Higher surface temperature in the WT + FF stands may have had direct influence on this change in variation. These results are in contrast to Allmér et al. (2009) who showed that following timber harvest in Sweden, the saprophytic taxa frequency was not altered. This disparity could be a function of geographic differences where cooler climatic conditions, as observed in Sweden, would be less likely to develop increases in heat- and desiccation-tolerant saprophytic taxa. At our site, the increase in saprophytic taxa can be directly related to heat-tolerant taxa such as *Aspergillus* and *Penicillium*, which are widely considered to be ruderals and germinate rapidly in response to readily available substrate. These and similar saprophytic taxa would be better adapted to persist in environments such as WT + FF, where substrate is lower and pulses occur randomly. It should also be noted that the significant reduction in root biomass observed in the WT + FF stands might be the simplest explanation for decreased abundance in ECM; however, no significant correlations were found between root biomass and any guild classification. It should be mentioned that the long-term effect of OMR may have robust implications for fungal-mediated ecosystem services; specifically, the reduction in ECM taxa in WT + FF stands, which may have directly impacted stand productivity by reducing the subsequent generation *P. taeda*'s ability to acquire nutrient resources leading to lower tree biomass. Theoretically, the effects observed here would be exacerbated by any subsequent timber stand rotations at this site using intensive OMR.

Additionally, the relative abundance of Basidiomycota was positively correlated to concentrations of $NO_2^- + NO_3^-$, which decreased with increasing OMR. This illustrates an interesting connection to the previously reported decrease in nitrifying bacteria and archaea with increasing OMR (Mushinski et al., 2017b, 2018) and may represent a key link between forest perturbation, fungi, and soil prokaryotes. It is possible that following OMR, the WT + FF stands experienced a large increase in N-mineralization; however, the N pool diminished over time due to decreased N inputs. We hypothesize that because of lower levels of mineralized N, the nitrifying bacteria and archaea as well as many mycorrhizal taxa began to decline and have not reestablished due to the low concentrations of mineralized N. However, this argument cannot be extrapolated to all ECM fungi due to low levels of correlation with this putative guild and inorganic-N.

It is not surprising that *Russula* was found to be the most abundant genera in our stands considering it is one of the primary ectomycorrhizal colonizers of *P. taeda*, which, similar to Hartmann et al. (2012) and Wilhelm et al. (2017b), decreased significantly with increasing OMR. Additionally, considering *Russula* was the dominant genus observed in this system, the significant decrease in the relative abundance of this genus with increasing OMR is the primary factor responsible for the decrease in Basidiomycota and ECM. The change in *Russula* with increasing OMR is likely a function of multiple factors including the loss of mature plant hosts as well as changes in edaphic variables. Of all the soil variables analyzed, soil pH had the largest negative correlation with the relative abundance of *Russula*, which may have had a significant impact of *Russula*'s ability to colonize growing *P. taeda*. It has been shown that within acidic soil, the availability of phosphorus (P) becomes more limited due to chemical fixation by soluble iron (Fe), aluminum (Al), and manganese (Mn) as well as fixation by hydrous oxides of Fe, Al, and magnesium (Mg). Forest trees can compensate for reduced mineral P availability by mycorrhizal colonization. ECM taxa, such as *Russula*, have the ability to produce extracellular enzymes that hydrolyze organic P, making it available for plant uptake. Previous studies have shown that increasing timber harvest intensity can lead to increased inorganic soil P pool size (Dickie et al., 2009). Whether this increase is related to the change in soil pH is unknown; however, the increase in inorganic soil P may lead to the reduced abundance of *Russula* due to the lessening of P limitation on the associated plant species. It has been shown that total P is not significantly altered with respect to increasing OMR intensity at the Groveton LTSP (Mushinski et al., 2018); however, Mehlich-III P, which is generally considered plant-available P, increased significantly with increasing OMR. Additionally, the relative abundance of *Russula* was negatively correlated with concentrations of Mehlich-III P, indicating a connection between this specific genus, soil pH and P availability. As with many of the other Basidiomycota taxa, *Russula* was also positively correlated with inorganic-N, which could be an alternative or concurrent explanation for the overall decline of this, and other Basidiomycete genera with increasing OMR.

ERM, represented primarily by fungi similar to *R. ericae* in our system, showed significant increase in relative abundance with respect to increasing OMR intensity, and reduced abundance with increasing soil depth. Vegetation surveys of the experimental plots and surrounding natural forest have not been able to identify any plant species in the Ericaceae family; however, all species in this plant family are well suited for acidic soils and have been reported throughout the southeastern US. There is also a large presence of *Callicarpa americana*, a berry producing asterid in the Lamiaceae family, throughout the surrounding forest, especially in more open canopy areas similar WT + FF stands. Many ERM taxa have been shown to increase in abundance in soil that have low substrate availability or are recently disturbed (Hansen and Pfister, 2006). Additionally, it has been previously demonstrated that ERM can occur in roots of ECM trees and it is likely that ERM are forming associations with *P. taeda* (Villarreal-Ruiz et al., 2004). Heinonsalo et al. (2007) showed that following clear-cut, *Pinus sylvestris* seedlings had a higher percentage of root-associated *R. ericae* than the control forest. This is even more likely considering that clearcutting and forest floor removal has been shown to reduce ECM ability to colonize regenerating seedlings (Simard, 2009). It is possible that this taxon colonized *P. taeda* seedlings immediately following implementation of the OMR treatments, and may be better adapted at persisting in harsher conditions such as the WT + FF stands, relative to ECM fungi.

Our results are consistent with general trends observed in microbial community analysis from other southern LTSP harvested stands (Ponder and Tardos, 2002; Busse et al., 2006). Based on PERMANOVA, fungal community composition was statistically altered by OMR in the 0–10 and10-30 cm increments. These differences in the upper soil profile are consistent with studies of Chinese pine plantations (Nie et al., 2012), aspen-dominated forests of Minnesota (Lewandowski et al., 2016), and spruce-dominated forests of Finland (Toivanen et al.,

2012). It is conceivable that observed OMR-based differences in these surface mineral soils may be due to unmeasured variables such as tree age and complexity of root networks; however, similar to other studies, concentrations of C and N, root biomass, and soil pH were shown to be major determinants of fungal community structure. The effects of these four variables are strongly linked where roots are a major source of C and N and soil pH influences the availability of these substrates. Additionally, the observation of no significant differences below 30 cm is consistent with our hypothesis that deeper soil fungal communities may be more resistant and/or resilient with respect to alterations in the quantity and/or quality of above- and belowground biomass inputs (Rumpel and Kögel-Knaber, 2011; Angst et al., 2016; Dietzel el al. 2017). This indicates that OMR-based disturbance may not lead to long-term fungal community structure changes deeper in the mineral soil, which is also evidenced by the functional guild analysis. As mentioned with the diversity analysis, this resistance and/or resiliency may be a function of the large variability in community structure observed in deeper soil. Increased fungal community variability with depth has been previously reported by Hartmann et al. (2009), who showed that in a British-Columbia LTSP site there were OMR-based differences in fungal (eukaryal) profiles up to 30 cm; however, these differences became non-significant at 55 cm due to high variability between biologically replicated stands. Alternatively, this constant observation of high fungal variability at depth may have led to statistical misrepresentation in our current study. Even though PERMANOVA states that OMR did not significantly impact fungal community structure at 30–60 cm or 60–100 cm, there is a similar NMDS distribution pattern as observed in the 0–10 cm and 10–30 cm increments, albeit with high variability. This suggests that OMR likely had some level of impact on deeper fungal communities but it was not statistically detected. Future studies investigating the impact of OMR on soil fungal communities should focus their attention on deeper soil depths with special attention paid to implementing an experimental design that accounts for this large variability at depth. Regardless of OMR differences, our work builds upon other studies who have suggested that fungal communities have differential distributions across a range of soil depths (Kramer and Gleixner, 2008; Hartmann et al., 2009), perhaps reflecting niche differentiation in response to variation in edaphic variables.

This study illustrates a long-term fungal response to increasing intensity of OMR, as has also been shown in previous studies. Additionally, this study considers whether or not differences incurred from these intensive OMR practices extends to soil depths beyond what is commonly explored. This study complements previous work done at this long-term experimental coniferous forest site, which has been instrumental in exploring how OMR can affect microbial community composition and biogeochemical cycling. To the best of our knowledge, this is one of the first studies to explore how OMR influences fungal community structure to a depth of 100 cm. We found significant alterations in many fungal taxa with respect to OMR and depth; however, OMR-based differentiation in fungal taxa, functional guilds, and community structure decreased substantially as depth increased. In surface soil, saprophytic taxa increased while ECM taxa decreased with increasing OMR intensity, most likely due to increased surface temperature and reduced N. Additionally, ERM increased in intensive OMR-stands indicating that following OMR, ERM could outcompete ECM for colonization of seedlings. Due to large heterogeneity, it is difficult to infer whether fungal community structure at depth is still showing variability with respect to OMR, was initially perturbed by OMR and is now fully recovered (resilient), or was simply not affected by tree harvest (resistant). We should also mention that many of the differences observed in the 0–10 cm of mineral soil could be due to a lack of re-development of the O-horizon, which, as postulated by Hartmann et al. (2009), might be the rate-limiting step in the reestablishment of a normal fungal community in mineral soil.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2018.09.019.

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