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Short Communication

Forest organic matter removal leads to long-term reductions in bacterial and fungal abundance

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ABSTRACT

Intensive organic matter removal (OMR) associated with forest harvest has been shown to significantly affect soil physical, chemical, and biological properties; however, the influence on microbial abundance has been neglected, especially at depth and over time. We used quantitative PCR to assess the long-term impact of two different intensities of OMR, soil depth, seasonality, and their interactions on bacterial and fungal abundances 18 years post-harvest, relative to an unharvested control. Both bacterial and fungal community sizes were significantly reduced by intensive OMR, and these effects were largest in surface soil (0–10 and 10–30 cm), and largest in summer and spring. The response ratios for bacteria and fungi were not statistically different, indicating that both taxonomic groups were susceptible to long-term changes in soil properties induced by intensive OMR. Furthermore, we found that root biomass was significantly correlated to bacterial and fungal abundance in unharvested, low-intensity harvest, and high-intensity harvest stands throughout time and across soil depths, suggesting that the input of organic matter through root exudation and turnover are key to controlling microbial community size. These reductions in bacterial and fungal community sizes suggest that microbial community functions related to biogeochemical processes may be altered for decades post-harvest, with potential implications for forest productivity and ecosystem function during subsequent rotations.

1. Introduction

Increases in the extent of organic matter removal (OMR) during timber harvest has become more prevalent due to economic gains from the commercialization of secondary harvest materials such as slash, sawdust, and the forest floor (Fox, 2000). High-intensity OMR has been shown to reduce soil carbon (C) and nutrients (Achat et al., 2015a, 2015b) which can directly influence the relative abundance, composition, and activity of soil bacteria and fungi. Furthermore, OMR-induced changes in aboveground composition, litter abundance, and observed decreases in soil C, create a strong potential for alterations in the quantity and quality of available C, possibly influencing the abundance of specific microbial groups. A recent meta-analysis showed that soil bacteria and fungi respond to forest disturbances in a similar manner (Holden and Treseder, 2013); however, there is limited experimental evidence regarding the long-term influence of high-intensity OMR on a quantitative measure of soil bacteria and fungi, which is exacerbated by a lack of data on bacterial and fungal dynamics over time as well as their response to depth.

Seasonality and depth both significantly affect soil microbes primarily through alterations in soil C and N (Eilers et al., 2012; Koranda et al., 2013; Siles and Margesin, 2017). However these dynamics are generally neglected leading to ambiguity in regards to ecological phenomena such as disturbance. Temporal variability in microbial activity is related to the availability of different substrates (e.g., root exudation during the growing season and increased litter production during the fall) as well as fluctuations in soil temperature and moisture. Changes in microbial community structure with depth are also related to soil C, N, water, and temperature as well as oxygen availability. In pine forest stands, the concentration of C and nutrients decrease substantially with soil depth (Mushinski et al., 2017a), which creates opportunity for microbial community differentiation (Mushinski et al., 2018a, 2018b). The combined effects of OMR, seasonality, and depth on soil bacterial and fungal abundance are difficult to predict. Considering intensive OMR has been shown to decrease soil C and N up to one meter, it is plausible that soil microbial abundance will follow suit. However, it may also be possible that differences in community composition between surficial and deeper mineral soil foster different levels of resiliency to intensive OMR. This resiliency may also fluctuate throughout the year with large differences observed during the growing

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season due to higher inputs of root exudates and litter in unharvested stands relative to intensive OMR stands.

Regardless of the outcome, if we simultaneously consider the role of soil depth and time, we should be able to develop a more comprehensive understanding of how OMR affects soil microbial populations. We hypothesized that high intensity OMR would reduce the size of both bacterial and fungal communities relative to control and low-intensity OMR stands and reduce community sizes of soil bacteria and fungi equivalently. Furthermore, we hypothesized that high-intensity OMR will have detrimental impacts on microbial community size that will be (i) most evident in the upper portions of the soil profile due to an assumed lower resiliency to changes in substrate availability and (ii) greatest during the growing season (spring and summer) due to large OMR-induced differences in substrate inputs relative to unharvested stands. We also hypothesize that fluctuations in unharvested, low-intensity OMR, and high-intensity OMR stands would be primarily driven by differences in soil C and N across time and depth.

2. Materials and methods

2.1. Study site

The study area was a *Pinus taeda* (loblolly pine) forest located in eastern Texas USA (31° 06′ 32.48″ N, 95° 09′ 59.15″W) and described in detail in Mushinski et al. (2017a). In brief, the experimental design consisted of two different OMR intensities (low-intensity: bole-only harvest, BO; high intensity: whole-tree harvest + forest floor removal, WT + FF) and unharvested control forest plots, with three replicates per treatment. Treatments were harvested in 1996, and replanted with containerized *P. taeda* seedlings at 2.5 m × 2.5 m spacing in 1997. Nearly two decades following harvest and replant, soil was sampled seasonally for one year (June 2014, September 2014, December 2014, and March 2015). At each sample date, 4 vertical soil cores (0–100 cm) within each replicate plot were sampled at 1.8 m from the base of a randomly selected mature *P. taeda* individual, and separated into depth increments (0–10, 10–30, 30–60, and 60–100 cm). No stumps from the initial harvest remained at the time of sampling. The four cores per plot were then pooled by depth increment and homogenized to create a single homogenous sample for each replicate plot, from which a representative subsample was removed and stored at −80 °C for molecular analyses. Microbial DNA was extracted from soil as noted in Mushinski et al. (2017b). Previous studies have shown that the high-intensity harvest treatment has resulted in significant decreases in soil carbon and nitrogen (Mushinski et al., 2017a), decreases in mineral nitrogen and nitrifying microbes (Mushinski et al., 2017b), and a complete reorganization of the soil bacterial and fungal communities (Mushinski et al., 2018a, 2018b).

2.2. Quantitative PCR

Quantitative-PCR (qPCR) targeting total bacteria and fungi was performed using primer pairs 1100F/1492R for bacteria (Lane et al., 1985; Dorsch and Stackerbrandt, 1992; Turner et al., 1999) and ITS1F/ITS5.8S for fungi (Vilgalys and Hester, 1990; Gardes and Bruns, 1993). The 25 µl reaction mixture contained 13 µl SYBR green real master mix (5Prime, Gaithersburg, MD), 0.5 µl of each primer (concentration 10 mM), 1 µl DNA template, and 10 µl molecular-grade water. Each analytical run included a set of standards, negative controls, and replicated samples (n = 3) on a 96-well plate. For the 16S rRNA gene, the qPCR conditions were as follows: 95 °C for 10 min; 95 °C for 30 s, 53 °C for 30 s (40 cycles); 72 °C for 1 min (Fierer et al., 2005). For the ITS region, the qPCR was run with the following conditions: 94 °C for 5 min; 94 °C for 30 s, 57 °C for 45 s (30 cycles); 72 °C for 1.5 min (Manter and Vivanco, 2007). The qPCR was performed using a Mastercycler® ep realplex thermal cycler (Eppendorf, Hamburg, Germany). Amplification efficiencies of 77–80% and 84–86% were obtained for bacteria and fungi, with r^2 values > 0.96. Plasmids containing 16S and ITS inserts were used for the standard curves (Hollister et al., 2013). Copy numbers are reported as 16S or ITS gene copies per gram of dry-weight soil.

2.3. Statistical design

Statistical analyses and graphic visualization were performed using JMP Pro 13 (SAS Institute, Inc., Cary, NC, USA), OriginPro (OriginLab, Inc., Northhampton, MA, USA), and R (R Development Core Team). A mixed model split-plot ANOVA with repeated measures was used to investigate the effect of OMR, soil depth, season (time), and their interactions. When differences were significant, Tukey's honest significant difference tests was performed to assess post hoc contrasts with significance inferred at $\alpha < 0.05$. A normal data distribution was obtained following $\log_{10}$ transformation to gene copy values for both 16S and ITS. The F/B (fungi/bacteria) ratio was calculated by dividing the number of $\log_{10}$-transformed ITS gene copies by the number of $\log_{10}$ transformed 16S gene copies. Bacterial and fungal response ratios for both OMR treatments were based on Hedges et al. (1999) and calculated using Eq. 1 (E1),

$$R = log_{10}\left(\frac{t}{c}\right) \quad (1)$$

where the response ratio (R) is a $\log_{10}$-transformed function of 16S or ITS gene copies in the low-intensity or high-intensity harvest treatments (t) divided by 16S or ITS copies in the control (c). To compare the relative combined response of bacteria and fungi to the two treatments, the response ratios were subjected to the same mixed model split-plot ANOVA mentioned previously. Additionally, to compare if one taxonomic group (bacteria vs. fungi) was more affected by OMR, a one-way ANOVA, for each treatment, was performed on all data points using taxonomic group as the single fixed effect. Correlation between gene copy numbers and soil properties were assessed using Spearman's rank correlation coefficient. Data from all seasons and soil depths were combined for this analysis. Soil properties used for correlation analyses have been previously reported (Mushinski et al., 2017a).

3. Results and discussion

Across all samples, the bacterial 16S copy number per gram of soil was $6.3 \times 10^8 \pm 8.0 \times 10^8$ (mean ± std. dev.). Bacterial community size was significantly influenced by OMR, soil depth, season, and the interaction of OMR × season (Table 1). The three OMR treatments were statistically different from each other, with the highest abundance of 16S gene copies in the control and the lowest in the high-intensity treatment. The abundance of 16S copies varied significantly with soil depth (0–10 and 10–30 cm > 60–100 cm > 30–60 cm) and season

Table 1
Effects of organic matter removal, soil depth, season, and their interactions on the size of soil bacterial and fungal communities. Results are derived from a mixed model ANOVA on $\log_{10}$-transformed 16S (bacteria) and ITS (fungi) gene copies, and the ratio of fungi to bacteria (F:B ratio).

	F-ratio		
	Bacteria	Fungi	F:B ratio
Organic matter removal (OMR)	**45.31*****	2.03	0.42
Soil depth (SD)	**26.28*****	**20.02*****	**13.57*****
Season	**79.88*****	**44.54*****	**35.66*****
OMR × SD	1.28	**3.12****	**2.75***
OMR × season	**14.89*****	**2.40***	1.81
SD × season	1.27	1.33	1.21
OMR × SD × season	1.11	0.75	0.65

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

(summer > spring, fall > winter). Additionally, the low-intensity and high-intensity harvest treatments during winter were significantly lower than any other treatment-season combination ($p < 0.001$).

Mean fungal ITS copies per gram of soil was $6.5 \times 10^6 \pm 1.5 \times 10^7$. Fungal community size was significantly affected by soil depth, time, and the two factor interactions of OMR with soil depth and time (Table 1). Across all data points, the control possessed significantly more ITS copies than either of the OMR treatments. When ITS data is analyzed for soil depth, independent of OMR or season, copy numbers in the two uppermost layers were significantly higher than in the two deepest ones. In regards to seasonality, the summer and spring samples possessed significantly higher ITS copy numbers than the fall and winter samples. For the interaction of OMR and soil depth, the control 0–10 cm, low-intensity harvest 0–10 cm, and control 10–30 cm increments possessed significantly higher abundances that any of the other OMR-soil depth combinations. Additionally, the low- and high-intensity harvest 60–100 cm increments possessed the lowest abundance of ITS copies. For the interaction of OMR and time, according with ITS copies: a) spring and summer samples of all treatments possessed the highest values; b) fall and winter samples of low- and high-intensity harvest possessed the lowest values; and c) fall and winter samples of control possessed intermediate values.

Results demonstrate clear changes in bacterial and fungal community size in response to OMR, soil depth, and seasonality. The most likely explanation for the significant decreases in the sizes of these microbial communities in the OMR treatments is the significant reduction in soil C and nutrients following tree harvest as reported previously for this site (Foote et al., 2015; Mushinski et al., 2017a) and other forest sites (Kellman et al., 2014; Achat et al., 2015a, 2015b; Dean et al., 2017; Menegale et al., 2016); however, a significant correlation between 16S and ITS copy number and soil C and N was only observed in the control stands (Table 2). The commonality between the two treatments plus control and gene copy number was actually root

Table 2
Results from Spearman's Ranked Correlation Analysis which compared the relationship between bacterial (16S) and fungal (ITS) abundance against soil properties.

	Spearman's rho	
	16S (copies per g-soil)	ITS (copies per g-soil)
Unharvested control		
Soil pH	0.22	0.05
Soil bulk density	**−0.33***	**−0.33***
Volumetric water content	−0.19	0.17
Total root biomass	**0.29***	**0.53*****
Soil C:N	**0.53*****	**0.37****
Bole-only harvest (*Low-intensity harvest*)		
Soil pH	0.19	**0.42****
Soil bulk density	−0.10	−0.27
Volumetric water content	0.11	0.06
Total root biomass	**0.37****	**0.60*****
Soil C:N	0.24	0.28
Whole-tree harvest + forest floor removal (*High-intensity harvest*)		
Soil pH	−0.02	0.15
Soil bulk density	−0.26	−0.25
Volumetric water content	**0.30***	**0.57*****
Total root biomass	**0.29***	**0.33***
Soil C:N	0.00	−0.05

Significance levels are indicated with asterisks.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

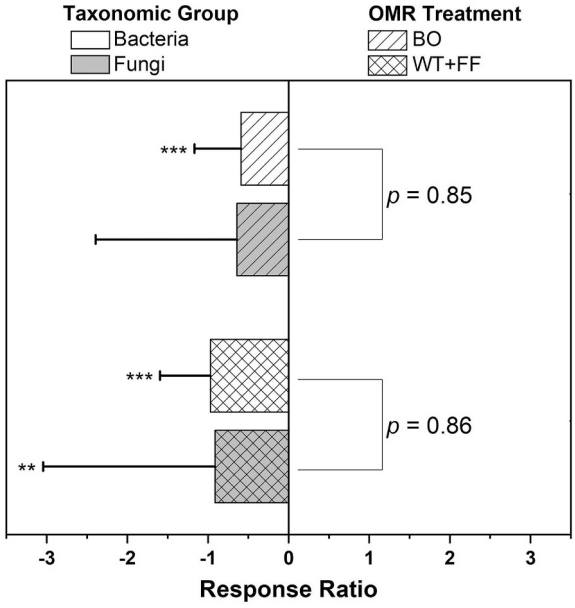

Fig. 1. Response ratio barplots of bacteria and fungi to bole-only harvest (BO; low-intensity harvest) and whole-tree harvest + forest floor removal (WT + FF; high-intensity harvest), summated from all soil depths and seasons, relative to control. Each bar and corresponding error represent mean ± standard deviation (n = 48). Asterisks to the left of bars indicate significance relative to zero (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

biomass, which lends credence to the idea that the input of labile organic matter from roots (e.g., root exudates) is critical in maintaining the abundance of both bacteria and fungi over time and at depth. Furthermore, the removal of living roots due to harvest significantly reduces both microbial groups to a similar degree as evidenced by the concurrent response to harvest (Fig. 1). Decreases in the size of bacterial and fungal communities with soil depth are also probably driven by the exponential decline in root biomass with increasing soil depth at this site (Foote et al., 2015; Mushinski et al., 2017a), as well as variations in oxygen and temperature (Eilers et al., 2012). Moisture is also thought to significantly influence microbial growth due to its influences on microbial nutrient acquisition; however, copy numbers were only correlated to volumetric water content in the high-intensity OMR treatment. Volumetric water content in the high-intensity treatment was slightly lower than in the control or low-intensity treatment over the course of these four sampling times (Mushinski et al., 2017a), indicating that the procurement of nutrients may have been more difficult for both bacteria and fungi which compounded the effect of root death and exacerbated their response to harvest. High 16S and ITS copy numbers in the summer and spring are most likely a microbial response to increased plant-derived organic matter inputs (e.g., root turnover, root exudates, litter) and warmer soil temperatures.

The mean F/B ratio (non $\log_{10}$-transformed) for all samples was 0.013 ± 0.035, which is consistent with F/B calculations based on DNA quantification from other forests (Lauber et al., 2008; Cheeke et al., 2017) as well as from PLFA analysis from forest (Bååth and Anderson, 2003) and grassland (Malik et al., 2016) soils. The F/B ratio did not differ by OMR; however, F/B ratios were significantly affected by soil depth (i.e., decreased with depth) and seasonality (i.e., highest in summer and spring) (Table 1). Mean response ratios of bacteria and fungi to OMR, regardless of soil depth and time, were negative, indicating an overall detrimental effect of OMR on microbial community size (Fig. 1). The response ratio for bacteria was significantly different from zero (e.g., the control) for both low- and high-intensity OMR, while fungal response ratios were significantly different only in the high-intensity plots. Furthermore, there was a significantly larger

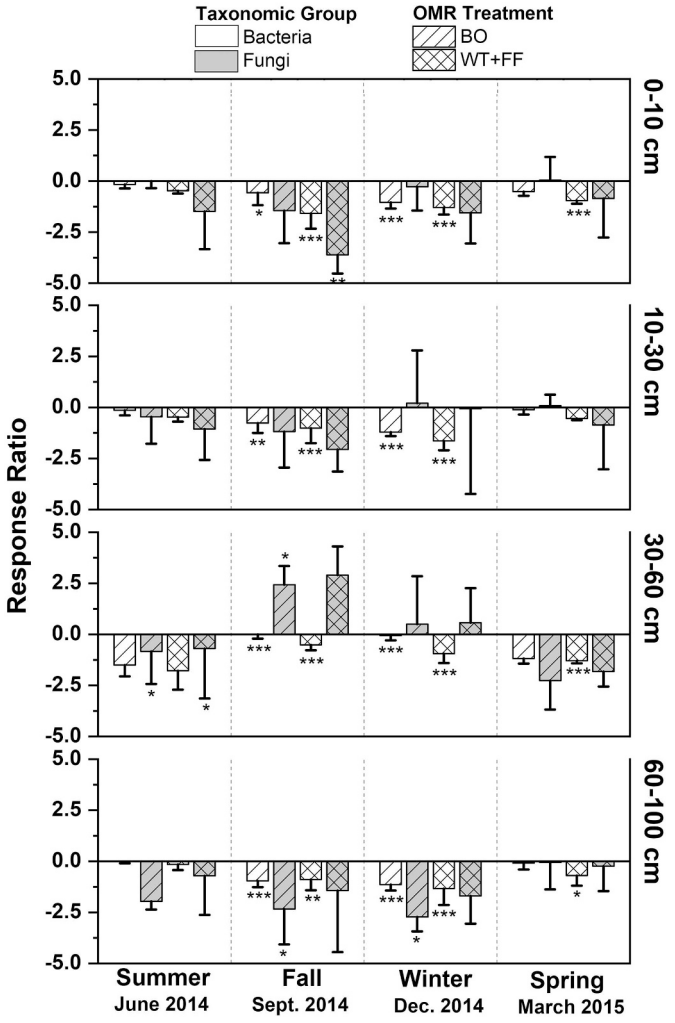

Fig. 2. Response ratio barplots showing, for each season and soil depth, the $\log_{10}$-transformed ratio of bacteria (16S) or fungi (ITS) gene copies in the harvested plots to the bacteria or fungi gene copies in the control plots. Key: BO, bole-only harvest (low-intensity); WT + FF, whole-tree harvest + forest floor removal (high-intensity). Each bar and corresponding error represent mean ± standard deviation (n = 3). Asterisks above or below bars indicate significance relative to zero (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

negative response for bacteria in the high-intensity treatment relative to the low-intensity treatment, which was not observed for fungi. Response ratios for bacterial and fungal groups showed no difference in either low-intensity ($p = 0.85$) or high-intensity ($p = 0.86$) treatments (Fig. 1). The similar responses of bacteria and fungi to intensive OMR, when integrated throughout the soil profile and over time, demonstrates that forest soil disturbances leading to long-term changes in key soil properties (such as root biomass) can have comparable effects on these two major soil microbial groups. Moreover, these differences persist nearly 2 decades after the timber harvest events occurred.

Contrary to our hypothesis, both bacteria and fungi in low- and high-intensity OMR stands had a greater negative response during the fall and winter seasons, indicating a lower tolerance to substrate limitation or changes in environmental properties such as temperature and/or moisture during the non-growing season. It is possible that intensive OMR provokes a shortage of available substrates derived from soil organic matter leading to bacterial and fungal abundance in the harvested plots to be more reliant on root exudates, which would, in theory, be lower during the non-growing season. That being said, there were outliers for fungal response at 30–60 cm during fall and winter where means were positive (Fig. 2), and significantly different from response ratios at 0–10 and 60–100 cm. This is rather interesting considering neither SOC, TN, nor microbial biomass were seen to increase in these OMR at this depth during this time of year (Mushinski et al., 2017a). A possible explanation is that fresh litter is shed during the fall leading to rapid decomposition and possible downward movement of dissolved organic matter (DOM) in OMR stands due to the smaller litter layer relative to the control. This DOM may accumulate in more compact or clay-rich soil layers below 30 cm leading to stimulation of fungal growth. Additionally, this litter-derived DOM may possess high concentrations of organic polymers and phenolic compounds which would differ from the simple compounds associated with root exudates and microbial turnover commonly found in summer and spring (Schadt et al., 2003), possibly stimulating a large scale shift in fungal community size and structure in OMR stands. Alternatively, this observation may be a function of our sampling capturing a microbial "hot-spot" (Kuzyakov and Blagodatskaya, 2015) where the environmental conditions favor high fungal growth and the drivers of these hot spots such as labile substrates are localized and not evident in the SOC or TN pool.

Implicitly, this long-term reduction in bacterial and fungal community size with increasing OMR may have direct consequences on soil biogeochemical processes. It has been previously shown that increasing OMR can lead to significant changes in the abundance of prokaryotic functional genes associated with C- and N-cycle processes (Mushinski et al., 2017b, 2018a, 2018b; Cardenas et al., 2018), and that fungal community composition as well as the relative abundance of fungal functional guilds can be altered by intensive OMR (Hartmann et al., 2009, 2012; Wilhelm et al., 2017; Mushinski et al., 2018b). Although it is difficult to infer specific functional differences from quantifying genes not associated with specific processes, especially considering that there are still many unknowns regarding the overlap of bacterial and fungal functionality in soil, it is possible that quantifying these groups can lead to generalizations regarding responses to disturbance. The observed decrease in the size of bacterial and fungal communities most likely indicates alteration in microbially-mediated ecosystem function, as demonstrated by previous work done at this and other sites with similar experimental designs.

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