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Forest harvest intensity and soil depth alter inorganic nitrogen pool sizes and ammonia oxidizer community composition

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ABSTRACT

Intensive forest harvest techniques have the potential to alter soil carbon and nutrient stocks and biogeochemical processes. We investigated how differing levels of organic matter removal (OMR) during timber harvest influenced the long-term stability of nitrification and the microbes regulating this process. Nitrification is limited by the activity of ammonia oxidizing bacteria (AOB) and archaea (AOA); however, reports on the relative contribution of each of these groups to forest soil nitrification have varied and have not been investigated in response to OMR. The influence of soil depth on the structure and function of the ammonia-oxidizing community has also been underreported and was included in this study. We quantified soil physicochemical properties including concentrations of ammonium (NH_4^+) and nitrite (NO_2^-) + nitrate (NO_3^-), and also coupled next generation sequencing and qPCR of the *amoA* gene to a whole-soil assay that stimulates nitrification and allows for the discrimination of AOA-from AOB-activity using 1-octyne, which inhibits bacterial ammonia monooxygenase activity. Soils were collected (1 m depth) from replicated loblolly pine (*Pinus taeda* L.) stands subjected to three different intensities of OMR (i.e., unharvested control, bole-only harvest, and whole-tree harvest + forest floor removal). Increasing intensity of OMR and increasing soil depth lead to significant reductions in concentrations of in situ NH_4^+ and NO_2^- + NO_3^-. Sequencing and subsequent annotation of the ammonia oxidizing community revealed that AOA were dominated by Crenarchaeota and AOB were dominated by *Nitrosospira* spp. The abundance of both bacterial and archaeal *amoA* were influenced by OMR and soil depth; furthermore, archaeal *amoA* was more abundant than bacterial *amoA* across all soil depths and the ratio of AOA to AOB increased with depth. Community structure of AOA and AOB were influenced by soil depth; however, only AOB were altered by OMR. Soil incubations revealed nitrification was N-limited in these forest soils. Furthermore, AOA- and AOB-contributions to total nitrification were nearly equivalent in surface soils; however, AOA contribution increased to 75% at 1 m. In general, the highest rates of nitrification occurred in the soils taken from unharvested control stands; however, OMR treatment differences were only significant when soils were amended with high levels of ammonia indicating that at ambient levels, intensive OMR may not lead to long-term alterations in nitrification potential.

1. Introduction

Coniferous forests of the southeastern USA comprise 9% of total North American forestlands (Oswalt et al., 2014) provide habitat for wildlife (Neu et al., 2014), contribute to carbon sequestration (Noormets et al., 2015), and provide economic output in the form of timber-related products (McNulty et al., 1996; Hodges et al., 2011; Brandeis et al., 2012). Recently there has been growing interest in utilizing intensive organic matter removal (OMR) techniques during timber harvest to increase economic output. Intensive techniques such as whole-tree harvest + forest floor removal result in the removal of all aboveground organic matter as well as forest byproducts such as downed woody debris, slash, sawdust, and forest litter. These byproducts have been utilized as substitute feedstocks in industrial processes, for bio-energy production, and sold as merchantable mulch (Janowiak and Webster, 2010; Dickens et al., 2012). Before being broadly adopted, the long-term

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biogeochemical consequences of these intensive OMR techniques should be investigated in order to determine if they are sustainable.

Nitrogen (N) is often the most limiting nutrient in terrestrial ecosystems (Binkley and Vitousek, 1989; Vitousek and Howarth, 1991; LeBauer and Treseder, 2008; Mitchell, 2011) and its availability is influenced by biogeochemical processes including plant-uptake, microbial-immobilization, ammonification, nitrification, and denitrification (Schlesinger and Bernhardt, 2013). Intensive OMR associated with timber harvest has been shown to impart decade-scale reductions in soil carbon (C) and nutrient stocks (Johnson and Curtis, 2001; Hazlett et al., 2014; Vario et al., 2014; Foote et al., 2015; Dean et al., 2017) and alter nutrient transformation rates (Yanai, 1998; Burns and Murdoch, 2005; Kreutzweiser et al., 2008; Wilhelm et al., 2013). It has been shown that shortly after harvest (i.e., 1 yr) soil nitrate (NO_3^-) can increase up to 8x pre-harvest conditions (Burns and Murdoch, 2005); however, the long-term effect of OMR on soil inorganic-N pool sizes and the processes that regulate these pool sizes has not been investigated, especially at soil depths that exceed 10–15 cm. Given that pine forest soils have large pools of root biomass and microbial biomass throughout the upper 1 m of the soil, there is a high potential for N-cycle activity deep in the profile (Mushinski et al., 2017). Considering that intensive OMR can result in significant long-term reductions in soil total nitrogen (TN) (Kellman et al., 2014; Achat et al., 2015a, 2015b), it is conceivable that inorganic-N stocks and process rates will follow suit. Furthermore, OMR-induced loss of inorganic-N may be exacerbated in the southeastern US where soils are often sandy, highly weathered, acidic, and possess a low cation exchange capacity.

Nitrification has been extensively studied because of the influence of inorganic-N pool size on plant productivity, soil fertility, water quality, and the release of greenhouse gases into the atmosphere. Ammonia (NH_3) oxidation to nitrite (NO_2^-), the initial step in nitrification is carried out by both chemolithoautotrophic ammonia-oxidizing archaea (AOA) and bacteria (AOB) (De Boer and Kowalchuk, 2001) and is considered rate limiting. Growing evidence suggests that AOA frequently outnumber AOB in a multitude of ecosystems (Leininger et al., 2006; Prosser and Nicol, 2008; Hatzenpichler, 2012; Norman and Barrett, 2014) indicating that AOA may contribute more to nitrification than AOB (Chen et al., 2008; Leininger et al., 2006; Prosser and Nicol, 2008); however, diverging reports have led to questions regarding the mechanisms controlling ammonia oxidizer niche differentiation (Yarwood et al., 2010; Hu et al., 2014). Many have suggested that nitrogen availability and pH are the major determinants of the abundance and functionality of AOA versus AOB (Offre et al., 2009; Stopnišek et al., 2010). Forest disturbances have been shown to affect the community composition of AOA and AOB through modifications of the aforementioned soil properties. Disturbances such as fire (Webster et al., 2005; Yeager et al., 2005; Ball et al., 2010; Tourna et al., 2010), tree girdling (Rasche et al., 2011), and forest clear-cutting (Hynes and Germida, 2012) have been investigated; however, the decade-scale influence of differing intensities of forest harvest on AOA and AOB community structure and function has not been investigated nor has the vertical distribution of ammonia oxidizers.

Although molecular methods have made it easier to determine the relative abundance and community structure of AOA and AOB, coupling functionality to community metrics has been difficult and often relies on gene expression methods. Recently, Taylor et al. (2013) described an assay for discriminating between AOA and AOB activities, which is based upon AOB ammonia oxidization being irreversibly inactivated by 1-octyne. This method has subsequently been applied to agricultural (Giguere et al., 2015) and forest systems (Lu et al., 2015). We utilized this method to link AOA and AOB community metrics to ammonia oxidation functionality in soil.

In this study, we attempt to determine the decade scale influence of OMR on inorganic-N stocks as well as the composition and potential activity of the ammonia oxidizing archaeal and bacterial communities in the upper 1 m of the soil profile in a southeastern US loblolly pine forest. We hypothesized that (i) increasing OMR intensity would impose significant reductions in inorganic-N resulting in altered community structure and abundance of AOA and AOB, (ii) AOA would constitute a significantly larger proportion of the ammonia oxidizing community as proxied by *amoA* gene copy number, (iii) AOA abundance would not be altered with soil depth while AOB *amoA* gene copy number would be reduced, (iv) AOA and AOB community composition would be altered by depth, and (v) rates of nitrification would be reduced by increasing intensity of OMR with AOA contributing a higher proportion to total nitrification potential than AOB.

2. Materials & methods

2.1. Study site description and experimental design

Field sampling was conducted in April 2015 at the Long-Term Soil Productivity (LTSP) site (Powers, 2006; Ponder et al., 2012) in Davy Crockett National Forest near Groveton, TX, USA (31°06′ 32.48″N, 95°09′ 59.15″W). The climate is subtropical with a mean annual temperature of 18.7 °C and mean annual precipitation of 1107 mm (1950–2010). Topography is relatively flat with slopes of 1–3% and elevation ranging from 101 to 110 m. Soil across the study area is a fine-loamy, siliceous, thermic Oxyaquic Glossudalf in the Kurth series which developed in loamy coastal plain sediments of the Yegua and Whitset geological formations (USDA/NRCS, 2003). The experimental design includes *Pinus taeda*-dominant unharvested control stands (tree age = 60–80 yrs), and two harvest treatments differing in the extent of organic matter removal. The harvest treatments consisted of low-intensity treatment, bole-only (BO) harvest, where only the bole of the tree was removed, and a high-intensity treatment, whole-tree harvest + forest floor removal (WT + FF), where the entire tree (bole, branches, leaves) was removed and the forest floor litter was removed by hand-raking. During harvest, trees were hand-felled and lifted off the plots with a loader to reduce soil compaction. Control and both harvest treatments were replicated 3X and each replicate was 0.2 ha. All plots are located within a 1.5 km radius. Treatment plots were harvested in 1996 and then replanted in 1997 with containerized *P. taeda* L. (loblolly pine) seedlings at 2.5 m × 2.5 m spacing.

2.2. Soil sampling

Soil cores were extracted with a JMC Environmentalist's Sub-Soil Probe PLUS (Clements Associates, Newton, IA, USA) (2.8 cm diameter x 120 cm depth). Cores were taken in both control and treatment plots 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. 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 6 g subsamples (3 sample^{-1}) were immediately stored at −80 °C for future DNA extraction. The remaining soil was stored at 4 °C for subsequent biogeochemical analysis.

2.3. Soil physicochemical analyses

Soil pH was analyzed using an Accumet Basic pH meter (Denver Instrument, Arvada, CO, USA) on a 1:2 solution of soil in a 0.01 M $CaCl_2$ solution (Minasny et al., 2011). Bulk soil was passed through a 2-mm sieve to remove large organic material and roots. A 25-g aliquot of sieved soil was dried at 60 °C for 48 h and subsequently pulverized. The pulverized soil was used to determine soil organic carbon (SOC) and total nitrogen (TN) concentration via combustion elemental analysis on a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ, USA).

Soil inorganic-N was extracted from 15 g of sieved, field moist, soil with 50 ml of 2 M KCl within 36-hrs of soil being taken from the ground. The soil + KCl solution was shaken for 1 h and then filtered over pre-leached (2 M KCl) #40 Whatman filter paper and analyzed immediately for concentrations of NH_4^+ and $NO_2^- + NO_3^-$ on a Seal Analytical AQ2+ Discrete Chemistry Analyzer (SEAL Analytical, Ltd., Southhampton, UK). Colorimetric-based chemistry for the determination of NH_4^+ was based on indophenol-blue chemistry, and determination of $NO_2^- + NO_3^-$ was based on cadmium-reduction and subsequent diazotization. To assess the availability of NH_3 for ammonia oxidizer consumption, pH-adjusted NH_3 levels were calculated as described by Norman and Barrett (2016).

2.4. Whole soil nitrification assay

A soil assay (Taylor et al., 2013) was used to measure total nitrification as well as the potential contributions of AOA and AOB to nitrification. Soil samples were incubated at three different NH_4^+ levels (equivalent to 3.5, 25, and 100 mg NH_4^+ kg^{-1} soil) achieved by adding sufficient anhydrous NH_3 gas to the headspace of a 125-ml Wheaton bottle fitted with a butyl stopper. NH_4^+ levels were verified by colorimetric analysis. The lowest level of NH_4^+ addition (3.5 mg NH_4^+ kg^{-1}) was selected because it represented the highest environmental level of NH_4^+ and was thereby the lowest possible normalized level for all samples. The moderate and high levels of NH_4^+ (i.e., 25 and 100 mg NH_4^+ kg^{-1}) were selected because they represented the initial stimulation of nitrification (i.e., 25 mg NH_4^+ kg^{-1}) and the maximum rate of nitrification (i.e., 100 mg NH_4^+ kg^{-1}) as evidenced by a preliminary experiment (Supplementary Figure 1). Prior to initiation of the experiment, soils were pre-incubated at 25 °C for 48-hrs to stimulate microbial activity. Three treatments were imposed to each sample at each NH_4^+ level: (i) acetylene amendment (6 μmol L^{-1}) to inhibit all autotrophic nitrification, (ii) 1-octyne amendment (4 μmol L^{-1}) to inhibit AOB nitrification, and (iii) positive control (no octyne or acetylene amendment) to determine autotrophic + heterotrophic nitrification. Following amendment, soil samples were incubated at 25 °C for 96 h. Subsequent $NO_2^- + NO_3^-$ concentrations were determined using a Seal Analytical AQ2+ Discrete Chemistry Analyzer (SEAL Analytical, Ltd., Southhampton, UK) as previously noted. Total chemoautotrophic nitrification rates were calculated after subtracting $NO_2^- + NO_3^-$ accumulation in the acetylene treatment and pre-incubation levels of $NO_2^- + NO_3^-$. Nitrification in the presence of 1-octyne (octyne-resistant) was attributed to AOA activity, with AOB activity (octyne-sensitive) calculated as the difference between total potential autotrophic nitrification and AOA potential nitrification.

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

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 (dry weight equivalent) 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 ammonia oxidizer communities was done by Molecular Research DNA Laboratory (www.mrdna.com, Shallowater, TX, USA) through target-based unidirectional amplification of the *amoA* gene with primers Arch amoA-1F (5′-STA ATG GTC TGG CTT AGA CG-3'; Francis et al., 2005) and Arch amoA-2R (5′- GCG GCC ATC CAT CTG TAT GT -3'; Francis et al., 2005) for AOA as well as amoA-1F (5′- GGG GTT TCT ACT GGT GGT -3'; Rotthauwe et al., 1997) and amoA-2R (5′- CCC CTC KGS AAA GCC TTC TTC -3'; Rotthauwe et al., 1997) for AOB. 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. PCR products were verified via gel electrophoresis (2% agarose gel). Samples were barcoded and subsequently pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified with calibrated AMPure XP beads (Agencourt Biosciences Co., Brea, CA, USA). The pooled and purified PCR products were then used to prepare an Illumina DNA library for each sample. Synthesis-based sequencing on an Illumina MiSeq followed the manufacturer's guidelines and resulted in single-end reads of 250.4 ± 25.9 (mean ± std. dev.) bp for AOA and 399.1 ± 135 bp for AOB.

2.6. Bioinformatic analysis

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 >1000 bp, barcode or primer sequence errors, and those with homopolymers or ambiguous base calls that exceed six nucleotides were discarded. Raw sequences were deposited in NCBI's sequence read achieves under the accession number SRR5218290. Operational taxonomic units (OTUs) were defined by clustering at 97% sequence identity using the QIIME implementation of UCLUST (Edgar, 2010). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

2.7. Quantification of amoA gene copy number

Quantitative-PCR (qPCR) targeting ammonia oxidizing bacteria and archaea were performed using primers amoA 1F/amoA 2R for bacteria (Rotthauwe et al., 1997) and Arch amoA 1F/Arch amoA 2R for archaea (Francis et al., 2005). The 25 μL reaction mixture contained 13 μL SYBR green real master mix (5Prime, Gaithersburg, MD), 0.5 μL of each primer (concentration 10 μM), 1 μL DNA template, and 10 μL molecular grade water. Each analysis run included a set of standards, negative controls, and replicated samples ($n = 3$) on a 96-well plate. For bacterial and archaeal *amoA*, the qPCR was run with the following conditions: 95 °C for 5 min; 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 1.5 min (30 cycles). All qPCR assays were performed using an Eppendorf Mastercycler® ep realplex thermal cycler (Eppendorf, Hamburg, Germany). qPCR products were length-verified via gel electrophoresis (2% agarose gel).

Amplification efficiencies of 89.7–99% were obtained for AOA and AOB, with r^2 values > 0.97.

AOB *amoA* standards were acquired from the lab of Raina Maier (Nelson et al., 2015). The archaeal *amoA* gene standard was prepared by amplifying soil DNA extracts using primers Arch amoA 1F/Arch amoA 2R (Francis et al., 2005). The PCR reaction followed the same conditions as listed above and produced 635 bp amplicons that were cloned using the TOPO® TA Cloning Kit (Life Technologies) with pCR™2.1-TOPO® vector and transformed into chemically competent *Escherichia coli* DH5α. The sequence of *amoA* clones was verified via sequencing with an ABI 2700 PCR sequencing system (IPGB, Texas A&M University). Copy numbers are reported as *amoA* gene copies g^{-1} dry-weight soil.

2.8. Statistical analysis

All statistical analyses on AOA and AOB communities were carried out using the sequence count within each OTU as an abundance value (Danzeisen et al., 2011). All datasets were tested for normality using Shapiro-Wilk's test. When data was not of normal distribution, non-parametric statistical tests or $\log_{10}$ transformations were applied. OTU data generated in QIIME were used to quantify the number of observed OTUs, richness, and diversity. Community metric calculations were analyzed using normalized sequence data set to 5352 reads for AOA and AOB. Unless otherwise noted, physicochemical properties, AO community metric estimates, and OTU abundance values were statistically analyzed using a linear mixed model ANOVA. Because of the inherent autocorrelation between differing soil depths, a split plot repeated measures statistical design was employed with OMR as the fixed main plot and soil depth designated as the fixed split plot (Derner et al., 2006). Soil depth was also designated as a repeated measure. Replicated plots were nested within harvest treatment and considered a random effect (Dai et al., 2006). When differences were significant, Tukey's honest significant difference (HSD) test was performed to assess *post hoc* contrasts with significance inferred at $p < 0.05$. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957) was performed on normalized OTU data. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using the Bray-Curtis matrix listed above was employed to characterize differences in AOA and AOB community structure based on OMR treatment and soil depth. PERMANOVAs were run using 999 permutations. Correlation analyses was performed using JMP (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Soil properties

Control stands possessed the highest mean SOC (10.1 ± 3.3 g C kg^{-1}) and TN (0.5 ± 0.1 g N kg^{-1}) concentration and the most acidic overall soil pH (3.5 ± 0.1) while the WT + FF stands possessed the lowest SOC (4.8 ± 1.5 g C kg^{-1}) and TN (0.3 ± 0.2 g N kg^{-1}) concentration and the least acidic soil pH (4.3 ± 0.1) (Table 1). BO stands fell between control and WT + FF stands in regards to SOC (6.4 ± 1.8 g C kg^{-1}), TN (0.4 ± 0.1 g N kg^{-1}), and soil pH (3.6 ± 0.1). Regardless of treatment, SOC and TN decreased with depth; however, soil pH was not different with depth. Extractable NH_4^+ and $NO_2^- + NO_3^-$ were lowest in WT + FF stands (NH_4^+: 1.0 ± 0.2 mg kg^{-1}; $NO_2^- + NO_3^-$: 0.3 ± <0.1 mg kg^{-1}) and highest in control stands (NH_4^+: 2.4 ± 0.2 mg kg^{-1}; $NO_2^- + NO_3^-$: 0.5 ± 0.1 mg kg^{-1}). Inorganic-N concentrations in BO stands were generally identical to the control stands (Table 1). Calculated NH_3 was statistically unaffected by harvest treatment, but decreased with increasing soil depth (0–10 cm: 13.9 ± 4.3 ng NH_3 kg^{-1}; 10–30 cm: 10.0 ± 2.2 ng NH_3 kg^{-1}; 30–60 cm: 10.3 ± 6.8 ng NH_3 kg^{-1}; 60–100 cm: 4.7 ± 1.3 ng NH_3 kg^{-1}). On average, NH_4^+ concentrations were 4.5x higher than $NO_2^- + NO_3^-$ concentrations across all treatments and depths. NH_4^+ accounted for 0.45% of TN while $NO_2^- + NO_3^-$ accounted for 0.10% of TN on a g kg^{-1} basis. Extractable NH_4^+ ($p < 0.001$) and $NO_2^- + NO_3^-$ ($p < 0.05$) decreased significantly with depth; however, $NO_2^- + NO_3^-$ was less affected by depth than NH_4^+. Soil pH was negatively correlated to concentrations of NH_4^+ and $NO_2^- + NO_3^-$ while SOC, TN, NH_4^+, and $NO_2^- + NO_3^-$ were all significantly positively correlated with each other (Table 2).

3.2. AOA and AOB community composition

Sequencing revealed that richness ($p < 0.001$) and diversity ($p < 0.001$) metrics were statistically higher for AOA (Chao1 Richness: 2186 ± 120; Simpson's Diversity: 0.92 ± < 0.1) than AOB (Chao1 Richness: 365 ± 24; Simpson's Diversity: 0.79 ± < 0.1) regardless of harvest treatment or soil depth. Counts of OTUs in AOA libraries were statistically lower in control treatment stands (435 ± 30 OTUs) than BO (537 ± 20 OTUs) and WT + FF treatment stands (528 ± 31), but did not vary with soil depth (Table 3). AOA and AOB OTU richness (Chao1) were statistically unaffected by treatment and depth; however, AOB OTU richness showed a general increase with increasing OMR and a reduction with depth. Furthermore, AOB richness was positively correlated to NH_3 ($R = 0.47$; $p < 0.01$). Simpson's diversity for AOA and AOB generally increased with increasing OMR and AOA diversity was negatively correlated to increasing concentrations of SOC ($R = -0.45$; $p < 0.01$), TN ($R = -0.48$; $p < 0.01$), and $NO_2^- + NO_3^-$ ($R = -0.38$; $p < 0.05$) (Table 2). AOB diversity was unaffected by depth; however, AOA diversity was significantly higher at depth (0–10 cm: 0.89 ± 0.02; 10–30 cm: 0.93 ± 0.01; 30–60 cm: 0.94 ± 0.01; 60–100 cm: 0.92 ± 0.01).

Phylum-level annotation of OTUs revealed that the ammonia oxidizing community was dominated by Crenarchaeota (>90% of all AOA sequences) and Thaumarchaeota (>9% of all AOA sequences) for AOA and Proteobacteria (>83% of all AOB sequences) for AOB (Supplementary Figure 2). The majority of AOA sequences were annotated to Crenarchaeota spp. (representing > 64% of all AOA sequences) and AOB sequences were in the *Nitrosospira* lineages (representing >31% of all AOB sequences). Harvest intensity did not alter the relative abundance of AOA phyla; however, increasing depth did lead to significant decreases in OTUs annotated to the phylum Crenarchaeota ($p < 0.01$) and significant increases in Thaumarchaeota ($p < 0.01$). Likewise, the relative abundance of AOB OTUs annotated at the phylum-level illustrated no response to harvest treatment; however, OTUs annotated as Proteobacteria did significantly decrease with increasing depth ($p < 0.05$).

Non-metric multidimensional scaling (NMDS) plots, based on Bray-Curtis distance matrices, of OTUs resulted in no statistical separation for AOA based on harvest treatments; however, AOB community composition was significantly affected by treatment ($p < 0.05$) (Fig. 1). AOA ($p < 0.01$) and AOB ($p < 0.001$) community composition was statistically altered by soil depth with unique clustering when soil depth was analyzed independent of OMR treatments; specifically, for AOA the 60–100 cm depth was statistically separated from the other 3 depths ($p < 0.01$), while for AOB the 0–10 cm and 10–30 cm increments were statistically different than the 30–60 and 60–100 cm increments ($p < 0.05$).

3.3. AOA and AOB amoA gene abundance

AOA *amoA* copy numbers (5.7 ± <0.01 $\log_{10}$ *amoA* copies) were

Table 1
Edaphic parameters of the three organic matter removal treatments for each of the four soil depth increments *Post hoc* contrasts (Tukey-Kramer) were computed on values for each depth nested within each treatment and indicated by differing letters within each column. For each soil depth in each treatment, n = 3. Statistical differences were inferred at $p < 0.05$. SOC: soil organic carbon, TN: soil total nitrogen.

Soil Depth (cm)	SOC (g kg^{-1} soil)	TN	Soil pH	NH_4^+	$NO_2^- + NO_3^-$	Calculated NH_3 (ng N kg^{-1} soil)
				(mg N kg^{-1} soil)		
Unharvested Control						
0–10	27.6 (5.1)a	1.1 (0.2)a	3.3 (0.2)a	3.4 (0.2)a	0.7 (0.1)a	4.7 (2.1)a
10–30	7.2 (1.3)bc	0.5 (0.2)bcd	3.6 (0.1)ab	2.7 (0.4)ab	0.5 (<0.1)abc	6.4 (0.5)a
30–60	3.3 (0.6)cd	0.3 (0.1)de	3.5 (0.2)ab	1.8 (0.3)bcd	0.4 (<0.1)bcde	4.3 (2.0)a
60–100	2.4 (0.1)d	0.3 (0.1)cde	3.4 (0.3)a	1.9 (0.3)bcd	0.3 (0.1)cde	3.5 (1.9)a
Bole-only Harvest						
0–10	16.5 (2.1)a	0.8 (0.1)ab	4.2 (0.1)ab	3.1 (0.4)a	0.6 (0.1)ab	26.9 (7.9)a
10–30	4.1 (0.3)cd	0.3 (<0.1)cde	3.7 (0.3)ab	2.8 (0.5)ab	0.5 (0.1)bcd	9.7 (3.8)a
30–60	2.6 (0.7)d	0.3 (0.1)cde	3.4 (0.2)a	2.4 (0.4)abc	0.4 (0.1)bcd	3.5 (1.2)a
60–100	2.4 (0.3)d	0.3 (<0.1)cde	3.3 (0.2)a	1.4 (0.4)cde	0.5 (0.1)bcd	1.6 (0.9)a
WT Harvest + FF Removal						
0–10	12.9 (1.3)ab	0.6 (<0.1)abc	4.0 (0.3)ab	1.5 (0.3)cd	0.4 (0.1)bcde	10.3 (5.0)a
10–30	4.0 (0.8)cd	0.2 (<0.1)de	4.4 (0.1)ab	1.1 (0.4)de	0.3 (0.1)cde	14.0 (5.2)a
30–60	1.2 (0.4)e	0.2 (<0.1)e	4.3 (0.4)ab	0.8 (0.2)de	0.2 (0.1)de	23.2 (20.4)a
60–100	1.3 (0.7)e	0.2 (0.1)e	4.7 (0.1)b	0.4 (0.1)e	0.2 (0.1)e	8.9 (1.3)a

Table 2
Spearman's ranked correlation analysis between soil physicochemical and biological properties. Bold values indicate significance with level of significance inferred by superscript symbol. AOA: ammonia oxidizing archaea, AOB: ammonia oxidizing bacteria. $^*p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

		SOC	TN	Soil pH	Calculated	Environmental	Environmental	*amoA* copy No.		Unique OTUs		Chao 1		Simpson's Index	
					NH_3	NH_4^+	$NO_2^- + NO_3^-$	AOA	AOB	AOA	AOB	AOA	AOB	AOA	AOB
	SOC														
	TN	**0.82**‡													
	Soil pH	−0.12	−0.30												
Calculated	NH_3	0.20	−0.04	**0.84**‡											
Environmental	NH_4^+	**0.57**‡	**0.52**‡	**−0.47**†	0.02										
Environmental	$NO_2^- + NO_3^-$	**0.62**‡	**0.55**‡	**−0.53**‡	−0.21	**0.68**‡									
amoA Copy No.	AOA	0.30	0.27	**−0.56**‡	−0.31	**0.59**‡	**0.55**‡								
	AOB	**0.66**‡	**0.45**†	−0.13	0.16	**0.53**‡	**0.55**‡	0.19							
No. Unique OTUs	AOA	−0.18	−0.17	0.03	0.12	−0.14	−0.10	−0.11	−0.08						
	AOB	0.20	0.06	**0.38***	**0.41***	−0.02	0.13	−0.31	0.32	−0.01					
Chao 1	AOA	0.02	0.09	−0.08	0.24	0.06	0.16	−0.07	0.21	**0.52**‡	−0.13				
	AOB	0.28	0.18	**0.39***	**0.47**†	0.01	**0.13**	−0.29	0.31	−0.01	**0.96**‡	−0.08			
Simpson's Index	AOA	**−0.45**†	**−0.48**†	0.27	0.17	−0.27	**−0.38***	−0.32	0.08	−0.05	**0.77**‡	−0.23	**0.74**‡		
	AOB	0.09	−0.02	0.27	0.23	−0.12	0.01	−0.32	**−0.38***	−0.03	−0.08	0.05	−0.14	−0.05	

Table 3
Summary of operational taxonomic units (OTUs), and their diversity and richness estimates. Datasets were normalized by setting each sample to 5352 sequences per sample. OTUs were defined as sequences sharing ≥97% similarity and served as the basis for number of unique OTUs, Chao1 richness estimate, and Simpson's Diversity Index. For each soil depth in each treatment, n = 3; for mean values per treatment, n = 12. AOA: ammonia oxidizing archaea, AOB: ammonia oxidizing bacteria. Mean values are bolded.

Soil Depth (cm)	No. Unique OTUs		Chao1 Richness Estimate		Simpson's Diversity Index	
	AOA	AOB	AOA	AOB	AOA	AOB
Unharvested Control						
0–10	470 (27)	248 (52)	1718 (415)	424 (29)	0.84 (0.03)	0.69 (0.2)
10–30	494 (15)	219 (79)	2644 (398)	343 (110)	0.91 (0.02)	0.87 (0.03)
30–60	452 (55)	162 (41)	2429 (250)	282 (70)	0.92 (0.03)	0.74 (0.1)
60–100	326 (86)	120 (9)	2079 (102)	224 (12)	0.89 (0.03)	0.66 (0.1)
Mean	**435 (30)**	**188 (27)**	**2218 (172)**	**318 (36)**	**0.89 (0.02)**	**0.74 (0.1)**
Bole-only Harvest						
0–10	456 (39)	262 (44)	2470 (176)	473 (81)	0.92 (0.01)	0.74 (0.1)
10–30	574 (46)	166 (24)	1185 (713)	300 (59)	0.93 (0.01)	0.76 (0.04)
30–60	574 (4)	239 (25)	2739 (668)	404 (70)	0.95 (0.01)	0.85 (0.04)
60–100	546 (29)	127 (33)	2420 (350)	251 (75)	0.94 (0.01)	0.64 (0.2)
Mean	**537 (20)**	**199 (22)**	**2203 (268)**	**357 (40)**	**0.94 (0.01)**	**0.75 (0.1)**
WT Harvest + FF Removal						
0–10	480 (72)	321 (14)	2165 (289)	549 (40)	0.88 (0.03)	0.95 (<0.01)
10–30	619 (79)	308 (83)	2646 (274)	497 (116)	0.94 (0.02)	0.84 (0.1)
30–60	546 (47)	201 (57)	1799 (314)	335 (88)	0.95 (0.01)	0.84 (0.1)
60–100	465 (21)	174 (21)	1941 (574)	295 (26)	0.93 (0.02)	0.85 (0.02)
Mean	**528 (31)**	**251 (29)**	**2138 (190)**	**419 (46)**	**0.92 (0.01)**	**0.87 (0.03)**

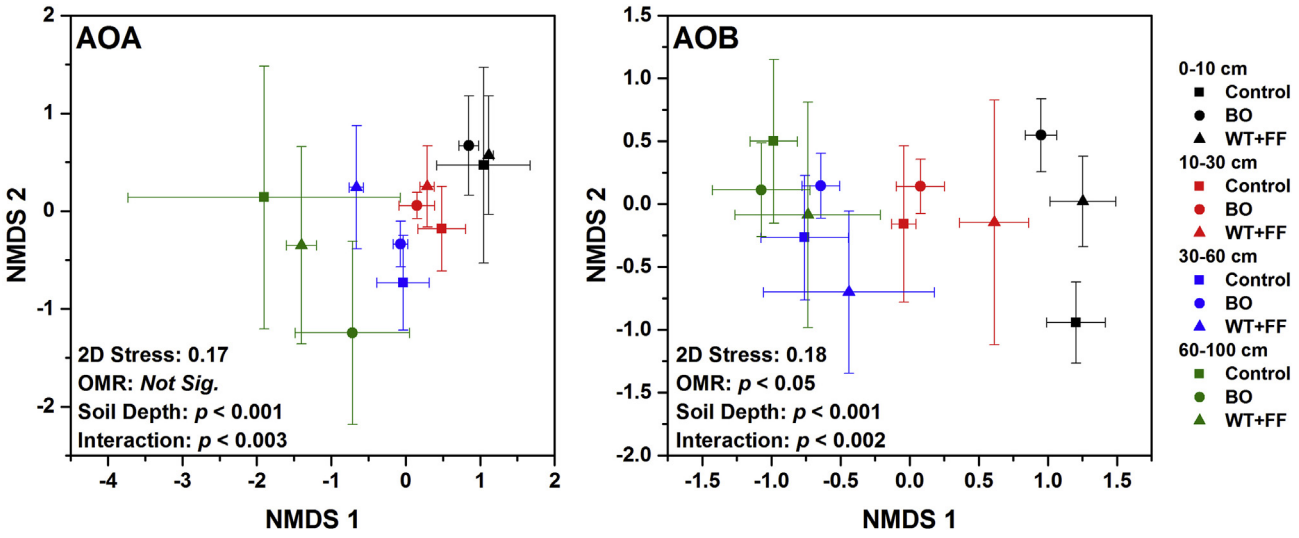

Fig. 1. Nonmetric multidimensional scaling (NMDS) ordinations of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) communities based upon their OTU composition derived from Bray-Curtis distances matrices. Each point and corresponding bars represent mean ± standard deviation (n = 3). Statistical differences in organic matter removal, soil depth, and their interaction were obtained using PERMANOVA. Control: unharvested control, BO: bole-only harvest, WT + FF: whole-tree harvest + forest floor removal.

significantly higher than AOB *amoA* (4.2 ± 0.01 $\log_{10}$ *amoA* copies) regardless of harvest treatment or depth (Fig. 2). Increasing harvest treatment significantly reduced AOA *amoA* ($p < 0.01$) and AOB *amoA* ($p < 0.05$) copy numbers. Specifically, control treatment stands possessed a statistically larger amount of AOA (5.8 ± <0.1 $\log_{10}$ *amoA* copies) and AOB (4.3 ± 0.2 $\log_{10}$ *amoA* copies) *amoA* copies than BO (AOA: 5.7 ± <0.1 $\log_{10}$ *amoA* copies; AOB: 4.0 ± <0.1 $\log_{10}$ *amoA* copies) and WT + FF (AOA: 5.6 ± <0.1 $\log_{10}$ *amoA* copies; AOB: 3.9 ± <0.1 $\log_{10}$ *amoA* copies) stands. Soil depth drove significant linear reductions in copies of AOB *amoA* but not AOA *amoA*. The ratio of AOA:AOB *amoA* copy number ranged from 3.6 to 356 and did not vary by treatment; however, this ratio significantly increased linearly with depth ($p = 0.001$) (Fig. 2). Copy number of AOA and AOB *amoA* were significantly positively correlated to concentrations of NH_4^+ (AOA: $R = 0.59$, $p < 0.001$; AOB: $R = 0.53$, $p < 0.001$) and $NO_2^- + NO_3^-$ (AOA: $R = 0.55$, $p < 0.001$; AOB: $R = 0.55$, $p < 0.001$) (Table 2). Congruently, AOA *amoA* was negatively correlated to soil pH ($R = -0.56$, $p < 0.001$) and AOB *amoA* was positively correlated to concentrations of SOC ($R = 0.66$, $p < 0.001$) and TN ($R = 0.45$, $p < 0.01$).

3.4. Whole-soil nitrification assay

Incubation in the presence of acetylene led to no significant accumulation of $NO_2^- + NO_3^-$ from soils at the Groveton-LTSP indicating that heterotrophic nitrification is not a major process in these soils. The small amount of $NO_2^- + NO_3^-$ that did accumulate (<0.1 mg N kg^{-1} soil) was subsequently subtracted before calculating potential autotrophic nitrification rates. Following incubation, accumulation of $NO_2^- + NO_3^-$ was detected for every sample. Regardless of harvest treatment or depth, total rates of $NO_2^- + NO_3^-$ accumulation were highest when soil was amended to 100 mg NH_4^+

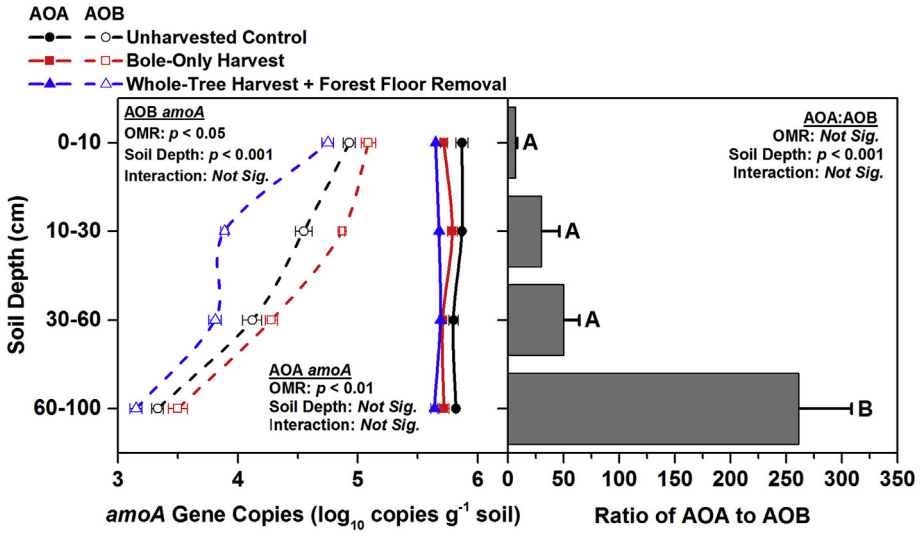

Fig. 2. Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) *amoA* quantification based on organic matter removal (OMR) treatment and soil depth as well as ratios of AOA to AOB *amoA* gene copies in response to soils depth.

kg^{-1} soil which was four-fold higher than the 25 mg NH_4^+ kg^{-1} normalization level ($p < 0.001$) and seven-fold higher than the 3.5 mg NH_4^+ kg^{-1} normalization level ($p < 0.001$) (Fig. 3). Total nitrification potential in the presence of 3.5 mg NH_4^+ kg^{-1} was statistically unaffected by harvest treatment, but significantly decreased linearly from 1.05 ± 0.07 mg NO_2^- + NO_3^- kg^{-1} day^{-1} at 0–10 cm to 0.81 ± 0.06 mg NO_2^- + NO_3^- kg^{-1} day^{-1} at 60–100 cm ($p < 0.001$). Significant total nitrification rate differences for harvest treatment and depth were observed for soils amended with 25 mg NH_4^+ kg^{-1} (harvest treatment: $p < 0.001$; depth: $p < 0.001$) and 100 mg NH_4^+ kg^{-1} (harvest treatment: $p < 0.001$; depth: $p < 0.001$). For both the 25 and 100 mg NH_4^+ kg^{-1} normalization levels, the control treatment (25 mg NH_4^+ kg^{-1}: 2.37 ± 0.05 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 8.23 ± 0.08 mg NO_2^- + NO_3^- kg^{-1} day^{-1}) possessed the highest accumulation rate which was significantly higher than the BO treatment (25 mg NH_4^+ kg^{-1}: 1.65 ± 0.05 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 6.90 ± 0.06 mg NO_2^- + NO_3^- kg^{-1} day^{-1}) and the WT + FF treatment (25 mg NH_4^+ kg^{-1}: 0.38 ± 0.04 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 4.32 ± 0.06 mg NO_2^- + NO_3^- kg^{-1} day^{-1}). As was observed for soils amended with 3.5 mg NH_4^+ kg^{-1}, total NO_2^- + NO_3^- accumulation decreased linearly for both the 25 and 100 mg NH_4^+ kg^{-1} normalization levels.

Potential nitrification rates in the presence of 1-octyne (octyne-resistant, AOA) statistically varied by treatment (3.5 mg NH_4^+ kg^{-1}: $p < 0.05$; 25 mg NH_4^+ kg^{-1}: $p < 0.001$; 100 mg NH_4^+ kg^{-1}: $p < 0.001$); however, only the 25 mg NH_4^+ kg^{-1} ($p < 0.01$) and the 100 mg NH_4^+ kg-1 ($p < 0.001$) varied by depth (Fig. 4). Specifically, 1-octyne-resistant (AOA) nitrification potential was highest in the control treatment (3.5 mg NH_4^+ kg^{-1}: 0.66 ± 0.03 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 25 mg NH_4^+ kg^{-1}: 1.49 ± 0.06 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 5.42 ± 0.23 mg NO_2^- + NO_3^- kg^{-1} day^{-1}) which

	NH_4^+ Normalized Level (mg NH_4^+ kg^{-1} soil)		
	3.5	25	100
	F-Ratio		
OMR	3.5	**224.5*****	**882.4*****
Soil Depth	**13.6*****	**14.8*****	**63.3*****
Interaction	2.1	2.6	2.2

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

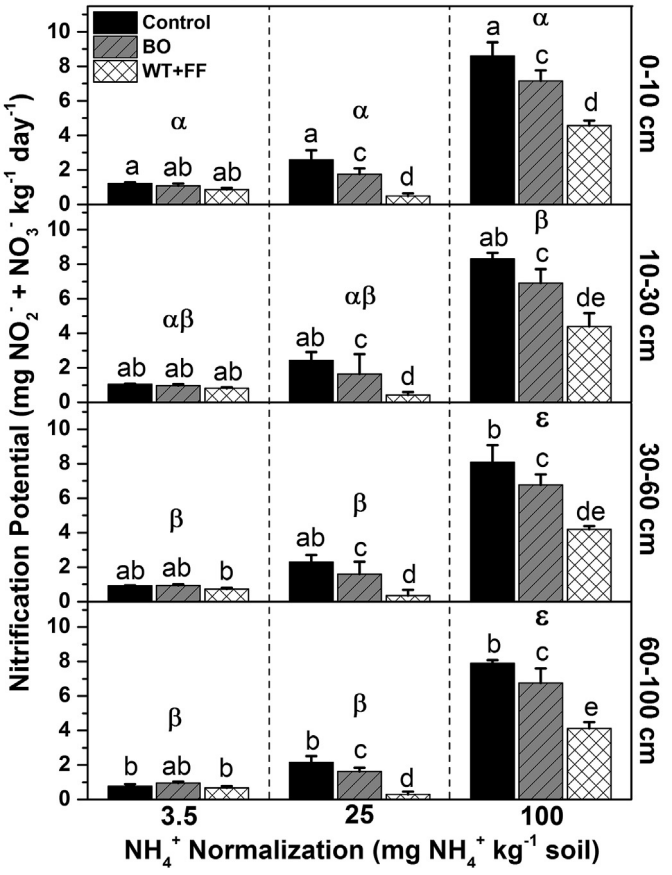

Fig. 3. Potential total nitrification rates in response to organic matter removal (OMR) and differing levels of NH_4^+ addition within each soil depth. Data are means ± standard error (n = 3). A repeated measures ANOVA table shows the statistical significance of OMR, soil depth, and their interaction for each NH_4^+ normalization level (i.e., 3.5, 25, 100 mg NH_4^+ kg^{-1} soil). *Post hoc* contrasts (Tukey-Kramer) were computed on values for each treatment within a depth profile (0–100 cm) for each NH_4^+ amendment level and significance is indicated by different letters above bars. *Post hoc* contrasts were also calculated for mean soil depth values and significance is indicated by different Greek letters in each box. Control: unharvested control, BO: bole-only harvest, WT + FF: whole-tree harvest + forest floor removal.

	Octyne-Resistant (AOA)			Octyne-Sensitive (AOB)		
	3.5	25	100	3.5	25	100
	F-Ratio					
OMR	**6.4***	**228.1*****	**97.2*****	0.5	**32.7*****	**35.6*****
Soil Depth	0.3	**7.0****	**18.8*****	**44.4*****	**69.6*****	**33.5*****
Interaction	1.6	2.0	**3.5***	1.0	**7.3*****	**2.9***

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

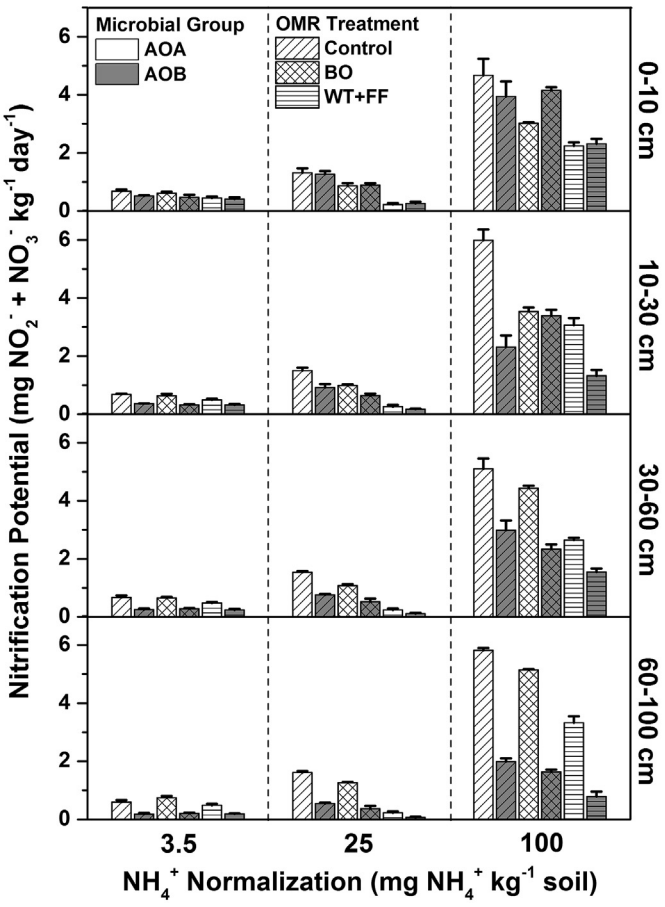

Fig. 4. Potential octyne-resistant (ammonia-oxidizing archaea, AOA) and octyne-sensitive (ammonia-oxidizing bacteria, AOB) nitrification rates in whole soil assays amended with differing levels of NH_4^+. Data are means ± standard error (n = 3). A repeated measures ANOVA table shows the statistical significance of organic matter removal (OMR), soil depth and their interaction for each NH_4^+ normalization level (i.e., 3.5, 25, 100 mg NH_4^+ kg^{-1} soil). Control: unharvested control, BO: bole-only harvest, WT + FF: whole-tree harvest + forest floor removal. AOA: ammonia oxidizing archaea, AOB: ammonia oxidizing bacteria.

was significantly higher than the BO treatment (3.5 mg NH_4^+ kg^{-1}: 0.66 ± 0.03 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 25 mg NH_4^+ kg^{-1}: 1.05 ± 0.05 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 4.03 ± 0.25 mg NO_2^- + NO_3^- kg^{-1} day^{-1}) and the WT + FF treatment (3.5 mg NH_4^+ kg^{-1}: 0.48 ± 0.02 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 25 mg NH_4^+ kg^{-1}: 0.24 ± 0.02 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 2.82 ± 0.15 mg NO_2^- + NO_3^- kg^{-1} day^{-1}); furthermore, the BO treatment had significantly more NO_2^- + NO_3^- accumulation than the WT + FF treatment ($p < 0.001$). In contrast, octyne-sensitive (AOB) nitrification was statistically unaffected by harvest treatment for the 3.5 mg NH_4^+ kg^{-1} normalization level; however, the 25 ($p < 0.01$) and 100 ($p < 0.001$) mg NH_4^+ kg^{-1} normalization levels were both statistically affected, with the control (25 mg NH_4^+ kg^{-1}: 0.87 ± 0.09 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 2.80 ± 0.28 mg NO_2^- + NO_3^- kg^{-1} day^{-1}) and BO (25 mg NH_4^+ kg^{-1}: 0.60 ± 0.07 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 2.87 ± 0.30 mg NO_2^- + NO_3^- kg^{-1} day^{-1}) treatments reporting higher rates of potential nitrification than the WT + FF treatment (25 mg NH_4^+ kg^{-1}: 0.15 ± 0.03 mg NO_2^- + NO_3^- kg^{-1} day^{-1}; 100 mg NH_4^+ kg^{-1}: 1.50 ± 0.18 mg NO_2^- + NO_3^- kg^{-1} day^{-1}). Octyne-sensitive (AOB) nitrification was significantly reduced with increasing soil depth for all levels of NH_4^+ normalization (3.5 mg NH_4^+ kg^{-1}: $p < 0.001$; 25 mg NH_4^+ kg^{-1}: $p < 0.001$; 100 mg NH_4^+ kg^{-1}: $p < 0.001$).

Unlike total nitrification, octyne-resistant (AOA) accumulation of NO_2^- + NO_3^- increased linearly with depth for the 25 mg NH_4^+ kg^{-1} ($p < 0.01$) and 100 mg NH_4^+ kg^{-1} ($p < 0.001$) normalization levels. Regardless of harvest treatment, the proportion of total nitrification attributed to octyne-resistant (AOA) activity at 0–10 cm decreased with increasing concentrations of NH_4^+ from 58% at 3.5 mg NH_4^+ kg^{-1} to 49% at 100 mg NH_4^+ kg^{-1}. Increasing depth resulted in an overall linear increase in the proportion of nitrification attributed to AOA activity. Regardless of treatment or NH_4^+ normalization level, AOA contributed from 51% at 0–10 cm to 76% at 60–100 cm. Octyne-resistant and -sensitive nitrification was significantly correlated to multiple biological and edaphic properties for each NH_4^+ normalization level. Specifically, octyne-resistant (AOA) nitrification was negatively correlated to soil pH and calculated NH_3 levels and positively correlated to AOA *amoA* copy numbers; congruently, octyne-sensitive (AOB) nitrification was positively correlated to SOC, TN, and AOB *amoA* levels (Table 4).

4. Discussion

4.1. Soil carbon and nitrogen concentrations

In this study we illustrate how differing intensities of OMR associated with timber harvest can influence concentrations of SOC, TN, and inorganic N as well as community structure, distribution, abundance, and activity of AOA and AOB. We found that intensive forest harvest led to decade-scale reductions in concentrations of SOC and TN which is consistent with what has been observed previously in surface soils at this site (Foote et al., 2015) and other sites (Johnson and Curtis, 2001; Li et al., 2003; Nave et al., 2010; Jones et al., 2011; Huang et al., 2013; Achat et al., 2015a, 2015b). However, our results indicate that forest harvest-induced losses can occur throughout the upper 1 m of the profile and persist for decades. Furthermore, concentrations of soil NH_4^+ and NO_2^- + NO_3^- were also significantly altered by increasing harvest intensity; specifically, the WT + FF treatment resulted in significantly lower concentrations of soil inorganic-N than the control and BO treatments. It is likely that lower concentrations of inorganic-N in the more severe organic matter removal treatment are at least in part attributable to higher rates of N-losses. For example, changes in microclimate conditions following harvest such as increases in solar radiation reaching the soil surface, decreases in transpiration and rainfall interception, and increases in the amount of precipitation reaching and infiltrating the forest floor and into the soil would favor higher rates of leaching (Vitousek et al., 1997; Carlyle et al., 1998; Holmes and Zak, 1999; Marchman et al., 2015) and denitrification (Brumme, 1995) following treatment application. Sustained reductions in inorganic-N may be attributable to reduced N-inputs (i.e., leaf litter, woody debris, root exudates, etc.) in the WT + FF treatment stands. To that point, the observed reduction in NH_4^+ and NO_2^- + NO_3^- concentrations were significantly correlated to the concurrent reduction of TN. Increasing soil depth lead to significant reduction in both NH_4^+ and NO_2^- + NO_3^-. This is consistent with most studies of inorganic-N in forest soils where ammonification typically decreases with depth (Liu et al., 2015; Tanner et al., 2016) and NO_2^- + NO_3^- concentrations decrease because of the mobility of NO_3^- in well drained soils.

4.2. amoA quantification

Both AOA and AOB *amoA* genes were detected in all treatments and at all depths. Based on *amoA* quantification, AOA were 95X more abundant than AOB throughout the soil profile which is consistent with what has been previously reported in arid ecosystems (Adair and Schwartz, 2008), agricultural plots (Nicol et al., 2008; Hai et al., 2009) and forest soils (Lu et al., 2015). AOB *amoA* gene abundance declined significantly with depth in all treatments while AOA *amoA* gene abundance remained relatively constant, resulting in a significantly larger AOA to AOB ratio at 60–100 cm. As postulated by Leininger et al. (2006), the high numbers of AOA (relative to AOB) at depth indicate that AOA are adapted to a broad range of growth conditions and may possess a more versatile

Table 4
Spearman's ranked correlation analysis between octyne-resistant (ammonia-oxidizing archaea, AOA) and −sensitive (ammonia-oxidizing bacteria, AOB) nitrification potential for each NH_4^+ addition level (3.5, 25, 100 mg NH_4^+ kg^{-1} soil) and select soil physicochemical and biological properties. Bold values indicate significance with level of significance inferred by superscript symbol. *p < 0.01, †p < 0.01, ‡p < 0.001.

	Nitrification Potential (mg NO_2^- + NO_3^- kg^{-1} day^{-1})					
	Octyne-Resistant (AOA)			Octyne Sensitive (AOB)		
	3.5	25	100	3.5	25	100
SOC	0.15	0.11	−0.03	**0.78‡**	**0.65‡**	**0.67‡**
TN	0.18	0.09	−0.01	**0.58‡**	**0.57‡**	**0.61‡**
Soil pH	**−0.68‡**	**−0.67‡**	**−0.58‡**	−0.05	**−0.51‡**	**−0.35***
Environmental (calculated) NH_3	**−0.47‡**	**−0.46†**	**−0.47†**	0.28	−0.10	0.09
Environmental NH_4^+	**0.51‡**	**0.48†**	0.31	**0.59‡**	**0.81‡**	**0.78‡**
Environmental NO_2^- + NO_3^-	**0.77‡**	**0.47†**	0.29	**0.70‡**	**0.80‡**	**0.66‡**
AOA *amoA* Copy No.	**0.58‡**	**0.76‡**	**0.65‡**	0.23	**0.73‡**	**0.53†**
AOB *amoA* Copy No.	0.19	0.06	−0.11	**0.68‡**	**0.58‡**	**0.67‡**

metabolism than AOB. Furthermore, the small cell size, small genome, and oligotrophic lifestyle associated with AOA (Hatzenpichler et al., 2008; Tourna et al., 2011; Hatzenpichler, 2012) may contribute to their high abundance deeper in the soil profile where energy sources are likely to be more limited. It has also been shown that some archaeal groups lack an enzyme homologous to hydroxylamine oxidoreductase (Schleper and Nicol, 2010) and therefore may oxidize NH_3 via a nitroxyl intermediate (Walker et al., 2010) instead of hydroxylamine as seen in AOB. This alternate nitroxyl pathway requires less oxygen than the hydroxylamine pathway, which may allow AOA to occur and function in soil horizons and microsites where oxygen concentrations may be low (Schleper and Nicol, 2010). Abundance of AOB *amoA* was significantly positively correlated to SOC and TN with the highest abundances being found in surface soils as well as control stands. It has been shown that AOB are likely to be more abundant in soils with high nutrient and substrate availability (Wessén et al., 2010; Rasche et al., 2011). Control and BO treatment stands possessed significantly higher AOA and AOB *amoA* gene copy numbers than WT + FF treatment stands which may be attributed to the harvest-induced differences in physicochemical soil properties. It has been hypothesized that soil pH drives niche differentiation of AOA and AOB (Nicol et al., 2008; Stempfhuber et al., 2014) with archaea being more competitive at low pH due to their high affinity for NH_3 (Martens-Habbena et al., 2009; Verhamme et al., 2011) and AOB's physiological inability to function at low pH (Frijlink et al., 1992; Gubry-Rangin et al., 2011; Zhang et al., 2012; Hu et al., 2014). Although it has been theorized that AOB are physiologically unable to oxidize NH_3 at pH < 5.5 (Hankinson and Schmidt, 1988; Jiang and Bakken, 1999) their presence in acidic soils is generally observed and leads to the hypothesis that they may contribute to nitrification in acidic soils, perhaps in less acidic microsites. AOA *amoA* abundance increased with increasing soil acidity while AOB *amoA* abundance was statistically unaffected; congruently, we observed the least acidic conditions in WT + FF treatment stands. From these results, we would expect the ratio of AOA:AOB would be lowest for the WT + FF treatment due to the less acidic pH; however, it is actually the highest. This suggests that harvest-induced reductions in SOC and TN are more detrimental to AOB abundance than concurrent increases in soil pH are to AOA abundance.

4.3. Community structure, diversity, and richness of ammonia oxidizers

Results show that AOB community structure is significantly different among the three OMR treatments with clear separation in the 0–10 cm increment. This indicates that the AOB community was less resistant and/or resilient to perturbation and has not yet recovered to pre-harvest conditions represented by the unharvested control, most likely because of reduced substrate availability. In contrast, AOA community structure was not statistically affected by harvest treatment, indicating community resistance and/or resilience to increasing harvest intensity. The lack of treatment differences for AOA is most likely related to their physiological ability to maintain functionality in nutrient-depleted conditions. This is similar to Pereira e Silva et al. (2012) who showed that ammonia oxidizer community structure can change in response to seasonal differences in substrate availability with AOB variability being higher than AOA. Regardless of harvest treatment, we observed distinct community composition clustering for both AOA and AOB in response to soil depth. This is similar to what was observed by Gan et al., 2015, who demonstrated that soil depth has more influence on AOA and AOB community structure than forest type.

We also observed that the diversity and richness of AOA OTUs were significantly higher than AOB regardless of OMR or soil depth which is similar to previous studies (Pester et al., 2012; Stahl and de la Torre, 2012). Although AOA richness was unaffected by depth, we did observe that OTU numbers were lowest in controls suggesting that disturbance promotes the cohabitation of AOA lineages with differing ecological strategies. Similar to what was observed with AOB *amoA* quantification, AOB OTU richness decreased with depth. Congruently, AOB OTU richness was positively correlated to calculated levels of NH_3. This implies that only select AOB lineages are able to function in soil horizons with lower resource levels. In contrast, AOA diversity increased with depth indicating that there is more AOA intraspecific competition when resources are low. This hypothesis is bolstered by the observation that AOA diversity was negatively correlated to SOC, TN, and $NO_2^- + NO_3^-$.

4.4. Total, AOA, and AOB nitrification potential

Potential total nitrification rates measured across all harvest treatments and soil depths fall within the range of 0.57–1.35 mg $NO_2^- + NO_3^-$ kg^{-1} soil day^{-1} (for soils amended with 3.5 kg NH_4^+ kg^{-1} soil) and 4.01–8.72 mg $NO_2^- + NO_3^-$ kg^{-1} soil day^{-1} (for soils amended with 100 kg NH_4^+ kg^{-1} soil). These values are consistent those observed previously in forest soils (Vitousek et al., 1982; Wertz et al., 2012; Lu et al., 2015). We found that both AOA and AOB activity was stimulated by the addition of anhydrous NH_3, indicating that both populations were N-limited in these soils. Although many studies dealing with acidic soils have reported the presence of AOB (Nicol et al., 2008; Gubry-Rangin et al., 2010; Stopnišek et al., 2010; Yao et al., 2011; Zhang et al., 2011; Lu et al., 2015), it has not been entirely clear if these populations are actively oxidizing NH_3. Similar to Lu et al. (2015), we observed that NH_4^+-stimulated activity was octyne sensitive in all treatments and at all depths indicating that AOB are potentially active in this system. Furthermore, regardless of harvest treatment, AOB contributed roughly 47% to total nitrification at 0–10 cm with 3.5 mg NH_4^+ kg^{-1}; however, this percentage increased to 51% contribution when 100 mg NH_4^+ kg^{-1} was applied. This indicates that AOB become more competitive when higher substrate concentration becomes available which is consistent with Giguere et al. (2015). AOB contribution decreased linearly to 23–25% at 60–100 cm which is similar to the trend that was observed with *amoA* gene copy number. Considering the acidity of this system it was surprising that AOB were not only functionally active but also contributed substantially to total nitrification. It is not entirely clear why the AOB contributed so greatly to nitrification in these soils; however, AOB might persist and be locally active in acidic forest soils via protective (less-acidic) aggregates and microsites as was maintained in this whole-soil assay. Slurry assays in which soil structure is lost have reported lower AOB contributions to nitrification than whole-soil assays (Lu et al., 2015). It is also possible that AOA phylotypes exist in the acidic forest soils that are more sensitive to inhibition by octyne, which could potentially alter the relative contributions of AOA and AOB to total nitrification (Lu et al., 2015).

Increasing OMR intensity did not result in significant potential total nitrification difference for the 3.5 mg NH_4^+ kg^{-1} normalization level; however differences were observed with higher levels of NH_4^+. This indicates that at lower NH_4^+ conditions (similar to what is observed in the environment), increasing OMR intensity may not lead to long-term changes in total rates of nitrification which is somewhat surprising considering that we observed significant treatment differences in both AOA and AOB *amoA* quantification; however, *amoA* quantification is only an estimate of potential activity. Coupling this nitrification assay to gene expression analysis may yield more accurate estimates of actual activity; however this was not done in this study because of the difficulty in extracting

quality mRNA from these acidic, humic soils. The observed treatment differences in total, octyne-resistant (AOA), and octyne-sensitive (AOB) nitrification at high levels of NH_4^+ may be an indication that high levels of AMO enzyme synthesis (mirroring *amoA* quantification levels) are activated only when substrate levels reach a certain threshold which exceeds environmental levels in this system. Contrary to total nitrification potential and AOB (octyne-sensitive) nitrification potential, AOA (octyne-resistant) nitrification was significantly reduced by increasing OMR intensity at 3.5 mg NH_4^+ kg^{-1}. Considering that AOA are dominant (especially at depth) in this system, increasing OMR intensity may be a solution to reducing total nitrification rates, and the subsequent loss of nitrogen from the soil system; however, the associated loss of total nitrogen with increasing OMR intensity most likely outweighs any positive contribution of reduced nitrification.

5. Conclusions

Our findings indicate that differing intensities of OMR associated with timber harvest can impart long-term reductions in concentrations of SOC, TN, NH_4^+, and $NO_2^- + NO_3^-$, and alter the abundance and community structure of AOA and AOB throughout the soil profile. The abundance of AOB *amoA* gene copy number was significantly positively correlated to SOC and TN, while AOA *amoA* was negatively correlated with soil pH indicating that the abundances of these two functional taxonomic groups are influenced by soil physicochemical properties which may be modified by different OMR treatments. Soil depth also strongly shapes AOA and AOB abundances and community composition, with an increasing ratio of AOA:AOB with increasing depth. Furthermore, total-, octyne-resistant (AOA), and octyne-sensitive (AOB) nitrification potential were all affected by increasing OMR intensity; however, only octyne-resistant (AOA) nitrification potential was significantly affected at low levels of NH_4^+ indicating that differing levels of OMR may not lead to significant differences in total rates of nitrification. Understanding the influence of differing intensities of OMR on key nitrogen cycle processes and the microbial communities that regulate those processes can yield important insights regarding the mechanism by which forest disturbance can modify biogeochemical cycling and influence forest productivity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.05.015.

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