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.

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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

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