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Relationship between ammonia oxidizing bacteria and bioavailable nitrogen in harvested forest soils of central Alberta

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ABSTRACT

Forest soils are commonly limited in nitrogen (N), and the removal of aboveground biomass in harvesting operations can exacerbate the problem. Thus, the soil organisms that facilitate the rate-limiting step in the N cycle, the oxidation of ammonium (NH_4^+), are of special interest in harvested environments. The objective of this study was to investigate the changes in ammonia oxidizing bacteria (AOB) communities that occurred in the years following clear cutting, and link those community shifts to availability of inorganic N forms NH_4^+ and nitrate (NO_3^-). Genetic fingerprinting targeting the *amoA* gene coupled with denaturing gel gradient electrophoresis was carried out over two summers on forest floor (LFH) and mineral (Ae) soils of three similar cutblocks harvested during different years. *In-situ* NH_4^+ and NO_3^- availability was measured over the growing seasons of 2009 and 2010, as well as a suite of physical soil characteristics. Results indicated that the AOB community composition differed in younger vs. older cutblocks, but not by soil horizon. The changes seen in the AOB paralleled the change in N bioavailability across sites, soil horizons, and sampling years, thus indicating that N bioavailability may be directly linked to AOB community composition. This link may provide the basis for the use of AOB as indicators of nutrient availability in the future.

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1. Introduction

Forest ecosystems are commonly limited in nitrogen (N) and the transformation of N from one form to the next is a tightly coupled cycle (Rennenberg et al., 2009). Nitrogen limitation is exacerbated in harvested soils, where not only has the N in the biomass itself been removed, but subsequent leaching, erosion, and increased N cycling rates deplete nutrient availability further (Chanasyk et al., 2003; Grigal, 2000). The rate-determining step in forest N cycles is often the oxidation of ammonia or ammonium (NH_4^+) to nitrite (Laverman et al., 2001), which is catalyzed by ammonia oxidizing bacteria (AOB) and archaea.

Chemolithotrophic AOB are aerobic, obligate autotrophs (Wrage et al., 2001) that use NH_4^+ as their sole source electron acceptor for respiration and carbon dioxide as their chief source of carbon (Kowalchuk and Stephen, 2003). They are responsible for the rate limiting step in nitrification in a wide variety of environments, thus they are crucial in the global N cycle (Kowalchuk et al., 1997). All AOB are categorized within the evolutionary lineage called *Proteobacteria*. Also known as purple bacteria, the *Proteobacteria* are separated into 5 subdivisions, two of which, β and γ, have AOB lineages. Within the β subclass, which is the terrestrial group and thus the relevant organisms in this study, there are two genera of ammonia oxidizers referred to as *Nitrosomonas* and *Nitrosospira* (Bäckman et al., 2004; Laverman et al., 2001). Both of these genera can be further subdivided into clusters that respond differently to environmental conditions such as pH, salinity, acidity and NH_4^+ availability (Kowalchuk et al., 1997, 2000; Laverman et al., 2001). Cluster groups 1, 2, and 4 of the *Nitrosospira* lineage are dominant in acidic forest soils with low NH_4^+ availability (Mintie et al., 2003), while cluster 3A have been identified in disturbed soils with high quantities of NH_4^+ (Boyle-Yarwood et al., 2008; Kowalchuk et al., 2000; Laverman et al., 2001; Mintie et al., 2003; Yeager et al., 2005).

Studies of forest soil AOB have identified a number of environmental factors that may influence AOB community composition. For example, in the only published study concerning AOB in harvested soils, Bäckman et al. (2004) found that AOB community composition shifts due to clear cutting. They concluded that the change in AOB community structure was driven by NH_4^+ availability and nitrification potential associated with harvesting disturbance. Irrespective of clear cutting disturbances, other forest soil studies have linked AOB community shifts to such environmental factors as NH_4^+ content (Hastings et al., 1997; Mintie et al., 2003), nitrification

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potential (Bäckman et al., 2004; Yeager et al., 2005), vegetation cover (Boyle-Yarwood et al., 2008; Nugroho et al., 2005), temperature (Avrahami and Conrad, 2005), changes in pH (Bäckman et al., 2003; Kowalchuk et al., 1997; Yeager et al., 2005), and C/N ratio (Nugroho et al., 2005). Contrastingly, Avrahami et al. (2002) concluded that AOB community composition did not respond to varying NH_4^+ concentrations and Laverman et al. (2005) found there was no relationship between AOB and nitrate (NO_3^-) or NH_4^+ production rates.

Molecular techniques offer a means of investigating AOB community composition. Polymerase chain reaction (PCR) coupled with denaturing gel gradient electrophoresis (DGGE) is one means of assessing community composition and does so by separating unique genetic sequences in a polyacrylamide gel based on nucleotide composition (Muyzer et al., 1993; Nicolaisen and Ramsing, 2002). In 1997, Rotthauwe and others developed a primer set to target a 491 bp fragment of the ammonia monooxygenase subunit A gene (*amoA*), thus enabling researchers to investigate bacteria responsible for NH_4^+ oxidation by targeting the enzyme that catalyzes the reaction, ammonia monooxygenase. This gene is present in all AOB, and is believed to contain enough information to make phylogenetic inferences based on its sequence (Nicolaisen and Ramsing, 2002; Rotthauwe et al., 1997).

Nitrogen limitations represent a challenge for the long-term productivity of managed forests and the response of AOB, the organisms catalyzing the rate limiting step in the N cycle, is scarcely understood. Increasing understanding of this functional group of bacteria may help characterize and delineate N deficiencies in the environment (Yeager et al., 2005). With this in mind, the objective of this study was to investigate the impact of clear cutting on AOB community composition using a chronosequential approach. The secondary objective was to identify whether N bioavailability could be linked with shifts in AOB community composition in these disturbed soils.

Soil from the forest floor (LFH) and the top 5 cm of the mineral horizon (Ae) were analyzed to account for the zones a) most rich in organic matter and bioavailable nutrients (Neville et al., 2002), and b) the most densely concentrated rooting zone (Strong and La Roi, 1985). Ammonia oxidizing bacteria community composition was determined using PCR-DGGE of the *amoA* primer set (Rotthauwe et al., 1997). To draw connections between changes in AOB and changes in N bioavailability, *in-situ* NO_3^- and NH_4^+ was measured, as were general soil characteristics. The response of AOB community composition, bioavailability of N, and general soil characteristics were assessed at three differently aged cutblocks of the Boreal Plain, Alberta.

2. Materials and methods

2.1. Site description

The study site was located in central Alberta (54°03′ N, 115°84′ W; 782 masl) on the Boreal Plain, 220 km northwest of Edmonton, Alberta. The region is underlain by Cretaceous and Tertiary sandstones, shales, clays and gravels ranging from <15 to >150 m thick (Pawlowski and Fenton, 1995). The predominant soil type is Orthic Gray Luvisol, which typically exhibits an organic LFH layer, an Ae horizon leached of clay, and a Bt horizon enriched in clay from the above Ae horizon. These Luvisols are relatively rich in phosphorus (P) and calcium (Ca) because of high apatite and Ca content of the parent material (Smith et al., 2003). The predominant trees in the area are lodgepole pine (*Pinus contorta*), jack pine (*Pinus banksiana*), white spruce (*Picea glauca*), black spruce (*Picea mariana*), balsam poplar (*Populus balsamifera*) and trembling aspen (*Populus tremuloides*) (Smith et al., 2003). The region experiences mean summer and winter temperatures of around 10 °C and −15 °C, respectively, and an average of 500–600 mm of precipitation annually (Environment Canada, 2000; Strong and Leggat, 1992).

Three cutblocks were established as sampling sites based on consistent soil type (Orthic Gray Luvisol) and vegetation cover of >60% lodgepole pine before harvesting. One cutblock was harvested in 2007 (HY, 2007), the second in 2005 (HY, 2005), and the third in 1991 (HY, 1991). All three cutblocks were clear cut in January (woody debris left onsite) to minimize soil compaction and the two more recently harvested sites (HY, 2005 and HY, 2007) were treated with glyphosate herbicide (Vision, Monsanto Canada Inc.) at a rate of 6 L per hectare during the first growing season after harvesting. Lodgepole pine seedling plugs were replanted in the first year after harvesting at each site. The slope of the sites were 15, 16, and 5%, respectively from youngest to oldest, and the average LFH thickness at each site in 2009 was 8, 5, and 9 cm listed in the same respective order.

2.2. Soil sampling methodology

Cutblocks were divided into a systematic sampling plan and sampled in June 2009 and 2010. Each cutblock was separated into 3 transects at least 90 m in length, with at least 15 m between each transect. Within a transect three sample plots (roughly a 5 m rectangle) were sampled in four randomly chosen subsamples and composited. Soil was collected using a JMC Backsaver probe (Clements Assoc. Inc, Newton, IA) with a 3.2-cm diameter tip. Samples were gathered from the LFH and Ae at each sample location for a total of 54 samples per year (i.e., 3 plots × 3 transects × 2 horizons × 3 cutblocks = 54 samples per year). Transects were set up along the slope gradient (if any) to account for any differences associated with slope position and spread across the entire cutblock to account for as much inter-site variability as possible. Soils were stored on ice until returning to the lab, at which point they were sieved with a <2 mm mesh and stored at 4 °C. One set of subsamples was air-dried and pulverization for general soil characterization, and a second set was stored at −80 °C for subsequent DNA extraction and amplification.

2.3. Characterization of soil properties and N availability

All soil analysis were carried out based on the Soil Sampling and Methods of Analysis Book (Canadian Society of Soil Science, 2008). Gravimetric water content (GWC) was determined by calculating the mass lost after drying a known quantity of soil at 105 °C for 48 h, and expressed as mass of water per unit mass of dry soil (%). Soil pH was measured in a 2:1 soil: double deionized water slurry. Total C (C_{total}) and N (N_{total}) were quantified by the combustion method using a LECO CNS-2000 and expressed as a percentage (%) of the total soil mass (Helgason et al., 2009). The carbon to nitrogen (C/N) ratio was calculated by dividing the C_{total} by the N_{total} and thus reported here unit-less.

In-situ NO_3^- and NH_4^+ fluxes were measured using Plant Root Simulator (PRS™) Probes (Western Ag Innovations, Saskatoon, SK). This technology consists of an ion exchange membrane that emulates the nutrient sorption and surface characteristics of plant roots, thus assesses potential nutrient supply rates. In other words, the flux of nutrient into the PRS™ probe supplies a surrogate measurement of plant available nutrient concentration in soil water, hereafter referred to as bioavailable N. Probes were installed on the same date, and in the same location as soil samples for a total of 54 separate measurements per summer. A 6-week burial time was utilized for the PRS™ probes to ensure saturation would not occur (Western Ag Innovation, PRS™ probe Operations Manual), thus probes were replaced mid-July of each summer with a fresh

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