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Contributions of ammonia-oxidizing archaea and bacteria to nitrification in Oregon forest soils

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ABSTRACT

Ammonia oxidation, the first step of nitrification, is mediated by both ammonia-oxidizing archaea (AOA) and bacteria (AOB); however, the relative contributions of AOA and AOB to soil nitrification are not well understood. In this study we used 1-octyne to discriminate between AOA- and AOB-supported nitrification determined both in soil-water slurries and in unsaturated whole soil at field moisture. Soils were collected from stands of red alder (*Alnus rubra* Bong.) and Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) at three sites (Cascade Head, the H.J. Andrews, and McDonald Forest) on acidic soils (pH 3.9–5.7) in Oregon, USA. The abundances of AOA and AOB were measured using quantitative PCR by targeting the *amoA* gene, which encodes subunit A of ammonia monooxygenase. Total and AOA-specific (octyne-resistant) nitrification activities in soil slurries were significantly higher at Cascade Head (the most acidic soils, pH < 5) than at either the H.J. Andrews or McDonald Forest, and greater in red alder compared with Douglas-fir soils. The fraction of octyne-resistant nitrification varied among sites (21–74%) and was highest at Cascade Head than at the other two locations. Net nitrification rates of whole soil without NH_4^+ amendment ranged from 0.4 to 3.3 mg N kg^{-1} soil d^{-1}. Overall, net nitrification rates of whole soil were stimulated 2- to 8-fold by addition of 140 mg NH_4^+-N kg^{-1} soil; this was significant for red alder at Cascade Head and the H.J. Andrews. Red alder at Cascade Head was unique in that the majority of NH_4^+-stimulated nitrifying activity was octyne-resistant (73%). At all other sites, NH_4^+-stimulated nitrification was octyne-sensitive (68–90%). The octyne-sensitive activity—presumably AOB—was affected more by soil pH whereas the octyne-resistant (AOA) activity was more strongly related to N availability.

1. Introduction

Nitrification, the oxidation of ammonia (NH_3) to nitrate (NO_3^-), is mediated by microorganisms and is a key component of the nitrogen (N) cycle. The importance of nitrification in forest soils has been debated for a long time because N turnover and availability limit net primary productivity. In the conifer-dominated forests of the Pacific Northwest, N turnover varies between red alder (*Alnus rubra* Bong.) sites and conifer sites (Binckley et al., 1992; Perakis et al., 2012) and a several-fold increase in the rates of N turnover by the inclusion of red alder has been reported (Hart et al., 1997; Boyle et al., 2008). As a result of red alder having the potential to fix 50–300 kg N ha^{-1} y^{-1} (Bottomley and Myrold, 2015), there is an increase in the N capital and N availability of the soil. A negative consequence is that red alder can reduce soil pH, to as low as 3.6–3.9 (Yarwood et al., 2010). The pH decline under red alder probably occurs as a result of increased nitrification (Binkley and Sollins, 1990). Although ammonia-oxidizing bacteria (AOB) have been isolated from soils of pH < 5 (De Boer and Kowalchuk, 2001), it remains equivocal whether or not they can effectively oxidize NH_3 at that pH. Heterotrophic nitrification, mediated by heterotrophic bacteria and fungi, has been suggested to be an alternate option (Hynes and Knowles, 1982; Stein, 2011; Zhang et al., 2011). Another possibility is the recent discovery of ammonia-oxidizing *Thaumarchaeota* (AOA) that have the potential for NH_3 oxidation and growth on CO_2. Recently, an acidophilic AOA has been isolated (Lehtovirta-Morley et al., 2011, 2013), and may represent a lineage of dominant AOA types in acidic soils (Nicol et al., 2008; Gubry-Rangin et al., 2010; Yao et al., 2011; Prosser and Nicol, 2012; Hu et al., 2014). Although no isolates of AOB have been found to grow at

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pH < 5 (De Boer and Kowalchuk, 2001), they exist in acidic soils and their potential for activity has been inferred by detection of AOB mRNA for *amoA* (Nicol et al., 2008). This might be due to residence in pH-neutral microsites (Hankinson and Schmidt, 1984), an ability to use urea and/or other organic N sources (Burton and Prosser, 2001), or through protective aggregation (De Boer et al., 1991; Spieck et al., 1992). In most soils, AOA outnumber AOB based on their *amoA* gene abundances, suggesting the potential for a greater role in nitrification (Leininger et al., 2006; Prosser and Nicol, 2008; Norman and Barrett, 2014). In some soils, however, AOB can be more abundant than AOA (Hu et al., 2014; Petersen et al., 2012; Yarwood et al., 2010). Thus, to date no clear consensus has emerged about the mechanism(s) controlling niche differentiation in acidic soils.

We designed a study using our newly developed assay, which is based upon AOB NH_3-oxidizing activity being quickly and irreversibly inactivated by low concentrations of 1-octyne, whereas AOA activity is unaffected by the same range of concentrations (Taylor et al., 2013). The study involved a comparison of the relative contribution of AOA and AOB to soil nitrification in acidic forest soils under different tree types. Three sites spanning a range of soil types and containing replicated stands of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) and red alder were selected. The soils varied in pH and organic C and N contents, in part because of the N inputs provided by the red alder-*Frankia* symbiosis. The aims of this study were to: (i) assess the relative contributions of AOA and AOB to nitrification; and (ii) determine what factors influence their distribution and contribution to nitrification. It was hypothesized that N input through N_2-fixation by red alder would increase both ammonia oxidizer abundances and nitrification potential, and would affect the relative contributions of AOA and AOB to nitrification.

2. Materials and methods

2.1. Site description and soil properties

Soils were collected from three forest sites containing plots of either pure stands of Douglas-fir or red alder. At two of the sites the stands were planted approximately 30 years ago (Radosevich et al., 2006). One site lies within the Cascade Head Experimental Forest (45°01′ N, 123°54′W, 330 m elevation), 1.6 km from the Pacific Ocean, and receives ~2400 mm y^{-1} of precipitation. Soils at the site are classified as Isomesic Fluvudands. A second site was located within the H.J. Andrews Experimental Forest (44°14′ N, 122°11′W, elevation 800 m), along the western slopes of the Cascade Range, and receives ~2300 mm y^{-1} of precipitation. Soils in the area are classified as Typic Haplumbrepts (Dyrness, 2001). The third site was located within the McDonald-Dunn Forest (44°42′ N, 123°20′W, 350 m elevation), on the eastern foothills of the Coast Range. The soils are classified as Xeric Palehumults. Annual precipitation averages ~1070 mm y^{-1}. At McDonald Forest, natural stands of red alder are found along streams within a matrix of Douglas-fir; both about 30 years old. All soil samples were collected during the late spring of 2013. Soil was collected from each of three field replicate plots of each tree type at each location, kept in separate bags, and stored at 4 °C and used within six months. Samples (10 g) were removed from each bag and frozen (−20 °C) for DNA extraction at a later date.

Soils spanned a relatively wide range of pH (3.9–5.5), a three-fold range in total C, a four-fold range of total N, and an eight-fold range of NH_4^+ and NO_3^- (Table 1). Soil pH was measured in deionized water; total C and N were measured by combustion; concentrations of NH_4^+ and NO_3^- plus NO_2^- (referred to simply as NO_3^- afterwards) in 2 M KCl extracts were determined colorimetrically (Hood-Nowotny et al., 2010; Kandeler and Gerber, 1988).

2.2. Nitrification potential assay

Nitrification potentials of soils were measured using a shaken soil-slurry method with supplemental NH_4^+ (1 mM NH_4Cl; Taylor et al., 2010). Field moist soil (2.5 g) was added to 150-ml serum bottles loosely capped by phenolic caps fitted with butyl stoppers, and pre-incubated at room temperature (22 ± 2 °C) for 2 d. Deionized water (15 ml) supplemented with 1 mM NH_4Cl was added to each bottle (final concentration ~140 mg NH_4^+-N kg^{-1} soil). Bottles were shaken at 200 rpm at 30 °C. Samples of soil slurries (1 ml) were taken after 15 min of shaking and at 24 and 48 h, centrifuged for 3 min at 13,000× g, and NO_3^- determined. At the beginning of the slurry assays, a set of bottles were amended with 2 kPa of either acetylene (6 μM aqueous) or octyne gas (4 μM aqueous) as described by Taylor et al. (2013). Acetylene was used to block NH_3 oxidation by AOA and AOB and to evaluate the possibility of acetylene-insensitive heterotrophic nitrification. Octyne was used to block AOB activity while allowing AOA activity to proceed (Taylor et al., 2013). For Cascade Head and McDonald Forest soils, nitrification potentials were calculated from NO_3^- accumulation during the first 24 h. For H.J. Andrews soils, nitrification potentials were calculated from NO_3^- accumulation occurring between 24 and 48 h due to lack of significant accumulation during 0–24 h. Nitrification potentials were calculated by subtracting the NO_3^- accumulation in the acetylene treatment from values measured in the treatments without acetylene to focus primarily on acetylene-sensitive AOA and AOB activities. The pH of the soil slurries was measured at the end of the incubation.

2.3. Whole soil nitrification assay

A whole soil assay was used to measure the potential contributions of AOA and AOB to soil nitrification. Soil samples (5 g) were incubated at three different NH_3 levels (equivalent to 0, 14, and 140 mg NH_4^+ kg^{-1} soil) achieved by adding sufficient anhydrous NH_3 gas to the headspace of 150-ml serum bottles sealed by phenolic caps fitted with butyl stoppers. Prior to initiation of the assay, soils were pre-incubated at room temperature (22 ± 2 °C) for 2 d. Three treatments were imposed at each NH_4^+ level: (i) positive control (no octyne or acetylene amendment), (ii) acetylene amendment (2 kPa = 6 μM aqueous), and (iii) octyne amendment (2 kPa = 4 μM aqueous). Soil samples were incubated at 25 °C, NH_4^+ and NO_3^- concentrations were determined at 2 and 7 d, and nitrification rates were calculated after subtracting NO_3^- accumulation in the acetylene treatment. The pH at the beginning of whole soil incubation was measured 12 h after NH_3 gas was added, when NH_3 gas had fully defused into soil samples and pH came to equilibrium. The pH of soil samples was measured at the end of the incubation.

2.4. Copy numbers of amoA genes

DNA was extracted from soil (0.25 g dry weight equivalent) using a MoBio PowerSoil™ DNA isolation kit (MoBio Laboratories Inc, Carlsbad, CA) according to the manufacturer's instructions. DNA extracts were quantified with a NanoDrop™ ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and diluted to 1 ng μl^{-1}. DNA extracts were stored at −20 °C for future use.

The quantitative polymerase chain reaction (qPCR) was used to determine AOA and AOB abundance by quantifying the number of *amoA* gene copies for each group. All qPCR reactions were performed in triplicate by using an ABI PRISM 7500 FAST (Carlsbad, CA)