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Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil

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ABSTRACT

In the majority of agricultural soils, ammonium (NH_4^+) is rapidly converted to nitrate (NO_3^-) in the biological ammonia and nitrite oxidation processes known as nitrification. The often rate-limiting step of ammonia oxidation to nitrite is mediated by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). The response of AOA and AOB communities to organic and conventional nitrogen (N) fertilizers, and their relative contributions to the nitrification process were examined for an agricultural silage corn system using a randomized block design with 4 N treatments: control (no additional N), ammonium sulfate (AS) fertilizer at 100 and 200 kg N ha^{-1}, and steer-waste compost (200 kg total N ha^{-1}) over four seasons. DNA was extracted from the soil, and real-time PCR and 454-pyrosequencing were used to evaluate the quantity and diversity of the *amoA* gene which encodes subunit A of ammonia monooxygenase. Soil pH, nitrate pools, and nitrification potentials were influenced by ammonium and organic fertilizers after the first fertilization, while changes in AOB abundance and community structure were not apparent until after the second fertilization or later. The abundance of AOA was always greater than AOB but was unaffected by N treatments. In contrast, AOB abundance and community structure were changed significantly by ammonium fertilizers. Specific inhibitors of nitrification were used to evaluate the relative contribution of AOA and AOB to nitrification. We found that AOB dominantly contributed to potential nitrification activity determined at 1 mM ammonium in soil slurries and nitrification potential activity was higher in soils treated with ammonium fertilizers relative to control soils. However, AOA dominated gross nitrification activity in moist soils. Our result suggests that AOB activity and community are more responsive to ammonium fertilizers than AOA, but that *in situ* nitrification rate is controlled by ammonium availability in this agricultural soil. Understanding this response of AOA and AOB to N fertilizers may contribute to improving strategies for the management of nitrate production in agricultural soils.

1. Introduction

Nitrification, the biological oxidation of ammonia to nitrite and subsequently to nitrate, influences the fate of N in terrestrial systems and often promotes the loss of nitrate from soils. In soils, the first step of autotrophic nitrification is mediated by ammonia oxidizing bacteria (AOB) of the *Betaproteobacteria* and ammonia oxidizing archaea (AOA) of the *Thaumarchaeota* (Schleper and Nicol, 2010; Norton, 2011). Since both AOA and AOB contain the ammonia monooxygenase enzyme, the *amoA* gene is frequently used as a molecular marker to explore the abundance and diversity of ammonia oxidizers. Leininger et al. (2006) first revealed that AOA were quantitatively dominant in a variety of soils from diverse ecosystems. The abundance and communities of AOA and AOB and their relative contributions to soil nitrification are influenced by complex factors in agricultural soils (Taylor et al., 2012; Giguere et al., 2015).

Comparisons of N dynamics under organic N sources versus mineral fertilizers have consistently shown changes in N transformation rates and associated microbial communities (Shi and Norton, 2000; Burger and Jackson, 2003; Habteselassie et al.,

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2006b; Chu et al., 2007; Reeve et al., 2010; Habteselassie et al., 2013; Geisseler and Scow, 2014). AOB and AOA have been found to co-exist in most agricultural soils and may have functional differences in their response to nitrogen management (Di et al., 2009; Offre et al., 2009; Xia et al., 2011; Habteselassie et al., 2013). The application of mineral fertilizers or urea changed the abundance and composition of AOB, but it did not significantly influence the AOA community in several agricultural soils (Shen et al., 2008; Wang et al., 2009; Xia et al., 2011; Ai et al., 2013). In contrast, AOA growth was detected when ammonia was supplied by mineralization of organic N derived from composted manure or soil organic matter (Offre et al., 2009; Schleper, 2010; Levicnik-Hoefferle et al., 2012; Jiang et al., 2014b). However, these studies were conducted in microcosms or after long term (>5 years) field fertilization experiments. We have limited information about how AOA and AOB population and community respond to mineral and organic fertilization temporally in the field.

While previous studies have shown that the archaeal *amoA* is often more abundant than bacterial *amoA* in soils (Leininger et al., 2006; Schleper and Nicol, 2010; Shen et al., 2012), the rate of ammonia oxidation may or may not be linked directly to current AOA and AOB populations (Nicol et al., 2008; Myrold et al., 2014). Xia et al. (2011) found AOB dominantly contributed to nitrification activity in an agricultural soil by using a stable isotope probing technique. However, AOA were found to be the more dominant players in nitrification in other agricultural soils (Offre et al., 2009; Zhang et al., 2010). Taylor et al. (2010) developed a short-term assay based on the recovery of the nitrification potential (RNP) after inhibition with acetylene in the presence and absence of bacterial protein synthesis inhibitors. They discovered that in recently N fertilized and cropped soils the majority of RNP activity was due to AOB, and that in pasture and grassland soils, RNP was due primarily to AOA or to a mixture of AOA and AOB. Recent findings by Taylor et al. (2013, 2015) showed that AOA pure cultures were more resistant to the C8 alkyne inhibitor, 1-octyne, in comparison to AOB, and that therefore octyne can be used to distinguish AOA and AOB contributions to soil nitrification in short-term assays. These differential inhibitors allow us to assess the relative contribution of AOA and AOB to nitrification.

In the present study, our goal was to investigate effects of conventional and organic N sources on AOA and AOB populations and community composition in a replicated field experiment over 4 years. Concurrently, we assessed nitrification rates and the relative contribution of AOA and AOB under these contrasting N treatments. We hypothesized that the AOA and AOB communities and their relative contributions to nitrification would respond differentially to these contrasting N sources. Our expectation was that AOB abundance and activity would increase in response to ammonium fertilizer while AOA would increase in response to an organic N source. Understanding the relative contribution of AOA and AOB to nitrification may contribute to improving strategies for the management of nitrate production in agricultural soils.

2. Materials and methods

2.1. Experimental field plots and soil sampling

Field plots were established in 2011 at the Utah Experiment Station Greenville farm located in North Logan, Utah (41°45′ N, 111°48′ W). The soil is an irrigated, very strongly calcareous Millville silt loam (Coarse-silty, carbonatic, mesic Typic Haploxeroll). Previously, the field was used for conventional small grain production with the annual application of 70 kg N ha^{-1} as urea. The experimental design is a randomized complete block with four blocks and four nitrogen treatments: control (no N fertilization), ammonium sulfate (AS100 and 200 kg N ha^{-1}), and steer-waste compost (200 kg total N ha^{-1}). Each plot is 3.8 m wide and 9.1 m long with a 4.6 m alley between each plot and a 1.2 m alley between each block. Composted steer manure (obtained from Miller Co. Hyrum, UT) was analyzed for moisture and N content immediately before application to determine the mass needed to supply the 200 kg total N ha^{-1} rate. Treatments were surface applied in May of each year (2011–2014), incorporated by tilling immediately after application, and silage corn was planted within one week after treatment application as previously described (Habteselassie et al., 2006a).

Soil samples were collected in May (0–30 cm depth) and August (0–15 cm depth) from 2011 to 2014. We collected 0–30 cm soil depth in May before tillage to coincide with standard practice for fertility determinations and to assess winter leaching of nitrate. In August, we sampled the 0–15 cm soil depth approximately 90 days after fertilization. Fertilizers are tilled into this depth and nitrification activity is generally higher in this surface layer (Habteselassie et al., 2006a, 2006b). Six soil cores were randomly taken from each plot, composited and mixed, placed on ice, and brought to the laboratory for immediate processing.

2.2. Soil chemical properties

Soil ammonium and nitrate were extracted immediately after sampling with 2 M KCl (1:5 of soil:solution by mass). Soil ammonium and nitrate were measured with a flow injection analyzer (QuikChem 8500, methods 12-107-06-1-A, 12-107-04-1-J Lachat Instrument, Loveland CO). The soil moisture was determined by drying soils at 105 °C for 24 h. Soil samples were then sieved (2.0 mm) and stored at 4 °C or air-dried for other measurements. Soil pH was determined on a 1:2 soil-water suspension. Soil organic C and total N were determined by dry combustion (Primacs^{SLC} for organic carbon, Primacs^{SN} for total N, Skalar, Inc, GA, USA).

2.3. Nitrification potential and recovered nitrification potential assays

Nitrification potential was determined by the shaken soil slurry method as described previously (Hart et al., 1994; Norton and Stark, 2011). Briefly, 15 g sieved fresh soil was placed into a 250 ml flask, and 100 ml 1 mM phosphate buffer (pH = 7.2) containing 1 mM NH_4^+ was added to the flask. Flasks were shaken for 24 h at 200 rpm and sampled four times (2, 4, 22, 24 h) after the beginning of shaking. The concentrations of nitrite (NO_2^-) and nitrate (NO_3^-) were measured with a flow injection analyzer (QuikChem 8500 method 10-107-04-1-C Lachat Instruments, Loveland CO).

We measured the recovery of nitrification potentials (RNP) as described by Taylor et al. (2010, 2012). Briefly, 9 g of moist soil were added to 60 ml of 30 mM TES buffer (pH 7.2) with 1 mM NH_4^+ in 150 ml bottles with caps fitted with gray butyl stoppers. Soil slurry was then exposed to acetylene (0.025 kPa) for 6 h. Acetylene was removed by placing the soil slurries under a vacuum and degassing for 6 min. After degassing, all bottles were shaken and incubated with caps loosened at 30 °C. Once acetylene is removed, AMO may be synthesized again allowing nitrification to resume and nitrite and nitrate to accumulate after a delay of approximately 24 h. Thus the RNP was calculated by assessing the rate of nitrite and nitrate accumulation 24–48 h after acetylene removal. In some samples, two bacterial protein synthesis inhibitors, kanamycin (at a final concentration of 800 μg/ml) and spectinomycin (at a final concentration of 200 μg/ml) were used together to prevent synthesis of ammonia monooxygenase (AMO) by AOB, and thus the recovery in these samples is due to AOA (RNP_{AOA}). RNP of AOB was calculated as the difference between total RNP and RNP_{AOA}.