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Uncoupling of ammonia oxidation from nitrite oxidation: Impact upon nitrous oxide production in non-cropped Oregon soils

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ABSTRACT

The factors controlling the relative contributions of ammonia- (NH_3) oxidizing archaea (AOA) and bacteria (AOB) to nitrification and nitrous oxide (N_2O) production in soil remain unclear. A study was conducted to examine the contributions of AOA and AOB to nitrification, nitrite (NO_2^-) accumulation, and NO_2^--affected N_2O production in three non-cropped Oregon soils. Nitrification potential rates in the three soils ranged seven-fold from 0.15 to 1.08 μmol N g^{-1} d^{-1}, with AOA contributing 64–71% of the total activity. AOA- and AOB-driven NO_2^- accumulation represented 8–100% of total NO_2^- + NO_3^- accumulation, persisted over 48 h, and was accompanied by acetylene-sensitive, ammonium- (NH_4^+) stimulated N_2O production. Ammonium- and NO_2^--dependent N_2O production occurred when both AOA and AOB, or AOA alone were active. By adding the NO_2^--oxidizing bacteria, *Nitrobacter vulgaris*, to soil slurries to increase NO_2^--oxidizing capacity, both NO_2^- accumulation and N_2O production were prevented, while the overall rate of nitrification was unaffected. Yields of N_2O-N amounted to 0.05 ± 0.01% of total NO_2^- + NO_3^--N accumulation in the presence of supplemental NH_4^+, and 0.28 ± 0.11% in the presence of both supplemental NH_4^+ + NO_2^-. Regression analysis of the N_2O production against NO_2^- accumulation over 24 h revealed a positive, non-linear relationship for N_2O production by both AOA plus AOB and by AOA alone. Values of V_{max} ranged 12-fold from 0.05 to 0.62 nmol N_2O g^{-1} d^{-1}, and predicted K_m values for NO_2^- ranged 15-fold from 0.02 to 0.30 μmol NO_2^- g^{-1} soil. These findings provide new insights into the impact of NO_2^- accumulation in soils on N_2O production by both AOA and AOB, and show that NO_2^- accumulation primarily drives N_2O formation in these soils, and increases N_2O yield by both AOA and AOB.

1. Introduction

Nitrification is the process whereby ammonia (NH_3) is oxidized sequentially to nitrite (NO_2^-) and nitrate (NO_3^-). The first step of nitrification is carried out by NH_3-oxidizing bacteria (AOB) and thaumarchaea (AOA) (Arp and Stein, 2003; Leininger et al., 2006; Vajrala et al., 2013). Several studies have shown that the process of NH_3 oxidation can be a major source of aerobically produced N_2O, and can contribute 36–57% of total N_2O production from soils (Kool et al., 2011; Wrage et al., 2001; Zhu et al., 2013). Whereas AOA and AOB are generally abundant and widely distributed in soils (Leininger et al., 2006; Prosser and Nicol, 2012; Taylor et al., 2012, 2013), few studies have examined the relative contributions of AOA and AOB to soil nitrification (Chen et al., 2013; Daebeler et al., 2015; Giguere et al., 2015; Taylor et al., 2010, 2013; Wessén et al., 2010; Lu et al., 2015). Furthermore, despite the activities of AOA and AOB having the potential to produce N_2O (Kozlowski et al., 2014; Poth and Focht, 1985; Santoro et al., 2011; Shaw et al., 2006; Stieglmeier et al., 2014; Stein, 2011), to our knowledge there is only one study in the literature that has examined the relative contributions of AOA and AOB to nitrifier-dependent N_2O production in soil (Hink et al., 2016). There is considerable interest in determining the factors that influence the proportion of NH_3 oxidized that is transformed to N_2O, and if the relative contributions of AOA and AOB might influence the latter value (Jung et al.,

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2013; Mørkved et al., 2007; Shaw et al., 2006; Stieglmeier et al., 2014).

There is a growing body of evidence that aerobic N_2O production in soil may be associated with NO_2^- accumulation (Maharjan and Venterea, 2013; Venterea, 2007; Venterea et al., 2015). Several studies have demonstrated that NO_2^- accumulates in soil under conditions where NH_3-oxidizing activity is stimulated (Müller et al., 2006), and/or NO_2^--oxidizing activity is negatively affected by additions of urea (Burns et al., 1995; Chapman and Liebig, 1952; Ma et al., 2015; Shen et al., 2003; Venterea, 2007) or anhydrous NH_3 (Maharjan and Venterea, 2013; Venterea et al., 2015). Production of N_2O by AOB has been demonstrated to be stimulated by NO_2^- (Shaw et al., 2006) and most AOB possess both NO_2^- nitrite reductase (NirK) and nitric oxide reductase (NorB) which enable them to carry out NO_2^--dependent N_2O production (Cantera and Stein, 2007; Kozlowski et al., 2014). In the case of AOA, although they possess the gene encoding for NirK (Spang et al., 2012; Walker et al., 2010), the gene encoding for nitric oxide reductase has not been detected (Hatzenpichler, 2012; Kozlowski et al., 2016). Although it has been suggested that AOA can abiologically produce N_2O, the isotopic signature of N_2O produced from AOA enrichments suggests that NO_2^- is involved in N_2O production (Jung et al., 2013; Stieglmeier et al., 2014), and a positive relationship was observed between NO_2^- concentration and N_2O production by marine AOA enrichment cultures (Santoro et al., 2011).

Nonetheless, only one study has examined the relative importance of AOA and AOB driven NH_3 oxidation to N_2O production (Hink et al., 2016), and no study has examined the importance of NO_2^- accumulation on AOA- and AOB-dependent N_2O production. Indeed, Hink et al. (2016) measured both AOA- and AOB-dependent N_2O production over a 28-d incubation of a cropped UK sandy loam soil and found KCl-extractable NO_2^- levels to be undetectable. We have identified Oregon soils with significant nitrification contributions from both AOA and AOB (Taylor et al., 2013; Giguere et al., 2015), and that also accumulate NO_2^- when nitrification is stimulated by NH_4^+ additions. In addition, with our recent discovery of the selective AOB inactivator, 1−octyne (Taylor et al., 2013), we have formulated the following objectives. These are: to determine to what extent AOA and AOB-driven NH_3 oxidizing activities contribute to N_2O production, and to determine the influence of NO_2^- accumulation on AOA and AOB-driven N_2O production.

2. Materials and methods

2.1. Soil sampling and location

Three locations in Oregon (Pendleton, Madras, and Klamath Falls) were selected for this study and are described in detail elsewhere (Giguere et al., 2015). At each location, 4 replicates of cropped and non-cropped soils were sampled from adjacent sites on the same soil series Pendleton (Walla Walla silt loam), Madras (Madras loam), and Klamath (Fordney loamy fine sand). A preliminary survey showed that non-cropped soils accumulated NO_2^- after nitrification was stimulated with 1 mM NH_4^+ additions as described elsewhere (Giguere et al., 2015; Taylor et al., 2012).

2.2. Soil slurry design

Soils were removed from 4 °C storage and composite 5-g portions of soil were added to 125-ml Wheaton bottles, wet to approximately field capacity, capped loosely with butyl stoppers, and pre-incubated at room temperature (21 °C) for 24 h. Each bottle received 15 ml of water, was amended depending on the experiment, and was capped tightly. Soil slurries were shaken continuously at 200 rpm at 25 °C. Gas samples for N_2O analysis were collected through the butyl stoppers at 24 and 48 h for all experimental incubations. Acetylene (6 μM_{aq}) was used to inhibit ammonia-oxidizing activity. Previous studies of these soils found no evidence of acetylene-insensitive nitrification, implying that all ammonia oxidation was chemolithotrophic (Giguere et al., 2015; Taylor et al., 2013). Octyne (4 μM_{aq}) was used to inactivate AOB activity, leaving AOA activity unaffected (Giguere et al., 2015; Hink et al., 2016; Lu et al., 2015; Taylor et al., 2013, 2015). Octyne was prepared by adding 40 µl liquid octyne to a Wheaton bottle with a 155 ml headspace, with several glass beads and over-pressured with 100 ml air, and a 2.8 ml aliquot was added to each sample bottle.

2.3. Analysis of NO_2^-, NO_3^-, NH_4^+, pH and N_2O

Initial pH measurements were made in a 2:1 soil water slurry and ranged from 7.2 to 7.6.

Concentrations of NO_2^- and NO_3^- were determined as described elsewhere (Miranda et al., 2001; Taylor et al., 2013). Briefly, aliquots of soil slurries were sampled from sealed Wheaton bottles, centrifuged, and were immediately analyzed. Nitrite was measured colorimetrically using Griess reagents, and NO_3^- was measured using a vanadium reduction assay in which NO_3^- is reduced to NO_2^- and the total $NO_2^- + NO_3^-$ measured (Miranda et al., 2001). The NO_3^- concentration was calculated as the difference between $NO_2^- + NO_3^-$ and NO_2^- accumulations. Nitrification rates were calculated as the net accumulation of $NO_2^- + NO_3^-$ above the acetylene controls. Detection limits for NO_2^- were 0.02 µmol NO_2^- g^{-1} soil, and 0.05 µmol NO_3^- g^{-1} soil for NO_3^-.

NH_4^+ extractions were conducted independently from NO_2^- or NO_3^- by extracting 5 g portions of soil in 15 ml 2 M KCl for 1 h. Extracts for NH_4^+ analysis were frozen until analysis and measured colorimetrically as described by Mulvaney (1996).

N_2O concentration in the gas phase was determined using a Varian Model 3700 gas chromatograph equipped with an electron capture detector as described previously (Mellbye et al., 2016). Total N_2O production from the soil was calculated as described by Tiedje (1994) using the equation

$$M = C_s(V_g + V_l{}^*\alpha) \quad [1]$$

where, M is total N_2O, C_s is N_2O concentration in the gas phase, V_g is total gas volume, V_l is volume of the liquid and α is the Bunsen absorption coefficient for N_2O at 25 °C (0.544). The detection limits for N_2O production were 0.015 nmol g^{-1} soil. Rates of N_2O formation were calculated as the difference between the acetylene control N_2O levels and N_2O accumulation at 24 h and 48 h N_2O yields were calculated using the equation

$$\frac{N_2O - N}{\left(NO_2^- - N + NO_3^- - N\right)} \quad [2]$$

2.4. Incubations to establish the impact of NH_4^+, and NO_2^- on N_2O production by AOB + AOA and AOA alone

An experiment was conducted to examine the effect of supplemental NH_4^+ and NO_2^- on nitrification activity and N_2O production by the combination of AOA + AOB (−octyne) and by AOA alone (+octyne). Soil slurry incubations for each of the 3 soils were conducted in the presence or absence of supplemental 1 mM NH_4^+ and in the presence or absence of supplemental 1 mM NO_2^-. NO_2^- and NO_3^-, concentrations were measured at 0, 6, 24, and 48 h.