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Differential responses of soil ammonia-oxidizing archaea and bacteria to temperature and depth under two different land uses

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ABSTRACT

Although soil nitrification has been studied intensively, with an effort to elucidate the relative contributions of both ammonia-oxidizing archaea (AOA) and bacteria (AOB), their relative contributions to N_2O production remain unclear. Understanding the temperature- and depth-dependent activities of AOA and AOB, as well as production of N_2O, is of great importance for predicting their responses to climate change. This work applied the recently discovered AOB inhibitor, octyne, to soil microcosms incubated at different temperatures (20, 30, 40 °C) in order to differentiate ammonia-oxidation potential and N_2O production by AOA and AOB, in soils from different land uses and depth. Our results showed that surface soils (0-15 cm) possessed significantly greater ammonia oxidation potential than subsurface soils (30-45 cm) at all temperatures tested, and that AOA-associated nitrification potential dominated at higher temperatures for both summer and autumn soils. The accumulation of N_2O was only detected in surface agricultural soil at 30 °C and positively correlated with nitrite accumulation within the incubation period. The detected N_2O production, along with most nitrification potential activity, were attributed to AOB, implicating AOB as major producers of this greenhouse gas in the tested agricultural soil. Higher ammonia-oxidation activity and N_2O production within surface agricultural soil reinforces the importance of agricultural surface soils as sources of nitrification and N_2O production, with potential implications for land management practices and responses to climate change.

1. Introduction

Nitrification is traditionally considered a two-step microbially driven process for oxidizing ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-). This process links reduced and oxidized nitrogen pools by the combined activities of ammonia-oxidizing bacteria (AOB; Prosser, 1990), ammonia-oxidizing archaea (AOA; Könneke et al., 2005), nitrite-oxidizing bacteria (NOB; Prosser, 1990), and the newly discovered complete ammonia oxidation "comammox" bacteria (Daims et al., 2015; van Kessel et al., 2015).

Although ammonia oxidation to nitrite has been studied intensively, the relative contributions of AOA and AOB to this process and factors that may influence their contributions are still unclear (Hatzenpichler, 2012; Prosser and Nicol, 2012; Schleper, 2010). The isolation of AOA and AOB cultures from different environments suggests that temperature may have a role in niche separation between AOA and AOB. Specifically, cultured AOA range from mesophiles to hyperthermophiles, with optimum growth temperatures ranging from 25 °C for *Candidatus* Nitrosotalea devanaterra Nd1 (Lehtovirta-Morley et al., 2014) to 72 °C for *Candidatus* Nitrosocaldus yellowstonii (de la Torre et al., 2008), with others in between these extremes (Daebeler et al., 2018; Jung et al., 2011, 2014a; Kim et al., 2012; Lehtovirta-Morley et al., 2016; Sauder et al., 2017; Stieglmeier et al., 2014a; Abby et al., 2018). Isolated AOB appear to have a narrower range, with the optimum temperatures for most strains ranging from 20 to 30 °C (Avrahami and Bohannan, 2007; Avrahami and Conrad, 2005; Groeneweg et al., 1994; Jiang and Bakken, 1999), but with growth of several also possible at 4 °C (Jones et al., 1988).

The influence of temperature on AOA and AOB community composition (Tourna et al., 2008) and activity (Horak et al., 2013; Taylor et al., 2017; Wu et al., 2013) has been assessed for various environmental samples. In soil, an increase of AOA-associated activity was observed for an agricultural soil in the warmer seasons of late summer and early fall (Taylor et al., 2012), with differential inhibition during soil incubations indicating that AOA possessed at least a 10 °C higher optimal temperature than AOB (Ouyang et al., 2017; Taylor et al., 2017). Whether these patterns are observed in contrasting land-use types and at different soil depths remains unclear. In addition, the effect

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of temperature on AOA- and AOB-associated nitrous oxide (N_2O) production associated with soil nitrification is poorly understood. Because previous studies examining the effects of temperature on N_2O production (Avrahami et al., 2003; Conrad et al., 1983; MacDonald et al., 1997; Mogge et al., 1998, 1999; Slemr et al., 1984) were conducted before the discovery of AOA, and a recent study only focused on greenhouse agricultural soils (Duan et al., 2018), the temperature-dependent relative contributions of AOA and AOB to soil N_2O production activity in a natural system requires further investigation.

Nitrous oxide, a trace gas with a ~300 fold greater global warming potential than CO_2, is a reactant capable of causing stratospheric ozone destruction (Ravishankara et al., 2009). Soil is considered the largest source of N_2O emissions (Schreiber et al., 2012; Syakila and Kroeze, 2011), of which microbial ammonia oxidation contributes approximately 80% (Gödde and Conrad, 1999; Kool et al., 2011; Wrage et al., 2001; Zhu et al., 2013). AOB produce N_2O enzymatically through two mechanisms: incomplete oxidation of hydroxylamine (NH_2OH) to NO_2^- and sequential reduction of NO_2^- to NO and N_2O by "nitrifier-denitrification" (Arp and Stein, 2003). Although the N_2O-producing mechanism within AOA has not been fully resolved, it is thought that AOA produce N_2O during NH_3 oxidation through an abiotic reaction between NH_2OH and NO, an intermediate of the AOA ammonia oxidation pathway (Stieglmeier et al., 2014b), which has been demonstrated for pure cultures (Jung et al., 2011; Kozlowski et al., 2016; Qin et al., 2017; Stieglmeier et al., 2014b). AOA-associated N_2O production in environmental samples has been reported in several studies (Giguere et al., 2017; Hink et al., 2017, 2018; Löscher et al., 2012; Peng et al., 2016; Santoro et al., 2011). Although some studies have determined the relative contributions of AOA and AOB to soil nitrification using selective inhibitors (Daebeler et al., 2015; Duan et al., 2018; Giguere et al., 2015; Lu et al., 2015; Ouyang et al., 2016; Taylor et al., 2013, 2010), and examined the relative contributions of AOA and AOB to nitrifier-dependent N_2O production in agricultural or non-cropped soils (Shi et al., 2017; Giguere et al., 2017; Hink et al., 2017; Wang et al., 2016), no microcosm study has yet examined the influence of temperature on N_2O production by AOA and AOB along a soil depth profile.

In our previous work, we characterized the influence of depth and land-use on thaumarchaeotal and bacterial community structures at the ***rare*** Charitable Research Reserve (Lu et al., 2017; Seuradge et al., 2017), showing depth- and land-use-associated community changes. Using this knowledge of thaumarchaeotal and bacterial biogeography, site-specific heterogeneity, and depth profiles, the aim of this study was to perform a targeted investigation into how AOA and AOB at different soil depths and land uses respond to temperature with respect to their relative contributions to ammonia oxidation and N_2O production.

2. Material and methods

2.1. Site selection and soil sampling

Soil samples were taken from the ***rare*** Charitable Research Reserve (Cambridge, Ontario) in early September ("summer" samples) and early November ("autumn" samples) in 2015. Soil samples were collected at two depths (0-15 and 30-45 cm) from either an agricultural site (Preston Flats; A) or forest site (Hogsback; F). According to our previous research, sites A and F possessed distinct bacterial and thaumarchaeotal communities that also varied with depth (Lu et al., 2017; Seuradge et al., 2017). A composite soil sample from each depth at each site was generated by randomly collecting 3-5 replicates from the same location as our previous sampling plots used for a bacterial and thaumarchaeotal biogeography survey (Lu et al., 2017; Seuradge et al., 2017). The agricultural site has been under no-till management since 2002, operated under a rotation of corn (*Zea mays*) and soybean (*Glycine max*) from 2002 to 2011, and a corn monocrop since 2011. The forest site is a mixture of northern hardwood and Carolinian tree species, maintained as pristine forest for over 100 years, and is thus classified as a mature forest. Soils from both sites are classified as Burford series, which is typical for the area along the Grand River. The soils are well-drained and calcareous, with a relatively thin A horizon. The B horizons of both soils have a sandy clay loam texture, wavily extending into a C horizon that contained over 50% gravel (Presant and Wicklund, 1971). Soil samples for the incubation experiment were sieved (4.75 mm) and stored at 4 °C prior to establishing microcosms, or at -20 °C prior to physicochemical analysis at the Agriculture and Food Laboratory (University of Guelph).

2.2. Whole soil nitrification assay

Similar to a previously published method (Lu et al., 2015), a modified 8-day whole soil assay was used to measure the nitrification potential (NP) attributed to AOA and AOB in composite soil samples. Triplicate subsamples from each composite soil were pre-incubated for two days in a 120-mL serum bottle (15 g field moisture soil per replicate) at room temperature (22 °C) with a loosely capped stopper prior to microcosm incubation. This room temperature pre-incubation minimized the influence of 4 °C storage (Giguere et al., 2015). An NH_4Cl solution was added to each microcosm, resulting in a final concentration of 200 mg-N kg^{-1} $soil_{dry}$ and a gravimetric water content of 30%. Serum bottles were sealed with silica stoppers and incubated in the dark at 20 °C, 30 °C, or 40 °C. At the beginning of the incubation, triplicate bottles were amended with either acetylene (0.02% v/v) or octyne gas (1.9% v/v) following a protocol published elsewhere (Taylor et al., 2013). Acetylene was used to irreversibly inactivate ammonia monooxygenase of both AOA (Offre et al., 2009; Vajrala et al., 2013) and AOB (Hyman and Wood, 1985), whereas octyne is a specific inhibitor of AOB only (Giguere et al., 2015, 2017; Hink et al., 2017; Lu et al., 2015; Taylor et al., 2013, 2015). To monitor net nitrification potential, the incubated soils were sampled at 0, 2, 4, 6, and 8 d by removing 2.5 g of soil, and then re-established inhibitor concentrations. Nitrification potential rates were determined by measuring the accumulation of NO_2^- + NO_3^- at each sampling point (Taylor et al., 2010). Total net nitrification potential rates were determined by the accumulation of NO_2^- + NO_3^- without inhibitors (i.e., acetylene or octyne). Nitrification potential in octyne-amended microcosms was attributed to AOA, with the difference between no inhibitor and octyne-amended microcosms attributed to AOB activity. To determine NO_2^- + NO_3^- production from autotrophic nitrification in the forest soil, NO_2^- + NO_3^- concentrations in acetylene-amended microcosms at each time point were subtracted from those in uninhibited microcosms, as described previously (Lu et al., 2015). Because forest soil microcosms showed a decrease in NO_2^- + NO_3^- concentrations after six days of incubation, indicating a possible increase in denitrification activity and/or immobilization (data not shown), only the first six days of data were used for analysis.

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

N_2O concentrations were determined in the headspace of microcosms immediately before each sampling point, as described previously (Coyotzi et al., 2017), with 1 mL gas sampled using a disposable syringe and injected into a GC-2014 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with a Porapak Q 80-100 column and an electron capture detector (ECD).

Soil NO_2^- concentrations were measured directly with Griess reagents, whereas soil NO_2^- + NO_3^- accumulation was determined using a vanadium reduction assay to convert NO_3^- to NO_2^- before adding Griess reagents to measure NO_2^- (Miranda et al., 2001; Sauder et al., 2017). All assays were conducted in clear flat bottom 96-well plates (Greiner, Frickenhausen, Germany) and absorbance measured at 550 nm using a Filtermax F5 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA).