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Thermodynamic responses of ammonia-oxidizing archaea and bacteria explain N_2O production from greenhouse vegetable soils

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ABSTRACT

Greenhouse vegetable soils are characterized by high N_2O emissions, but the functional importance of potential ammonia (NH_3) oxidation (PAO) and nitrite (NO_2^-) consumption (PNC) by ammonia-oxidizing archaea (AOA) and bacteria (AOB) is poorly understood. Inhibitors of 1-octyne and 2-pheny l-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) in combination with potassium chlorate were used to examine the importance of AOA and AOB in NO_2^- production and consumption, to evaluate the soil-specific effects on N_2O production under six long-term fertilization greenhouse vegetable soils across mainland China, and to predict their thermodynamic responses in a temperature gradient of 5–45 °C using the square root growth (SQRT) and macromolecular rate theory (MMRT) models. The ammonia oxidizers-driven PAO and PNC exhibited a strong response to temperature with maximum rates attained at 35 °C or 40 °C by AOA compared to 30 °C or 35 °C by AOB. Concomitantly, compared with ammonium amendment, NO_2^- addition stimulated N_2O production by approximately 2–4-fold for AOA and 2–9-fold for AOB at 20–40 °C in four of six soils. AOA exhibited a broader range of temperatures (−33.2–56.8 °C *vs* −16.1–52.3 °C) and lower relative temperature sensitivity (Q_{10}) than AOB ($p < 0.05$). The heat capacity ($\Delta C_P^{\ddagger}$) of AOA and AOB in PAO and PNC increased in relation to the optimum temperature (T_{opt}) and exhibited a greater negative value by AOB than AOA, indicating that specialized ammonia-oxidizer populations of PAO and PNC adapted to *in situ* soil environmental changes. Structural equation modeling revealed that potential AOA-driven N_2O production was influenced by $\Delta C_P^{\ddagger}$ and Q_{10} and C/N, while AOB-driven N_2O production by $\Delta C_P^{\ddagger}$ and Q_{10} and soil nitrate (NO_3^-) content, all of which is influenced directly and indirectly by *in situ* climate parameters. Findings suggest the important roles of specific *in situ* climate and soil properties and provide new insights into the NO_2^- consumption in soil N_2O production by ammonia-oxidizers.

1. Introduction

Nitrogen (N) fertilizer is generally overused on Chinese croplands, resulting in soil secondary salinization and acidification, low N-use efficiency, and a decrease in soil microbial functional diversity (Zhang et al., 2013; Chen et al., 2014). N-fertilization rates from chemical fertilizer and manure in greenhouse vegetable ecosystems are 3–5 times higher than those used for cereal grain cultivation in China (Diao et al., 2013). The increase in N fertilizer application would result in much higher greenhouse gas nitrous oxide (N_2O) emissions from Chinese agricultural soils in the future (Tian et al., 2016).

Agriculture soil contributes approximately 60% of total N_2O emissions (Hu et al., 2015a). During nitrification in soil, ammonia (NH_3) oxidation by ammonia-oxidizing bacteria (AOB) and archaea (AOA) is generally thought to be rate limiting (Pester et al., 2012), and could contribute up to 80% of total N_2O emissions (Gödde and Conrad, 1999); Nitrite (NO_2^-) oxidation, catalyzed by autotrophic, slow-growing nitrite-oxidizing bacteria (NOB), is the second step of nitrification. NO_2^- does not usually accumulate in soil under natural conditions (Robertson and Groffman, 2007). However, there is a growing body of evidence that NO_2^- can accumulate in soil under certain conditions (Maharjan and Venterea, 2013; Müller et al., 2014; Giguere et al., 2017) due to the decoupling of NH_3 oxidation from NO_2^- oxidation. This may increase the frequency of NO_2^- flux peaks, with potential ecosystem consequences that include increased NO_2^- toxicity and NO_2^--dependent N_2O production, *e.g.*, (1) abiotic chemodenitrification (Venterea, 2007); (2) N-nitrosation hybrid N_2O production ($NH_2OH + NO_2^- + H^+ \rightarrow N_2O + 2H_2O$) by ammonia oxidizers (AOs, so called co-denitrification) (Stieglmeier et al., 2014; Frame et al., 2016; Kozlowski et al., 2016) or abiotic reaction (Samarkin et al., 2010; Rubasinghege et al., 2011); (3) nitrifier denitrification (ND) performed by AOs (Jung et al., 2014; Stieglmeier et al., 2014); and (4) bacterial heterotrophic denitrification

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(Hallin et al., 2018). Evidence is emerging that ND may contribute considerably to N_2O fluxes in agricultural soils (Venterea and Rolston, 2000; Maharjan and Venterea, 2013; Zhu et al., 2013; Huang et al., 2014; Giguere et al., 2017; Shi et al., 2017). ND can be ecologically important in terms of soil N_2O emissions in greenhouse vegetable soils, which are characterized by high N, low organic C content, and low oxygen pressure induced by intensive microbial activities under high temperature and frequent irrigations. Hence, it is imperative to understand the responses of NH_3 oxidation and NO_2^- consumption by ND under varying conditions. Notably, it was recently discovered that some species of *Nitrospira* are capable of complete ammonia oxidation (comammox) in water systems (Daims et al., 2015; van Kessel et al., 2015) and also in soils (Kits et al., 2017; Pjevac et al., 2017).

The activity of AOA and AOB in the soil has been intensively studied, and mechanistic models have been recently adopted to assess N_2O emission from soils (Hergoualc'h and Verchot, 2014; Jones et al., 2014; Hu et al., 2015b). However, information about the relative contribution of each group to soil nitrification rates, especially N_2O production, is currently scarce and contradicting results have been reported (Lu et al., 2015; Hink et al., 2017; Ouyang et al., 2016; Wang et al., 2016; Taylor et al., 2017). AOA and AOB are physiologically diverse, resulting from a range of soil environmental conditions under which they can function and driven NH_3 oxidation in different proportions (Prosser and Nicol, 2012; Taylor et al., 2012; Yao et al., 2013). Previous studies suggest that AOB dominate both ammonia oxidation and N_2O production in soils amended with NH_4^+ under moist soil conditions (Hink et al., 2017; Wang et al., 2016). However, Giguere et al. (2017) reported that there was no significant differences in N_2O production between AOA and AOB. Soil temperature is recognized as one of the most important factors influencing nitrification (Szukics et al., 2010) and shaping the large-scale distribution patterns of AOA and AOB (Fierer et al., 2009; Gubry-Rangin et al., 2017), which may further influence the relative contributions of AOA and AOB to N_2O production in soils (Ouyang et al., 2017; Taylor et al., 2017). Various studies have been carried out to attempt to quantify the different temperature responses of AOA and AOB in soils. For example, the majority of thaumarchaeotal enrichment cultures and isolates are mesophilic, or even moderately thermophilic and have optimum growth temperatures of 25 °C for *Candidatus Nitrosotalea devanaterra* (Lehtovirta-Morley et al., 2011) and 40 °C for *Candidatus Nitrososcosmicus franklandus* (Lehtovirta-Morley et al., 2016), and 46 °C for *Candidatus Nitrososphaera gargensis* from temperate soil (Hatzenpichler et al., 2008), whereas the hyperthermophilic strain *Candidatus Nitrosocaldus yellowstonii* can grow at temperatures up to 74 °C (Jr et al., 2008) or 15 °C for AOA in Arctic soil (Alves et al., 2013). Compared to AOA, the growth of AOB isolates is usually optimal ≤30 °C (Jiang and Bakken, 1999); furthermore, *Nitrosomonas europaea* and *Nitrososphira* can grow at 0–3 °C in agricultural soils (Groeneweg et al., 1994; Jiang and Bakken, 1999). While the high diversity of AOA and AOB in soils could explain their ubiquity in environments with a range of temperature, pH and substrate concentrations (NH_4^+, NO_2^-, NO_3^- and soil organic carbon), the optimal range of temperature for AOA- or AOB-driven NH_3 oxidation and NO_2^- consumption remains unknown. Furthermore, there exists a significant knowledge gap about whether AOA- or AOB-driven nitrification is affected by multiple environmental factors, including temperature and N substrate.

Long-term fertilization has been shown to affect nitrification and the community abundance and composition of ammonia oxidizers (He et al., 2007; Wang et al., 2015; Segal et al., 2017). Recently, a target inhibitor of AOB combined with a model was used to fit or explain the relative contribution of AOA and AOB to nitrification and their response to increasing temperatures over the short-term (< 5 years) in neutral and alkaline agricultural soils (Ouyang et al., 2017; Taylor et al., 2017). The linear correlation between the soil characteristics and community structures or gene abundances may not necessarily represent the functional importance of nitrifying populations in complex soil (Prosser and Nicol, 2012; Breuillin-Sessoms et al., 2017). The responses of ammonia oxidizers in NO_2^- accumulation and N_2O production to temperature remains unknown. Therefore, we conducted temperature-controlled shaken soil-slurry incubation experiments using field soils receiving long-term fertilizer inputs (> 10 years). Our objective is to determine the responses of AOA and AOB to temperature in specific soils, to evaluate the consistency between the measurements and the square root growth (SQRT) and macromolecular rate theory (MMRT) models, and then to extrapolate the NH_3 oxidation and NO_2^- consumption under varying conditions. Our hypotheses were: 1) AOA- and AOB-driven NH_3 oxidation and NO_2^- consumption will respond differently to temperature due to their significant divergence in thermodynamic characteristics; 2) AOA and AOB will exhibit distinct thermodynamic characteristics in different soils, and this will affect the relative contributions of AOA and AOB to N_2O production.

2. Materials and methods

2.1. Soil collection and preparations

Six typical greenhouse fields with a long history (> 10 years) of conventional vegetable cultivation were selected ranging from cold temperate to subtropical regions across mainland China (Fig. 1) as follows: 1. a Phaeozem in JiaMuSi (JMS)/Heilongjiang Province (46°48′ N, 130°12′ E); 2. an Anthrosol in YangLing (YL)/Shanxi Province (34°18′ N, 108°2′ E); 3. an Acrisol in ChangSha (CS)/Hunan Province (28°32′ N, 113°23′ E); 4. a Cambisol in ShouGuang (SG)/Shandong Province (36°56′ N, 118°38′ E); 5. a Fimi-Orthic Anthrosol in NanJing (NJ)/Jiangsu Province (32°01′ N, 118°52′ E); and 6. a Ferralic Cambisol in JianNing (JN)/Fujian Province (26°45′ N, 116°32′ E) (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012). Detailed information is provided in Table S1. Soil samples were manually collected from the layer of cultivation (0–20 cm) after the local vegetable harvest in April of 2015. The fresh soil samples were air-dried, sieved through ≤2 mm mesh to carefully remove stones and plant debris, and then stored at 4 °C for soil slurry assays.

2.2. Ammonium sorption isotherms study

Ammonium sorption isotherms were obtained using a batch equilibrium method in triplicate with modifications (Venterea et al., 2015). In brief, NH_4^+-N solutions at 0, 10, 50, 100, 200, 300 and 400 mg NH_4^+-N L^{-1}were prepared in a 0.01 M $CaCl_2$ solution and adjusted to pH 6.20 with a few drops of chloroform. Then, 0.75 g soil was added to the corresponding NH_4^+-N solutions in a 50 ml Falcon tube and adjusted to pH 6.20. The suspensions were first shaken for 18 h on a rotary shaker and then centrifuged and filtered. The supernatant NH_4^+-N was measured on an ultraviolet spectrophotometer (Hitachi, UV-2900, Tokyo, Japan) using indophenol blue method. The adsorption data were fitted using the Langmuir model:

$$srNH_4^+ = \frac{a \times \mu \times slNH_4^{+(1-b)}}{1 + a \times slNH_4^{+(1-b)}} \quad (1)$$

Where $srNH_4^+$ (mg N kg^{-1}) represents ammonium adsorption capacity at solution-phase ammonium concentration ($slNH_4^+$) (mg N kg^{-1}); μ is the maximum sorption capacity (mg N kg^{-1}); a (mg N L^{-1}) and b are Langmuir constants. The model parameters were derived from the nonlinear fitting with a user defined Langmuir function using OriginPro 8.1 (OriginLab, USA).

2.3. Soil slurry assays

2.3.1. Specific inhibitors for AOB, AOA and NOB

1-octyne, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) and potassium chlorate ($KClO_3$) are specific inhibitors of AOB, AOA and NOB, respectively, as described in previous studies (Taylor