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Overdose fertilization induced ammonia-oxidizing archaea producing nitrous oxide in intensive vegetable fields

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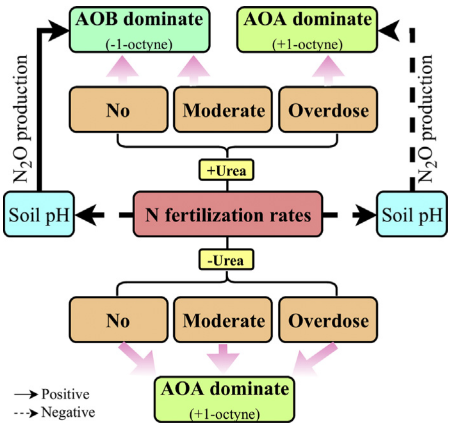
HIGHLIGHTS

- Soil pH induced by N gradients controlled the abundance/contribution of AOA and AOB.
- AOA-derived N_2O were greater than AOB in the control and overdose fertilized soils.
- AOB-derived N_2O dominated in conventionally fertilized vegetable soils.

GRAPHICAL ABSTRACT



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ABSTRACT

Little is known about the effects of nitrogen (N) fertilization rates on ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) and their differential contribution to nitrous oxide (N_2O) production, particularly in greenhouse based high N input vegetable soils. Six N treatments (N1, N2, N3, N4, N5 and N6 representing 0, 293, 587, 880, 1173 and 1760 kg N ha^{-1} yr^{-1}, respectively) were continuously managed for three years in a typically intensified vegetable field in China. The aerobic incubation experiment involving these field-treated soils was designed to evaluate the relative contributions of AOA and AOB to N_2O production by using acetylene or 1-octyne as inhibitors. The results showed that the soil pH and net nitrification rate gradually declined with increasing the fertilizer N application rates. The AOA were responsible for 44–71% of the N_2O production with negligible N_2O from AOB in urea unamended control soils. With urea amendment, the AOA were responsible for 48–53% of the N_2O production in the excessively fertilized soils, namely the N5–N6 soils, while the AOB were responsible for 42–55% in the conventionally fertilized soils, namely the N1–N4 soils. Results indicated that overdose fertilization induced higher AOA-dependent N_2O production than AOB, whereas urea supply led to higher AOB-dependent N_2O production than AOA in conventionally fertilized soils. Additionally, a positive relationship existed between N_2O production and NO_2^- accumulation during the incubation. Further mechanisms for NO_2^--dependent N_2O production in intensive vegetable soils therefore deserve urgent attention.

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1. Introduction

China accounts for about 52% of the world's vegetable production. The area devoted to vegetable cultivation increased from 3.3×10^6 ha in 1976 to 24.7×10^6 ha in 2012 (FAO, 2015). It has become a common phenomenon for excessive fertilizer application to produce higher yield in greenhouse vegetable field. According to the review by Hu et al. (2017), fertilization in greenhouse vegetable areas were 2–5 times higher than those in open fields. The nitrogen (N) amount of urea could be up to 510–948 kg N ha^{-1} two crops yr^{-1} in the greenhouse vegetable system in Nanjing, far beyond the nutrient demands for cropping (Yang et al., 2016; Zhong et al., 2016), which has greatly contributed to serious environmental consequences such as soil acidification (Guo et al., 2010) and nitrous oxide (N_2O) emissions (De Rosa et al., 2016; Li et al., 2016; Fan et al., 2017). The direct N_2O emissions from intensive greenhouse vegetable fields are believed to contribute approximately 20% of the total N_2O emissions from Chinese cropland (Wang et al., 2011) and the main source of global warming potentials judged by the life cycle assessment (Jia et al., 2012). To predict the consequences of this input, there is a pressing need to understand the basic mechanisms that underlie microbial N transformations (Kuypers et al., 2018).

Various processes are associated with N_2O production in soils following urea or organic N addition with or without enzyme catalysis, i.e. nitrification (Beeckman et al., 2018), nitrifier denitrification (Stieglmeier et al., 2014), codenitrification (Spott et al., 2011), chemodenitrification (Homyak et al., 2017) and heterotrophic denitrification (Hallin et al., 2018). The ammonia (NH_3) oxidation pathway converts NH_3 to nitrite (NO_2^-) as the first and rate-limiting step in nitrification and is therefore the main contributor to the ammonium (NH_4^+):nitrate (NO_3^-) balance in soil (Beeckman et al., 2018). There are three distinct groups of aerobic autotrophic microorganisms that oxidize ammonia: NH_3 oxidizing bacteria (AOB) (Prosser, 1990), NH_3 oxidizing archaea (AOA) (Konneke et al., 2005) and the newly discovered complete ammonia oxidation "comammox" bacteria (Daims et al., 2015; van Kessel et al., 2015; Kits et al., 2017). However, their relative contribution is difficult to estimate, as different environmental factors can affect their abundance and activity (Prosser and Nicol, 2012). For example, although the relative importance of AOA might be low in N-rich soils, their nitrification rate is at maximum and, therefore, should not be ignored (Beeckman et al., 2018; Li et al., 2018), especially in acidic soils, where the NH_3 concentration is low, high substrate affinity might give them a competitive advantage (Prosser and Nicol, 2012). Recently, Kits et al. (2017) have indicated that oligotrophic AOB, many AOA and comammox bacteria are adapted to low ammonium concentrations and are inhibited by higher concentrations, but comammox bacteria has a higher ammonia affinity than all cultured terrestrial ammonia-oxidizing archaea. There remains a paucity of evidence regarding how the amendment of soil with different N fertilization rates influences their relative importance in NH_3 oxidation.

The niche differentiation of AOA, AOB and comammox bacteria associated with NH_4^+ supply has the potential to influence, significantly, N_2O emissions due to their apparently distinct physiological processes (Beeckman et al., 2018; Lehtovirta-Morley, 2018). Hink et al. (2017, 2018) reported that the N_2O production was lower following ammonia oxidation by AOA and the N_2O production of AOB and the N_2O/NO_2^- product ratio of nitrification increased by increasing ammonium concentration in agricultural soils. Traditionally, the AOB produce N_2O enzymatically by two mechanisms: incomplete oxidation of hydroxylamine (NH_2OH) to NO_2^- (Arp and Stein, 2003), and via nitrifier denitrification, the sequential reduction of NO_2^- to nitric oxide (NO) and N_2O (Kozlowski et al., 2014). However, recent studies revealed two other routes for the N_2O production from the AOB *N. europaea* under anaerobic conditions: the direct oxidation of NH_2OH to N_2O by the enzyme cytochrome (cyt) P460 and nitrification intermediate NO (Caranto et al., 2016; Caranto and Lancaster, 2017). Moreover, the mechanisms of N_2O production by AOA appear to differ from that of AOB, as AOA lack genes encoding a canonical hydroxylamine dehydrogenase and NO reductase, which are involved in N_2O production by AOB (Walker et al., 2010; Tourna et al., 2011). It is thought that AOA produce hybrid N_2O (with one N sourced from ammonium and the other from nitrite) during NH_3 oxidation through an abiotic reaction between NH_2OH and NO, an intermediate of the AOA ammonia oxidation pathway (Stieglmeier et al., 2014; Lehtovirta-Morley, 2018), which has been demonstrated for pure cultures (Kozlowski et al., 2016).

Given that nitrite and nitrous acid are in a pH-dependent equilibrium, AOA strains grown in acidic pH media produce proportionally much more N_2O than in neutral pH, although it is unclear whether this is due to effects of pH or strain physiology (Jung et al., 2014). This indicates that acidic soil has a higher potential to cause AOA-dependent N_2O production. However, the mechanisms of N_2O production by AOA under oxic conditions remain unclear. Hence, considering the soil pH as an important factor and the different mechanisms of AOA and AOB in N_2O production can provide more insights for the associated kinetic properties influencing N_2O pathways in soils with different N fertilization rates.

As described by Taylor et al. (2013), 1-octyne is a newly developed inhibitor, which specifically inhibits AOB growth and activity while acetylene can effectively inhibit both AOA and AOB at low concentrations. Therefore, 1-octyne and acetylene are adopted to assess the relative contribution of AOA and AOB to N_2O production from six levels of N fertilization treated vegetable soils after 3-years. We hypothesize that the AOA would be responsible for the majority of N_2O production, given the acidic conditions which induced by intensive and overdose fertilization.

2. Materials and methods

2.1. Site description, field treatment and soil collection

The field experiment was conducted in a typically managed vegetable agroecosystem in a suburban area of Nanjing, Jiangsu Province, China (32°01 N, 118°52 E) from April 30, 2013 to May 10, 2016 covering eleven crops. Vegetables have been cultivated continuously at this site for more than ten years. The region has a subtropical monsoon climate with an annual mean precipitation of 1107 mm and a mean air temperature of 15.4 °C. The soil is classified as a Fimi-Orthic Anthrosol (RGCST 2001) consisting of 64.7% silt (0.002–0.05 mm), 30.1% clay (<0.002 mm) and 5.2% sand (>0.05 mm). The soil bulk density is 1.22 g cm^{-3} with an initial soil pH of 5.1. The soil has a total carbon content of 19.2 g kg^{-1} $soil_{dw}$, a total N content of 1.4 g kg^{-1} $soil_{dw}$, and a cation exchange capacity of 31.2 cmol kg^{-1}. The six field fertilizer treatments (each replicated three times) included 0 kg N ha^{-1} yr^{-1} (N1), 293 kg N ha^{-1} yr^{-1} (N2; 1/3 of the conventional N rate), 587 kg N ha^{-1} yr^{-1} (N3; 2/3 of the conventional N rate), 880 kg N ha^{-1} yr^{-1} (N4; the conventional N rate), 1173 kg N ha^{-1} yr^{-1} (N5; 4/3 of the conventional N rate) and 1760 kg N ha^{-1} yr^{-1} (N6; 6/3 of the conventional N rate). These urea rates were selected to cover a range of N fertilizer application practices across different vegetable production patterns. Phosphate (P) and potassium (K) fertilizers as calcium/magnesium phosphate and potassium chloride, respectively, were applied at a rate of 880 kg P_2O_5 ha^{-1} yr^{-1} and 880 kg K_2O ha^{-1} yr^{-1}. These basal fertilizers were evenly distributed to each of the eleven crops and incorporated into the soil.

Soil samples from each replicated plot at a depth of 0–20 cm in November 2016, were divided into three parts: the first part was stored at −80 °C for DNA extraction, the second part was stored at 4 °C prior to determination of exchangeable NH_4^+, NO_3^- and NO_2^- and other chemical properties, the third part was used for the incubation.