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Nitrogen fertilization induced changes in ammonia oxidation are attributable mostly to bacteria rather than archaea in greenhouse-based high N input vegetable soil

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ABSTRACT

Little is known about the effects of nitrogen (N) fertilization rates on ammonia-oxidizing bacteria (AOB) and archaea (AOA) and their differential contribution to ammonia oxidation, particularly in greenhouse-based high N input vegetable soils. Potential ammonia oxidation (PAO) of these vegetable soils was evaluated under five levels of N fertilization (with urea) in the presence or absence of the bacterial protein synthesis inhibitors kanamycin and gentamicin. Abundance and community composition of AOB and AOA were evaluated by quantitative polymerase chain reaction (PCR) and clone libraries. The five annual N fertilization rates studied were 100% (N870: 300, 270 and 300 kg N ha^{-1} for tomato, cucumber and celery, respectively), 80% (N696), 60% (N522), 40% (N348) and 0% (N0) of the conventional N rate. The PAO decreased significantly with increasing N fertilization rates irrespective of the presence of kanamycin plus gentamicin. PAO was significantly lower in the presence than in the absence of kanamycin plus gentamicin, and was decreased by 71.9% under N0, 77.2% under N348, 54.9% under N522, 49.9% under N696, and 51.6% under N870. The abundance of bacterial and archaeal *amoA* genes was significantly decreased at the highest N fertilization rate. The clone sequences of AOB and AOA were mostly affiliated with the genus *Nitrosospira* and group 1.1b thaumarchaeota. Changes in community composition were more pronounced in AOB than in AOA after long-term (6-year) N fertilization. Our results suggest that AOB may play a more important role than AOA in NH_3 oxidation in such high N input vegetable soils.

1. Introduction

Land use change and agricultural intensification are directly related to food security, water and soil quality, soil organic matter and carbon sequestration, and biodiversity and ecosystem services. The Yangtze River Delta in eastern China, covering a total of 3.6 million hectares of agricultural land, has been the most intensive rice (*Oryza sativa* L.) with wheat (*Tritucum aestivum* L.) or rape (*Brassica napus* L.) cropping system for several centuries. Recently, to meet ever-increasing vegetable demand over 10% of these traditional croplands have been changed into polythene tunnel or "polytunnel" greenhouse vegetable lands or soils, which are able to yield three or four harvests of vegetables per year that are more profitable than the traditional rotation of rice-wheat (or rice-rape). The conversion of traditional rice/wheat (or rape) rotation lands or open field vegetable lands to greenhouse-based intensive vegetable lands has resulted in a decrease in soil microbial diversity (Shen et al., 2008b). Moreover, the high nitrogen (N) application rate in such polytunnel greenhouse vegetable soils (up to 570–948 kg N ha^{-1} two crops yr^{-1}) has resulted in soil secondary salinization and acidification, lower N use efficiency, and a decrease in soil microbial functional diversity, including in the population and diversity of ammonia-oxidizing bacteria (AOB, Shen et al., 2010, 2011; Min et al., 2011). Yet little is known about the effects of N fertilization rates on the abundance and composition of AOB and

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AOA, and the NH_3 oxidation in these vegetable soils. In addition, it is also not known which group are more important in NH_3 oxidation and what the effects of N fertilization rates are on their differential contribution to NH_3 oxidation.

Oxidation of NH_3 to nitrite (NO_2^-) by AOB and AOA is the primary and rate-limiting step in nitrification and is therefore important in the global nitrogen cycle in terrestrial ecosystems (Kowalchuk and Stephen, 2001; He et al., 2012). The AOB and AOA are considered central to N turnover in soil and its availability to plants, which may affect NO_3^- leaching to groundwater and nitrous oxide (N_2O) emissions from a soil system. It is controversial whether NH_3 oxidation is exclusively or predominantly linked to AOA as is implied by their exceptionally high abundance in soils (Leininger et al., 2006; Prosser and Nicol, 2008; Hatzenpichler, 2012; He et al., 2012). The AOB rather than the AOA functionally dominate NH_3 oxidation in N-rich grassland soils (Di et al., 2009), despite the population of AOA being more abundant than AOB. The relative importance of AOB and AOA in NH_3 oxidation is not well understood in high N input agricultural soils.

NH_3, rather than NH_4^+, is used as substrate by ammonia monooxygenase (AMO) during ammonia oxidation (Kowalchuk and Stephen, 2001; He et al., 2012). The concentration of NH_3 declines exponentially with decreasing pH ($NH_3 + H^+ \leftrightharpoons NH_4^+$, pKa = 9.25 at 25 °C), thus the NH_3 availability is under the substrate threshold of AOB in low-pH environments (Stopnisek et al., 2010; He et al., 2012). Recent studies have shown that AOA play a more important role than AOB in NH_3 oxidation in low-pH soils, probably due to higher substrate affinity (Nicol et al., 2008; Yao et al., 2011; He et al., 2012; Huang et al., 2012; Zhang et al., 2012). For example, archaeal *amoA* gene abundance and transcriptional activity increased with decreasing soil pH, whilst bacterial *amoA* gene abundance was generally lower and transcriptional activity decreased with decreasing pH after a 46-y soil pH manipulation with lime or aluminum sulfate (Nicol et al., 2008). As a result, further studies on the effects of N fertilization rates on the differential contribution of AOB and AOA to NH_3 oxidation are needed in low-pH soils created by high N fertilization in intensive agricultural scenarios.

We therefore conducted a 6-year tomato/cucumber/celery rotation field plot experiment to study whether the abundance and composition of AOB and AOA, and NH_3 oxidation, were changed by N fertilizations after the conversion of traditional lands to polytunnel greenhouse lands in the Yangtze River Delta, eastern China. We also studied which group of microbes were more important in NH_3 oxidation, and what the effects of N fertilization rates were on their differential contribution to NH_3 oxidation. Our objectives were to investigate the effects of five N fertilization rates (Table 1) on 1) soil NH_3 oxidation in the presence or absence of the bacterial protein synthesis inhibitors kanamycin and gentamicin; 2) population sizes; and 3) community composition of AOB and AOA. We then related these effects to the relative importance of bacteria and archaea in nitrification, and better N management strategies for such greenhouse-based intensive vegetable soils.

Table 1
Crops and nitrogen fertilization rates of urea (kg N ha^{-1}) in the field plots.

Treatment	Tomato	Sweet corn	Cucumber	Celery
N0	0	0	0	0
N348	120	0	108	120
N522	180	0	162	180
N696	240	0	216	240
N870	300	0	270	300

All treatments during each vegetable (tomato, cucumber or celery) growing season received 18 (September 2005–July 2006) or 78 kg N ha^{-1} (September 2006 onwards) of commercial organic manure as basal fertilizer.

2. Materials and methods

2.1. Field site

The field site was located in Yixing County (31°14′N, 119°53′E), Jiangsu, China, which has a subtropical monsoon climate. With around 240 frost-free days, the annual mean and accumulated temperature, and precipitation, are 15.7 and 5418 °C, and 1177 mm, respectively. The traditional open land use for rice-wheat (or rape) rotation has been transformed to a polytunnel greenhouse covered tomato-cucumber rotation since 2001. The soil (Stagnic Anthrosol) in the polytunnel greenhouse was fertilized at the conventional N rate between 2001 and 2004, and then five different N fertilization rates have been created since September 2005 (Table 1). Before the experimentation, in 2005, the soil at 0–20 cm depth had pH (H_2O) of 5.89, electrical conductivity (EC) of 0.51 dS m^{-1}, total N and organic C of 1.04 and 14.4 g kg^{-1}, and $NO_3^- - N$, Olsen-P and NH_4OAc-K of 42.7, 61.4 and 56.2 mg kg^{-1}.

2.2. Plant growth and fertilization

For the present experiments, cucumber (*Cucumis sativus* cv. Jinchun 4), tomato (*Solanum lycopersicum* cv. Jinpeng 1) and celery (*Apium graveolens* cv. Xiqin 5) were cultivated between December 2006 and July 2011 in twenty 7.0 × 2.5 m plots in two 36 × 12 m polytunnel greenhouses. The rotation schedule was tomato from April to July, cucumber from September to November, and celery from December to next March. To increase N use efficiency and/or reduce N leaching during fallow periods, a catch crop of sweet corn (*Zea mays* L. ssp. *saccharata* Sturt) was grown on equally-split half-size plots for 1–1.5 months from July to August between 2008 and 2011. During the sweet corn growth period, the polytunnel greenhouses were uncovered and no fertilization was employed. Meanwhile, the other halves of the split plots lay fallow.

The five annual N fertilization treatment rates (four replicates of each) were 100% (N870: 300, 270 and 300 kg N ha^{-1} for tomato, cucumber and celery, respectively), 80% (N696), 60% (N522), 40% (N348) and 0% (N0) of the conventional N rate (Table 1). The N fertilizer was urea, which was used basally (50%) before transplanting and for supplementary (50%) fertilization during the flowering/fruit stage. Calcium-magnesium phosphate (120 kg ha^{-1} P_2O_5), potassium sulfate (150 kg ha^{-1} K_2O) and commercial organic manure (made from composted chicken manure, 29.7% moisture, total C and N of 261 and 20 g kg^{-1} fresh weight, $NH_4^+ - N$ and $NO_3^- - N$ of 527.4 and 174.9 mg kg^{-1} fresh weight, and pH (H_2O) of 6.12) were used in addition to the urea as basal fertilizer for each plot before transplanting. In addition, the soil received 3900 kg ha^{-1} of commercial organic manure at the start during each vegetable (tomato, cucumber or celery) growing season. Chemical fertilizers and organic manures were spread evenly onto the soil surface by hand and incorporated into the soil through ploughing and irrigation.

2.3. Soil sampling and physico-chemical analyses

On 20 July 2011, soil samples were collected from three randomly selected plots for each of the five N fertilization rates about 2 months after the supplementary N fertilization. Six soil cores at 0–20 cm depth per plot were randomly taken by a hand ‘push-in’ auger (20 mm diameter × 200 mm depth) prior to cultivating the catch crops, and the soil was then mixed to yield one composite sample per plot. The soil was transported from the field to the laboratory in Nanjing in an ice-cooled container. Fresh composite samples were stored at 4 °C for ammonia oxidation