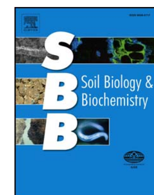
Soil Biology and Biochemistry 119 (2018) 83–89

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



# Neutrophilic bacteria are responsible for autotrophic ammonia oxidation in an acidic forest soil

Xueru Huang[a,b], Jun Zhao[c], Jing Su[a], Zhongjun Jia[c], Xiuli Shi[d], Alan L. Wright[e], Xia Zhu-Barker[f], Xianjun Jiang[a,*]

[a] *College of Resources and Environment, Southwest University, 2 Tiansheng Road, Beibei, Chongqing 400715, China*
[b] *College of Land and Environment, Shenyang Agricultural University, Shenyang, Liaoning 110866, China*
[c] *State Key Laboratory of Soil and Sustainable Agriculture Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu 210008, China*
[d] *College of Horticulture and Landscape Architecture, Southwest University, 2 Tiansheng Road, Beibei, Chongqing 400715, China*
[e] *Indian River Research & Education Center, University of Florida-IFAS, Fort Pierce, FL 34945, USA*
[f] *Biogeochemistry and Nutrient Cycling Laboratory, Department of Land, Air and Water Resources, University of California Davis, CA 95616, USA*

ARTICLE INFO

*Keywords:*
Nitrification
DNA stable isotope probing
*Nitrosospira* AOB
Neutrophilic
Nitrosospira multiformis

ABSTRACT

Increasing evidence suggests that autotrophic ammonia oxidation in acidic soils is largely performed by archaea. However, ammonia oxidizing bacteria (AOB) are also found in acidic soils, and mechanisms have been demonstrated that enable the growth of AOB in low pH soils. This work used DNA stable isotope probing to examine the response of autotrophic ammonia oxidizers in an acidic forest soil (pH 5.0) following urea amendment. After application, increases in nitrate concentrations correlated only with increases in AOB and not ammonia oxidizing archaeal (AOA) amoA gene abundance, indicating that AOB may be responsible for urea-derived ammonia oxidation. To identify growing autotrophic populations associated with ammonia oxidation, qPCR and high throughput sequencing analysis of 16S rRNA gene amplicons demonstrated that $^{13}CO_2$ was assimilated by *Nitrosospira* AOB rather than archaeal ammonia oxidizing populations, and *Nitrospira*-associated nitrite-oxidizing bacteria (NOB). Phylogenetic analysis of amoA genes demonstrated that the active ammonia oxidizers were closely related to neutrophilic Nitrosospira multiformis within *Nitrosospira* cluster 3a.2. These results demonstrate that AOB can dominate ammonia oxidation in acidic soils under specific conditions.

## 1. Introduction

The discovery of ammonia oxidizing archaea (AOA) over a decade ago (Konneke et al., 2005; Treusch et al., 2005; Venter et al., 2004) resulted in a reevaluation of the contribution on ammonia oxidizing bacteria (AOB) to nitrification processes in many terrestrial and marine environments. This included examining the major contributors to ammonia oxidation in acidic soils, which represent approximately 30% of all ice-free land (Von Uexküll and Mutert, 1995). AOB are found in many acidic soils, including populations that are phylogenetically distinct from those found in neutral pH soils, AOB cultivated from acidic soils typically do not grow in standard batch culture at pH range < 6.5 (Jiang and Bakken, 1999). However, mechanisms including growth in biofilms, aggregate formation and urea hydrolysis have been demonstrated as mechanisms that facilitate growth of neutrophilic AOB at low pH (Allison and Prosser, 1993; Burton and Prosser, 2001; De Boer et al., 1991; De Boer and Kowalchuk, 2001).

The cultivation of obligately acidophilic AOA belonging to the genus *Nitrosotalea* (Lehtovirta-Morley et al., 2014, 2011) provided a more parsimonious explanation for high rates of ammonia oxidation that are often be observed in acidic soils (Booth et al., 2005). AOA possess a greater substrate affinity for ammonia compared to AOB which may confer a competitive advantage in environments with low concentrations of free ammonia (Martens-Habbena et al., 2009). In addition, while most cultured AOB possess Rhesus-type ammonia transporters, AOA possess AMT-type putative ammonium transporters that may further enable growth in acidic environments where ammonium, rather than ammonia, is the dominant form (Lehtovirta-Morley et al., 2016). As such, it seems that AOA are primarily responsible for ammonia oxidation in acidic soils.

The recent discovery of *Nitrospira* 'comammox' that perform complete oxidation of $NH_3$ to nitrate ($NO_3^-$) (Daims et al., 2015; Van Kessel et al., 2015), contributes to a growing appreciation that nitrifiers are more diverse than originally thought (Santoro, 2016). Viable bacteria of a specific functional type can be active in almost any environment, even in environments which cannot support their growth (Falkowski et al.,

* Corresponding author.
*E-mail address:* jiangxj@swu.edu.cn (X. Jiang).

https://doi.org/10.1016/j.soilbio.2018.01.016
Received 9 November 2017; Received in revised form 8 December 2017; Accepted 20 January 2018
0038-0717/ © 2018 Elsevier Ltd. All rights reserved.

2008). Therefore, AOB cannot be excluded in driving nitrification in acidic soils, though the cultivated acid-sensitive or -tolerant AOB does not exhibit ability to oxidize $NH_3$ below pH of 6.0 (De Boer and Kowalchuk, 2001; Hu et al., 2014; Prosser and Nicol, 2012).

DNA-SIP is a powerful means to link functional process to taxonomic identities of active microorganisms in complex environments because specific functional groups of organisms incorporate particular substrates identified without the prerequisite of cultivation (Radajewski et al., 2003). The success of DNA-SIP relies entirely on genome replications and necessitate active propagation of target microorganisms grown on the labeled substrates. This division of microbial cell can only be achieved through substantial incorporations of macronutrients into the newly generated cells, in addition to harvesting energy source from the microbial-mediated process. Here we reported neutrophilic Nitrosospira multiformis-like strains within the *Nitrosospira* cluster 3a.2 lineage that drive $NH_3$-oxidation in an acidic forest soil after experimental urea addition. DNA-based stable isotope probing was used to identify the dominant autotrophic ammonia oxidizing communities that assimilated $^{13}CO_2$ for growth.

## 2. Material and methods

### 2.1. Site description and soil sampling treatments

Soil samples were collected in September 2015 from Tanghongling forestry center (48°21′N, 129°11′E) in the Xiaoxing'a Mountain, located in Hongxing County, in the northeast of China. The site has temperate zone monsoon climate with an annual mean temperature of –4 °C, precipitation of 500–600 mm, and frost-free period of 90–110 days. The dominant vegetation consists of Korean pine, larch, and spruce fir. Soil samples were composited from five cores at the surface layer (0 − 20 cm), air-dried to reach 15% moisture content (equal to 24% of water holding capacity (WHC)), passed through a sieve of 2 mm, and kept in a 4 °C freezer. The soil is a dark burozem and classified as Inceptisols Cryepts (Soil Survey Staff, 2014) with characteristics of 5.53 mg $NO_3^-$-N $kg^{-1}$, 40.2 mg $NH_4^+$-N $kg^{-1}$, 32.3 g organic matter $kg^{-1}$, 1.10 g total N $kg^{-1}$, 11.3 g total Fe $kg^{-1}$, 46.1 mg available Fe $kg^{-1}$, and 5.0 pH (water to soil ratio: 2.5).

### 2.2. DNA-SIP soil incubation

A DNA-SIP incubation was carried out using the method described in previous research (Jia and Conrad, 2009). Three treatments were applied: 5% (vol/vol) $^{13}CO_2$, 5% (vol/vol) $^{12}CO_2$ and 5% (vol/vol) $^{13}CO_2$ + 0.1% (vol/vol) acetylene ($C_2H_2$). Each treatment was replicated three times and received urea at 50 μg N $g^{-1}$ on a weekly basis. The enrichment of $^{13}CO_2$ is 99 atom% (Shanghai Engineering Research Center of Stable Isotopes, Shanghai, China) and $^{12}CO_2$ was prepared through the acidification of sodium carbonate. For each treatment, 6.0 g dry weight per gram soil (d.w.s.) was placed in a 120 ml serum bottle and incubated at 28 °C with soil moisture 60% of WHC for 28 days. The serum bottles were sealed with butyl rubber stoppers during the 28 days incubation. Before the weekly addition of urea, $CO_2$, and $C_2H_2$, each serum bottle was flushed with synthetic air (80% $N_2$, 20% $O_2$) for 1 min to maintain oxic conditions in the bottles. To keep the soil-respired $CO_2$ at a consistently low concentration during the 28 days incubation, a 2-week soil pre-incubation at 40% of WHC was conducted (Zhao et al., 2015). For the $C_2H_2$ treatment, an additional one week soil pre-incubation with 0.1% (vol/vol) $C_2H_2$ was performed to inhibit the activity of ammonia oxidizers in soils. At the end of 28 days incubation, 2.0 g fresh soil from each bottle was collected and stored in a –20 °C freezer before molecular analysis.

### 2.3. Nitrification activity

For each treatment, 4 g of soil was collected at the end of incubation and extracted with 2 M KCl (ratio of solution and soil = 5:1), and ultracentrifugated at 200 r.p.m. for 30 min. The contents of $NH_4^+$ and $NO_3^-$ in the extractant were measured using a GENESYS 10 UV spectrophotometer (ThermoScientific, Madison, WI) by the method of salicylate and single reagent, respectively (Doane and Horwáth, 2003; Verdouw et al., 1978).

### 2.4. Soil DNA extraction and SIP gradient fractionation

A FastDNA spin kit for soil (MP Biomedicals, Cleveland, OH, USA) was used for the extraction and purification of soil extracted DNA. The quantity of the soil DNA was measured by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Isopycnic density gradient centrifugation of the extracted total DNA was carried out to isolate $^{13}$C-DNA and native $^{12}$C-DNA in the SIP microcosms (Xia et al., 2011). For each treatment, the mixture of 3.0 μg DNA extract in CsCl stock solution was progressed in a ~5.5 ml Beckman polyallomer ultracentrifuge tube. CsCl buoyant density was adjusted to 1.725 g $ml^{-1}$, and then the mixture was ultracentrifuged at 177,000 × *g* for 44 h at 20 °C in a Vti65.2 vertical rotor (Beckman Coulter, Palo Alto, CA, USA). 15 fractioned DNA samples (~380 ml) was collected using NE-1000 single syringe pump (New Era Pump Systems Inc. Farmingdale, NY, USA) with a flow rate of 0.38 ml $min^{-1}$ according to the method of Wang et al. (2014). The measurement of refractive index was performed by 60 μl aliquot of each DNA fraction using an AR200 digital hand-held refractometer (Reichert, Inc. Buffalo, NY, USA) in order to calculate the CsCl buoyant density. To empirically standardize the conversion of refractive index to buoyant density of each DNA fraction, a series of pre-defined CsCl/gradient buffer mixture ranging from 1.68 to 1.76 g $ml^{-1}$ was repeatedly weighted with 1.0 ml aliquot from a 6.0 ml standard, and compared with temperature-corrected refractive index readings (Lueders et al., 2004; Xia et al., 2011). The 'light' DNA fractions showing a disturbed linear density gradient were discarded (Freitag et al., 2006). Nucleic acids were separated from CsCl solution by precipitation using 2 vol polyethylene glycol 6000 at 37 °C for 1 h, followed by centrifugation at 13,000 × *g* for 30 min. The DNA fraction was diluted in 30 ml TE buffer after PEG 6000 precipitation and 70% ethanol purification.

### 2.5. Real-time quantitative PCR (qPCR) of the amoA genes

Real-time quantitative PCR of both bacterial and archaeal amoA genes in the total DNA extracts and each fractionated DNA sample were quantified on a CFX96 Optical RealTime Detection System (Bio-Rad, Laboratories Inc. Hercules, CA, USA). The PCR primers and reaction conditions of archaeal and bacterial amoA genes were shown in Table S1. PCR standard curves were obtained from one typical clone including amoA genes of archaea and bacteria, and composed with seven orders of magnitude standard template per plasmid DNA. Blank design was carried out by replacing soil DNA extract with sterilized water as the template. For qPCR of the archaeal and bacterial amoA genes, the total reaction system was 25 μl, and included 12.5 μl SYBR Premix Ex Taq™ (Takara Biotech, Dalian, China), 0.25 μl primer per each one, 1 μl of DNA template (1–10 ng), and 11 μl sterilized water. The quantification operation had three biological and technical replicates per each. The qPCR amplification efficiencies and the obtained $R^2$ values were 96%–105% and 0.996–0.999, respectively. A single peak always occur in the melting curve was used for checking the specificity amplification of amoA genes.

### 2.6. Cloning libraries of amoA genes

Construction of clone libraries of bacterial amoA genes were carried out for $^{13}$C-labeled heavy fractions DNA (day 28) to retrieve the active ammonia oxidizers, and bacterial and archaeal amoA gene libraries from the total soil DNA extracts at day-0 were constructed for

Download English Version:

https://daneshyari.com/en/article/8362886

Download Persian Version:

https://daneshyari.com/article/8362886

Daneshyari.com