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Abundance and community structure of ammonia-oxidizing bacteria and archaea in purple soil under long-term fertilization

Zhifeng Zhou [a], Xiaojun Shi [a,b], Yong Zheng [c], Zixian Qin [a], Deti Xie [a], Zhenlun Li [a], Tao Guo [a,b,*]

[a] College of Resources and Environment, Southwest University, Chongqing 400716, China
[b] The National Monitoring Base for Purple Soil Fertility and Fertilizer Efficiency, Southwest University, Chongqing 400716, China
[c] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

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ABSTRACT

Ammonia-oxidizing microorganisms, which drive the first and rate-limiting step in the process of nitrification, play an important role in soil N-cycling processes. However, little is known about the ammonia-oxidizing bacteria (AOB) and archaea (AOA) in purple soils, which are characterized as lithologic soils without distinct pedogenic horizons and are mainly distributed in the Sichuan basin of Southwestern China. Here, the abundance and community structure of AOB and AOA in a 21-year fertilization experiment were investigated by real-time PCR and terminal restriction fragment-length polymorphism (T-RFLP), respectively. In the present study, nine different fertilization treatments were examined: NPK fertilization plus pig manure (NPK + M), NPK fertilization (NPK), without fertilization (CK), pig manure fertilization (M), P with NH_4Cl and KCl plus pig manure (Cl + M), PK fertilization (PK), NK fertilization (NK), NP fertilization (NP), and N fertilization (N). Our results indicate that N-fertilized treatments have higher AOB abundances than the control (CK) and treatments without N amendment. The lowest AOA abundance and AOA/AOB ratios were observed in the treatment (Cl + M) with long-term application of NH_4Cl plus KCl. The AOB community structure under combined addition of N and P differed from that under other treatments. The Cl + M treatment had a distinct AOA community structure and higher diversity compared with other treatments. Moreover, higher potential nitrification rates (PNR) were found in the combined N and P addition treatment groups than that in the other treatment groups, and the lowest PNR and pH (<6) were detected in the Cl + M treatment. These results highlight that not only nitrogen but also phosphorus may be a crucial factor affecting soil ammonia-oxidizing activity and triggering changes of AOB community composition. Moreover, soil pH might have an essential role in controlling the AOA community structure and ammonia-oxidizing activity in purple soil.

1. Introduction

As the first and rate-limiting step in the process of nitrification, soil ammonia oxidation driven by soil ammonia-oxidizing microorganisms plays an essential role in global N-cycling [1]. Ammonia-oxidizing bacteria (AOB) were long considered as the main contributors to soil ammonia oxidation. However, the discovery of ammonia-oxidizing archaea (AOA) challenged this traditional concept about soil ammonia-oxidation process [2]. An increasing number of studies have confirmed the important role of AOA. High AOA abundances have been reported in different soils and marine environments [3–7]. It was assumed that soil AOA might be the primary participant in the N-cycling under some harsh environmental conditions, such as low pH, extremely low or high temperature, high salinity, low oxygen, and low NH_4^+ concentration [8]. The use of DNA enrichment detection with stable $^{13}CO_2$ has verified the direct relationship between the autotrophic nitrification activity and AOA in acidic soils [9,10]. However, to date, although the role of soil AOA has been confirmed, the mechanisms of the environmental factors determining the relative contributions of soil AOB and AOA to ammonia oxidation is still debated [11].

Nitrification in agroecosystems can lead to large N losses following ammonia-based fertilizer applications by converting the positively charged ammonia into the negatively charged nitrate [12]. Consequently, leaching of nitrate may result in the pollution of streams and groundwater, and denitrification may increase the greenhouse gas nitrous oxide [13,14]. Thus, ammonia-oxidizing microorganisms, essential in nitrification, undoubtedly play an

* Corresponding author. Tel.: +86 23 68251249; fax: +86 23 68250444.
E-mail address: taoguo78@163.com (T. Guo).

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important role in the sustainable development of the agricultural ecosystem as well as the environmental protection. For arable soil, the utilization of N fertilizer decreased the abundance and changed the community structures of soil AOB and AOA in an acidic soil [15], whereas long-term fertilization only exhibited detectable effects on soil AOB in an alkaline soil [6]. Similarly, long-term N input changed the AOB community dramatically but displayed no obvious effects on the AOA community in grassland soil in Inner Mongolia [16]. Therefore, it appears that soil pH and N availability might be important factors affecting the response of AOB and AOA to fertilization. Soil pH might have a direct effect on the availability of ammonia [17]. In fact, AOA displays a stronger adaptive capacity to soil pH than dose AOB [15,18–20], and autotrophic ammonia oxidation might be attributable largely to archaea in acidic soils [21]. In addition, as the energy substrate of autotrophic ammonia-oxidizing microorganisms, ammonia might be the other important factor affecting the growth of AOB and AOA. The different affinity to ammonia of AOB and AOA might lead to differential growth of AOB and AOA [22]. AOB exhibited high activity in the environments with high N availability, but the growth of AOA might be associated with nitrification in environments with low N availability [22–24]. Collectively, soil AOB and AOA have different responses to anthropogenic activities, which might be directly related to the complex environmental factors and soil parameters.

Purple soils, which are classified as Orthic Entisols in the Chinese Soil Taxonomic System, Regosols in FAO Taxonomy or Entisols in USDA Taxonomy, developed from the fast physical weathering of sedimentary rocks of the Trias-Cretaceous system. They are characterized by lithologic soils without distinct pedogenic horizons and are mainly distributed in the Sichuan basin of Southwestern China [25]. In the National Monitoring Base for Purple Soil Fertility and Fertilizer Efficiency, the soil is the most representative neutral purple soil, which occupies almost 80% of the purple soil. In this base, a long-term fertilization experiment was established in 1991.

The objective of this study was to fingerprint the differences in abundance and community structure of AOB and AOA in purple soil under long-term treatment with different fertilizers, to study the relations among soil basic properties, ammonia-oxidizing activity, and community composition of ammonia-oxidizing microorganisms, and to explore potential factors in determining the ecological characteristics of soil ammonia-oxidizing microorganisms in purple soil.

2. Materials and methods

2.1. Site description and soil sampling

The National Monitoring Base for Purple Soil Fertility and Fertilizer Efficiency is located in the Beibei district, Chongqing, China (N29°48′, E106°24′). This region has a subtropical monsoon moist climate with a mean annual temperature of 18.2 °C and precipitation of 1106 mm, and the soils are classified as Orthic Entisols (Chinese taxonomy) or Regosols (FAO taxonomy). At this monitoring base, a long-term fertilization experiment with rice-wheat rotation was initiated in 1991. In the present study, nine different fertilization treatments were examined: NPK fertilization plus pig manure (NPK + M), NPK fertilization (NPK), without fertilization (CK), pig manure fertilization (M), P with NH_4Cl and KCl plus pig manure (Cl + M), PK fertilization (PK), NK fertilization (NK), NP fertilization (NP), and N fertilization (N). Urea, P_2O_5, and K_2O were used as the N, P, and K fertilizer source, respectively, with the exception of the Cl + M treatment, in which the urea and K_2O were replaced by the NH_4Cl and KCl, respectively, and pig manure was used as the organic fertilizer. Except for the pig manure, the other fertilizers were applied twice each year in the rice and wheat season individually, and the application amounts of N, P, and K were 135 kg (wheat season) plus 150 kg (rice season), 60 kg, and 60 kg ha^{-1}, respectively. The pig manure was applied at 22.5 ton ha^{-1} once a year before the wheat season.

Top soil samples (0–20 cm) were collected from each plot in June 2012, after the wheat had been harvested. Six soil cores (5 cm diameter) were taken and mixed thoroughly from each plot to form one composite sample. Each treatment contained three replicate soil samples. After removing the fine roots and visible organic debris by passing through a 2-mm sieve, soil samples were stored at 4 °C and −80 °C for the chemical analysis and DNA extraction, respectively.

2.2. Analysis of soil properties and potential nitrification rates (PNR)

Soil pH was determined with a ratio of 2:5 (soil to water). Soil organic matter (OM) was measured using the $K_2Cr_2O_7$ oxidation method. The soil total N (TN), P (TP), and K (TK) were determined using micro-Kjeldahl digestion, colorimetric analysis, and a dissolution-flame photometer, respectively [26]. Soil available N (AN), P (AP), and K (AK) were determined by routine methods recommended by the Chinese Society of Soil Science [26]. Soil properties are listed in Table 1. Soil PNR was measured by the chlorate inhibition method [27]. In detail, $(NH_4)_2SO_4$ was added to catalyze the nitrite formation, and $KClO_3$ was added to inpress the last step in which the nitrite is transformed to nitrate. Subsequently, the accumulation of nitrite was measured to calculate the PNR value.

2.3. DNA extraction

Soil DNA was extracted from 0.5 g of fresh soil using a Fast DNA® SPIN Kit for Soil (Q BIOgene Inc., Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted DNA was checked using a 1% agarose gel electrophoresis and stored at −20 °C prior to use.

2.4. Quantification of bacterial and archaeal amoA gene by real-time PCR

Real-time PCR was performed on an iCycler iQ5 thermocycler (Bio-Rad, USA). Amplification was performed in 25-μL reaction mixtures by using SYBR® Premix Ex Taq™ following the manufacturer's instructions (Takara Bio, Otsu, Shiga, Japan), with the original DNA extracts as template in each reaction mixture. The primer sets (AOB: *amoA*-1F/*amoA*-2R; AOA: Arch-*amoA*F/Arch-*amoA*R) and thermal profiles used in the amplification of each target gene are listed in Table 2. Following the thermal profile, a melting curve analysis was performed to confirm the specificity of the PCR product for each real-time PCR amplification by measuring the fluorescence continuously as the temperature increased from 55 to 95 °C. Data analysis was performed with iCycler software (version 1.0.1384.0 CR). The parameter C_t (threshold cycle) was determined as the cycle number at which the start of exponential increase in the reporter fluorescence was detected.

Standard curves for real-time PCR assays were developed as described previously [15]. Briefly, the AOA and AOB *amoA* genes were PCR-amplified from extracted DNA with the primer sets (AOB: *amoA*-1F/*amoA*-2R; AOA: Arch-*amoA*F/Arch-*amoA*R) listed in Table 2, and then the PCR products were cloned into the pGEM-T Easy Vector (Promega Madison, USA). The plasmids were extracted from the correct insert clones of each target gene and then were used as standards for quantitative analyses. The concentration of plasmid DNA was determined on a Nanodrop® ND-1000 UV–vis Spectrophotometer (NanoDrop Technologies, USA) to calculate the