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Responses of soil ammonia oxidation and ammonia-oxidizing communities to land-use conversion and fertilization in an acidic red soil of southern China

Huifeng Liu [a, b], Xing Wu [a, c, *], Qing Wang [a, b], Shuai Wang [a, c], Dan Liu [a, b], Guohua Liu [a, c, **]

[a] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] University of Chinese Academy of Science, Beijing 100049, China
[c] Joint Center for Global Change Studies, Beijing 100875, China

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ABSTRACT

Ammonia oxidation, the conversion of ammonium (NH_4^+-N) to nitrite (NO_2^--N), is the critical step of nitrification and performed by ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, the effects of land-use conversion and fertilization on the AOA and AOB are not well documented, and the contribution of these two groups to soil ammonia oxidation is still debatable. This study aimed to explore how land-use conversion from rice paddies (RP) to citrus orchards (OR) and fertilization affect the abundance and communities of AOA and AOB, and whether AOA and AOB were correlated to ammonia oxidation. The potential ammonia oxidation (PAO) was measured by chlorate inhibition method, and the abundance and communities of AOA and AOB were quantified using quantitative real-time polymerase chain reaction (qPCR), terminal restriction fragment length polymorphism (T-RFLP) and cloning and sequence analysis. Land-use conversion from RP to OR tended to increase PAO, whereas fertilization increased PAO only in the OR. qPCR results demonstrated that land-use conversion significantly increased AOA abundance, but failed to affect the AOB abundance. The AOA abundance was increased in the OR but decreased in the RP after fertilization, whereas fertilization distinctly stimulated AOB abundance in the OR and RP. T-RFLP analysis showed that the communities of AOA and AOB were significantly changed after land-use conversion. Meanwhile, fertilization significantly affected the communities of AOB both in the OR and RP and only affected the communities of AOA in the OR. Phylogenetic analyses of *amo*A genes showed that group 1.1 a like lineage predominated archaea ammonia oxidizers in the acidic red soil, while all AOB clones were belonged to the *Nitrosospira*. Besides, soil ammonia oxidation was positively correlated to AOA and AOB abundance both in the OR and RP. Our findings imply that land-use conversion and fertilization could potentially alter the ammonia oxidation by regulating the growth of AOA and AOB in this acidic red soil.

1. Introduction

Ammonia oxidation, the conversion of ammonia (NH_3) to nitrite (NO_2-), is the first and rate-limiting step of nitrification and plays an important role in global nitrogen cycle [1]. Ammonia oxidation has important agricultural and environmental consequences, such as the availability of nitrogen, nitrate (NO_3^--N) leaching to groundwater and the releases of nitrous oxide [2]. Therefore, this step has been considered to be “pinhole” of nitrogen turnover and is an important process to be studied in different ecosystems.

It has long been accepted that ammonia oxidation is performed by autotrophic ammonia oxidizing bacteria (AOB) of the β- and γ-subgroups of *Proteobacteria* [1,3]. This viewpoint has been rapidly changed by the discovery of new archaeal groups in the AOA strain,

* Corresponding author. State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.
** Corresponding author. State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.
E-mail addresses: xingwu@rcees.ac.cn (X. Wu), ghliu@rcees.ac.cn (G. Liu).

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which also possess the homologs of AOB-like ammonia monooxygenase gene [4,5], thus raising the prospect of the existence of AOA in different ecosystems [6,7]. Though it has been widely assumed that ammonia oxidation is typically performed by the ammonia-oxidizers [6,8], our knowledge of the relative contribution of AOA and AOB in ammonia oxidation is still limited and inconsistent [9,10]. In fact, quantification of the *amo*A gene showed that AOA copy numbers were found to be more abundant than AOB in different ecosystems, supporting a possibly major contribution for AOA than AOB to ammonia oxidation, in particular in acidic soils or low-ammonia soils [3,11,12]. However, extensive previous studies showed that most of the ammonia oxidization was predominated by autotrophic AOB in nitrogen-rich soils [13–15]. In addition, though the huge phylogenetic members, which were found in the AOA and AOB, only specific groups could transform NH_3 to NO_2- [16]. Therefore, there is necessary to improve our understanding of the relative roles of AOA and AOB in the ammonia oxidation process.

Generally, abundance and communities of ammonia-oxizing communities vary with land-uses, fertilization and soil microconditions [17,18]. AOA and AOB have been found to co-exist in most agriculture soils and might be controlled by soil characteristics (*e.g.* soil moisture, pH and NH_3) [19–22]. In the microcosm studies, the soil moisture might distinctly alter the communities of AOA and AOB [22,23]. Meanwhile, higher ammonia oxidation rates were found in the dry period than the wet period [24,25]. Moreover, soil pH was known to be a main factor determining the activity and communities of soil AOA and AOB [26–28]. Some AOA and AOB phylogeny have been found to be related to soil pH, with increases in the ratio of *amo*A transcript/abundance accompanied by decreasing and increasing pH for AOA and AOB compositions, respectively [26]. In addition, ammonia (NH_3) provide energy sources for ammonia oxidizers and might influence the abundance of AOA and AOB [14,29]. Land use conversion from rice paddies to citrus orchards represented niche specialization with environmental factors (e.g. soil moisture, ammonia, pH, et al.,) that are key factors influencing AOA and AOB [30–32]. Moreover, such conversion would be definitely affected the ammonia oxidation rates. However, very little is known about how soil ammonia oxidizers and ammonia oxidation rates might respond to the conversion from rice paddies to citrus orchards.

Fertilization is one of the most common agronomic practices for rice paddies and orchards throughout the world. Generally, nitrogen fertilization may change NH_3 availability and thereby affect ammonia oxidizers and regulate the ammonia oxidation in soil [10,33]. Moreover, nitrogen inputs into soils are generally accompanied by a decrease in soil pH, which tend to have contradictory effects on the abundance and activity of AOA and AOB [10]. Therefore, differences in physiology and metabolic pathways imply that the responses of AOA and AOB to the change of soil properties induced by fertilization are different [34]. For example, long-term fertilization was shown to significantly affect AOB abundance, but not for AOA in the alkaline soil [14,35]. In contrast, previous findings have also reported that fertilization could increase the abundance of AOA, but has minor impact on AOB in the acidic soils [36,37]. It has also been suggested that fertilization could affect the soil ammonia oxidation rates by affecting the ammonia oxidizers [25,29]. As a result, it is necessary to obtain an understanding of how fertilization affects the abundance and communities of AOA and AOB and the ammonia oxidation rates.

Over the past few years, the acidic red soil regions have undergone great changes in land-uses, especially the conversion of rice paddies to upland cultivations for growing vegetables, fruits and economic forest due to the increasing market demands and higher economic returns. This has resulted in a diverse range of land-uses with different cultivation, irrigation and fertilizer rate, which in turn could cause significant changes in soil ammonia oxidizers with different abundance, compositions and activities. However, litter study is available on the responses of soil ammonia oxidation and ammonia oxidizers to land-use conversion and fertilization in this acidic red soil of southern China. Therefore, the present study aimed to (1) evaluate the effects of land-use conversion and fertilization on soil potential ammonia oxidation rates (PAO); (2) assess the relative changes in the abundance and compositions of AOA and AOB; and (3) explore the relationships between the PAO and AOA and AOB. We hypothesized that (1) the PAO would be increased after the land-use conversion from rice paddies to orchards and fertilization; (2) land-use conversion and fertilization would alter the abundance and compositions of AOA and AOB; and (3) changes in abundance and compositions of AOA and AOB are intimately related to soil PAO.

2. Material and methods

2.1. Field site and soil sampling

The study fields were conducted in the Qianyanzhou Ecological Research Station of Chinese Academy of Sciences (26°44′46″ N, 115°04′05″ E) of Jiangxi province, southern China. The experimental site is a subtropical monsoon climate with an average annual air temperature and precipitation of 17.9 °C and 1489.0 mm, respectively. Double cropping of rice is one of the important cropping systems in the region. The soils in this area are sandy loam and classified as Cambosols (Ultisols classification).

The two common land-use types (*i.e.* rice paddy and citrus orchard) were used for the present study. The experimental site was cultivated with rice paddies for more than 10 years and had been converted to citrus orchards in June 2012. Each land-use had two fertilization treatments: conventional fertilization and non-fertilization. Then, four treatments were included, referred as, citrus orchards with fertilization (OR-F) and without fertilization (OR-NF), and rice paddies with fertilization (RP-F) and without fertilization (RP-NF). All treatments were arranged in a randomized block design with four replications, totaling 16 plots. Each plot was 168 m^2 and was isolated by a buffer strip (12 m length and 2 m width). In the OR-F, compound fertilizer and urea were applied, whereas in the RP-F, compound fertilizer was applied as basal fertilization during the transplanting and urea was applied at the tillering stage. In order to ensure survival and yield, a floodwater layer of 5–7 cm in depth was maintained in the RP till to the mid-season drainage, which began from April 18 and ended on June 10, 2014 for the early rice. Details about descriptions of the experimental site and planting density were recorded in our former studies [38].

Soil samples were collected at four times representing a paddy cycle: T1 = April 28, transplantation and water logging for RP and fertilizer applied for OR-F and RP-F; T2 = May 13, fertilizer applied for RP-F and water logging for RP; T3 = June 24, drainage and pre-crop; T4 = July 23, harvest. For each time point, samples comprised 5 cores (diameter = 3 cm) from the top layer (0–10 cm) were acquired, pooled together, passed through a 2 mm sieve, and any visible material was removed. Thus, 64 soil samples were collected. Subsequently, subsamples of each soil sample were stored at 4 °C for analyses of soil properties or at −80 °C for molecular analysis. The details of the cultivation, fertilization schemes and time points of sampling are presented in Table 1.

2.2. Soil chemical analyses

The soil pH was measured by suspending 8 g of dry soils in 20 ml