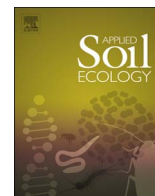




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## Ammonia-oxidizing archaea are more sensitive than ammonia-oxidizing bacteria to long-term application of green manure in red paddy soil

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## ABSTRACT

Growing winter green manure is an effective method for improving nitrogen (N) management in paddy fields to enhance rice production. Ammonia oxidization is a key process in N cycling in these soils, but effects of green manuring on ammonia oxidizers have not been fully addressed for red paddy soils. The objective of this study was to investigate long-term impacts of winter green manure on abundance and diversity of ammonia oxidizers in rice paddy soils of Southern China. The field experiment established in 1982 included four treatments: rice-rice-winter fallow (RRF), rice-rice-ryegrass (RRG), rice-rice-rape (RRP) and rice-rice-milk vetch (RRV). The abundance and diversity of *amoA* genes from ammonia-oxidizing archaea (AOA) and bacteria (AOB) were quantified using quantitative PCR and 454 pyrosequencing, respectively. The AOA were more abundant than AOB in red paddy soils, with ratios of AOA to AOB from 36 to 1686. Long-term application of milk vetch increased the abundance of both AOA and AOB after green manures were incorporated. Some of the relative abundances of most abundant operational taxonomic units (OTUs) of AOA increased and others decreased after application of green manures, while most abundant OTUs of AOB remained unaffected. Redundancy analysis (RDA) found a clear separation between milk vetch and winter fallow, indicating that the community structure of AOA was influenced by application of milk vetch. Phylogenetic analysis showed that the most dominant OTUs of AOA and AOB were affiliated with *Nitrososphaera* and *Nitrosospira*, respectively. In conclusion, in red paddy soil, long-term application of milk vetch increased the abundances of AOA and AOB after incorporated. Moreover, long-term application of green manures had more profound influences on AOA community than on AOB in red paddy soils.

## 1. Introduction

Ammonia oxidization is the first and limiting step in nitrification, and a key process in the soil N cycle (Kowalchuk and Stephen, 2001). Both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) contribute to ammonia oxidization (Leininger et al., 2006). Ammonia monooxygenase (AMO) is the key enzyme that catalyzes the first reaction of ammonia oxidization, and the alpha subunit of

the enzyme is encoded by *amoA* gene (Kowalchuk and Stephen, 2001). Both AOA *amoA* gene and AOB *amoA* gene are frequently used as molecular biomarkers to determine the abundance and diversity of ammonia oxidizers (Prosser and Nicol, 2012).

There is still much debate about the relative contribution of each group to ammonia oxidation activity in different types of agricultural soils (Rudisill et al., 2016). Previous studies have showed that the quantity and diversity of AOA and AOB and their relative contributions

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