

Efficiency Strategies for Community Assembly Processes of Soil Ammonia Oxidisers

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Abstract - Directly, by providing nitrite and, after further oxidation, nitrate to denitrifiers, the bacterial and archaeal nitrification of ammonia is a substantial source to worldwide NO_x emissions. Due to the huge and continuous increases in the usage of ammonia-based fertilisers, which have been driven by the demand for higher food production but also serve as a source of energy for ammonia oxidizers, terrestrial settings are the primary contributors to growing N₂ O emissions (AO). Many metabolic processes in AO, sometimes in conjunction with abiotic mechanisms, lead to the direct synthesis of N₂ O. Ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and comammox bacteria all have their own unique physiological traits and methods for producing N₂ O, which set them apart from one another. In terms of N₂ O production, AOB much outstrips the other two types. Many studies have shown that AOA and AOB live in discrete ecological niches because of the differences between their natural and man-made habitats. For instance, low soil pH and a slow rate of ammonium delivery promote AOA, which is similar to the use of delayed-release fertiliser, while a rapid rate of supply promotes AOB, which is similar to the use of highly concentrated inorganic ammonium or urea. These differences between AOA and AOB provide a potential avenue for improved fertilisation strategies, which might lead to greater fertiliser use efficiency and reduced N₂ O emissions from agricultural soils. In this piece, we examine how community-assembly processes form the basis of effective treatments for soil ammonia oxidizers.

Keywords - Efficiency strategies, Ammonia oxidizers, Soil

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INTRODUCTION

The process of nitrification starts with the bacterial oxidation of ammonia. This slow stage of the nitrification process reduces the plant's ability to take up nutrients. It was previously believed that only members of the Proteobacteria - and - subclasses were involved in ammonia-oxidizing bacteria (AOB). However, this notion has been put into doubt by the transcription of the amoA gene in the archaeal ammonia-oxidizer (AOA). After further investigation, it was found that AOA abundance outnumbered AOB abundance in the majority of soils. With the help of mixotrophy, AOA may outnumber AOB, even though AOB have far higher specific cell activity (Prosser and Nicol, 2012). This means that the relevance of AOA and AOB for a given function may rely heavily on the specific setting. In low-ammonia, high-acid, low-oxygen soils, AOA may dominate nitrification (Zhang et al., 2010, 2012), whereas AOB may contribute more in neutral or alkaline soils, and soils with a larger

ammonia concentration (Shen et al., 2012; Ke et al., 2013).

Because AOB are so sensitive to changes in their environment, their existence is often interpreted as proof of soil disturbance (Silva et al., 2013). Most arable soils are dominated by Nitrosospora from Clusters 2, 3, and 4, with Cluster 3 being most ubiquitous (Prosser and Nicol, 2008). Several researches have shown this (Ai et al., 2013). AOB phylotypes also differed in their responsiveness to fertiliser treatments. The abundance of AOA is less responsive to changes in fertilisation regimes in arable soils than that of AOB, according to multiple studies (Peng et al., 2013). Treatment with organic fertilisers often results in a rise in archaeal 16S rRNA abundance, hence increases in AOA should be anticipated under these circumstances. Further research by (Xu et al. 2012) shown that the 1.1b clade was more abundant when root extract was

added as an organic augmentation to the AOA enrichment culture.

Through the use of crop rotation, hundreds of millions of people were guaranteed a constant supply of food all year long. This rotation system has the potential to serve as a model ecosystem for studying the ecology of microbial communities and biogeochemical processes due to the frequent oxic/anoxic alternations brought about by water management and the rice rhizosphere. Soil ammonia-oxidizers have been drastically altered due to years of nitrogen (N) fertiliser inputs in this climate. In most Chinese paddy soils, the amount and composition of AOB communities were significantly affected by various fertilisation treatments, but AOA communities were not (Shen et al., 2012). Fertilization responses from AOA communities were similarly favoured in low pH environments (Shen et al., 2012). Ammonia-oxidizer communities' distributions and abundances were affected by the stages of the wheat-rice cycle due to shifts in a number of environmental factors. Soil temperature, wheat and rice root exudates, and waterlogged rice crop management were also crucial. However, there is a dearth of data on the dynamic changes of ammonia-oxidizers communities in the wheat-rice rotation system, taking into account both plant growth stage and fertilisation regime. Particularly, the amount of time that AOA and AOB contributed to nitrification over the whole wheat-rice cycle is unknown.

ACTIVITY AND FUNCTION OF AOB AND AOA IN THE SOILS

The communities of AOB and AOA in different soil systems have varied responses to the same changes in environmental variables. However, in alkaline soils from the Fengqiu experiment station, where fertilisation practises were comparable to those at the Qiyang station, it was discovered that the AOB community composition differed considerably across the three fertilisation treatments. At this location, PNR was positively linked with AOB *amoA* gene copy numbers but not with AOA *amoA* gene copy numbers. These results suggest that in acidic soils AOA may be more active than AOB, whereas in alkaline soils the situation may be reversed. Two recent investigations, one an in-situ field study of Chinese tea orchard soils (Yao et al., 2011) and the other a laboratory microcosm experiment, both show that AOA plays a preeminent role in ammonia oxidation in acidic soils (Zhang et al., 2012). In addition, albeit not alone, the research demonstrated a substantial association between nitrification capability and the prevalence of AOA in the area. The function of AOA in autotrophic nitrification in acidic soil microcosms was shown by the findings of Zhang et al. (2012), who discovered that AOA, but not AOB, substantially absorbed soil inorganic carbon at the same time as soil nitrate accumulated. The results of this study, coupled with those from upland acidic soils, show that AOA communities are more able to adapt to low pH settings than AOB communities. Recently, an obligate acidophilic thaumarchaeal ammonia oxidizer from a nitrifying acidic soil was

cultured and characterised, providing more evidence that this hypothesis is correct (Lehtovirta-Morley et al., 2011).

According to research conducted at the Fengqiu experiment station on alkaline soils, although changes in fertilisation regime had no effect on AOA abundance, they did enhance AOB abundance in soils treated with N fertiliser. This finding agrees with the discovery that N fertiliser amendment altered the abundance and composition of AOB in a neutral pH semi-arid temperate grassland soil, but had no appreciable effect on the abundance and composition of AOA communities (Shen et al., 2011). This indicates that AOB may be actively engaged in nitrification, which provides a growth advantage over AOA in alkaline and N-rich neutral soils. These results are consistent with previous studies conducted in New Zealand's neutral pH, N-rich grassland soils, which discovered that AOB was more important than AOA for ammonia oxidation. These investigations add to the growing body of evidence that AOB and AOA may fill distinct ecological niches in soil ammonia oxidation, one that is sensitive to N supply and another that is more strongly influenced by soil pH (He et al., 2012).

INHIBITION OF AMMONIA OXIDATION BY AOB AND AOA

Nitrification may have negative effects on agriculture by causing the loss of nitrogen when N fertiliser is no longer available to feed crops and by contributing to the release of greenhouse gases via the formation of N₂O gas. The creation of nitrification inhibitors has been significant because of the importance of these compounds in controlling ammonia oxidation rates, reducing N losses through nitrate leaching, and halting N₂O emissions. Here we take a look back at how dicyandiamide (DCD) and acetylene altered the AOB and AOA ecosystems, in terms of their make-up, quantity, and ability to operate (C₂H₂). DCD, an accessible non-volatile chemical, blocks nitrification by creating a covalent bond with the AMO enzyme's active site. Recent microcosm study in China's acidic 4.5 pH soil showed that DCD greatly suppressed AOA but not AOB. Several researchers (Zhang et al., 2012) came to this conclusion. Similar findings demonstrated that DCD successfully suppressed nitrification in the soil under study, since AOA were functionally dominant over AOB. To counter this, discovered that high rates of ammonium substrates promoted AOB development and that DCD treatment in urine-rich pasture soil significantly stifled AOB growth. One study found that when applied to N-rich New Zealand grassland soils, DCD decreased NO₃ leaching and N₂O emissions from animal urine patches by 59% and 64%, respectively. It's fascinating to see the varying ways in which AOB and AOA persons are constrained by DCD. The result suggests that structural and/or functional changes may influence how bacterial and archaeal AMO enzymes respond to DCD inhibition, but further research is obviously needed before any firm

statements can be made. Since DCD is able to deactivate the AMO enzyme produced by the actively nitrifying microbial population, it is often regarded as an effective nitrification inhibitor regardless of whether the activity is dominated by AOA or AOB. Increased understanding of soil nitrification may lead to decreased N₂O emissions.

C₂H₂ is a critical nitrification inhibitor and an effective AMO suicide inhibitor. In an alkaline Chinese soil, Xia et al. (2011) used molecular studies in conjunction with C₂H₂'s suppression of nitrification to determine that the growth of both AOA and AOB was inhibited by C₂H₂, with the inhibition of AOB growth being greater. Various studies using C₂H₂ as a nitrification inhibitor have come to different conclusions about the microbial communities primarily responsible for ammonia oxidation, which is an intriguing finding. In microcosms using Scottish agricultural soil, for instance, AOA dominated ammonia oxidation, and its growth could be reduced by treatment with C₂H₂. Using carbon dioxide and hydrogen, showed that in German agricultural soils, AOB, a by-product of ammonia oxidation, was more functionally important than AOA.

Nitrous oxide (N₂O) emissions from soil are mostly attributed to nitrifier denitrification under aerobic conditions (Kool et al., 2011). The role of AOB in mediating soil N₂O emissions has been widely assumed; however, it is not known whether or not AOA also contributes to this process. Recent study analysing stable isotopic signatures of N₂O emissions suggests that AOA is likely responsible for the majority of N₂O emissions from marine environments. However, further research is required to fully understand this mechanism in soil environment (Santoro et al., 2011).

ABUNDANCE AND COMPOSITION OF AOB AND AOA

The amoA gene, which encodes the alpha (A) component of the AMO enzyme, is found in both bacteria and archaea. Culture-independent molecular ecology techniques, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), cloning, sequencing, and quantitative polymerase chain reaction, have allowed for rapid progress in understanding the diversity and distribution of AOB and AOA targeting the amoA genes in different Chinese soils over the past five years (qPCR). Example: scientists in China compared the ratio of acid-loving to acid-hating organisms in acidic red soils at a field station in Qiyang that had been continuously fertilised for 16 years (pH 3.7-6.0). That was the consensus of a group of researchers (He et al., 2007). Soil samples from each of the eight fertilisation methods showed a ratio of AOA to AOB amoA gene copy numbers between 1 and 12. Along with a group, he (2007). These results backed with those of a research done in 2006 by Leininger and colleagues, who discovered that AOA predominated over AOB in agricultural soils. Numerous studies conducted on alkaline agricultural soil with pH levels

between 8.3 and 8.7 yielded similar findings. Researchers Shen et al. The presence of AOB and AOA in agricultural soils of various pH levels in China was first shown by He et al. (2007) and Shen et al. (2008). Grassland (Shen et al., 2011) and paddy (Chen et al., 2008, Wang et al., 2009, Ying et al., 2010, Yao et al., 2011, Zhang et al., 2012) are only two examples of the many habitats and management techniques that have been studied in comparison to AOB and AOA. Archaeal amoA genes were more common than bacterial amoA genes throughout the majority of soils examined, with the exception of two long-term fertilisation treatments, where the ratio was 0.25 and 0.39, respectively. It was determined by Wu et al (2011).

To further illustrate the distribution of AOB and AOA throughout China's soils, we reanalyzed previously published data on the community makeup of these organisms in 23 soil samples from five different regions of the nation (Table 1). Clusters 10, 11, and 12 of Nitrosospira amoA were the most abundant in the AOB community of acidic red soils, whereas clusters 3a.1, 3a.2, and 6 and 7 of Nitrosomonas amoA were more prevalent in alkaline and neutral soils. Review Figure 1A. We detected two main classes of archaeal amoA sequences in these soils, designated group 1.1a-associated and group 1.1b (Figure 1B). Soil pH was shown to be negatively associated to the relative abundance of group 1.1a (r = 0.553, n = 23, p 0.05) and group 1.1b (r = 0.357, n = 23, p 0.05). It was shown in a 2011 meta-analysis and high-throughput sequencing research by Gubry-Rangin et al. that soil pH was the only observable physicochemical component that substantially altered the AOA community structure. We used soil sequences from a pH range that was similar to that of the soils. New studies conducted on Chinese soils have shed light on the factors that contribute to the global dispersal patterns of AOB and AOA.

Table 1: Physical And Chemical Properties of The Twenty-Three Soils Used For The Meta-Analysis

Location and experimental design	Soil type	Sample name	Treatment	pH	Organic matter (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Longitude (°)	Latitude (°)	Altitude (m above sea level)	Mean temperature (°C)	Reference
Toupan(TV), Hunan, Land utilization patterns	Acidic red soil	TV PM	Pine plantation	4.1	11.6	0.0	18.5	111.433	28.917	110	16.5	Ying et al., 2009
		TV CM	Cropland	4.1	9.5	2.4	45.3	111.433	28.917	110	16.5	
		TV DM	Degradation	4.3	14.0	1.6	0.5	111.433	28.917	110	16.5	
		TV RM	Restoration	4.2	16.5	6.2	13.2	111.433	28.917	110	16.5	
Toupan(TVPL), Hunan, Different fertilizations	Acidic paddy soil	TVP NPK+C	N, P, K fertilizers and organic matter	5.3	41.6	85.9	0.9	111.433	28.917	110	16.5	Chen et al., 2011
		TVP NK	N plus K ¹	5.3	30.4	55.9	0.7	111.433	28.917	110	16.5	
		TVP CK	No fertilization	5.4	29.2	27.1	0.6	111.433	28.917	110	16.5	
		Qiyang(QY), Hunan, Different fertilizations	Acidic red soil	QY NK	N plus K fertilizers	3.8	14.3	15.5	35.2	111.887	26.750	
QY NP	N plus P fertilizers	4.0		16.4	13.4	21.5	111.887	26.750	100	18.0		
QY N	N fertilize	3.7		15.2	21.0	43.6	111.887	26.750	100	18.0		
QY PK	P plus K fertilizers	5.0		14.6	19.7	6.0	111.887	26.750	100	18.0		
Duzhuni(DL), Inner Mongolia	Chernozem soil	DL NPK	N, P, K fertilizers	4.0	16.0	27.9	8.7	111.887	26.750	100	18.0	Shen et al., 2011
		DL CK	No fertilization	5.5	13.6	19.1	5.1	111.887	26.750	100	18.0	
		DL Fallow	Fallow	5.8	13.7	13.4	0.7	111.887	26.750	100	18.0	
		DL NPK+OM	N, P, K fertilizers and organic matter	5.8	21.3	13.7	18.7	111.887	26.750	100	18.0	
Fengzhu(FZ), Henan, Different fertilizations	Sandy loam soil	FZ NPK	N plus K fertilizers	8.4	7.4	5.4	35.2	114.400	35.000	675	13.9	Shen et al., 2006
		FZ PK	P plus K fertilizers	8.5	8.4	4.7	2.4	114.400	35.000	675	13.9	
		FZ NP	N plus P fertilizers	8.3	9.5	4.3	33.4	114.400	35.000	675	13.9	
		FZ CK	No fertilization	8.5	16.7	5.5	4.9	114.400	35.000	675	13.9	
Fengzhu(FZ), Henan, Different fertilizations	Sandy loam soil	FZ CK	No fertilization	8.7	7.3	6.1	6.5	114.400	35.000	675	13.9	Shen et al., 2006
		FZ NPK	N, P, K fertilizers	8.4	9.8	4.7	22.6	114.400	35.000	675	13.9	
		FZ 100%PK	100%PK fertilizers plus organic matter	8.5	12.8	4.2	10.9	114.400	35.000	675	13.9	
		FZ 100%PK	100%PK fertilizers plus organic matter	8.5	12.8	4.2	10.9	114.400	35.000	675	13.9	

¹ Sample name was designated as the sampling location, followed by the treatment.
² Fertilization abbreviation: chemical fertilizer nitrogen (N), phosphorus (P) and potassium (K). "": no data.

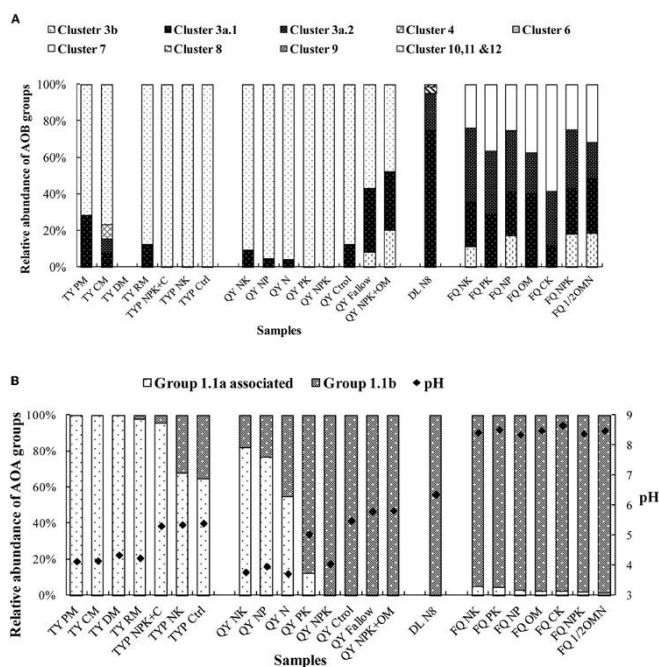


Figure 1: Relative abundances of bacterial and archaeal ammonia-oxidizer groups in selected Chinese soils. (a) AOB community composition; (b) AOA community composition.

PHYSICOCHEMICAL PROPERTIES OF THE SOIL

Soil $OXC=5 [NO_3]+2 [Mn(IV)]+[Fe(III)]+8 [SO_4]$ was used to calculate the soil's oxidation capacity (OXC). Taking oxidising agents into account, the soil OXC value reveals the soil's capacity to accept electrons, but it does not take electron suppliers into consideration (such as soil organic matter). The brackets around the equation contain the calculated millimolar concentrations (mmol kg⁻¹). The "free" forms of manganese (IV) and iron (III) oxides in soil are the ones that can be extracted using $NH_2OH \cdot HCl$.

In an effort to simulate the in-situ flooding conditions on the soil, microcosms of paddy soil were created. Approximately 1.5 kg of dirt and 2-3 cm of standing water were placed into each 1500 ml polyethylene container (height: 30 cm; diameter: 8.0 cm) and then kept at 25 °C for 60 days. An oxygen microelectrode sensor was used to get a read on the oxygen levels in the air at different heights (Unisense OX 50; Science Park, Aarhus, Denmark). Soluble oxygen concentrations were measured by inserting the microsensors tip 100 μm at a time into the soil to a depth of 5 mm. We recorded the entire oxygen profile of the soil to a depth of 20 cm with a spatial resolution of 5 mm.

CONCLUSION

Recently, fascinating new creatures involved in ammonia oxidation have been found, and their ecology and genomes have been extensively studied. The processes governing the distribution and activity of these microbes in the environment are still substantially

unknown, even if many of the ideas emerging from genomic analysis have not yet been tested. Future study is needed to evaluate the possibility of a new ammonia oxidation route in AOA, which has been described in this review. Recent discoveries of AOA and comammox *Nitrospira* culture models provide insight into the adaptation and response mechanisms of ammonia-oxidizing microbes.

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