Review

Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils

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ABSTRACT

Recent studies of ammonia-oxidizing archaea (AOA) suggested their significant contributions to global nitrogen cycling, and phylogenetic analysis categorized AOA into a novel archaeal phylum, the Thaumarchaeota. AOA are ubiquitous in terrestrial ecosystems, have unique mechanisms for nitrification, better adaptation to low-pH pressures, and strikingly lower ammonia requirements compared with ammonia-oxidizing bacteria (AOB). Previous perceptions that microbial ammonia oxidation in acidic soils was minimal, and entirely mediated by autotrophic bacteria and occasionally by heterotrophic nitrifiers have been dramatically challenged, and the dominant nitrifying groups urgently called for reassessment. Controversially, the relative contributions of AOA and AOB to autotrophic ammonia oxidation have been reported to vary in different soils, but ammonia substrate availability, which was largely restricted under acidic conditions, seemed to be the key driver. Theoretically predicted ammonia concentrations in acidic soils below the substrate threshold of AOB and remarkably high ammonia affinity of AOA raised the supposition that thaumarchaea could represent the dominant ammonia-oxidizing group in ammonia-limited acidic environments. Recently, the functional dominance of thaumarchaea over its bacterial counterpart and autotrophic thaumarchaeal ammonia oxidation in acidic soils has been compellingly confirmed by DNA-stable isotope probing (SIP) experiments and the cultivation of an obligate acidophilic thaumarchaeon, *Nitrosotalea devanaterrea*. Here, we review the currently available knowledge concerning the history and progress in our understanding of the ammonia-oxidizing microorganisms (AOB and AOA) and the mechanisms of nitrification in nutrient-depleted acidic soils, present the possible mechanisms shaping the distinct niches of AOA and AOB, and thus strengthen the assumption that AOA dominate over AOB in ammonia oxidation of acidic soils.

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1. Introduction

Acidic soils (defined as pH < 5.5 in the surface layers) are globally distributed and occupied approximately 30% of the world's total ice-free lands (Vonuexkull and Mutert, 1995). Besides natural soil acidity and acidification, anthropogenic perturbations in the past several decades, such as huge inputs of artificial nitrogen-based fertilizers in intensive agricultural systems, have greatly accelerated world-scale soil acidification (Vitousek et al., 1997) and global nitrification rates (Le Roux et al., 2003). Nitrification, oxidizing ammonia to nitrate via nitrite, is a crucial process in the global nitrogen cycle. Nitrification and denitrification could significantly contribute to atmospheric emissions of greenhouse

and ozone-depleting gas nitrous oxide (N₂O) (Wrage et al., 2001), nitrate leaching to groundwater, and losses of nitrogen-based fertilizers. In addition, nitrification in acidic soil ecosystems could lead to further soil acidification and then increase the exposure risks of plant roots to toxic metals like Al³⁺ (Carver and Ownby, 1995). Therefore, it is crucial to understand the possible mechanisms of nitrogen transformation in acidic soil ecosystems.

The first observation of nitrification in acidic soils was reported a century ago (Houzeau, 1872). For a long time, this important biogeochemical cycling step of ammonia oxidation was thought to be performed exclusively by bacterial ammonia oxidizers, belonging to two monophyletic groups within β- or γ-proteobacteria (Prosser, 1989; De Boer and Kowalchuk, 2001). However, acidic soils were generally considered detrimental to AOB due to soil acidity pressure, low ammonia substrate availability, presence of natural inhibitive compounds, and increasing toxicity of nitric and nitrous acids (De Boer and Kowalchuk, 2001). Recently,

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metagenomic studies and cultivation-dependent analyses revealed a novel archaeal group capable of oxidizing ammonia, which was completely overlooked in previous studies (Venter et al., 2004; Könneke et al., 2005; Treusch et al., 2005). Physiological characterization of the first AOA isolate, *Nitrosopumilus maritimus*, and environmental investigations strongly indicated that bulk nitrification in nutrient-depleted open oceans was presumably performed by the abundant thaumarchaea, a deep-branching archaeal phylum (Beman et al., 2008; Brochier-Armanet et al., 2008; Martens-Habben et al., 2009). Furthermore, genomic studies of *N. maritimus* revealed remarkably divergent pathways of thaumarchaeal ammonia oxidation from bacteria, which potentially offered a significant advantage for thaumarchaea in nutrient-poor environments (Walker et al., 2010). These new findings attracted considerable scientific interest, and raised questions about the relative contributions of thaumarchaea and bacteria to the autotrophic ammonia oxidation in terrestrial environments, particularly in acidic soils.

More and more studies have shown that nitrification can occur in a wide range of acidic soils (Killham, 1990; Degrange et al., 1998; Bottomley et al., 2004; He et al., 2007). It is generally accepted that ammonia (NH_3) rather than ammonium (NH_4^+) could serve as the direct substrate to ammonia monooxygenase (AMO), but ammonia availability is exponentially reduced by decreasing pH (Suzuki et al., 1974). The theoretically predicted ammonia concentrations in low-pH environments (according to the formula $\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$;

$\text{pK}_a = 9.25$ at 25°C) were significantly below the substrate threshold of cultured AOB, but seemed favorable to AOA which have higher substrate affinity (Stopnišek et al., 2010; Zhang et al., 2012). Competition between AOA and AOB for limited energy sources significantly intensifies under acidic conditions, when ammonia substrate availability becomes extremely limited. Therefore, acidic soil could be considered as an ideal ammonia-limited oligotrophic environment for thaumarchaea, and this hypothesis has been very recently supported by several laboratory microcosm incubations, which indicated a predominant role of thaumarchaeal ammonia oxidation under acidic conditions (Gubry-Rangin et al., 2010; Stopnišek et al., 2010; Zhang et al., 2012). Thus, it is of great importance to understand the possible mechanisms of microbial ammonia oxidation in acidic soils, and reconsider the role of thaumarchaea to mediate nitrification which has been largely underestimated.

2. Ammonia-oxidizing bacteria and nitrification in acidic soils

For a long time, nitrification was thought only to be present in neutral and alkaline soils, because many investigations did not detect nitrate in acidic soils, raising the supposition that nitrification could not take place in these low-pH environments (Noyes and Conner, 1919). The first observation of nitrification in acidic soils was reported in 1872 (Houzeau, 1872) (Fig. 1). In 1908, the detection

Fig. 1. A general timeline of researches on the nitrification in acidic soils, with emphasis on thaumarchaeal ammonia oxidation.

of nitrate accumulation in acidic soils was attributed to the probable presence of isolated calcium carbonate particles, which kept restricted neutral and alkaline micro-sites suitable for the nitrification activity (Hall et al., 1908). Since then, pronounced evidence contradicted the previous opinions that nitrification could not exist in acidic soil environments. However, these widely-observed phenomena were entirely attributed to some purely chemical agents, before the discovery of bacterial ammonia oxidizers in actively nitrifying soils (Winogradsky, 1890). Although the responsible microorganisms and relevant mechanisms still remained elusive, the positive correlations between aerobic bacterial numbers and nitrate concentrations began to be noticed (Fred and Graul, 1916; Noyes and Conner, 1919). These early discoveries paved the way for more convincing studies confirming that nitrification was a universal phenomenon in a wide range of acidic soils, and stimulated scientific interests to identify the responsible nitrifiers in soils (De Boer and Kowalchuk, 2001).

AOB were subsequently detected using the Most Probable Number (MPN) method in some natural acidic soils with the numbers in the range of 10^2 – 10^4 g⁻¹ soil, and were recognized as the responsible nitrifiers in acidic soils, even though the abundance was much lower than that commonly observed in neutral or alkaline agricultural soils (10^5 – 10^7 g⁻¹ soil) (Hankinson and Schmidt, 1984; De Boer et al., 1988, 1992; Pennington and Ellis, 1993; Carnol and Ineson, 1999; Hastings et al., 2000). PCR-based strategies also detected a wide diversity of AOB-like 16S rRNA and *amoA* genes from a variety of acidic soils (Stephen et al., 1996; Laverman et al., 2001), but still no studies could directly link AOB existence with *in situ* acidic soil nitrification.

Several postulated mechanisms to interpret bacterial ammonia oxidation under low-pH conditions have been proposed, including restricted pH-neutral micro-sites (Prosser, 1989), intracellular urea hydrolysis (De Boer and Laanbroek, 1989), acid-tolerant AOB cells (De Boer et al., 1989), high cell density or aggregation, and close associations between AOB and nitrite-oxidizing bacteria (NOB) (De Boer et al., 1991). However, such mechanisms could not convincingly support the supposition that AOB dominated the autotrophic nitrification under acidic conditions, when considering the usually narrow pH range for optimal growth of bacteria around neutral or alkaline conditions. The optimal pH range for bacterial communities is generally constrained between 3 and 4 pH units in pure culture (Rosso et al., 1995). A reduction of 1.5 pH units would significantly impede bacterial activity and net growth, leaving them in an inferior position rapidly outcompeted by other less-influenced microorganisms (Rousk et al., 2010). By now, most isolated AOB strains from acidic soils could not be able to oxidize ammonia in liquid batch culture below pH 5.5 (Hankinson and Schmidt, 1984). In some cases, there was evidence for selection of particularly acidophilic AOB strains in low pH soils, but this was not universal (Nicol et al., 2008).

A number of controversial findings indicated that AOB did not sustain the ammonia oxidation under acidic conditions. Molecular analyses showed that AOB community structure remained relatively stable irrespective of changing nitrification rates (Laverman et al., 2001) and nitrogen mineralization (Mintie et al., 2003) in acidic forest soils. Also in grassland soils, community structure of AOB did not shift between different vegetation types, whereas potential nitrification rates changed considerably (Smits et al., 2010). AOB in acidic soils subjected to various levels of nitrogen and sulfur deposition revealed a minor contribution of AOB to autotrophic nitrification (Schmidt et al., 2007). High sensitivity of AOB to low pH and inertia to oxidize ammonia in acidic soils were reflected by a significant decrease in abundance with decreasing pH and a weak correlation between AOB *amoA* gene copies and nitrification potential in Chinese acidic red soils

(He et al., 2007; Ying et al., 2010). In some strongly nitrifying acidic soils, AOB was even found to be below the detection limit using the MPN method (Killham, 1990; Papen and Von Berg, 1998). Jordan et al. (2005), who ran an acetylene inhibition experiment, also speculated that some undefined nitrifiers rather than AOB contributed more to the nitrification processes in acidic forest soils receiving high inputs of nitrogen deposition (Jordan et al., 2005). Although heterotrophic nitrifiers, including some bacteria and fungi, could account for a small proportion (usually less than 20%) of total nitrification in acidic soils (Pedersen et al., 1999; Islam et al., 2007), their minor contribution to nitrification and absence of AOB in a number of actively nitrifying acidic soils led to a growing hypothesis that some unrecognized ammonia-oxidizing microorganisms were more likely to explain the acidic soil nitrification.

3. Active thaumarchaea in nitrification of acidic soils

The existence of a novel archaeal group possessing homologs of bacterial ammonia monooxygenase gene was first suggested by metagenomic investigations (Venter et al., 2004; Treusch et al., 2005), and then AOA capacity for oxidizing ammonia was confirmed using the isolated pure strain *N. maritimus* in 2005 (Könneke et al., 2005) (Fig. 1). This breakthrough discovery opened up a novel perspective to reconsider the mismatch between AOB sensitivity to low pH and active nitrification in acidic soils. A number of molecular investigations demonstrated that thaumarchaea numerically dominated over their bacterial counterparts in various acidic soils (Leininger et al., 2006; He et al., 2007; Yao et al., 2011), and thaumarchaeal *amoA* gene abundance and transcriptional activity were found to increase with decreasing pH across a soil pH gradient ranging from 4.9 to 7.5, with the ratio of AOA versus AOB transcriptional activity reaching the maximum value in the lowest pH soil (Nicol et al., 2008). A similar observation for the trend of the ratios of AOA versus AOB *amoA* gene abundance was observed in Chinese acidic tea orchard soils, where a significant correlation was only found between AOA abundance and potential nitrification rates, with some specific AOA populations well-adapted to strongly acidic soils (Yao et al., 2011). Community composition and abundance changes of AOA, rather than AOB, responded sensitively to long-term fertilization in an acidic paddy soil, from which a predominant role of AOA could be inferred (Chen et al., 2011). Moreover, in an acidic forest soil where AOB were below the detection limit, AOA appeared to dominate the active nitrification and preferred to use ammonia produced by mineralization (Stopnišek et al., 2010).

Very recently, ¹³CO₂-DNA-SIP experiments convincingly linked the autotrophic nitrification activity in acidic soils with thaumarchaeal ammonia oxidizers (Lehtovirta-Morley et al., 2011; Zhang et al., 2012), and *Nitrosotalea devanattera*, a thaumarchaeon enriched from an acidic agricultural soil, was discovered to be chemolithotrophic and obligately acidophilic with optimal growth pH in the range 4–5 (Lehtovirta-Morley et al., 2011). In addition, pH selection of soil thaumarchaeal ammonia oxidizers at local, regional and global scales was demonstrated by 454 barcoded pyrosequencing, which identified some well-supported phylogenetic lineages with specific adaptation to acidic, acido-neutral and alkaliphilic soils (Gubry-Rangin et al., 2011). These studies provide a powerful explanation for high rates of nitrification in acidic soils and conclusively confirm the vital role played by thaumarchaea to mediate ammonia oxidation in acidic soils.

The isolated and enriched cultures of Group I.1a *N. maritimus*, *Cenarchaeum symbiosum*, *Nitrosoarchaeum limnia*, and *Nitrosoarchaeum koreensis*, Group I.1a-associated *N. devanattera*, Group I.1b *Nitrososphaera viennensis* and *Nitrososphaera gargensis*, ThAOA/

HWCG III *Nitrosocaldus yellowstonii* have shown a capacity to oxidize ammonia (Könneke et al., 2005; Hallam et al., 2006; de la Torre et al., 2008; Hatzenpichler et al., 2008; Blainey et al., 2011; Jung et al., 2011; Lehtovirta-Morley et al., 2011; Tourna et al., 2011). Group I.1a-associated and I.1b thaumarchaea were shown to participate in the ammonia oxidation processes of strongly acidic soils using the $^{13}\text{CO}_2$ -SIP approach in soil microcosms (Zhang et al., 2012). In addition, an unrecognized *amoA*-cluster has been identified from the *amoA*-gene-based phylogenetic trees, which has not been definitely linked with 16S rRNA-based thaumarchaeal lineage, but was speculated to include the Group I.1c archaea (Pester et al., 2011).

Group I.1c archaea was first described in a boreal forest soil (Jurgens et al., 1997), and was then found to predominate the archaeal 16S rRNA genotypes in two temperate acidic forest soils (Kemnitz et al., 2007). Furthermore, an apparent preference for lower pH could be inferred from a series of molecular surveys with the greatest gene abundance occurring in the lowest pH soil and even below detection limit at near-neutral pH (Nicol et al., 2005; Kemnitz et al., 2007). In the long-term pH-manipulated Craibstone soils, Group I.1c was only detected by cloning library in pH 4.9 soil plots (Nicol et al., 2008). Lehtovirta-Morley et al. (2009) found that soil pH was the key driver regulating the abundance and community structure of Group I.1c in a wide range of soil habitats, and this distinct lineage dominated the archaeal communities in most of acidic soils (Table 1). Community structures of soil-inhabiting Group I.1c was influenced by Scots pine ectomycorrhizae, leading to a supposition that it was heterotrophic (Bomberg and Timonen, 2007). However, despite of the widespread distribution of Group I.1c archaea in acidic soils, little is known of its particular ecological function, and its abundance was still not directly associated with the ammonia-oxidizing activity. Furthermore, as yet no cultured thaumarchaea belonged to Group I.1c. In a recent DNA-SIP microcosm incubated with acidic soils, Group I.1c archaea was retrieved in both light and heavy fractions during 30 days' active nitrification, however, no quantitative PCR was performed to detect the labeling of $^{13}\text{CO}_2$, thus its potential involvement in ammonia-oxidizing function of acidic soils still remained unclear (Zhang et al., 2012).

4. Possible mechanisms of thaumarchaeal ammonia oxidation in acidic soils

4.1. Ammonia-limited acidic soils and high affinity for ammonia by thaumarchaea

The ammonia oxidation step provides the sole energy source for the slow-growing nitrifying microorganisms, so ammonia substrate availability was thought to be a dominant factor dictating the differential growth and ecological niches of AOA and AOB (Martens-Habben et al., 2009; Schleper, 2010; Verhamme et al., 2011). Previous studies suggested that the presence of some unknown nitrifiers might be responsible for the ammonia oxidation in some low ammonia-containing ecosystems, because the *in situ* substrate concentrations were significantly below the growth requirement of AOB cultures (Prosser, 1989). Similarly, in a survey of non-fertilized, mineral-fertilized and organic + mineral-fertilized soils, the highest ratios of AOA to AOB *amoA* gene copies were found in the nutrient-limited non-fertilized soils (Leininger et al., 2006). In a series of soil microcosms with low background ammonia concentrations and incubated with no external nitrogen amendments, AOA was found to primarily control the nitrification (Offre et al., 2009; Gubry-Rangin et al., 2010; Zhang et al., 2010, 2012). In contrast, in some soils regularly supplied with nitrogen fertilizers, AOB was thought to be mainly responsible for the ammonia oxidation (Jia and Conrad, 2009; Xia et al., 2011), and AOB growth was also observed to benefit from increased ammonium availability (Schauss et al., 2009; Verhamme et al., 2011). AOB abundance responded positively to the amendment of nitrogen fertilizer and correlated well with nitrification activity in heavily fertilized soils (Di et al., 2009, 2010) or wetland (Levicnik-Höfferle et al., 2010), whereas AOA growth was suppressed by high levels of ammonium in soil microcosms (Verhamme et al., 2011).

The questions about their relative contributions should be much clearer if the actual ammonia concentrations could be taken into account. Generally assuming the soil water content of 25% and the ionization equilibrium constant pK_a 9.25 at 25 °C, ammonia concentrations in several soil microcosms could be theoretically predicted from the total ammonium concentrations (Table 2). It is

Table 1
Studies reporting Group I.1c thaumarchaea in soil ecosystems.

Soil sample	pH range	Findings	References
Scottish pH-manipulated plots	4.5–7.5	Group I.1c was only detected in pH 4.5–6.0, and increased steadily as pH decreased, with the highest abundance occurring in the lowest pH soil.	Lehtovirta-Morley et al., 2009
Various types of soils	3.9–6.6	High abundance of Group I.1c was observed in acidic soils with distinct characteristics at pH 3.9–5.7, accounting for 36%–69% of total Crenarchaeota.	Lehtovirta-Morley et al., 2009
A temperate acidic forest soil	3.8–4.3	T-RFLP and clone libraries analysis demonstrated that Group I.1c constituted a significant proportion of the archaeal phenotypes in the tested acidic forest soil, implying a crucial functional role.	Kemnitz et al., 2007
A glacier foreland soil	4.2–7.5	Group I.1c was the primary component of archaeal community only in more developed soil with a pH value of 4.2, and its diversity was not influenced by plant species rhizosphere.	Nicol et al., 2005
An organic acidic forest soil	4.1	29% of archaeal 16S rRNA genes were placed into Group I.1c but without evidence for their involvement in nitrification.	Stopnišek et al., 2010
Various soil ecosystems	5.0–9.5	The relative abundance of Group I.1c in Marburg acidic forest soil was much higher than that in sandy meadow soil.	Ochsenreiter et al., 2003
Chinese acidic red soils	4.0–4.3	Group I.1c and I.1c-associated sequences prevailed in the degradation plot (pH = 4.33), but no amplified archaeal <i>amoA</i> genes could be linked with Group I.1c.	Ying et al., 2010
Chinese strongly acidic soils	4.2–4.5	$^{13}\text{CO}_2$ -DNA-SIP revealed that Group I.1c archaea might be labeled during 30 days' active nitrification, indicating an autotrophic growth lifestyle. Although no direct evidence confirmed the presence of <i>amoA</i> genes in Group I.1c, its potential involvement in ammonia oxidation could not be overlooked.	Zhang et al., 2012

Table 2
Ammonia oxidizers and nitrification activity in various soil microcosms.

Soil type	Soil pH	Ammonia concentration		The dominant nitrifier	References
		The initial NH_4^+ concentration (mg kg^{-1})	Theoretic NH_3 concentration ^a		
Agricultural, pH-regulated plots	7.0	0.6	0.964 μM	Thaumarchaea	Offre et al., 2009
Agricultural, pH-regulated plots	7.5	3.2	1.22 μM	Thaumarchaea	Zhang et al., 2010
Agricultural, pH-regulated plots	4.5	0.86	0.00437 μM	Thaumarchaea	Gubry-Rangin et al., 2010
Agricultural, pH-regulated plots	6.0	0.59	0.0948 μM	Thaumarchaea	Gubry-Rangin et al., 2010
Agricultural, tea orchard	4.2	39.2	0.0246 μM	Thaumarchaea	Zhang et al., 2012
Agricultural, rotated maize, wheat and barley	7.0	4.19	6.73 μM	Bacteria	Jia and Conrad, 2009
Agricultural, sandy	8.0	1.13	323 μM	Bacteria	Xia et al., 2011

^a NH_3 concentrations in this table are theoretically predicted based on the equilibrium ($\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$; $\text{pK}_a = 9.25$) at 25 °C.

clear that the low-level ammonia concentrations ranging from 0.00437 μM to 1.22 μM selectively favored the growth of AOA. However, when the initial ammonia concentrations were higher (6.73–323 μM) and weekly satisfied by high-level amendments ranging from 643 μM to 6428 μM (four times of weekly 100 $\text{mg NH}_4^+ \text{kg}^{-1}$ soil inputs), AOB was observed to primarily control the nitrification activity (Table 2).

In addition to environmental investigations and laboratory microcosms, more evidence supporting the thaumarchaeal preference for low ammonia comes from ecophysiological features of the cultivated AOA strains, involving the lower inhibitory ammonia concentrations and higher affinity for ammonia. Observed inhibitory ammonia concentrations (0.89–356 μM) in all the currently available cultures or enrichments of thaumarchaea from marine and terrestrial ecosystems are considerably lower than the substrate threshold of cultured AOB (394–40,000 μM) (Table 3), indicating a narrow and lower substrate span for AOA. The bulk nitrification in nutrient-depleted open ocean has been presumed to be linked with the abundant AOA, where the typical ammonium concentrations ranged between 0.03 and 1 μM (Beman et al., 2008; Erguder et al., 2009). This range is very similar to the apparent K_m -value (0.13 μM ammonium) of the marine-isolated *N. maritimus* which is by now the lowest half-saturation constant among all the

cultured ammonia oxidizers (Martens-Habbena et al., 2009). Further, two enriched AOA strains also have a lower K_m -value from 0.61 to 0.69 μM ammonium (Park et al., 2010; Jung et al., 2011). In comparison, the half-saturation constant K_m of cultured AOB ranged from 50 μM to 1780 μM ammonium (Table 3). Therefore, AOA showed a much higher affinity for ammonia, more than 200-fold higher than that of reported AOB strains (Martens-Habbena et al., 2009), accounting for the specific ability of AOA to use low-level ammonia and compete efficiently for the limited substrate with AOB. Therefore, the higher capacity to use ammonia substrate, and low-pH-reduced ammonia availability in acidic soils, provide major advantages for AOA dominance over AOB in these environments.

4.2. Nitrogen sources for thaumarchaea in acidic soils

Despite increasing evidence supporting the thaumarchaeal preference for ammonia-poor environments, of particular interest is which kind of ammonia can serve as the nitrogen source for thaumarchaea, since different types of (inorganic or organic) fertilizer have contrasting influences on the corresponding ammonia-oxidizing groups (Hai et al., 2009). A broad survey of soils demonstrated a positive relationship between mineralization rates

Table 3
Parameters for several enriched or pure cultures of thaumarchaeal or bacterial ammonia oxidizers.

Cultured nitrifiers	Descriptions	pH	Observed inhibitory concentrations		Half-saturation constant (K_m)		References
			NH_4^+ (μM)	NH_3 (μM) ^a	NH_4^+ (μM)	NH_3 (μM) ^a	
<i>Nitrosopumilus maritimus</i>	AOA from marine aquarium	7.5	2000	35.6	0.13	0.0023	Martens-Habbena et al., 2009
<i>Nitrososphaera gargensis</i>	Enriched thermophilic AOA	7.8	3080	109	NA ^b	NA	Hatzenpichler et al., 2008
<i>Nitrosocaldus yellowstonii</i>	Enriched thermophilic AOA	7.5	NA	NA	NA	NA	de la Torre et al., 2008
<i>Nitrososphaera viennensis</i>	Soil isolated AOA	7.5	20,000	356	NA	NA	Tourna et al., 2011
<i>Nitrosotalea devanaterri</i>	Obligate acidophilic AOA	4.5	50,000	0.89	NA	NA	Lehtovirta-Morley et al., 2011
<i>Nitrosoarchaeum koreensis</i>	Soil enriched AOA	7.0	20,000	112	0.69	0.04	Jung et al., 2011
AR enrichment culture	Marine sediment AOA	7.0	40,000	224	0.61	0.02	Park et al., 2010
<i>Nitrosomonas europaea</i> ATCC 19718	Obligate lithoautotrophic AOB	8.2	263,000	40,000	NA	NA	Sato et al., 1985
<i>Nitrosomonas</i> Cluster 6a G5-7	AOB from freshwater sediment	7.8	NA	NA	50–100	1.77–3.55	Bollmann et al., 2002
<i>Nitrosomonas oligotropha</i>	AOB from river estuary	7.8	50,000	1774	50–116	1.77–4.12	Stehr et al., 1995
<i>Nitrospira</i> strains 40K1, AF, B6, L115	Soil-isolated AOB	7.8	NA	NA	208–380	7.38–13.5	Jiang and Bakken, 1999
<i>Nitrosomonas</i> sp. GH22	AOB in activated sludges	7.4	NA	NA	1780	25	Suwa et al., 1994
<i>Nitrosomonas europaea</i> Nm35	Soil-isolated AOB	8.0	7000	394	460	25.9	Groeneweg et al., 1994
<i>Nitrospira briensis</i> ATCC 25971	Soil-isolated AOB	7.5	NA	NA	167	3.0	Bollmann et al., 2005
<i>Nitrosomonas europaea</i> , <i>N. eutropha</i> , <i>N. halophila</i> and <i>N. mobilis</i>	AOB from sewage, freshwater and brackish water	NA	NA	NA	420–750	30–61	Koops and Pommerening-Roser, 2001
<i>Nitrosomomas cryotolerans</i>	Obligately halophilic AOB	NA	NA	NA	NA	42–59	Koops and Pommerening-Roser, 2001

^a NH_3 concentrations in this table are theoretically predicted based on the equilibrium ($\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$; $\text{pK}_a = 9.25$) at 25 °C.

^b NA, not available.

and net nitrification rates, indicating an important role of mineralized ammonia substrate for the following nitrification processes (Booth et al., 2005). There is also evidence that potential nitrification rates were only enhanced by manure treatment but suppressed by mineral fertilizers (Fan et al., 2011) in some soils. Actually, nitrification correlated with nitrogen mineralization rather than ammonium availability in acidic soils has also been indicated in several previous environmental investigations (Kretinger et al., 1985; Strong et al., 1997; Frank et al., 2000) and gross nitrogen mineralization in acidic forest soils has been recognized as a good index of ammonia availability for autotrophic ammonia oxidizers (Hart et al., 1997). Similar observations were also made in Chinese acidic red soils where potential nitrification rates were substantially reduced by long-term additions of mineral fertilizers but increased by manure addition (He et al., 2007).

Further evidence came from a number of microcosm experiments aiming to identify the dominant ammonia-oxidizing agents. Thaumarchaeal growth was significantly increased by root exudation in a paddy rhizosphere soil, but less affected by addition of inorganic fertilizers (Chen et al., 2008). In a low-ammonia acidic forest soil, continuous ammonia flush from mineralization of organic nitrogen, but not added inorganic nitrogen, was thought to be the major substrate source for thaumarchaeal nitrification processes (Stopnišek et al., 2010). This hypothesis was verified by measuring the effects of inorganic and organic nitrogen resources on acidic soil nitrification, where thaumarchaeal abundance was unaffected by inorganic ammonium, but significantly stimulated by organic nitrogen addition (Levicnik-Höfferle et al., 2012). Similar observations that ammonia resources generated from mineralization of organic matter stimulated nitrification were also recorded in soil incubations without external nitrogen addition (Offre et al., 2009; Gubry-Rangin et al., 2010; Zhang et al., 2010). Active nitrification dominated by thaumarchaea in a strongly acidic soil, showed by our recent DNA-SIP work, was also positively associated with the high rates of nitrogen mineralization (Zhang et al., 2012). On the contrary, high rates of inorganic ammonium supply did not stimulate thaumarchaeal ammonia oxidation in a number of neutral and alkaline soils (Jia and Conrad, 2009; Levicnik-Höfferle et al., 2010; Verhamme et al., 2011) as well as in acidic soils (Stopnišek et al., 2010).

In fact, ammonia produced from organic matters is not functionally different from ammonia localized in soil water ($\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$), even though numerous evidence revealed that thaumarchaea preferred to utilize mineralized ammonia resources. A possible explanation is that continuous flux of low-level ammonia substrate via mineralization under acidic environments is sufficient to satisfy the low-ammonia requirement of thaumarchaea, but provides a selective pressure for both thaumarchaea and bacteria. Better adaptation of archaea to chronic energy stress than bacteria (Valentine, 2007), and their specific preference for low ammonia ensure that thaumarchaea outcompete bacteria under such a chronic energy stress. Another possible explanation is that ammonia released by mineralization is more available to thaumarchaea cells than inorganic ammonia from $\text{NH}_3/\text{NH}_4^+$ equilibrium in soil water. Because mineralizer may directly transport ammonia to thaumarchaea to enable rapid oxidation of ammonia, thus aggregates of ammonia oxidizers and mineralizers might form in acidic soils (Levicnik-Höfferle et al., 2012). However, the diversity and abundance of nitrogen-mineralizing microorganisms are rarely known, nor is their possible aggregate formation with ammonia oxidizers.

Another proposed mechanism was a mixotrophic lifestyle of thaumarchaea, which is assimilation of organic matters, followed by the intracellular mineralization and ammonia oxidation in thaumarchaea cells (Levicnik-Höfferle et al., 2012) (Fig. 2). Genomic analyses of *C. symbiosum* and *N. maritimus* identified the presence of genes required for the oxidative TCA cycle and potential organic substrate transporters, indicating the thaumarchaeal potential to use other reduced nitrogen as an energy source and a mixotrophic or heterotrophic lifestyle (Hallam et al., 2006; Walker et al., 2010). Actually, thaumarchaea can use some alternative substrates than ammonia for an energy source, as revealed by the recently soil-isolated archaeon, *N. viennensis*, which could grow mixotrophically on urea or ammonia with addition of pyruvate (Tournier et al., 2011), and as indicated by assimilation of organic matters through marine thaumarchaeal lipids (Ingalls et al., 2006). Also in a microcosm study, when autotrophic ammonia oxidation was completely inhibited by acetylene, thaumarchaeal growth was still observed, strongly suggesting a mixotrophic or heterotrophic catabolism (Jia and Conrad, 2009). Therefore, a broader substrate spectrum does

Fig. 2. Possible ammonia sources for thaumarchaea and proposed pathways of thaumarchaeal ammonia oxidation based on genomic analysis of *N. maritimus* (Walker et al., 2010). AMO: Ammonia monooxygenase; CuHAO: periplasmic multicopper oxidase; NXOR: nitroxyl oxidoreductase.

exist for thaumarchaea and their actual nitrogen resources under acidic conditions remain to be fully investigated. At the same time, these mixotrophic thaumarchaeal characteristics are also encountered in AOB, because previous investigations have revealed that *Nitrosomonas europaea* could use fructose (Hommes et al., 2003) and amino acids (Frijlink et al., 1992) as their carbon sources. Thus, AOA dominance over AOB under this mechanism in acidic soils still needs careful consideration.

4.3. Unique biochemical and genetic features of thaumarchaea

Ecological niche separation between thaumarchaea and bacteria basically originates from their different biochemical and genetic features. Although numerous findings have demonstrated the broad distributions of archaea in diverse moderate environments, they are still mainly considered as extremophiles, well-adapted to thrive in some unfavorable habitats, such as high-temperature, salt-saturated and strongly acidic environments (Schleper et al., 2005). Thaumarchaeal *amoA* gene copy numbers, but not bacterial, remained almost stable along a changing salinity gradient, indicating a high tolerance of AOA to salinity pressures (Santoro et al., 2008). Moreover, a sedimentary AOA strain was selectively enriched over AOB from reduced oxygen levels caused by coculture with sulfur-oxidizing bacteria (SOB), suggesting a favorable niche of AOA in oxygen-limited environments (Park et al., 2010). Valentine (2007) proposed that the key biochemical basis for archaeal adaptation to chronic energy stress could be primarily attributed to their specific tetraether-based membrane composition. Compared with bacterial membranes, archaeal membranes are commonly less permeable to ions thereby reducing the futile ion cycling and enabling an energetic advantage for archaea (Valentine, 2007). Such low-permeability membrane may help thaumarchaeal cells keep intracellular pH close to neutral, enabling an undisturbed ammonia oxidization process. Simultaneously, Valentine (2007) contended that in order to outcompete bacteria, archaea have evolved to possess some unique catabolic pathways, like ammonia oxidation activity of thaumarchaea, whose ability under specific conditions (e.g. low-pH environment) is much stronger than their bacterial counterpart.

Presently, complete genome sequences of only three cultured thaumarchaeons, *N. maritimus*, *C. symbiosum* and *N. limnia*, are available for further analysis of their unique features (Hallam et al., 2006; Walker et al., 2010; Blainey et al., 2011). Genome features of these AOA strains suggested a distinctly divergent pathway for ammonia oxidation from known AOB, because they lacked the genes encoding a homolog of AOB-like hydroxylamine oxidoreductase (HAO) and cytochrome *c* proteins, and structural differences between archaeal and bacterial *amo* genes. Thus, it remains to be known whether AOA may use hydroxylamine as an intermediate, or contain some novel enzymes for the oxidation of hydroxylamine. In fact, a hypothetical model has been proposed simultaneously without using hydroxylamine as intermediate. This model proposes that two oxygen atoms are inserted into ammonia to form HNOHOH and then decayed to the intermediate nitroxyl (HNO) by archaeal AMO, in this way eliminating the reductant recycling in the initial oxygenase reaction, and indicating an ecological advantage of AOA over AOB in nutrient-limited environments (Walker et al., 2010) (Fig. 2). Further ecological advantages of AOA in acidic soils could be inferred from their highly copper-dependent systems to oxidize ammonia and transport electrons (Hallam et al., 2006; Walker et al., 2010; Blainey et al., 2011), because of the increasing copper availability in acidic soils (Goedert et al., 1997). Thirdly, the 3-hydroxypropionate/4-hydroxybutyrate pathway for carbon fixation by AOA is more energy-efficient than the bacterial Calvin–Bassham–Benson cycle

(Walker et al., 2010). Moreover, as in the AOB genomes, the existence of ammonium transporter genes was also identified in all three AOA genomes (Hallam et al., 2006; Walker et al., 2010; Blainey et al., 2011). It has been proposed that such active transport of ammonium might facilitate the regulation of AMO and improve the ammonia uptake into AOB cells (Arp et al., 2002). If the active assimilation of ammonium was a necessary function for AOA, it would enable AOA effectively competing with AOB under ammonia-limited acidic conditions, apart from the passive diffusion of ammonia into the cells. Taken together, these advantageous intrinsic features possessed by AOA put them in a superior position when competing with AOB in acidic soils.

The above proposed mechanisms still need careful verification tests to check their suitability to drive the thaumarchaeal dominance in various acidic soil environments. On the other hand, some other mechanisms used to explain the bacterial ammonia oxidation in acidic environments can also be employed to interpret the thaumarchaeal nitrification, such as increased ureolytic activity (Burton and Prosser, 2001), aggregated ammonia oxidizers (De Boer et al., 1991), pH-neutral microenvironments (Prosser, 1989), and the existence of acidophilic ammonia oxidizers. The last mechanism has been demonstrated by the cultivation of the first obligately acidophilic thaumarchaeon *N. devanatterra* (Lehtovirta-Morley et al., 2011). It can only grow in low pH liquid batches and 0.18 nM ammonia, and is inhibited at neutral pH and high ammonia concentration. Future characterization of the mechanisms for acidophilic ammonia oxidation requires more deep studies on physiology and evolution of *N. devanatterra*.

5. Concluding remarks and future perspectives

Despite the fact that accumulated evidence and collected data strongly demonstrated the dominant role of thaumarchaea in ammonia oxidation of acidic soil ecosystems, it should also be taken into account that a wide range of physiological diversity of AOB could also contribute to nitrification in acidic environments. We still need more environmental investigations and microcosm incubations to differentiate the maximum contribution of AOB or other possible microorganisms to nitrification, and to explore the underlying mechanisms of AOA-driven nitrification in a wide range of acidic soils. What is more, we have to admit that factors and mechanisms driving the corresponding ammonia-oxidizing populations are still not fully understood. We do not know whether pH itself has directly shaped the ecological niches of AOA and AOB in acidic soils, or whether the differences are due to indirect influences induced by low pH. Actually, reduced availability of ammonia in acidic soils did not occur in isolation, as low-pH conditions could alter many other elements as well. Acidic soils are usually constrained for crop production by poor fertility, due to an integration of mineral toxicities and element deficiencies. The low-pH soil conditions could increase exposure to toxic metals such as aluminum, manganese, copper, iron, and zinc, simultaneously decreasing the bioavailability of elements like phosphorus, sulfur, and calcium (Goedert et al., 1997). Such acidity-induced effects would influence the growth of plants as well as soil-inhabiting microorganisms, certainly including thaumarchaeal and bacterial ammonia oxidizers. It was reported that some other elements in addition to nitrogen could also influence the community structure and abundance of ammonia oxidizers (Mertens et al., 2009; Liu et al., 2010). Erguder et al. (2009) reviewed the researches on the effects of phosphate and sulfide on autotrophic ammonia oxidation, and suggested that AOA ecotypes might thrive in low phosphate availability and low sulfide-containing environments. Therefore, it is of great importance to investigate further whether direct acidity pressure or the availability of ammonia and some other necessary

elements influenced by low pH values select the dominating ammonia oxidizers.

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