Review

Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation

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Autotrophic archaeal and bacterial ammonia-oxidisers (AOA and AOB) drive soil nitrification. Ammonia limitation, mixotrophy, and pH have been suggested as factors providing niche specialisation and differentiation between soil AOA and AOB. However, current data from genomes, cultures, field studies, and microcosms suggest that no single factor discriminates between AOA and AOB. In addition, there appears to be sufficient physiological diversity within each group for growth and activity in all soils investigated, with the exception of acidic soils (pH <5.5), which are dominated by AOA. Future investigation of niche specialisation in ammonia-oxidisers, and other microbial communities, requires characterisation of a wider range of environmentally representative cultures, emphasis on experimental studies rather than surveys, and greater consideration of small-scale soil heterogeneity.

Ammonia-oxidising archaea and bacteria

Soil nitrification, the oxidation of ammonia to nitrate, results in enormous commercial losses of ammonium-based fertilisers, with associated atmospheric and groundwater pollution by nitrous oxide and nitrate, respectively. The process is usually limited by the first step, ammonia oxidation to nitrite, which was thought to be driven by AOB, first isolated in the 19th century. Metagenome [1,2] studies and cultivation of *Nitrosopumilus maritimus*, a marine AOA [3], suggested a role for AOA (now placed within the *Thaumarchaeota* [4]) in ammonia oxidation. AOA and AOB abundances were subsequently inferred by qPCR of the *amoA* gene, which encodes subunit A of ammonia monooxygenase that performs the first step in ammonia oxidation by both groups. Quantification indicated AOA to be abundant in soil (e.g., [5–7]), with a potentially greater role in soil nitrification [8]. These developments demanded reassessment of soil ammonia-oxidiser (AO) community ecology and its consequences for soil nitrification rates. In particular, they initiated a search for physiological characteristics distinguishing between AOA and AOB, indicating their evolution and adaptation to particular sets of abiotic and biotic characteristics within the soil (i.e., niche specialisation) and consequent different patterns of resource utilisation (i.e., niche differentiation) [9,10]. Three potentially distinguishing characteristics have been suggested: ammonia affinity, mixotrophy, and pH growth optimum. Recent analyses of cultivated ammonia-oxidisers and experimental approaches, rather than correlation studies, provide a sounder foundation for assessment of niche specialisation. They suggest that no single soil characteristic (with the possible exception of soil pH) explains the relative abundances of AOA and AOB, and that analysis of soil heterogeneity and microenvironments is necessary to understand the mechanisms controlling ammonia-oxidiser community composition and activity. This article will review the evidence for niche specialisation and differentiation associated with these three factors obtained from genome and physiological analyses of cultivated organisms, soil microcosms and field studies.

Methodology and approach for investigating AOA and AOB

An implicit assumption in the niche specialisation concept is physiological adaptation to the environment and consequent correlation between physiological and environmental characteristics. Physiological characterisation is therefore important but it is notoriously difficult to obtain pure cultures of AOB and, particularly, AOA. Physiological studies have been performed on several soil AOB, but only four soil AOA have been cultivated [11–14], and only *Nitrososphaera viennensis* has been purified [11]. Cultures enable invaluable quantitative analysis of kinetics and provide strong evidence of function and of links between function and specific genes. This information is only useful if isolates are representative both of the phylogenetic groups to which they belong and of environmentally relevant populations. The latter can be determined using molecular techniques, but little is known of the degree to which all phylotypes share particular physiological characteristics. It is therefore dangerous to infer the properties of soil communities from those of a few cultivated strains, whether comparing AOA and AOB or comparing phylotypes within these groups. Cultivation also facilitates genome sequencing, and genomes of two soil AOB (*Nitrosomonas europaea* [15] and *Nitrosospira multiformis* [16]) and one soil AOA (*Nitrosoarchaeum koreensis* MY1 [17]) have been sequenced. Genome analysis can indicate some

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physiological characteristics, but its ability to predict those with ecological significance and those that might define the niche (e.g., specific growth rate, optimal pH or temperature for growth, substrate conversion rate, product formation rate) is severely limited.

The favoured approach to hunting for niche specialisation of AOA and AOB has been to determine the presence/absence, abundance, and relative abundance of respective *amoA* genes and gene transcripts in soils with different characteristics or subjected to different treatments. Although relatively easy to perform, this approach provides limited information. Measured soil characteristics are often autocorrelated and chosen for convenience and studies are often descriptive. Statistical analysis is exploratory only and the heterogeneity and uncontrolled nature of soil physicochemical characteristics make interpretation difficult. Experimental approaches, testing specific proposed environmental drivers in directed and controlled experiments, are arguably better and have been used to test specific hypotheses using manipulated field experiments and soil microcosms.

Ammonia concentration and supply

Ammonia oxidation is the major source of energy for AOA and AOB, and the only known energy source under aerobic conditions. Niche specialisation may arise through differences in ammonia affinity, tolerance of high ammonia concentration, and the relatively wide range of ammonia concentrations found in soil, but other factors, such as the source of ammonia, may also be more important.

Studies of cultures and genomes

The influence of ammonia concentration on AO growth and activity is quantified, respectively, as maximum rates (μ_{max}, v_{max}) and associated half-saturation constants (K_s, K_m). Cultivated AOA [12,18] have much higher substrate affinity than AOB (Table 1). This may be related, in part, to the significantly smaller size (and greater surface area:volume ratio) of AOA (Table 1), and has led to suggestions that AOA and AOB will dominate low and high ammonia soils, respectively. This is only predicted if AOB have both higher K_s and higher μ_{max} (Box 1, Figure Ib), for which there is little evidence (Table 1) (or if AOA and AOB are inhibited differently at high ammonia concentration). μ_{max} measured in four AOA (three from soil) ranges from 0.015 to 0.027 h^{-1} (mean 0.0019 h^{-1}). AOB values range from 0.005 to 0.088 h^{-1}, but higher values are for nitrosomonads, which are rare in soil. The highest μ_{max} for a soil *Nitrosospira* is 0.044 h^{-1} [19] but most nitrosospiras fall within the range 0.005–0.013 h^{-1} (e.g., [20]) (overall mean, 0.017 h^{-1}).

Cultivated 'typical' soil AOA therefore appear to have both lower K_s and higher μ_{max} (Box 1, Figure Ia) and will outcompete AOB at all ammonia concentrations. If soil ammonia concentration is the major factor limiting specific growth rate, AOB will not dominate soils with high ammonia and AOA:AOB ratios >1 are likely, as observed in most soils [5–7]. In this respect, substrate affinity will not explain niche differentiation of AOA and AOB because it suggests that AOB will be outcompeted; we need to look for other factors that explain their presence. AOB do have significantly (approximately 10-fold) greater specific cell activity than AOA, another probable consequence of their larger cell volume. If AOA and AOB utilise ammonia with equal efficiency, and are equally competitive in soil, and if *amoA* abundance reflects activity, we would expect an AOA:AOB ratio of at least 10 (Box 2). AOA will therefore only dominate activity if this ratio is >10.

Ammonia (rather than ammonium [21,22]) also inhibits AOA and AOB at high concentrations. In the laboratory, AOB are routinely enriched in medium containing approximately 1 μM NH_3 and most cultivated strains can grow at concentrations several-fold higher (although selection for ammonia-tolerant AOB by such media should not be ruled out). AOA appear to be more sensitive to NH_3, being inhibited in the range 0.04–0.36 μM NH_3 (Table 1), and a possible soil AOA enrichment culture (see below) has a K_i value of 1.28 μM NH_3, suggesting greater sensitivity than

Table 1. Kinetic constants and other properties of soil AOA and AOB cultures and communities

Characteristic	Soil AOB (*Nitrosospira*) cultures[a]	Soil AOA cultures or *N. maritimus*[b]	Soil
Maximum specific growth rate (μ_{max})	0.005–0.044 h^{-1} [19,20]	0.015–0.027 h^{-1} [11–13]	
Saturation constant for growth (K_s)[c]	4–125 μM NH_3 (including values from some non-soil AOB) [20,33,69]	Not determined for soil AOA	
Maximum specific cell activity (v_{max})	4–23 fmol NH_3 $cell^{-1}$ h^{-1} [69]	0.57 fmol NH_3 $cell^{-1}$ h^{-1} [12][d]	
Saturation constant for activity (K_m)	6–11 μM NH_3 [70]	0.0036–0.019 μM NH_3 [12,18] 0.036 for soil enrichment [28]	0.11–1.94 μM NH_3 [70] 0.012 μM NH_3 for soil slurry [28]
Maximum specific biomass activity	30–80 nmol NH_3 g $protein^{-1}$ h^{-1} [12]	51.9 nmol NH_3 g $protein^{-1}$ h^{-1} [12][d]	
Cell size	120–650 fg protein $cell^{-1}$ [12]	10.2 fg protein $cell^{-1}$ [12] [d]	
Inhibition constant (K_i)	Not determined for soil AOB but 39–4500 μM NH_3 for other AOB [22,70] Inhibition observed at 7–50 mM NH_3	K_i not determined for cultivated AOA but inhibition observed at 2–20 μM NH_3 [70] 1.28 μM NH_3 for soil enrichment and inhibition observed at 1.6 mM NH_3 [28]	932–1388 μM NH_3 [70] 1.1 μM NH_3 for soil slurry [28]

[a]Values for soil AOB (nitrosospiras), if available, from original papers or reviews.

[b]Values for soil AOA, if available, from original papers or reviews.

[c]All concentrations are presented as ammonia (NH_3), rather than ammonium (NH_4^+).

[d]Value for *N. maritimus*.

Box 1. Ammonia-limited growth and competition between AOA and AOB

The effect of substrate concentration (s) on the specific growth rate (μ) of a microbial population is most frequently described using the Monod equation:

$$\mu = \frac{\mu_{max} s}{K_s + s} \quad \text{[Equation I]}$$

where μ_{max} = maximum specific growth rate (when substrate is in excess) and K_s = the saturation constant (i.e., the substrate concentration at which $\mu = \mu_{max}/2$). High substrate-affinity is reflected in a low K_s value. Monod kinetics are best determined in chemostat cultures, where the substrate that limits growth and specific growth rate can be controlled.

Knowledge of μ_{max} and K_s values of AOA and AOB enables prediction of the outcome of competition when growth is limited by ammonia. Values for growth and activity constants have been determined in AOB, but AOA have been studied only in cell suspensions; direct comparisons are only possible of v_{max} and K_m measured for very few strains. Simulations below are therefore based on the assumption that AOA K_s and K_m values are similar in magnitude, as is the case for AOB, but utilise measured values of μ_{max}. The basic values for simulations are 'typical' μ_{max} values for soil AOA and AOB (see text), typical K_s values for AOB and estimates of K_s for AOA based on measured K_m.

If AOA have higher μ_{max} and lower K_s than AOB, they will grow faster than AOB at all ammonia concentrations (Figure Ia), AOA will eventually dominate, regardless of ammonia concentration, and AOB will become extinct. Simulations in Figure Ia assume μ_{max}, K_s and K_m values at the centre of ranges determined experimentally for soil AOA and AOB (see text).

If AOA have lower μ_{max} and lower K_s than AOB, AOB will grow faster at high ammonia concentration, but slower at lower ammonia concentration (Figure Ib) and the outcome of competition will depend on ammonia concentration. AOA will dominate environments in which ammonia concentration is usually low, and AOB will dominate high ammonia environments. Values for growth constants are those for Figure Ia, with the exception of AOB μ_{max} which is increased approximately threefold.

Obviously the outcome of competition between AOA and AOB in soil will depend on other factors; for example, temporal and spatial variation in ammonia concentration, ammonia inhibition, variation in growth constants within each group, and many other environmental factors. Nevertheless, differences in these growth constants in cultivated AOA and AOB have been used to predict the effect of soil ammonium on relative abundances of AOA and AOB.

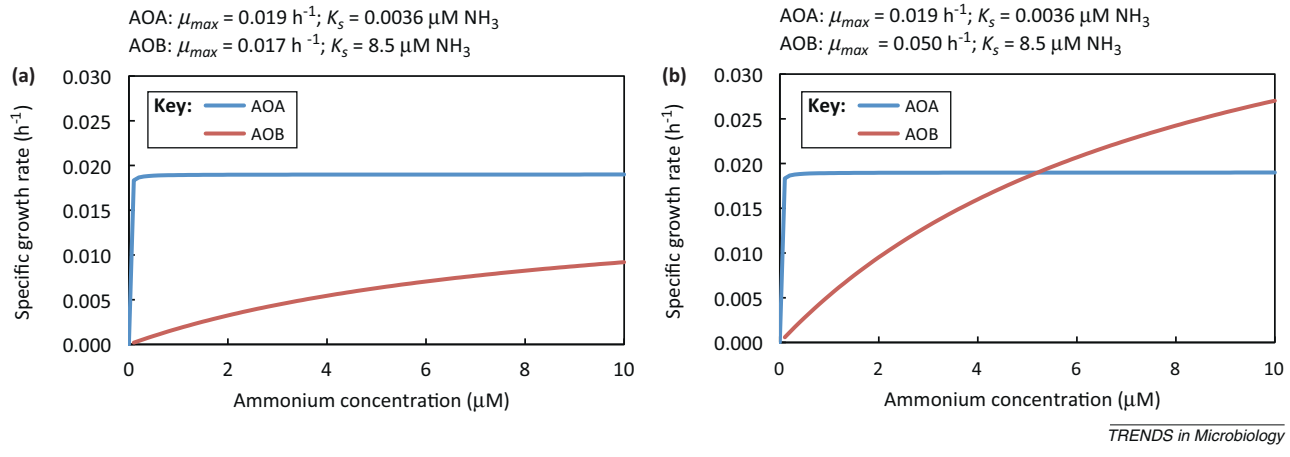

Figure I. The influence of ammonia concentration on specific growth rates of AOA and AOB described by Monod kinetics. Both simulations assumed AOA μ_{max} = 0.019 h^{-1} and K_s values of 0.0036 and 8.5 μM NH$_3$ for AOA and AOB, respectively. AOB μ_{max} values are 0.017 and 0.050 h^{-1} in **(a)** and **(b)**, respectively.

AOB (Table 1). Although limited, these data suggest that AOA are more sensitive to ammonia inhibition than AOB.

Results from field studies

Many field studies (discussed in detail below) infer the influence of ammonia concentration and fertilisation on AOA and AOB abundances and ratios, and/or their relative contributions to ammonia oxidation, by quantifying *amoA* genes. However, soil ammonia concentration is often not reported and the significance of relationships is not clear; low ammonia concentration will result from high activity, whereas sustained high ammonium concentration suggests inhibition, or limitation by other factors. This approach gives no information on dynamics or the relevance of concentrations to abundance. Saturation constants alone do not determine competitive ability (Box 1) and soil ammonia concentrations are rarely compared with K_m, K_s, or K_i values. In addition, functional gene abundance is not a reliable measure of activity (Box 2) and provides only limited information on relative activities of AOA and AOB. As a consequence, consistent patterns are rarely obtained from such studies. Potential nitrification (Box 2) is also frequently measured; but, again, conflicting relationships are obtained. For example, potential nitrification has been found to correlate with AOB *amoA* gene abundance (but not ammonium concentration) [23], AOB and ammonium concentration [24] and AOA abundance [25], but with neither AOA nor AOB *amoA* abundance [26,27].

Results from microcosm and mesocosm studies

Assessment of the influence of growth-limiting ammonia concentration on AO in soil is difficult because of background ammonia levels and production and utilisation by other functional groups. Nevertheless, K_m (0.012 μM NH$_3$) calculated for AO in a Californian soil slurry [28] was similar to those for AOA: only threefold greater than for *N. maritimus* and close to the K_m value (0.036 μM NH$_3$) of an enrichment culture obtained from the same soil (Table 1). This study pre-dated knowledge of AOA but, on the basis of substrate affinity, could represent the first reported cultivated AOA.

Box 2. Archaeal and bacterial ammonia-oxidiser activity and potential soil nitrification

The effect of ammonia concentration on AO activity, v (ammonia oxidised per unit time), can be analysed in a similar manner to growth (Box 1) using the Michaelis–Menten equation:

$$v = \frac{v_{max} s}{K_m + s} \quad \text{[Equation I]}$$

where v_{max} = maximum activity and K_m = saturation constant (ammonia concentration at which $v = v_{max}/2$). Kinetics are typically determined in short-term activity assays of cell suspensions; growth is presumed to be negligible. Relative activities of AOA and AOB will depend on relative values of v_{max} and K_m. Activity kinetics do not necessarily reflect growth kinetics, which depend on other cell processes and factors, and therefore do not, in themselves, predict the outcome of competition between different strains. They do quantify the relative activities of each strain at a particular ammonia concentration.

In describing enzyme kinetics, v relates to the product of enzyme concentration and specific activity. For microbial activity, v is the product of total cell or biomass concentration and specific cell or biomass activity, respectively (i.e., the activity per cell or per unit biomass). This is crucially important in assessing the relative activities of AO in the environment, as these will depend, not only on ammonia affinities and ammonia concentration, but also on cell or biomass concentrations and specific activity, which is approximately 10-fold greater for AOB than AOA (Table 1). AOA will therefore dominate activity only if the ratio of AOA:AOB biomass or cell abundance is >10. Assessing relative activity in terms of relative abundances of AOA and AOB *amoA* genes is also limited by potential biases associated with cell extraction efficiency, by the use of different primers, and differences in gene copy-numbers within the genome.

For soil activity, v_{max} is equivalent to potential nitrification, and is the product of total biomass or cell concentration (*amoA* abundance) and 'mean' activity across all active AO. It is typically measured in shaken soil slurries under 'optimal' conditions (e.g., neutral pH and non-limiting ammonia concentration). Potential nitrification is frequently used as a correlate of *amoA* abundance or a surrogate for *in situ*, gross ammonia-oxidiser activity. In this respect, it has several limitations:

- Incubation conditions will be selective; for example, some acidophilic strains will not grow at neutral pH.
- A single set of conditions will not reveal niche specialisation.
- A single initial ammonia concentration will not reveal differences in ammonia limitation or sensitivity.
- High ammonia concentration will inhibit sensitive strains.
- Loss of soil structure destroys potential interactions.
- Potential nitrification is sometimes assessed measuring increases in nitrite concentration after inhibition of nitrite oxidation by additional chlorate: this approach will select against nitrite sensitive strains.

Potential nitrification therefore gives no information on *in situ* activity, the identity of active AO or niche specialisation.

Microcosm studies indicate greater growth and activity of AOB in soils treated with high levels of inorganic ammonium [29,30]. Di *et al.* [31,32] reported increases in AOB, but not AOA *amoA* genes and transcripts, following addition of cow urine supplemented with urea to grassland soils. In both studies, nitrate concentration increased at a constant linear rate, which is predicted by a non-growing population with constant cell activity. However, AOB *amoA* gene abundance increased with nitrate concentration as a linear function [32] or a negative exponential function [31]. In both cases, this suggests a decrease in specific cell activity during incubation, possibly due to the decrease in pH observed in these incubations, or to growth limitation through other factors.

'Low' and 'high' ammonia soils are not well defined, but generally relate to intermittent increases in concentration due to heavy fertilisation, rather than total ammonia input. Ammonia inhibition may therefore better explain greater activity and abundance of AOB in heavily fertilised soils, given the possible greater ammonia sensitivity of cultivated AOA (Table 1), although few strains have been examined, and enrichment approaches may select against ammonia-sensitive strains of both groups.

Microcosm studies indicate, however, that the situation may be more complex in two ways. First, ammonia tolerance varies within both AOB [33,34] and AOA. Verhamme *et al.* [35] found growth of AOA in a pH 7.5 soil repeatedly amended with 0, 1.1 or 11 mM ammonium, whereas AOB grew only at the highest concentration (Figure 1). AOA communities changed, with replacement of 1.1b with 1.1a phylotypes. At least some soil AOA are therefore tolerant of the high ammonium concentrations typical of fertilised soils.

Second, it is important to consider physicochemical heterogeneity, interactions with other functional groups, and the source of ammonia. This is illustrated by a managed, acid peat soil with rapid AOA-ammonia oxidation, driven by mineralisation of organic N. AOA ammonia oxidation was not inhibited or stimulated by addition of 11 mM ammonium [36], but was stimulated by addition of mineralisable nitrogen: urea, glutamate, yeast extract [37]. Mineralisation led to accumulation of inorganic ammonium in the soil solution, which was subsequently oxidised, whereas added inorganic ammonium was not (Figure 2). This suggests close interactions between mineralisers and AOA and a need for greater consideration of the origin of ammonia and its transport within soil.

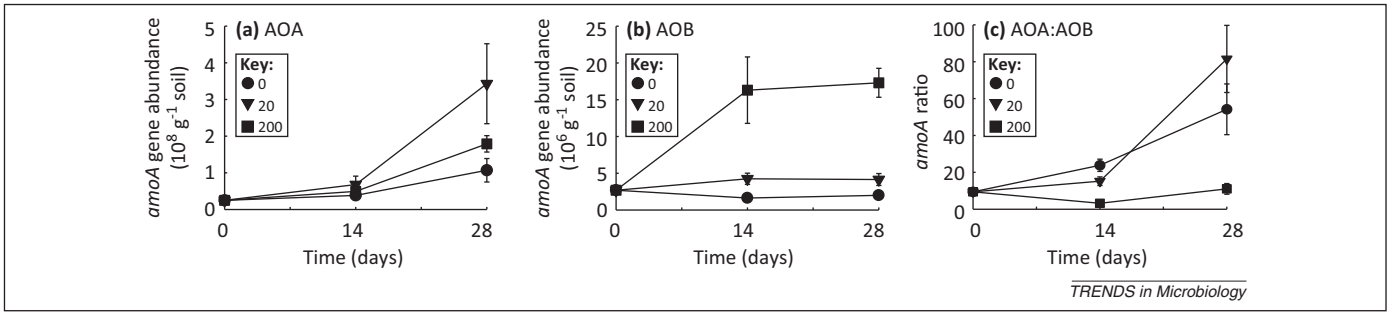

Figure 1. Changes in abundance of **(a)** AOA and **(b)** AOB *amoA* genes and **(c)** changes in AOA:AOB *amoA* abundance ratio in soil microcosms receiving ammonium amendments to maintain target ammonium concentrations of 0, 20, and 200 μg NH_4^+-N g^{-1} soil. Reproduced, with permission, from [30].

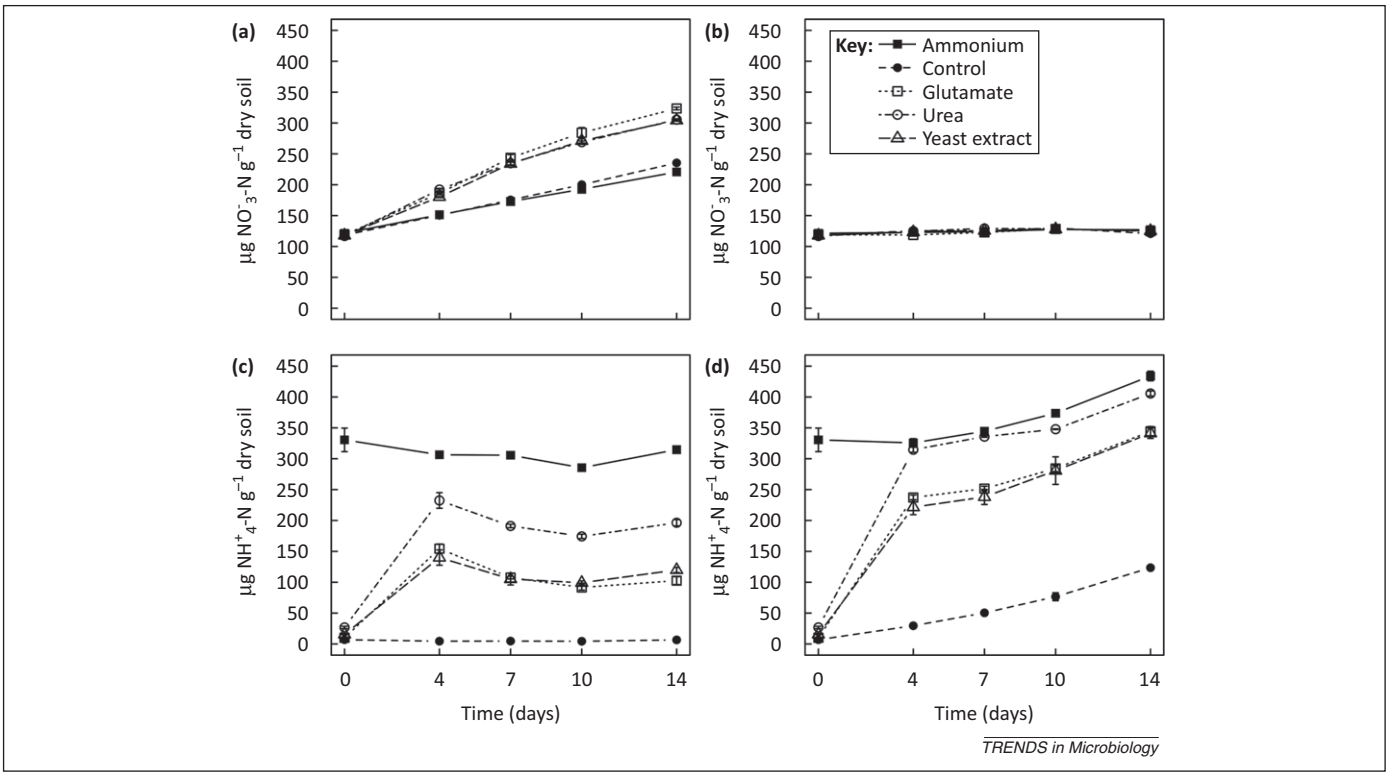

Figure 2. Changes in **(a,b)** nitrate and **(c,d)** ammonia in soil microcosms during incubation for 14 days after amendment with inorganic and organic nitrogen compounds in the absence (a,c) and presence (b,d) of the nitrification inhibitor acetylene. Reproduced, with permission, from [32].

Thus, although cultivated AOA and AOB differ in ammonia affinity, there is little evidence that this leads to niche differentiation in soil. Growth constants of cultivated strains predict that AOA will outcompete AOB if ammonia limitation is the major factor determining specific growth rate. This potentially explains the frequently observed higher abundance of AOA in soil. If cultivated strains are representative of natural communities, the challenge is to explain the presence of AOB in soil. If they are not representative, and other factors, discussed below, contribute to competition, high substrate-affinity may provide an advantage in microenvironments where ammonia concentration is low, for example, in acidic soils and where ammonia is produced through mineralisation of organic matter.

Analysis of specific activity, rather than specific growth rate, suggests that AOB will dominate ammonia-oxidising activity in soil unless AOA are significantly outnumbered by AOB, with the AOA:AOB ratio at least >10. This ratio, and differences in specific cell activity, reflect differences in cell size of cultivated AOA and AOB. The ratio may be lower if AOB genomes contain more copies of the *amoA* gene, as appears to be the case [17,38–40]. Our analysis of growth and activity kinetics questions the value and relevance of potential nitrification measurements and of correlations between *amoA* gene abundance and ammonia concentration. The few cultures investigated indicate greater sensitivity of AOA to ammonia inhibition, but microcosm studies demonstrate AOA growth at high ammonium and suggest a need both for greater analysis of physiological diversity within AOA and AOB and for analysis of the effects of soil heterogeneity on ammonia community development and their activity.

Heterotrophic and mixotrophic growth within ammonia-oxidiser populations

Ammonia oxidisers are traditionally considered to be obligate autotrophs, but the media used in their enrichment and isolation are designed to select for autotrophs. There is evidence for assimilation of organic compounds by ammonia oxidisers and this characteristic, and the potential for mixotrophic and heterotrophic growth, may be important in niche differentiation.

Results from cultures and genomes

AOA and AOB genomes contain genes encoding enzymes involved in, respectively, the modified 3-hydroxypropionate/4-hydroxybutyrate pathway [41], and the RuBisCO pathway [42] for carbon fixation. All soil AO isolates grow autotrophically in media containing inorganic ammonium and only low, contaminating levels of organic carbon.

All AOB and AOA genomes analysed contain genes encoding enzymes for metabolic pathways required for organotrophic growth [39,40,43]. Inability to grow mixotrophically or heterotrophically may therefore indicate inability to transport organic substrates and/or regulatory mechanisms suppressing organotrophy. In fact, some AOB can assimilate low molecular-weight organic compounds [43], and *N. europaea* and *Nitrosomonas eutropha* can grow anaerobically on several organic compounds using nitrite as the terminal electron acceptor [44]. AOB should therefore not be considered as obligate chemolithoautotrophs [43]. Mixotrophy also occurs in soil AOA, and *N. viennensis* grows 10-fold faster in the presence of >0.05 mM pyruvate than on ammonium alone [11]. Genome analysis currently provides little information on which organic substrates

might be assimilated, due to difficulties in assessing the specificity of membrane transporters. As a result, laboratory cultures are required to determine which, if any, organic compounds can be utilised and their influence on physiology.

Results from field studies

The traditional belief that AOB are obligate autotrophs, and evidence for mixotrophic growth of AOA, have led to suggestions that AOA would preferentially colonise roots or mycorrhizas, where organic C flux is greatest [45]. However, root exudates reduced both AOA abundance and the AOA:AOB *amoA* gene ratio, and the effects of mycorrhizal fungi were variable, with colonisation of mycorrhizas colonising ponderosa pine but not Sitka spruce or Western hemlock [45]. Mixotrophic growth of AOA, but not AOB, might also favour AOA following addition of biosolids to soil, but high doses of biosolids stimulated both AOA and AOB, whereas lower doses only stimulated AOA [46].

Results from microcosms

Stable isotope-probing (SIP) microcosm studies provide strong evidence for autotrophic growth of AOA in a neutral agricultural soil [47] and two acidic soils [13,48]. In soil microcosms receiving no input of inorganic ammonium, SIP data were consistent with increases in archaeal *amoA* gene abundance and changes in AOA community composition, whereas AOB communities did not change. SIP also provided evidence of autotrophic growth of AOB in an upland agricultural soil [24]. Further evidence of AOB autotrophy was obtained by mRNA-SIP, which demonstrated assimilation of CO_2 into AOB *amoA* transcripts. However, assimilation into AOA *amoA* transcripts (but not *amoA* genes) was also observed following addition of 15 μM, but not 100 μM ammonium [30]. Xia *et al.* [49] also found greater CO_2 assimilation by AOB in agricultural soil microcosms amended with ammonium, but some assimilation by AOA. This suggests that AOB dominate oxidation of added ammonium and that AOA are inhibited at higher ammonium concentration.

Jia and Conrad [29] investigated surface (0–20 cm) and subsurface (40–50 cm) soil. In the subsurface soil, potential nitrification and AOA abundance were both 10-fold lower, whereas AOB abundance was 1000-fold lower. There was therefore greater correlation of potential nitrification and AOA abundance, despite conditions (high ammonium concentration) favouring AOB. When nitrification was inhibited by acetylene, AOA (in both soils) and AOB (in surface soil) increased. These increases have been interpreted as indicative of heterotrophic growth. If so, they support cultivation-based studies in that both AOA and AOB may be capable of mixotrophic or heterotrophic growth.

There is therefore evidence for mixotrophy in both soil AOA and AOB, and currently little evidence of niche specialisation in this respect. Assessment of the importance of mixotrophy is hampered by difficulties in designing enrichment approaches favouring mixotrophs and analysis of mixotrophy in soil, but it raises interesting evolutionary and ecological questions. For example – does mixotrophy present specific advantages to autotrophic AO? If so, do these arise through benefits for carbon assimilation, energy production, or both? Are mixotrophs chemolithoautotrophs that are losing the ability to oxidise ammonia and fix CO_2 after gaining the ability to grow organotrophically? Or can chemolithoautotrophy benefit heterotrophs under particular environmental conditions? These questions, in turn, have implications for soil AO ecology. Higher growth rate and yield of mixotrophs may increase competitiveness, physiological versatility may extend periods of activity and habitable niches, and potential differences in assimilable organic substrates between AOA and AOB may provide additional scope for niche differentiation.

Soil pH

The discovery of AOA was surprising because there were few apparent gaps in our knowledge of soil nitrification, at least in qualitative terms, no major inexplicable phenomena and no 'missing' AO. One exception was nitrification in acid soils. Although mechanisms had been proposed for AOB activity in low pH soil, recent data indicate that AOA may represent the predominantly active populations in these environments.

Genomes and cultures

AOB are readily enriched from acid soils, but all cultivated AOB are neutrophilic and none grows in liquid batch-culture below pH 6.5. Nevertheless, autotrophic ammonia-oxidation occurs in soils with pH as low as 3.5 and gross nitrification rate does not appear to correlate with soil pH [50]. In fact, some of the highest gross nitrification rates are found in soils with pH <5.5 [50]. Ammonia oxidation in acid soils may be explained by reductions in pH minima for growth and activity in laboratory cultures in which AOB grow in aggregates or on surfaces [51,52]. Ureolytic growth of AOB also occurs at low pH [53,54]. It is, however, difficult to demonstrate the importance of surface growth and ureolysis *in situ*.

Some AOB phylotypes are selected in moderately acidic soils, but no acidophilic AOB has been isolated. The enrichment of the first acidophilic, autotrophic, ammonia-oxidiser, *Nitrosotalea devanaterra* [13], provides an explanation for nitrification in acidic soils, which does not require consideration of surface or aggregate growth or ureolysis. This organism grows at pH 4.0–5.5 on inorganic medium containing ammonium with μ_{max} = 0.0015 h^{-1}. The organism has high nitrite-sensitivity, suggesting a requirement for close proximity to acidophilic nitrite-oxidisers, or other mechanisms of nitrite removal.

Supporting field studies

AOA *amoA* genes appear to be ubiquitous in soil, suggesting potential activity over the full pH range investigated. By contrast, AOB *amoA* genes could not be detected in acidic forest soils, tea soils, or peat [25,36,55]. The ease with which soil pH can be measured has led to many surveys exploring correlations between pH and *amoA* gene abundance and sequence composition. Relationships vary between different sites, with examples of positive, negative, or no correlation between pH and AOA or AOB abundance [7,24–27,56–60]. This variation reflects the limitations of single or few-site studies and potential diversity within both AOB and AOA.

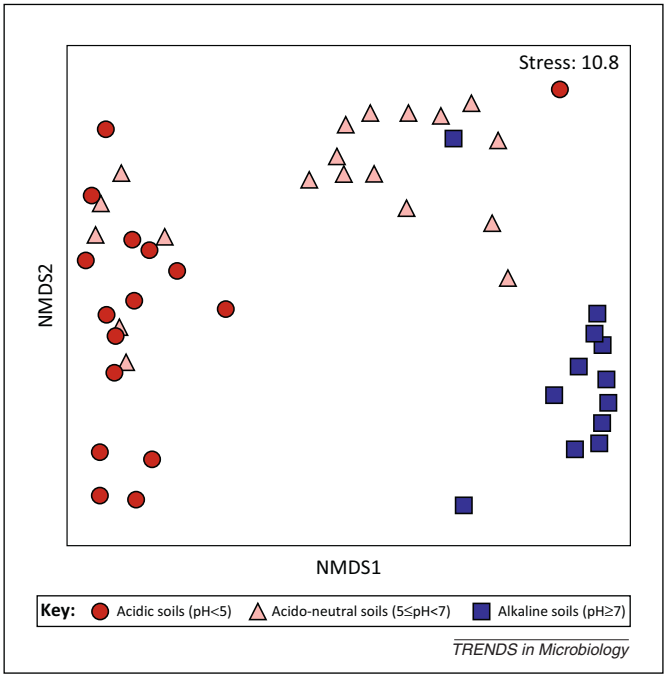

Figure 3. Non-metric multidimensional scaling (NMDS) plot of autotrophic archaeal ammonia-oxidiser (AOA) *amoA*-defined community structure in 47 soils based on the relative abundance of sequences within 19 *amoA* clusters in each soil. The first principal axis is dominated by soil pH effect and each soil is placed within acidic, acido-neutral, or alkaline categories. Reproduced, with permission, from [32].

Three surveys involve sufficient sites to investigate distribution patterns. In 107 Burgundy soils (pH 4.2–8.3), pH showed highest correlation with AOA:AOB ratio and AOA, but not AOB abundance [61]. In 19 fertilised tea soils [25], and an adjacent acid pine-forest soil (pH 3.58–6.29], AOB abundance correlated with pH whereas AOA abundance was largely unaffected by pH; the AOA:AOB abundance ratio was therefore negatively correlated with pH. The abundance of AOA phylotypes dominating the most acid soils correlated with potential nitrification rate, suggesting acidophilic and/or acid-tolerant AOA; sequences of T-RFs (terminal restriction fragments) obtained from the most acidic soils fell within the *N. devanaterra* cluster [62], which contains the cultivated obligate acidophile discussed above.

Gubry-Rangin *et al.* [63] clustered globally distributed archaeal *amoA* gene sequences into acidophilic, acido-neutral, or alkalinophilic groups, dominating soils with pH <5.5, pH 5.5–7.5, or pH >7.5, respectively, providing strong evidence for pH-based adaptation and selection (Figure 3). These data were then used to predict successfully the pH-associated distribution of most phylotypes in 47 UK soils. One of the two major acidophilic lineages falls within the *N. devanaterra* cluster [63]. Long-term soil pH manipulation also leads to selection of both AOA and AOB phylotypes, indicating pH-adaptation in both groups, and analysis of transcript:gene abundance ratio indicated dominance of AOA in these soils at low pH [7].

Examples from microcosms

Abundance or dominance of AOA or AOB does not necessarily reflect activity, and most surveys do not measure *in situ* activity; furthermore, cultivation conditions are selective and are not necessarily representative of *in situ* activity. Microcosm studies provide more direct evidence of activity in acidic soils and associations between nitrification and increases in archaeal, but not bacterial, *amoA* gene and transcript abundances have been demonstrated in acidic agricultural soils [7,64], organic, acidic forest peat soil (pH 4.1) [36,37], and neutral agricultural soils [35,65]. Lehtovirta-Morley *et al.* [13] demonstrated autotrophic growth (CO_2 assimilation) of *N. devanaterra*-associated phylotypes in rapidly nitrifying pH 4.5 soil using stable isotope probing. Growth of both AOA and AOB was observed in neutral agricultural soil, and the AOA:AOB ratio was dependent upon added ammonium [35], whereas AOB growth and AOA transcriptional activity were observed in an acidic agricultural soil supplied with ammonium sulfate [29,30].

These studies provide strong evidence that AOA are the dominant AO in acidic soils, and may be the sole drivers of nitrification in soils with pH <5.5. They also demonstrate that AOA are not restricted to acidic soils and that some phylotypes are selected in acido-neutral and alkaline soils, with high ammonia availability, which is considered to favour AOB. Soil pH selects for different groups within AOA and AOB, but does not provide niche differentiation between the two groups except, possibly, below pH 5.5. Acidophily within AOA, represented by *N. devanaterra*, may provide an explanation for nitrification in acid soils, but it would be unwise to rule out the existence of acidophilic AOB or the importance of mechanisms such as ureolysis or biofilm formation in acidophilic ammonia-oxidation.

Other potential factors

As indicated above, urease activity enables AOB growth at low pH, and has been observed also in *N. viennensis*. Although AOA and AOB share many traditional inhibitors of nitrification, there is some evidence of different levels of sensitivity between the two groups [12,66]. This may influence AOA:AOB ratios in agricultural soils treated with inhibitors. Interest in nitrous oxide production by AOB has been driven by concerns over atmospheric pollution, and no ecological advantage has been demonstrated. Growth of *N. maritimus* is accompanied by N_2O production [67], and in marine environments AOA may generate more N_2O than less abundant AOB [68]. Although evidence may be equivocal, mixed enrichment cultures containing soil AOA also produce N_2O [14]; it would be surprising if AOA do not contribute to soil N_2O production, but there is no evidence for its role in niche differentiation.

Concluding remarks

The discovery of thaumarchaeal ammonia-oxidisers led to a search for key environmental drivers of AOA and AOB, and for factors leading to niche specialisation and differentiation. This article challenges the view that single environmental factors distinguish between AOB and AOA in soil. Difficulties in interpreting data using a single approach, and those relying solely on correlations, highlight the need for a combination of approaches and, in particular, the increasing need for analysis of cultured

organisms representative of dominant environmental communities and for more controlled, experimental studies. Integration of data from different approaches suggests broad physiological diversity within both AOA and AOB and a combination of environmental drivers of relative abundance and community composition of both groups. The isolation of an acidophilic AOA has provided a new solution to the longstanding paradox of nitrification in acid soils, which is the only current example of niche differentiation in soil. This analysis also demonstrates the care needed in analysing the effects of substrate concentration on microbial communities, and the importance of soil heterogeneity in assessing links between communities and their ecosystem functions.

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