

ORIGINAL ARTICLE

Nitrification rates in Arctic soils are associated with functionally distinct populations of ammonia-oxidizing archaea

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The functioning of Arctic soil ecosystems is crucially important for global climate, and basic knowledge regarding their biogeochemical processes is lacking. Nitrogen (N) is the major limiting nutrient in these environments, and its availability is strongly dependent on nitrification. However, microbial communities driving this process remain largely uncharacterized in Arctic soils, namely those catalyzing the rate-limiting step of ammonia (NH₃) oxidation. Eleven Arctic soils were analyzed through a polyphasic approach, integrating determination of gross nitrification rates, qualitative and quantitative marker gene analyses of ammonia-oxidizing archaea (AOA) and bacteria (AOB) and enrichment of AOA in laboratory cultures. AOA were the only NH₃ oxidizers detected in five out of 11 soils and outnumbered AOB in four of the remaining six soils. The AOA identified showed great phylogenetic diversity and a multifactorial association with the soil properties, reflecting an overall distribution associated with tundra type and with several physico-chemical parameters combined. Remarkably, the different gross nitrification rates between soils were associated with five distinct AOA clades, representing the great majority of known AOA diversity in soils, which suggests differences in their nitrifying potential. This was supported by selective enrichment of two of these clades in cultures with different NH₃ oxidation rates. In addition, the enrichments provided the first direct evidence for NH₃ oxidation by an AOA from an uncharacterized Thaumarchaeota–AOA lineage. Our results indicate that AOA are functionally heterogeneous and that the selection of distinct AOA populations by the environment can be a determinant for nitrification activity and N availability in soils.

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Introduction

Arctic and boreal ecosystems cover 22% of the land surface on Earth and are currently undergoing dramatic changes that are predicted to increase drastically in the following century, as a result of the rising temperatures disproportionately affecting these regions (Chapin *et al.*, 2000; Sala *et al.*, 2000; Intergovernmental Panel on Climate Change, 2007). Estimates indicated that the carbon (C) stored in permafrost soils in Arctic and boreal regions is more than twice the atmospheric C (Tarnocai *et al.*, 2009).

Higher emissions of carbon dioxide (CO₂) from soils through respiration are predicted to become a positive feedback to climate warming, as a result of increased soil organic matter decomposition and consequent higher nutrient availability (Binkley *et al.*, 1994; Mack *et al.*, 2004; Biasi *et al.*, 2008; Schuur *et al.*, 2008; Schuur *et al.*, 2009). However, the extent of possible feedback effects will depend greatly on the response of the microbial populations driving the biogeochemical processes underlying nutrient fluxes (Singh *et al.*, 2010; Xu *et al.*, 2011).

A vast body of evidence shows that N is the major limiting nutrient in Arctic soils (Shaver and Chapin, 1980; Nordin *et al.*, 2004), and its availability is dependent on decomposition processes and transformations of N compounds catalyzed by microorganisms (Canfield *et al.*, 2010). Nitrification, the conversion of NH₃ via nitrite (NO₂⁻) to nitrate

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(NO_3^-), has been documented in Arctic soils (for example, (Binkley *et al.*, 1994; Chapin, 1996)), where it can consume up to half of the N mineralized annually (Giblin *et al.*, 1991). Nitrification is also directly and indirectly involved in the production of the potent greenhouse and ozone-depleting gas nitrous oxide (N_2O) (Conrad, 1996; Ravishankara *et al.*, 2009), and it has been shown to contribute most of the N_2O emitted from some soils (for example, (Martikainen, 1985; Mathieu *et al.*, 2006)), including Arctic soils (Ma *et al.*, 2007; Siciliano *et al.*, 2009). However, nitrification is strongly dependent on the environmental conditions (Booth *et al.*, 2005) and so are the fluxes of N compounds that it mediates.

Oxidation of NH_3 , the rate-limiting step of nitrification, is performed by members of both Bacteria and Archaea, although their relative contributions to nitrification in natural environments remain unclear (Prosser and Nicol, 2008; Schleper and Nicol, 2010). Archaea encoding the ammonia monooxygenase (AMO) and belonging to the phylum Thaumarchaeota (Brochier-Armanet *et al.*, 2008) are widely distributed and highly abundant in several environments, often greatly outnumbering ammonia-oxidizing bacteria (AOB; for example, (Leininger *et al.*, 2006; Wuchter *et al.*, 2006)). In contrast to the knowledge on the physiology and metabolic properties of AOB (Arp *et al.*, 2007), the ecophysiology and functional diversity of ammonia-oxidizing archaea (AOA) remains largely uncharacterized. Two isolates (Könneke *et al.*, 2005; Tourna *et al.*, 2011) and few enrichment cultures of AOA (Blainey *et al.*, 2011; de la Torre *et al.*, 2008; Hatzenpichler *et al.*, 2008; Jung *et al.*, 2011; Lehtovirta-Morley *et al.*, 2011) confirmed the capability of NH_3 oxidation by certain lineages of Thaumarchaeota, preferably at low ammonium (NH_4^+) concentrations. However, recent environmental studies suggested that not all Thaumarchaeota are NH_3 oxidizers or that they might be capable of using alternative energy (Mußmann *et al.*, 2011; Pratscher *et al.*, 2011; Xia *et al.*, 2011) and C sources (Ingalls *et al.*, 2006; Tourna *et al.*, 2011). It remains thus unknown whether AOA population structure affects their contribution to nitrification, as it was shown for other functional groups (Reed and Martiny, 2007; Strickland *et al.*, 2009), including AOB (Horz *et al.*, 2004).

Although AOA have been identified in Arctic permafrost-affected soils (Siciliano *et al.*, 2009; Lamb *et al.*, 2011; Banerjee and Siciliano, 2012), their populations and role in nitrification remain largely uncharacterized, despite the critical importance of N cycling in these ecosystems. Therefore, we aimed to characterize AOA populations and activity in 11 different Arctic soils through a polyphasic approach, integrating qualitative and quantitative marker gene analyses, determination of gross *in situ* and potential nitrification rates and cultivation attempts. We found distinct phylogenetic clades of AOA in different soil types, where they were a determinant of nitrification activity and,

thus, of potential response mechanisms to environmental changes in these ecosystems. Our observations have implications not only for Arctic permafrost-affected soils but also for other environments where AOA are the dominant NH_3 oxidizers.

Materials and methods

Sampling sites and soil physico-chemical parameter determination

Soil samples were collected from three distant Arctic geographic locations: Spitsbergen, in the Svalbard archipelago (78°N), the main study-site; Zackenberg, in the eastern coast of Greenland (74°N), and Tazovskiy, in western Siberia (67°N). Sampling was performed in August 2009. The sampling sites covered some of the most common Arctic terrestrial landscapes, each characterized by distinct geomorphologies, water regimes and vegetation type: dry and wet moss tundra sites (Longyearbyen, Spitsbergen), shrub tundra (Tazovskiy, Zackenberg and Hotelneset in Spitsbergen), tussock tundra (Adventalen in Spitsbergen), tundra fens (Solvatnet and Knudsenheia in Spitsbergen) and frost boil upwellings (Solvatnet and Knudsenheia in Spitsbergen). Field sites' and description, as well as sampling procedures are described in detail in Supplementary Information. Soil pH, gravimetric water content (moisture %) and NH_4^+ , NO_3^- , NO_2^- , dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations were determined following standard procedures, as described in Hood-Nowotny *et al.* (2010) (see Supplementary Information for details). Dissolved inorganic nitrogen was calculated as the sum of NH_4^+ and NO_3^- , and dissolved organic nitrogen (DON) was obtained by subtracting the dissolved inorganic nitrogen from the TDN.

In situ and potential gross nitrification

Gross nitrification rates were determined by a ^{15}N pool dilution assay, as described by Inselsbacher *et al.* (2007). *In situ* gross nitrification rates were measured in soil slurries under native N conditions, and potential rates were determined after amendment with NH_4Cl to a final concentration of 1.7–2.5 mM. Five replicate reactions were performed for each soil and nitrification rates were determined after incubation over a period of 20 h at 15 °C. This temperature is similar to the highest values measured in the region during the warm season (Westermann *et al.*, 2011). Detailed procedures of the ^{15}N pool dilution method are described in Supplementary Information. Net nitrification rates were calculated from the NO_3^- pools measured during the ^{15}N pool dilution assay, which were also used for modeling the gross rates.

Enrichment of AOA in laboratory cultures

Soil samples from the top soil layers of all Spitsbergen sites were used to inoculate 48 initial

enrichment cultures. Four cultures were initiated from each soil by inoculating 20 ml of fresh water medium (see Supplementary Information for medium composition) with 1 g of soil in sterile plastic vials. Initial cultures were incubated in pairs at 20 or 32 °C with either 0.2 or 0.5 mM NH₄Cl. All subsequent sub-cultures were supplemented with 0.5 mM NH₄Cl. All cultures were supplemented with NaHCO₃ (2 mM) as sole C source and with streptomycin (50 µg ml⁻¹). In a second enrichment stage, 30 sub-cultures were incubated at 14 or 20 °C, and in a third enrichment stage, 32 sub-cultures were incubated in groups of nine at 4, 20 or 28 °C and treated with either ampicillin (100 µg ml⁻¹) or lysozyme (16.7 mg ml⁻¹), in addition to streptomycin. NH₄⁺ and NO₂⁻ concentrations were measured in all cultures at several time points with the methods described above. Cultures with stable NH₃ oxidation activity were continuously sub-cultured at 20 °C in the laboratory for approximately 3 years. Detailed incubation conditions of all sub-cultures and acetylene treatment are described in Supplementary Information.

Cloning PCR, sequencing and quantitative PCR

Amplification of archaeal *amoA* genes for cloning and sequencing was performed with the newly designed primers Arch-*amoA*-7F (5'-ATGGTCTGGB TDAGAMG-3') and Arch-*amoA*-638R (5'-GCRGCCA TCCATCTRTA-3') (see Supplementary Information for primer design details). Detection of bacterial *amoA* genes was performed with the primers *amoA*-1F*/*amoA*-2 R (Rotthauwe *et al.*, 1997; Stephen *et al.*, 1999). Thaumarchaeal 16S rRNA genes were amplified with primers A109F (Großkopf *et al.*, 1998) and Cren-957 R (Ochsenreiter *et al.*, 2003). Quantitative PCR of archaeal *amoA* genes was performed with modified versions of primers 104F (Tourna *et al.*, 2011) and CrenamoA-616r (Tourna

et al., 2008), hereafter named Arch-*amoA*-104F (5'-G CAGGAGACTAYATHTTCTA-3') and Arch-*amoA*-616R (5'-GCCATCCATCTRTADGTCCA-3'), respectively. Modifications were made in order to cover most nucleotide base variation in the sequences obtained from the soils studied here. Quantitative PCR of bacterial *amoA* and thaumarchaeal 16S rRNA genes were performed with primers *amoA*-1F/*amoA*-2R (Rotthauwe *et al.*, 1997) and 771F/957R (Ochsenreiter *et al.*, 2003), respectively. All molecular, phylogenetic and statistical analyses are described in detail in Supplementary Information.

Data deposition

The sequences reported in this paper have been deposited in the GenBank database (accession nos. JX426266-JX426615 and KC559122-KC559431).

Results

Soil physico-chemical properties

Eleven tundra soil samples were collected mainly from Spitsbergen (Svalbard), and also from Tazovskiy (Siberia) and Zackenberg (Greenland), and categorized according to ecosystem type (Table 1 and Supplementary Table S1; see Supplementary Information for site descriptions). The majority of the soils were dry, with the exception of the waterlogged tundra fen peat sites (Sol-tf and Knu-tf) and one moss tundra site (Lon-mt1). Soil pH values ranged between 3.7 and 8.4, with the lowest values observed in shrub tundra soils (Hot-st and Taz-stol) (Supplementary Table S1). Concentrations of DOC and TDN were low in the mineral soils (frost boils and moss, shrub and tussock tundra), ranging between 38 and 85 µg C g⁻¹ dw soil and 10–30 µg N g⁻¹ dw soil, respectively, whereas the fen peat samples contained DOC and TDN concentrations within a much higher range (465–939 µg C g⁻¹

Table 1 Soil sample description and NH₃ oxidizer abundance

| Sample | Location | Ecosystem type | <i>amoA</i> copies ^{a,b} | |
|---------|-----------------------------|--|--|---|
| | | | Archaea | Bacteria |
| Hot-st | Hotelneset (Svalbard) | Shrub tundra (mineral soil) | 8 × 10 ⁶ ± 1 × 10 ⁶ | ND |
| Adv-tt | Adventalen (Svalbard) | Tussock tundra (mineral soil) | 4 × 10 ⁷ ± 2 × 10 ⁶ | ND |
| Lon-mt1 | Longyearbyen (Svalbard) | Moss tundra (mineral soil in wet ridge) | 3 × 10 ⁶ ± 1 × 10 ⁶ | ND |
| Lon-mt2 | | Moss tundra (mineral soil in dry mount) | 5 × 10 ⁷ ± 8 × 10 ⁶ | 5 × 10 ⁵ ± 1 × 10 ⁵ |
| Lon-mt3 | | | 2 × 10 ⁸ ± 2 × 10 ⁷ | 5 × 10 ⁵ ± 7 × 10 ⁴ |
| Sol-tf | Solvatnet (Svalbard) | Tundra fen peat (organic soil) | ND | 2 × 10 ⁶ ± 3 × 10 ⁵ |
| Sol-fb | | Frost boil in tundra fen (mineral soil) | 2 × 10 ⁷ ± 8 × 10 ⁶ | 2 × 10 ⁶ ± 2 × 10 ⁵ |
| Knu-tf | Knudsenheia (Svalbard) | Tundra fen peat (organic soil) | < 4 × 10 ^{5c} | 2 × 10 ⁶ ± 1 × 10 ⁵ |
| Knu-fb | | Frost boil in tundra fen (mineral soil) | 2 × 10 ⁶ ± 3 × 10 ⁵ | 4 × 10 ⁵ ± 6 × 10 ⁴ |
| Taz-sto | Tazovskiy (Western Siberia) | Shrub tundra (cryoturbated organic soil) | 1 × 10 ⁷ ± 3 × 10 ⁶ | ND |
| Zac-st | Zackenberg (Greenland) | Shrub tundra (mineral soil) | 4 × 10 ⁷ ± 3 × 10 ^{5d} | ND |

Abbreviations: dw, dry weight; fw, fresh weight; ND, not detected.

^aValues given as per g dw soil.

^bValues expressed as arithmetic mean ± s.d. from triplicate reactions.

^cBelow estimated quantification limit in the highest amount of template tested but detected with the end point PCR assay.

^dValue given as per g fw soil, as moisture content was not determined for the sample.

dw and 139–192 µg N g⁻¹ dw soil, respectively) (Supplementary Table S1). However, the relative contribution of the different N compounds to the TDN pools was very variable (Supplementary Table S1). In shrub, tussock and moss tundra soils, NH₄⁺ comprised most of the dissolved N (50–95%), followed by DON (2–41%) and very low fractions of NO₃⁻ (3–9%). By contrast, NO₃⁻ fractions were much higher in the fen peat and frost boil soils (24–64%), accounting for most of the extractable N in the Sol-fb and Knu-tf samples (64% and 60%, respectively). Nitrite was below the detection limit in all the samples.

In situ and potential gross and net nitrification rates

Gross nitrification under *in situ* N conditions was determined in eight distinct Arctic soils with a ¹⁵NO₃⁻ pool dilution assay and showed considerable rate variability between most soils, with great differences observed within small spatial scales (for example, tundra fen peat and frost boils) (Figure 1). Although also highly variable, *in situ* net nitrification rates were negative or close to zero in most soils, indicating a prompt consumption of the NO₃⁻ produced (Supplementary Figure S1). *In situ* gross nitrification rates were lower in the dry mineral soils, with a rate 4–17-fold higher in the wet moss tundra soil and the overall highest rates in the waterlogged tundra fen peat soils, when compared on a dry soil/peat basis (3–7-fold higher than the highest rate in mineral soils) (Figure 1). Potential gross nitrification rates, assessed after amendment with NH₄Cl to a final concentration of ~2 mM, were also variable and were similar to the *in situ* rates in half of the soils, indicating that the nitrifier populations were not NH₄⁺-limited (Figure 1). However, potential rates were over sevenfold higher in the Sol-tf peat soil and 3–4-fold higher in the Hot-st shrub tundra and Knu-fb frost boil soils, thus showing a variability in response to NH₄⁺ input that was irrespective of soil ecosystem type or moisture content (Figure 1 and Supplementary Table S1). Exclusively in the wet moss tundra soil (Lon-mt1), nitrification was almost completely inhibited by NH₄⁺ addition (Figure 1). *In situ* gross and net nitrification rates were strongly negatively correlated ($R^2 = 0.96$, $P < 0.0001$; Supplementary Figure S2), showing that gross NO₃⁻ consumption and production rates varied proportionally between the different soils. By contrast, potential gross and net nitrification rates were not correlated, showing that NO₃⁻ production and consumption responded differently to NH₄⁺ addition ($R^2 = 0.01$, $P = 0.82$; Supplementary Figure S2).

Detection and quantification of NH₃ oxidizers

Archaeal *amoA* genes (encoding the α-subunit of the AMO enzyme) were detected in 10 of the 11 soils with newly designed PCR primers based on an

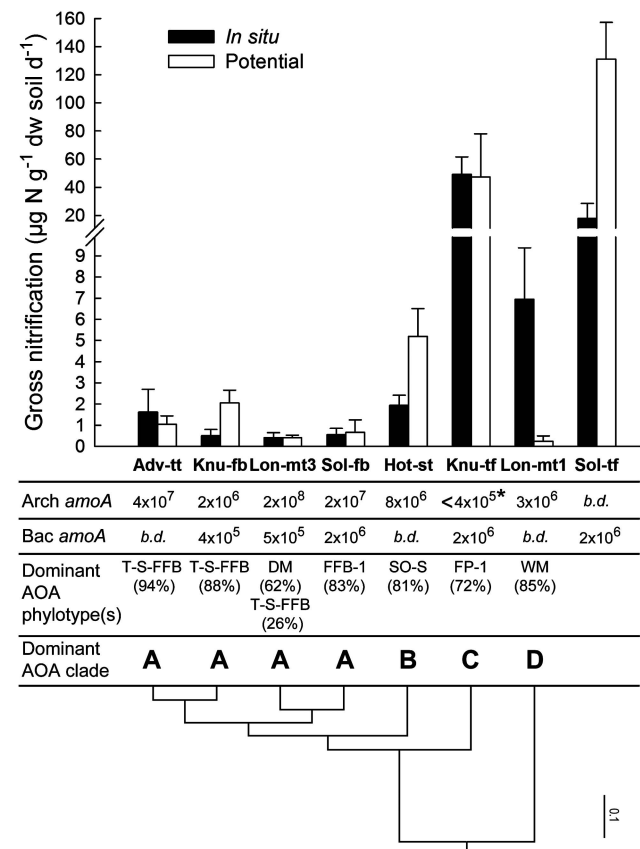


Figure 1 Relationship between gross nitrification and NH₃ oxidizer populations. *In situ* and potential gross nitrification rates (without or with amendment with NH₄Cl to a final concentration of 1.7–2.5 mM, respectively), abundance of AOA and AOB (*amoA* copies g⁻¹ dw soil) and proportion of archaeal *amoA* sequences associated with the dominant AOA clade in the clone library from each soil. The hierarchical clustering dendrogram represents relationships between AOA populations and is based on weighted UniFrac pairwise distances (with normalized branch weights). All nodes of the dendrogram have Jackknife support values of 100% (after 1000 permutations). The scale represents the distance between populations in UniFrac units. Arch, archaeal; Bac, bacterial; ND, not detected. *Below estimated quantification limit in the highest amount of template tested but detected with the end point PCR assay.

updated *amoA* gene database (Supplementary Information). By contrast, β-proteobacterial *amoA* genes were detected only in six of the soils. Archaeal and bacterial *amoA* gene copy numbers ranged between 10⁶–10⁸ and 10⁵–10⁵ copies g⁻¹ dw soil, respectively (Table 1). AOA outnumbered AOB by 1–3 orders of magnitude in soils where both were detected, namely in the frost boil and dry moss tundra soils. AOB dominated only the tundra fen peat soils, although AOA were detected in the Knudsenheia fen by end point PCR but were below quantification range of the quantitative PCR assay (Table 1).

AOA diversity and phylogenetic analysis

A total of 499 cloned archaeal *amoA* genes were sequenced from 10 soil samples (50 clones per

sample on average) and grouped into AOA phylogenetic clades at $\geq 93\%$ nucleotide identity level. Phylogenetic analysis revealed an overall broad phylogenetic representation of AOA in the sample set analyzed (Figure 2). The AOA phylogenetic clades were distributed between five robust phylogenetic clades, mainly within the *Nitrososphaera* cluster (Pester *et al.*, 2012), associated with the thaumarchaeal lineage I.1b. Based on DNA sequences, all clades had a bootstrap support $\geq 93\%$ (Figure 2). Analysis on the protein level further supported all clades with bootstrap values between 64% and 86% (Supplementary Figure S4). Despite the broad phylogenetic diversity of the AOA phylogenetic

detected, the intra-sample diversity was generally low, even when coverage of the clone libraries was identical to, or approached, the predicted phylogenetic richness (Supplementary Figure S3; Supplementary Table S2). This was also evident in the low phylogenetic diversity (PD) indices, with the exception of the Lon-mt1, Knu-fb and Knu-tf samples, which harbored rare phylogenetic clades associated with more dissimilar clades (Supplementary Table S2). The low diversity indexes reflected an uneven population composition resulting from the clear dominance of a single AOA phylogenetic clade in each population (Figure 2; Supplementary Table S2). Most dominant phylogenetic clades showed great local

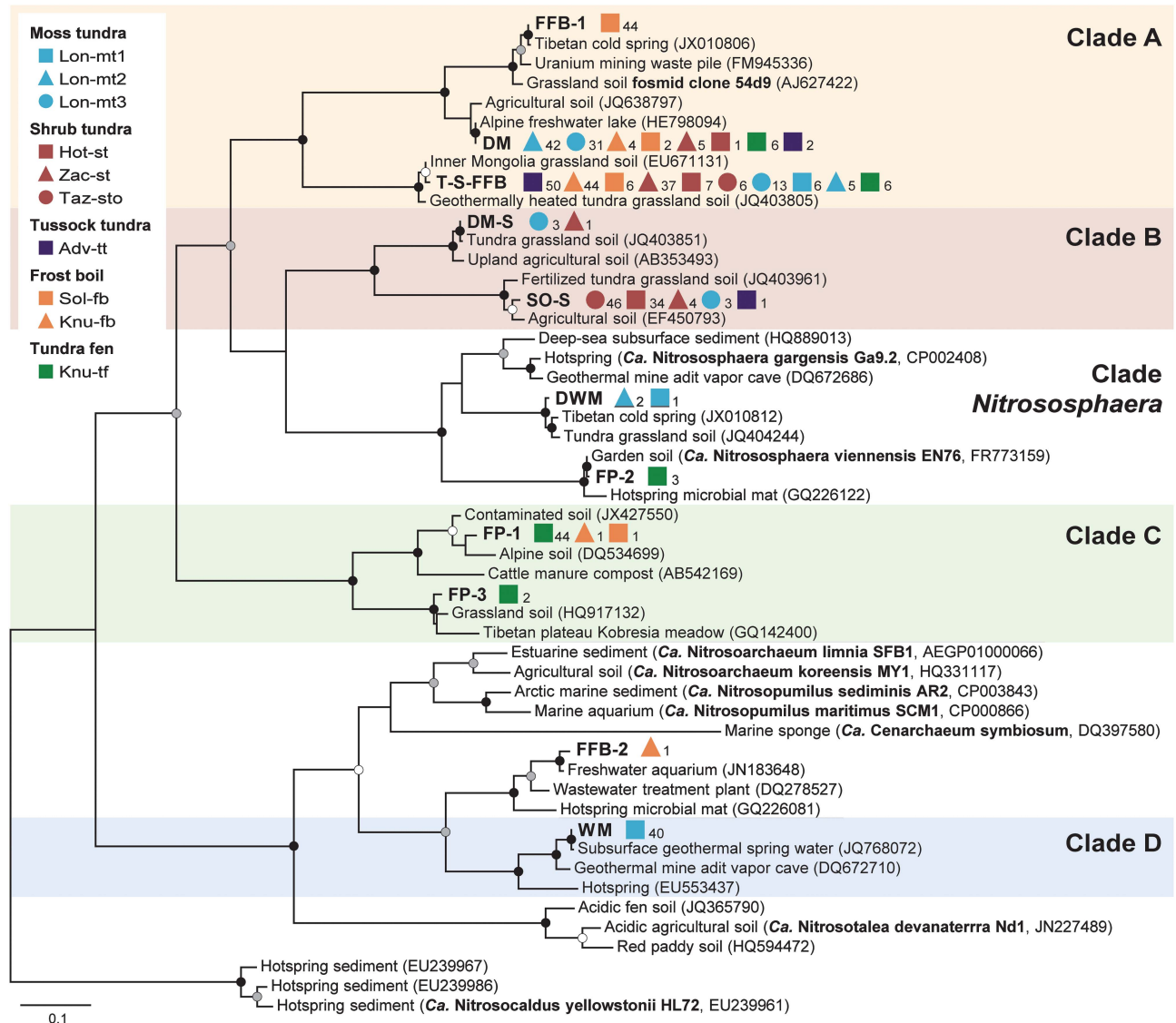


Figure 2 Maximum-likelihood phylogenetic tree of archaeal *amoA*. Phylogeny is based on 51 sequences (590-bp long) and includes a representative of each phylotype ($\geq 93\%$ nucleotide identity), closest BLAST hits with GenBank and major reference sequences. Tree was calculated based on the GTR+I+G model of DNA evolution (see Supplementary Information for details). Percentages of bootstrap replicates (1000 replicates) supporting the nodes are represented by filled circles: black, $\geq 90\%$; gray, $\geq 80\%$; and white, $\geq 70\%$. Phylotypes from this study, sequences from cultured organisms and metagenomic clones are indicated in bold. Reference sequences are named as 'environmental source (accession number)'. Different symbols represent different samples and are color-coded according to soil ecosystem. Numbers following the symbols represent the number of sequences obtained from the sample associated with the respective phylotype. Names on the right-hand side of the tree represent the main AOA clades defined in this study.

specificity, even between similar tundra soils, and few were shared only by a particular soil ecosystem, regardless of spatial scale or geographic location (that is, Hot-st and Taz-sto; Lon-mt2 and Lon-mt3). The majority of the sequences grouped within clade A, represented by the soil fosmid clone 54d9 (Treich *et al.*, 2005), and included the most abundant and widespread phylotype (T-S-FFB). This clade comprised nearly all sequences from the Spitsbergen frost boil, tussock and dry moss tundra samples, as well as from the Zackenberg shrub tundra. Clade B comprised, almost exclusively, sequences from shrub tundra, namely those dominating the Tazovski and Hotelneset samples. Clade C dominated the tundra fen peat from Knudsenheia and represents the only major AOA cluster without cultured representatives to date or 16S rRNA gene taxonomic affiliation (Pester *et al.*, 2012). Clade D was associated with the *Nitrosopumilus* lineage and was only detected in the wet moss tundra soil, where it dominated the AOA population, and by a single sequence from the Knudsenheia frost boil (Figure 2). Only two rare phylotypes in moss tundra samples and in the tundra fen were affiliated with clade *Nitrososphaera*, which includes the cultured *Ca. Nitrososphaera viennensis* (Tourna *et al.*, 2011) and *Ca. Nitrososphaera gargensis* (Hatzenpichler *et al.*, 2008).

AOA distribution in relation to soil physico-chemical properties

Potential relationships between AOA population composition and soil physico-chemical properties were inferred through unconstrained (that is, BIO-ENV) (Clarke and Ainsworth, 1993) and constrained (that is, CCA) multivariate methods, based on the relative abundance of the AOA phylotypes. Despite potential biases associated with the relatively small data set, the dominance of a single phylotype in most AOA populations suggested that the expected increase in diversity, as a result of a bigger data set, would exert little effect on the analysis. The BIO-ENV analysis showed that DOC/DON, moisture and DON had the best individual association with the composition of AOA distribution (Supplementary Table S3). The combination between moisture, pH, NO_3^- and DON showed the best association with AOA population structure, although with lower rank correlation coefficients than DOC/TDN alone (Supplementary Table S3). CCA analyses of all measured physico-chemical parameters also showed that moisture, pH, NO_3^- and DON combined yielded the most significant model explaining the highest percentage of variance in AOA distribution (model 1; Figure 3; Supplementary Tables S4 and S5). Analyses of ratio variables and non-redundant measured parameters yielded a significant model with $\text{NO}_3^-/\text{NH}_4^+$, DOC/TDN, moisture and pH (model 2), explaining a percentage of AOA distribution variance similar to that explained by model 1

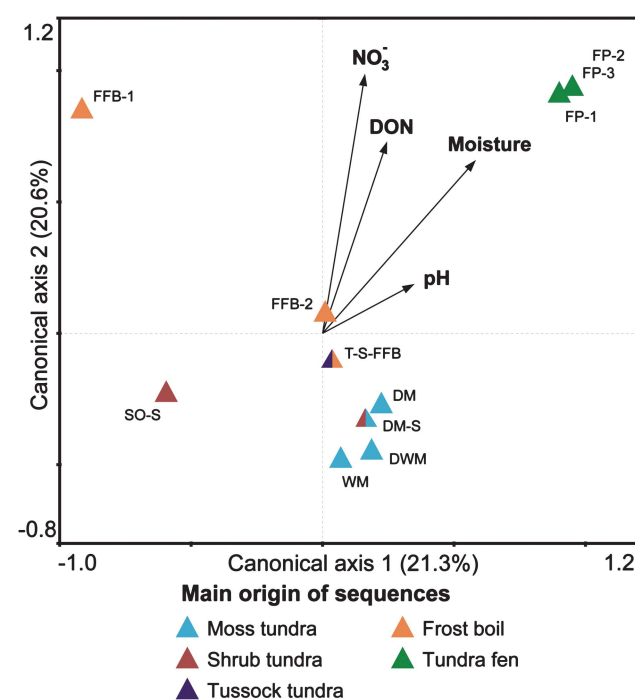


Figure 3 Relationship between AOA distribution and soil properties. CCA biplot of AOA phylotype relative abundances and selection of physico-chemical parameters (model 1). Symbols represent the 11 AOA phylotypes and are color-coded according to major origin of sequences. Symbols with two colors correspond to phylotypes dominating two distinct soil types. Symbols with more than one phylotype name represent overlapping phylotypes. The percentages of phylotype distribution variance explained by the two principal canonical axes are represented close to the axes. Conditional variables are represented by the black arrows.

(69.4% and 72.5%, respectively) (Supplementary Figure S5; Supplementary Tables S4 and S5). Although none of the variables had individual significant explanatory power (Supplementary Table S5), both CCA models showed a similar separation between AOA phylotypes, which reflected a general distribution according to the combined effect of pH, moisture and the N content of the soils, namely NO_3^- (Figure 3 and Supplementary Figure S5).

AOA growth and NH_3 oxidation activity in enrichment cultures

A total of 48 initial AOA enrichment cultures were set up from all the different Spitsbergen soils and sub-cultured at three different temperatures (4, 20 and 28 °C). Cultures from a frost boil soil (Knu-fb) and from a dry moss tundra soil (Lon-mt2) showed stable NH_3 oxidation activity, with AOA being the only NH_3 oxidizers detected after three enrichment stages (that is, no detectable AOB; Figure 4). The *amoA* sequences detected in five enrichment cultures from these two soils clustered exclusively with phylotypes from clades A and C (Table 2; Supplementary Figure S6). Although clade

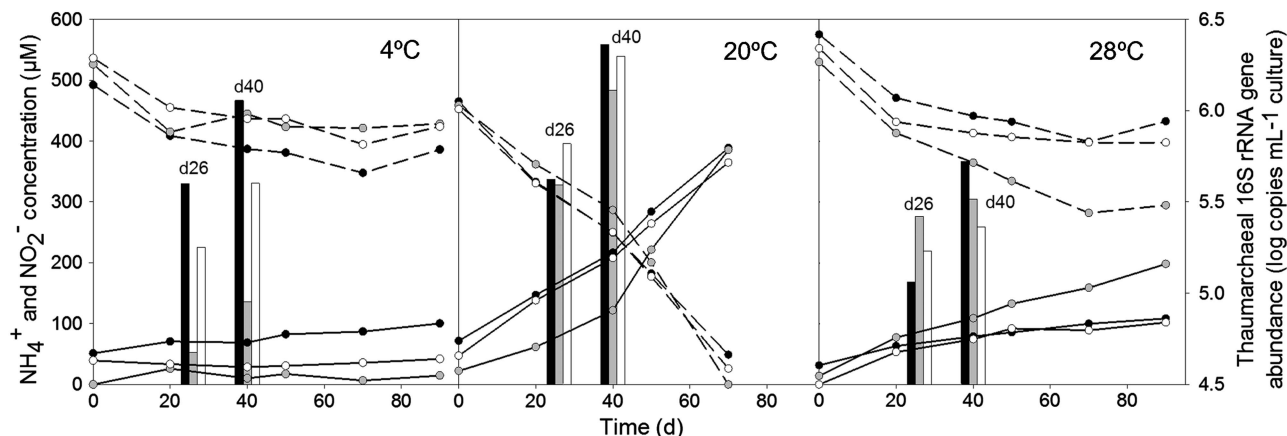


Figure 4 Growth and activity of AOA in enrichment cultures. NO_2^- production (solid lines), NH_4^+ consumption (dashed lines) and thaumarchaeal 16S rRNA gene copies at days 26 and 40 (bars) in nine enrichment cultures from moss tundra (Lon-mt2) and frost boil (Knu-fb) soils incubated at 4, 20 or 28 °C. Figures depict three example cultures out of nine cultures incubated at each temperature, exhibiting identical NH_3 oxidation activity. AOB were not detected in any of the cultures. Filled circles and bars: black, Knu-fb cultures; gray and white, two different Lon-mt2 cultures, respectively.

Table 2 Thaumarchaeal growth, AOA clade affiliation and NH_3 oxidation activity in nine enrichment cultures from moss tundra (Lon-mt2) and frost boil (Knu-fb) soils incubated at different temperatures

| Incubation temperature | Original soil | 16S rRNA gene copies fold increase | NH_4^+ consumption ($\mu\text{M NH}_4^+ \text{d}^{-1}$) | NO_2^- production ($\mu\text{M NO}_2^- \text{d}^{-1}$) | AOA clade (no. of sequences) | |
|------------------------|---------------|------------------------------------|--|---|------------------------------|----------|
| | | | | | A | C |
| 4 °C | Knu-fb | 2.8 | 1.07 | -0.10 | 17 (94%) | 1 (6%) |
| | Lon-mt2 | 2.4 | 0.93 | -0.24 | 10 (53%) | 9 (47%) |
| | Lon-mt2 | 1.8 | -1.48 | -0.79 | NA | NA |
| | AM \pm SD | 2.3 ± 0.5^a | 0.17 ± 1.43^a | -0.38 ± 0.36^a | | |
| 20 °C | Knu-fb | 5.4 | 4.18 | 3.48 | 8 (42%) | 11 (58%) |
| | Lon-mt2 | 3.0 | 4.02 | 3.48 | 3 (15%) | 17 (85%) |
| | Lon-mt2 | 3.3 | 3.75 | 3.02 | NA | NA |
| | AM \pm s.d. | 3.9 ± 1.3^a | 3.98 ± 0.22^b | 3.33 ± 0.27^b | | |
| 28 °C | Knu-fb | 4.5 | 1.49 | 1.58 | 19 (100%) | 0 (0%) |
| | Lon-mt2 | 1.4 | 0.92 | 1.05 | NA | NA |
| | Lon-mt2 | 1.2 | 2.44 | 0.79 | NA | NA |
| | AM \pm s.d. | 2.4 ± 1.9^a | 1.62 ± 0.77^a | 1.14 ± 0.40^c | | |

Abbreviations: AM \pm SD, arithmetic mean \pm s.d.; NA, not available.

Thaumarchaeal 16S rRNA gene copy increase was determined between days 26 and 40, and NH_4^+ consumption and NO_2^- production rates were determined between days 20 and 40. Proportion of sequences affiliated with the two AOA clades detected (A and C) are expressed as percentage in each clone library. Lowercase letters indicate significant differences between temperatures: 16S rRNA gene copies increase, $P = 0.3297$; NH_4^+ consumption, $P < 0.01$; NO_2^- production, $P < 0.0001$.

A dominated the cultures at 4 °C and 28 °C, all cultures at 20 °C were dominated by a single phylotype from clade C (Supplementary Figure S6). Thaumarchaeal growth, assessed by the increase in 16S rRNA gene copy numbers, was observed in all the cultures tested and not significantly different between cultures at the three different temperatures (Table 2). However, stable and continuous NH_4^+ oxidation occurred only at 20 °C, as shown by the conversion of $\sim 500 \mu\text{M NH}_4^+$ to NO_2^- over a period of 70–100 days in nine independent cultures (Figure 4). No conversion of NH_4^+ to NO_2^- was observed in cultures at 4 °C, while at 28 °C very slow rates were observed only during the initial 70 days (50–150 μM). AOA enrichment cultures with stable NH_3 oxidation activity at 20 °C were continuously sub-cultured and maintained in the laboratory over

a period of almost 3 years (Supplementary Figure S7). Forty-five identical archaeal *amoA* sequences obtained from several replicate sub-cultures yielded only the clade C phylotype detected in earlier enrichment cultures (Supplementary Figure S6). This organism was enriched up to 39–49% of all prokaryotes present in several cultures (that is, 16S rRNA gene copies ml^{-1} : 1.6×10^5 and 2.8×10^7 archaea out of 4.1×10^5 and 5.6×10^7 prokaryotes, respectively). NO_2^- production and NH_3 consumption were inhibited upon addition of acetylene, confirming the AMO-dependent oxidation of NH_4^+ to NO_2^- (Supplementary Figure S7). Twenty identical thaumarchaeal 16S rRNA gene sequences ($\geq 99.6\%$ identity) amplified from the same cultures showed that the *amoA* identified in clade C belongs to an organism closely associated with the reference

soil fosmid clone 29i4 (Quaiser *et al.*, 2002; 98.4–98.7% 16S rRNA identity; Supplementary Figure S8). Clone 29i4 represents a thaumarchaeal lineage widespread in soils, although without cultured representatives to date.

Discussion

The regulation of N-cycling processes, such as nitrification, is crucial for Arctic soil ecosystem functioning. Here, we have characterized AOA populations dominating NH_3 oxidizer communities in a wide range of Arctic permafrost-affected soils through a polyphasic approach, which combined phylogenetic and abundance analyses of AOA with physico-chemical parameters, gross nitrification rates and laboratory cultivation. This integrated approach yielded five major outcomes: (1) high thaumarchaeal β -diversity and abundance were associated with most Arctic soil ecosystems analyzed, outnumbering AOB in most samples; (2) the composition of AOA populations was associated with several combined physico-chemical parameters; (3) AOA populations were linked to different gross nitrification rates; (4) AOA populations reacted differently to changes in NH_4^+ availability; and (5) both environmental and cultivation analyses supported different nitrification capabilities among AOA clades.

Prevalence of AOA among NH_3 oxidizers in Arctic soils

Our study showed a widespread distribution, high abundance and differential phylogenetic diversity of AOA across different Arctic soils. By contrast, β -proteobacterial NH_3 oxidizers were surprisingly undetectable in nearly half of the soils analyzed, and orders of magnitude less abundant than AOA in most others, with the exception of the AOB-dominated peat soils. Although it is possible that unknown AOB groups might have not been detected in the soils sampled, the primer set used here was recently shown to cover the groups of AOB typically found in soils (Junier *et al.*, 2008). The overall prevalence of AOA and the presence of representatives of most major groups of soil AOA known strongly suggest that soils from cold environments might harbor common features that favor the general prevalence of thaumarchaea, as previously observed in alpine (Nemergut *et al.*, 2008) and Canadian Arctic soils (Lamb *et al.*, 2011; Banerjee and Siciliano, 2012). However, this needs to be confirmed by an extended survey.

Physico-chemical determinants of AOA population composition

The distribution of AOA phylotypes did not show geographic patterning between Svalbard, Greenland and Siberia sites or between the Svalbard sites but rather followed an 'ecosystem-type' (that is,

habitat) distribution. However, further sample replication would be required to confirm geographical or ecosystem distribution patterns, which were not the main aim of the present work. Independent multivariate analyses showed a multifactorial association between overall AOA distribution and the soil properties. The combined effect of pH and soil moisture and N contents, namely NO_3^- and DON, suggested a general niche partitioning between AOA clades according to: dry (clade A and B) or wet (clade D) mineral soils with low N (particularly NO_3^-) and high DOC/TDN, and wet organic soils with high N (particularly NO_3^-) and low DOC/TDN (clade C). Although pH has been shown to be an important determinant of AOA niche partitioning in other (non-Arctic) soils (Gubry-Rangin *et al.*, 2011), individually, it had no significant explanatory power for overall AOA distribution in our analyses (Supplementary Tables 3 and 5). Despite the apparent pH-associated selection of clade B in the acidic soils (Hot-st and Taz-sto; Figure 2 and Supplementary Table S1), pH only contributed to the resolution of AOA population composition when combined with several other soil properties. The particular association between AOA distribution and $\text{NH}_4^+/\text{NO}_3^-$, or NO_3^- alone (Supplementary Table S5), was consistent with previous studies from non-Arctic soils reporting significant correlations between AOA population structure, or abundance, and NO_3^- leaching (Wessén *et al.*, 2011) and fertilization (Glaser *et al.*, 2010). However, similar to these studies, the association between AOA and $\text{NH}_4^+/\text{NO}_3^-$, or NO_3^- , did not reflect their clustering according to nitrification rates (Figures 1 and 3), and thus the relationships between AOA and these two factors appear to be independent. Our results indicate that the composition of soil AOA populations are likely not explained by single physico-chemical properties and is rather dependent on the interplay between several environmental factors.

Differences in activity among AOA clades

The role in nitrification and niche adaptations of AOA (as compared with AOB) have been disputed since the discovery of their high abundances in soils (Leininger *et al.*, 2006), with environmental studies often yielding contrasting results (for example, (Di *et al.*, 2009; Jia and Conrad, 2009; Schauss *et al.*, 2009; Kelly *et al.*, 2011; Wessén *et al.*, 2011; Szukics *et al.*, 2012)). However, such studies have usually addressed AOA as a functionally homogeneous group although their diversity and distribution suggest a broader metabolic and physiological potential (Gubry-Rangin *et al.*, 2011; Pester *et al.*, 2012), as known for AOB (Arp *et al.*, 2007). In line with this AOA heterogeneity, gross nitrification rates did not reflect the overall NH_3 oxidizer abundance in the present Arctic soils. Rather, both *in situ* and potential nitrification rates were linked to AOA population structure in the AOA-dominated

soils. For example, soils dominated by clade A exhibited the lowest nitrification rates, in spite of harboring the largest AOA populations, which was further supported by enrichment cultures of clade A with little or no nitrification activity. In soils where no AOB were detectable, we estimated 'cell-specific' nitrification rates for AOA based on *amoA* gene abundances and *in situ* gross nitrification rates. In soil Adv-tt, dominated by clade A, the rate was comparably low with $0.18 \text{ fmol N cell}^{-1} \text{ h}^{-1}$, whereas the soils dominated by clade B (Hot-st) and by clade D (Lon-Mt1) had 5.6-fold ($1.0 \text{ fmol N cell}^{-1} \text{ h}^{-1}$) and 55-fold ($9.9 \text{ fmol N cell}^{-1} \text{ h}^{-1}$) higher cell-specific activities, respectively. The nitrification rate of AOB calculated with data from the Sol-tf soil was $30 \text{ fmol N cell}^{-1} \text{ h}^{-1}$, in line with the higher rates reported for this group.

The different responses to NH_4^+ amendment in soils where no AOB were detectable indicated also a niche partitioning between AOA clades regarding NH_4^+ concentration. For instance, addition of NH_4^+ induced a threefold higher nitrification rate in the soil dominated by clade B but did not affect the soil dominated by clade A, although *in situ* rates were similar in both (Figure 1). This indicated that clade B was NH_4^+ -limited and that it has a higher nitrification potential than clade A. Moreover, the low NH_4^+ amount added (up to $\sim 2 \text{ mM}$) strongly inhibited nitrification in the Lon-mt1 soil dominated by clade D, which showed rates among the highest under *in situ* NH_4^+ concentrations. This is in agreement with the fact that clade D is associated with the lineage represented by the isolate *Ca. Nitrosopumilus maritimus*, which is inhibited at NH_4^+ concentrations of $2\text{--}3 \text{ mM}$ (Martens-Habbena *et al.*, 2009).

Taken together, our data indicated that AOA from clade A, which are represented by the fosmid clone 54d9 (Treusch *et al.*, 2005) and constitute the most abundant and widespread AOA in soils (Bates *et al.*, 2011), possibly do not obligately or exclusively grow as NH_3 oxidizers. This was consistently supported by their very low nitrification activity *in situ* (that is, Adv-tt soil) and in enrichment cultures, as well as their lack of response to NH_4^+ amendment. In line with this, studies to date showing direct evidence for autotrophic NH_3 oxidation by soil AOA (Jung *et al.*, 2011; Lehtovirta-Morley *et al.*, 2011; Pratscher *et al.*, 2011; Tourna *et al.*, 2011; Xia *et al.*, 2011; Zhang *et al.*, 2012) could not detect activity by organisms associated with clade A. By contrast, the closely related clades B and *Nitrososphaera*, as well as clade D, could be linked to NH_3 oxidation. However, the possibility of heterotrophic nitrification cannot be excluded, as no selective inhibitor tests (with for example, acetylene) were performed. One also has to keep in mind that urea represents an alternative substrate for AOA, as was recently shown for *Ca. N. viennensis* (Tourna *et al.*, 2011) and for AOA in acidic soils (Lu *et al.*, 2012; Lu and Jia, 2012). Although urea was not tested in the

potential nitrification assays or enrichment cultures, our observations based on *in situ* gross nitrification rates are independent of the nature of the substrate.

Although none of the NH_3 oxidizer communities studied here was dominated by AOA from clade C, their continuous culturing under selective conditions for autotrophic NH_3 oxidizers provides evidence for NH_3 -dependent growth in this so far uncharacterized major lineage of AOA ('*Nitrososphaera*-sister cluster' in (Pester *et al.*, 2012)). The estimated cell-specific nitrification rate of $0.21 \text{ fmol N cell}^{-1} \text{ h}^{-1}$ in the enrichment cultures is comparable, albeit lower, than the value for *Ca. N. viennensis* ($1.63 \text{ fmol N cell}^{-1} \text{ h}^{-1}$), which is also reflected by the longer growth period ($\sim 500 \mu\text{M NO}_2^-$ produced in ~ 70 days) (Figure 4). Our results demonstrate, for the first time, NH_3 oxidation in this widespread thaumarchaeal lineage represented by the reference fosmid clone 29i4 (Quaiser *et al.*, 2002), which has not been previously linked to any activity or functional gene marker, such as *amoA*. Growth of organisms from this lineage was recently suggested to be stimulated by organic substrate amendment (that is, root extracts) in temperate soils (Xu *et al.*, 2012). However, the authors doubted their capability to oxidize NH_3 , as they could not associate *amoA* genes to their respective 16S rRNA sequences, possibly owing to PCR primer biases.

In conclusion, our study provides evidence that the distinct AOA clades defined here, which encompass the great majority of the AOA diversity found in soils, were not functionally redundant. Therefore, categorical assumptions on the whole-group level (that is, AOA) (for example, (Di *et al.*, 2009; Banerjee and Siciliano, 2012)) might lead to inconclusive or misleading conclusions.

Role of AOA in nitrification and functioning of Arctic soil ecosystems

Nitrification is a critical process for N availability in N-limited Arctic soils (Giblin *et al.*, 1991; Jonasson and Michelsen, 1996), although net nitrification has been shown to be low in these low-temperature ecosystems (Atkin, 1996). By contrast, gross nitrification rates in the present soils were surprisingly high and comparable to rates in soils from warmer climatic zones (Booth *et al.*, 2005). In addition to temperature, pH has been suggested to regulate NH_4^+ and NO_3^- pools in Arctic soils through nitrification (Atkin, 1996). By contrast, the gross nitrification rates here exhibited a high variability that was irrespective of differences in pH and $\text{NO}_3^-/\text{NH}_4^+$ ratios. Furthermore, the highest gross nitrification rates were observed in the wet soils, in contrast to what has been reported for net nitrification in other Arctic soils (Chapin, 1996). However, these and most other Arctic soil studies to date analyzed only net nitrification and, to our knowledge, only one previous study determined gross nitrification through a ^{15}N pool dilution method, as done here,

showing also great discrepancies with the corresponding net rates (Binkley *et al.*, 1994). Furthermore, gross and net nitrification responded differently to NH_4^+ amendment in the different soils dominated by distinct AOA clades, showing that, together with the N preferences of the soil microbiota, the activity potential of the nitrifier populations strongly affects the balance between the two major bio-available inorganic N-forms (that is, NH_4^+ and NO_3^-). Together, these observations support the idea that nitrification rates are possibly more directly dependent on nitrifier population structure and physiologic, or metabolic, properties than on abiotic factors and underline the importance of linking gross biochemical processes to their microbial catalysers. The different responses of the AOA clades to higher NH_4^+ availability further suggested that the distribution of functionally heterogeneous AOA is likely to play a critical role in the response of soil nitrification to environmental changes, not only in the Arctic but also in soils from other regions. It will, therefore, be crucial to differentiate the AOA populations in future studies assessing the effect of environmental changes on soil nitrification, as well as the direct and indirect contribution of these populations to N_2O emissions (Santoro *et al.*, 2011).

Conflict of interest

The authors declare no conflict of interest.

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