

Characteristics of ammonia oxidation potentials and ammonia oxidizers in mineral soil under *Salix polaris*–moss vegetation in Ny-Ålesund, Svalbard

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Abstract Although nitrification is a unique and important process in the nitrogen cycle with respect to ammonium consumption and nitrate production, limited information on this process is available for high-Arctic soils. We elucidated the ammonia oxidation potentials (AOPs) and characteristics of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in mineral soils under climax vegetation, i.e., *Salix polaris* (polar willow)–moss vegetation, on a coastal hill in Ny-Ålesund, Svalbard. AOPs at 10 °C were determined by incubation with sufficient substrate (2 mM ammonium). The ammonia monooxygenase subunit A (*amoA*) genes of AOB and AOA were analyzed by using quantitative polymerase chain reaction and pyrosequencing. AOPs ranged from 1.1 to 14.1 ng N g⁻¹ dry soil h⁻¹—relatively low but of a similar order to the gross nitrification rates reported in another Svalbard study. AOP

was positively correlated with thickness of the moss layer ($P < 0.01$), soil water content, and ammonium nitrogen content ($P < 0.05$). The population sizes of both AOB and AOA were not significantly related to AOP or edaphic factors. For AOB-*amoA*, six major operational taxonomic units (OTUs) were identified, all of which were classified into the *Nitrosospora* Mount Everest cluster. For AOA-*amoA*, six major OTUs were also identified, five of which were grouped with sequences from cold environments within clade A of the *Nitrososphaera* cluster, i.e., species known to have low, or no, AOP. It is, therefore, possible that the AOPs measured at the study site were driven mainly by psychrotolerant AOB.

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Polar willow · Regosolic cryosol · Tundra

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Introduction

Arctic ecosystems are characterized by low primary productivity, low element inputs, and slow element cycling. However, they tend to accumulate soil organic matter (SOM), carbon (C), nitrogen (N), and other elements because decomposition and mineralization processes are even more strongly limited than productivity by the cold and wet environment (Callaghan et al. 2005). A polar semidesert tundra in Adventdalen, Svalbard, Norway, stored 373 g N m⁻² in the soil (90 % of the ecosystem N) in a dry meadow and 463 g N m⁻² (92 %) in a moist meadow (Bardgett et al. 2007). The soil N pool in a wet-sedge tundra in Barrow, Alaska, was more abundant, at 937 g N m⁻² (97 % of the ecosystem N) (Shaver et al. 1992). The soil N pool differs among structural units in

patterned ground. For example, a frost-boil tundra on the Gydansky Peninsula in central Siberia had a structure consisting of polygons of bare soil, rims surrounding the polygons, and troughs between the rims; their N pools were 320, 840, and 1680 g N m⁻², respectively (Kaiser et al. 2005). In addition to this microscale variability, the soil N pool varies at the catchment scale, as shown in forest tundra soils of the Little Grawijka Creek catchment, central Siberia, where the soil N pool ranges from 500 to 6000 g N m⁻² (Rodionov et al. 2007).

Decomposition of SOM provides inorganic N as ammonium to the soil environment, i.e., N mineralization. Although the flow rate of mineralized N is smaller than the standing N stock in Arctic ecosystems (e.g., corresponding to only 0.2 % of the standing N stock in the case of Alaska; Shaver et al. 1992), this flow N is precious because it is available to microbes and plants. Biasi et al. (2005) evaluated gross N mineralization rates at ambient temperatures (1.0–6.2 °C) in mineral soils of hummock tundra on the Taymyr Peninsula in central Siberia. Hummock tundra is a kind of patterned ground and consists of hummocks and interhummock areas. Rates were higher in the interhummock areas [122 and 263 ng N g⁻¹ dry soil (ds) h⁻¹ for mesic and wet conditions, respectively] than in the hummocks (65 and 64 ng N g⁻¹ ds h⁻¹ for mesic and wet conditions, respectively), except under waterlogged conditions, in which case the rates were similar to each other (51 and 48 ng N g⁻¹ ds h⁻¹ in the interhummocks and hummocks, respectively). Wild et al. (2013) also evaluated gross N mineralization rates at 7 °C in organic, cryoturbated, and mineral soils of three types of tundra, i.e., heath tundra in Greenland and tussock tundra and shrub tundra in northeastern Siberia. The rates were 310 (organic soils), 86 (cryoturbated soils), and 64 ng N g⁻¹ ds h⁻¹ (mineral soils) on the heath tundra; similarly, they were 63, 22, and 21 ng N g⁻¹ ds h⁻¹ on the tussock tundra and 526, 68, and 73 ng N g⁻¹ ds h⁻¹ on the shrub tundra. The organic soils thus had larger rates of N mineralization than did the mineral soils.

Nitrification is a two-step microbial process unique to the N cycle for oxidizing ammonia to nitrate via nitrite, and it is the primary process governing the fate of available ammonia in the soil. Some bacteria and archaea have ammonia-oxidizing ability (Prosser and Nicol 2012); they are, respectively, called ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) and are collectively known as ammonia oxidizers. Ammonia oxidation is the rate-limiting step of nitrification (Klotz and Stein 2011). Ammonia oxidation produces nitrous oxide (N₂O) as a by-product (Prosser 1990), and ammonia oxidizers in Arctic ecosystems contribute to N₂O production (e.g., Ma et al. 2007; Siciliano et al. 2009). N₂O is a potent greenhouse gas and is currently a major stratospheric

ozone-depleting substance (Myhre et al. 2013). To our knowledge, the study by Alves et al. (2013) is the only one ever to have elucidated the nitrification properties of Svalbard's high-Arctic soils. Alves et al. (2013) measured both gross and net rates of in situ and potential nitrification at 15 °C by using an ¹⁵N dilution technique on eight types of tundra soils in Svalbard (mineral soils of shrub tundra, tussock tundra, moss tundra on a wet ridge, moss tundra on a dry mound, and in two plots of frost-boil tundra; organic soils in two plots of tundra fen peat). Potential gross nitrification rates of the mineral soils with the addition of 1.7–2.5 mM ammonium as substrate ranged from about 10–200 ng N g⁻¹ ds h⁻¹. Potential net nitrification rates ranged from about –70 to 35 ng N g⁻¹ ds h⁻¹, the negative value indicating that nitrate consumption exceeded production. According to Alves et al. (2013), the nitrification rates of soils in Svalbard were directly dependent on functionally distinct populations of AOA, rather than on abiotic factors. They also revealed that clade A of AOA was dominant in many cases (in the mineral soils of tussock tundra and moss tundra on the dry mound, and in two plots of frost-boil tundra); these sequences within clade A were low-temperature adapted and had low, or no, nitrification potential. Banerjee and Siciliano (2012) evaluated the ammonia oxidation potential (AOP) at 20 °C by incubation, adding sufficient substrate (8 mM ammonium) for mineral soils in three high-Arctic sites on the Canadian Arctic tundra, namely Truelove Lowland, Simpson Lake, and Ross Point. The AOPs they obtained ranged from 21 to 178 ng N g⁻¹ ds h⁻¹. They also evaluated the effect of the spatial scale (fine, 0–1 m; medium, 1–10 m; and large, 10–100 m) of edaphic and genetic factors on ammonia oxidation; they concluded that edaphic, rather than genetic, factors were an important controller of ammonia oxidation in Arctic ecosystems. Sanders et al. (2010) reported AOPs at in situ temperatures by incubation adding substrate (1.5 mM ammonium) for polygonal tundra soils on Samoylov Island in northeastern Siberia. In mineral soils of the polygon centers under water-saturated conditions, an AOP of 30 ng N g⁻¹ ds h⁻¹ was detectable. In the relatively dry polygon rims, the AOP was about 100 ng N g⁻¹ ds h⁻¹. Wild et al. (2013) also evaluated gross nitrification rates at 7 °C for organic, cryoturbated, and mineral soils of three types of tundra, namely heath tundra in Greenland and tussock tundra and shrub tundra in northeastern Siberia. The rates were 433, 99, and 101 ng N g⁻¹ ds h⁻¹ in organic, cryoturbated, and mineral soils, respectively, on the heath tundra, and similarly 896, 95, and 107 ng N g⁻¹ ds h⁻¹ on the tussock tundra and 1240, 390, and 231 ng N g⁻¹ ds h⁻¹ on the shrub tundra. The organic soils had higher nitrification rates than the mineral soils. Despite these studies, our knowledge of ammonia oxidation, its controlling factors, and ammonia

oxidizers in the polar semidesert of Svalbard, in the European High Arctic, is still limited.

It is clear that decomposition of SOM and soil N availability are pivotal to the responses of high-Arctic ecosystems to changes in climate (Robinson 2002). According to climate models (Ciais et al. 2013), there are medium levels of agreement that warming will increase land C uptake at high latitudes, but none of these models includes representation of permafrost C pools, which are projected to decrease under warmer conditions; therefore, confidence is low regarding the sign and magnitude of the future high-latitude land C response to climate change. Although warming essentially enhances both production and consumption of organic matter, differences in the effects of warming on each process could result in either net accumulation or loss of land C. In this regard, supply of N and other nutrients is necessary for C accumulation (Callaghan et al. 2005). Enhanced organic matter consumption, particularly through SOM decomposition, might enhance the attendant N mineralization and then stimulate nitrification. Nutrient limitation dominates multi-year responses and is linked to changes in other factors (e.g., temperature and water regime) through their indirect effects on nutrient mineralization and availability to plants (Callaghan et al. 2005). An 8-year snow manipulation experiment on the Alaskan tundra showed that changes in small-scale snow depth affected plant species abundance, canopy height, and diversity. An increase in small-scale snow depth also affected microclimate by insulating vegetation from winter wind and temperature extremes, modifying winter soil temperatures, and increasing spring runoff (Wahren et al. 2005). Changes in temperature and water regimes also strongly control microbial activity in the high Arctic (Bardgett et al. 2007) and thus might perturb N-related processes both directly and indirectly. Information on current nitrification status is therefore needed as a benchmark for assessing the responses of nitrification in Arctic ecosystems to future climate change.

Here, we focused on mineral soils under the climax vegetation of a polar semidesert in Svalbard, namely *Salix polaris* (polar willow)–moss vegetation (Nakatsubo et al. 2005; Muraoka et al. 2008; Uchida et al. 2010). According to Hodkinson et al. (2003), a high ground cover of *S. polaris* (more than 10 %) in the foreland of the Midre Lovén Glacier on the Brøgger Peninsula, Svalbard, took about 150 years to develop after the glacier's retreat. Hence, we expected that the soil under the climax vegetation at our study site was well developed and rich in SOM owing to long-term plant–soil interactions and consequently that the soil also had high nitrification activity. We also selected an area with a bare surface as a reference plot not directly influenced by plants on a long-term basis. We aimed to evaluate AOPs, to compare AOPs with soil

properties, and to elucidate the characteristics of ammonia oxidizers (abundance and diversity) in the mineral soils at the study site.

Materials and methods

Study site and soil sampling

The study site was on an ice-free coastal hill named Hamnerabben in Ny-Ålesund, Svalbard (Fig. 1). The annual mean air temperature in 2009, when the study was conducted, was -4.3 °C, and the precipitation was 398 mm. In the summer of 2009 (July and August), the mean air temperature was 5.2 °C and the precipitation was 46 mm (Norwegian Meteorological Institute 2015).

Despite its semidesert category, this land, where the snow and ice melt in summer, is covered by patchy vegetation consisting of bryophytes and vascular plants or a biological soil crust consisting of algae, cyanobacteria, and lichen (Yoshitake et al. 2010). Vegetation was classified into the High-Arctic *Dryas octopetala* (mountain avens) zone (Adachi et al. 2006); however, the distribution of *D. octopetala* was limited to relatively dry locations, and *S. polaris*, *Saxifraga oppositifolia* (purple saxifrage), graminoids, and bryophytes were also common. At the study site, vascular plants, mostly consisting of *S. polaris*, covered about 20 % of the ground (Muraoka et al. 2008), and the typical vegetation type was *S. polaris*–moss. The vegetation at our study site was similar to that at the study site of moss tundra on a dry mound in Longyearbyen, Svalbard, in the work of Alves et al. (2013), though the locations were different.

The soil at the study site is a Regosolic cryosol (Bekku et al. 2004). Note that neither obvious patterned ground nor water erosion was seen at the study site. Forman and Miller (1984) reported that the area lower than 40 m a.s.l. on the western part of the Brøgger Peninsula, Svalbard, originated from beach deposit during the last 12 ka. We therefore inferred that our study site—a coastal hill with a height of about 40 m a.s.l. and located on the same peninsula (Fig. 1)—consisted of a similar beach deposit. Figure 2 gives a schematic profile of the soil at the study site. Plants affect the underlying soil via their functions of water absorption, water retention, heat insulation, organic matter input, and nutrient uptake. For example, Uchida et al. (2002) showed that the water contents of fresh mosses ranged from 20 to 500 % (w/w); the lower of these values corresponded to dry conditions, even under which the mosses could hold water at 20 % or more by weight owing to their high water-retention capacity. The higher of these values resulted from high levels of absorption of rainwater owing to the mosses' strong water-absorption capacity.

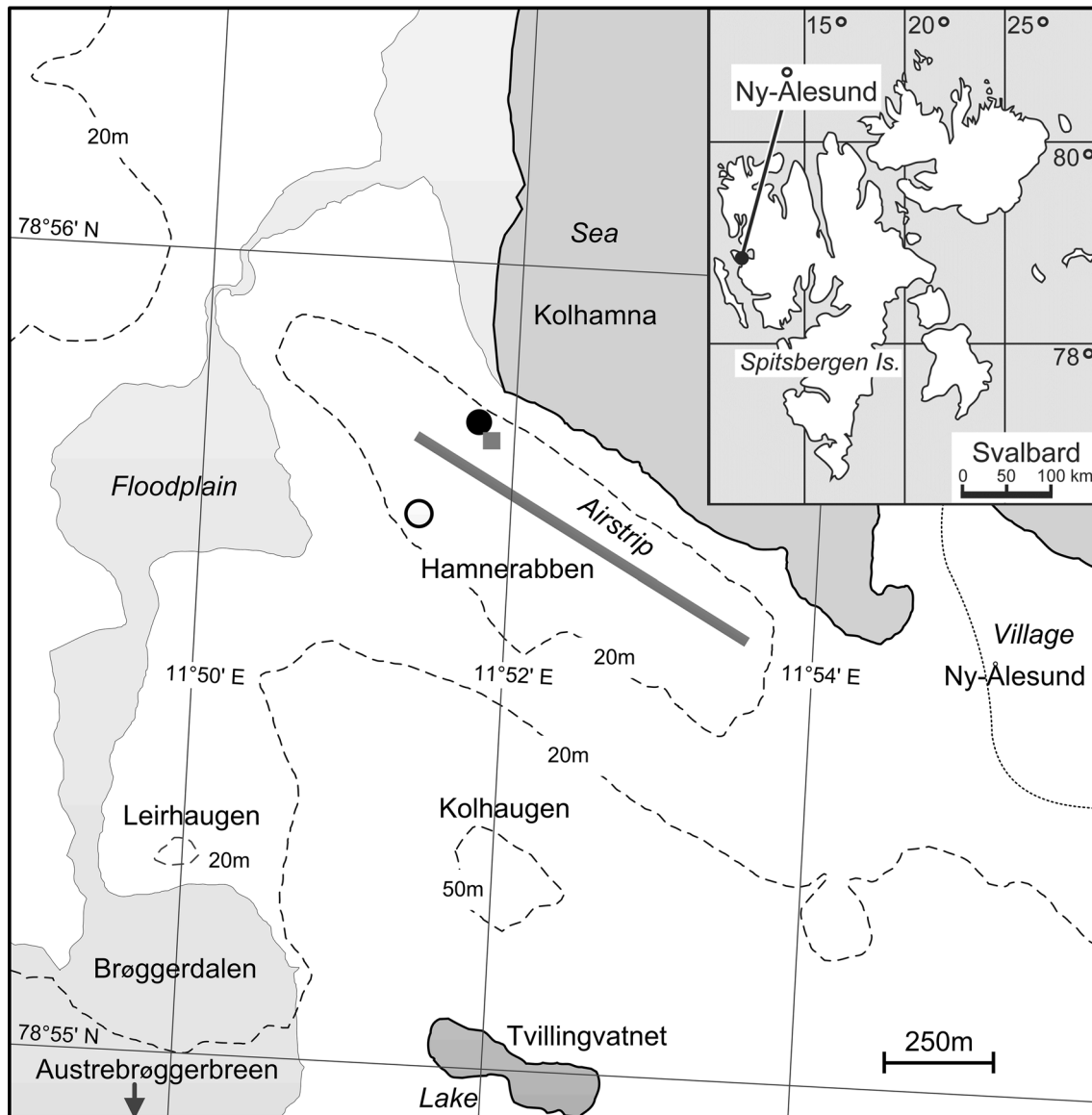


Fig. 1 Location of the study site. The *black* and *white circles* denote the sites where mineral soils under *Salix polaris*–moss vegetation and at a bare soil surface are collected, respectively. The *gray square* denotes the location of the Japanese research station

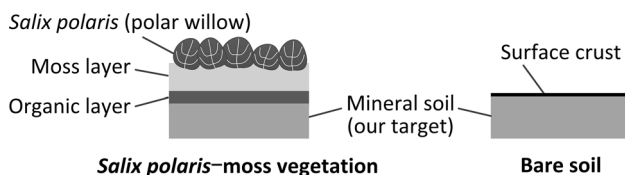


Fig. 2 Schematic view of soil profiles at the sampling sites

Gornall et al. (2007) found that the moss layer retained water within its structure and reduced evaporation from the soil surface; the deeper moss delayed the onset of soil thaw for several weeks, reduced the soil temperature during the growing season, and decreased the amplitude of diurnal

soil temperature fluctuations. It also buffered the soil against low temperatures for a month at the beginning of the cold season. Gross ecosystem photosynthesis on tundra is also strongly related to the biomass of mosses, but it is subject to moisture conditions; the contribution of mosses decreases under dry conditions (Sjögersten et al. 2006).

Mineral soil was collected at 12 points with different types of coverage within an area of about 10×10 m under *S. polaris*–moss vegetation (Lat. $78^{\circ}55'48''$ N, Lon. $11^{\circ}51'46''$ E; Fig. 1) (Table 1). The vegetation in this area was composed mostly of a thin covering of *S. polaris*–moss, mosses, or soil crust. A quadrat of 10×10 cm was set at each point on August 3, 2009. The soil crust or the

Table 1 Characteristics of the sampling points

Point	Overall condition	Thickness over mineral soil (cm)	
		Moss layer	Organic layer
<i>Salix polaris</i> (polar willow)–moss vegetation			
C01–03	Bare soil with surface crust	0–0.9	0–0.7
C04	Fully covered with moss	4.1	0
C05	Half <i>S. polaris</i> –moss and half crust	1.0	1.2
C06	Fully covered with moss	1.8	0
C07	Fully covered with <i>S. polaris</i> –moss	3.5	0
C08	Fully covered with <i>S. polaris</i> –moss	3.5	0.7
C09	Fully covered with <i>S. polaris</i> –moss	3.0	6.3
C10	Fully covered with <i>S. polaris</i> –moss	3.1	2.3
C11	Fully covered with moss	2.3	1.3
C12	Fully covered with moss; relatively dry	2.0	0.8
Reference plot of bare soil			
REF	Bare soil with surface crust	0	0

plants and the organic (humus) layer (if any) inside the quadrat were removed after we had measured their thicknesses at the four corners of the quadrat. The mineral soil was then collected from a volume of $5 \times 5 \times 5$ cm (or until we reached the bedrock, in cases where the depth was less than 5 cm). The collected soil was immediately mixed well, passed through a 2-mm sieve, and then vacuum freeze-dried for analysis of soil properties. Fresh mineral soil was also collected from each quadrat on August 9, 2009, and brought back to the laboratory in cold storage at about 4 °C to measure AOP. Soil samples collected at the three points covered by only soil crust (C01 to C03; Table 1) were mixed in equal weight to make a composite sample to reduce the costs of analysis. The fresh soil samples collected from three points under well-grown *S. polaris*–moss or mosses (C06, C07, and C08; Table 1) were stored at –80 °C until analysis for ammonia oxidizers.

A plot with a bare soil surface in patchy vegetation located on the same hill (Lat. 78°55'41"N, Lon. 11°51'25"E; Fig. 1) was selected as a reference plot (REF; Table 1). We expected that the mineral soil at the reference plot would have the characteristics of soil less influenced by plants on a long-term basis. On July 22, 2009, a plot of surface crust was chosen and the upper 2–3 cm, including the surface crust, was removed. Subsequently, the mineral soil down to a depth of 15 cm from the surface was collected from three points, mixed well, and passed through a 2-mm sieve. It was then vacuum freeze-dried for analysis of soil properties as a composite sample. Fresh composite soil for ammonia oxidizer analysis was also collected from the same plot on August 9, 2009, and processed similarly to the mineral soil under the *S. polaris*–moss vegetation.

Measurement of soil properties

Freeze-dried soil samples were analyzed to determine the soil pH (1:2.5 H₂O), total C (TC), total organic C (TOC), total N (TN), and exchangeable ammonium N (NH₄-N) and nitrate N (NO₃-N) ($n = 3$ replications per sample). Before the analysis of TOC, carbonate in the samples was gasified and removed as carbon dioxide by adding phosphoric acid (Robertson et al. 1999). Note that this method cannot remove elemental carbon (e.g., coal particles). TC, TOC, and TN were quantified with an NC analyzer (Sumigraph NC-22F, Sumika Chemical Analysis Service, Tokyo, Japan) in solid samples. Exchangeable NH₄-N and NO₃-N were quantified with a flow injection analyzer (AOLA-1000, Aqualab, Tokyo, Japan) after extraction with a solution of 10 % potassium chloride (w/v).

Measurement of AOP

AOP was determined as the nitrite production rate by incubation with the use of sufficient substrates ($n = 3$). This method originated from the work of Belser and Mays (1980) and is common practice for evaluating the activity of ammonia monooxygenase (AMO) (e.g., Öquist et al. 2004; Koper et al. 2010; Prosser and Nicol 2012). AOP was measured on August 18, 2009, i.e., 9 days after the sampling. The incubation solution contained 1 mM ammonium sulfate (2 mM ammonium), 10 mM sodium chlorate, and 1 mM monopotassium phosphate buffer (pH 7). Chlorate inhibits nitrite oxidation to nitrate (Belser and Mays 1980); therefore, AOP approximates the potential gross ammonia oxidation rate. Fresh soil with a weight of 2.5 g (2 mm sieved) was added to a 50-mL centrifugation tube, and 10 mL of the incubation solution was added. The tube was

shaken well in a tube shaker to create a suspension. Then 1 mL of the suspension was immediately added to a 1.5-mL tube, which was centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was added to another 1.5-mL tube and stored at 4 °C as the sample at zero time. After sampling, the incubation tube was shaken (150 rpm) at 10 °C for 8 h (soils from points C01–C12) or 24 h (soil from point REF). After the shaking incubation, a second supernatant was obtained. The nitrite concentration in the supernatant was determined by using a colorimetric method, and the nitrite production rate per unit weight of dry soil ($\text{ng N g}^{-1} \text{ ds h}^{-1}$) was then calculated as the AOP by using the measured soil water content (w/w) of the fresh soil.

DNA extraction from soil

Soil total DNA was extracted by using a FastDNA spin kit for soil (Qbiogene/MP Biomedicals, Solon, OH, USA) ($n = 3$) without skim milk, as described previously (Morimoto et al. 2008). DNA was finally eluted with 80 μL of the DNase/pyrogen-free water (DES) included in the kit. The extracted soil DNA was then purified by using a DNA Clean and Concentrator-25 kit (Zymo Research Corp., Orange, CA, USA). The purified DNA was eluted with 80 μL of DES and stored at -20 °C until analysis.

Quantification of AOB- and AOA-*amoA* genes

AOB- and AOA-*amoA* gene copy numbers were quantified by using a SYBR Green I-based real-time PCR technique and the primer sets *amoA*-1F (5'-GGG GTT TCT ACT GGT GGT-3') (Rotthauwe et al. 1997)/*amoA*-2IR (5'-CCC CTC IGI AAA GCC TTC TTC-3') (Avrahami et al. 2003) (for AOB-*amoA*) and AOA *amoA*19IF (5'-ATG GTC TGG CTI AGA CG-3') (Morimoto et al. 2011)/CrenamoA616r (5'-GCC ATC CAT CTG TAT GTC CA-3') (Tourna et al. 2008) (for AOA-*amoA*). Real-time PCR was performed on a StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). Quantitative PCR for AOB-*amoA* was performed under the same conditions as described previously (Shimomura et al. 2012). PCR for AOA-*amoA* was performed in 20- μL reaction mixtures containing 1 \times SYBR Premix Ex Taq (Takara, Kyoto, Japan), 1 μM of each primer, 0.2 mg mL^{-1} of bovine serum albumin (BSA), 0.4 μL of ROX Reference Dye (Takara), and about 10 ng of soil DNA (1 μL). Thermal cycling conditions for AOA-*amoA* were 10 min at 95 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, and a final elongation step for 10 min at 72 °C (Levičnik-Höfferle et al. 2012). To create a standard curve for AOA-*amoA* quantification, tenfold serial dilutions of linearized pGEM-T Easy vector (Promega, Madison, WI, USA) including a soil AOA-*amoA* gene fragment

(AB713533) were prepared. The standard curve was generated by plotting the threshold cycle for each standard calculated by StepOne software, ver. 2.1 (Applied Biosystems). The determination coefficients and amplification efficiencies of the standard curves were more than 0.99 and 90 %, respectively.

Pyrosequencing analysis of AOB- and AOA-*amoA*

We used a pyrosequencing technique to analyze AOA- and AOB-*amoA* gene diversity. Fusion primers that included adapter and key sequences for the GS Junior Titanium emPCR Kit (Lib-L) (Roche Diagnostics, Branford, CT, USA), followed by multiplex identifiers (MIDs) and then the *amoA*-specific sequences, were synthesized and purified in a reverse-phase column (Greiner Bio-One, Frickhausen, Germany). The *amoA*-specific sequences were the same as *amoA*-1F and *amoA*-2R-GG (5'-CCC CTC GGG AAA GCC TTC TTC-3') (Nicolaisen and Ramsing 2002) in the case of AOB-*amoA* and the same as AOA *amoA*19IF and CrenamoA616r in the case of AOA-*amoA*. Amplification was performed in 50- μL reaction mixtures consisting of 1 \times Premix Ex Taq (Takara), 0.4 μM of each primer, 0.2 mg mL^{-1} of BSA, and about 40 ng of DNA extract (1 μL). PCR cycling conditions for AOB-*amoA* were 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 1 min at 72 °C. The conditions for AOA-*amoA* were 10 min at 94 °C, followed by ten cycles of 30 s at 94 °C, 30 s at 54 °C, and 1 min at 72 °C, followed by 20 cycles of 30 s at 92 °C, 30 s at 54 °C, and 1 min at 72 °C, and a final elongation step for 10 min at 72 °C. The amplicons were purified with a QIAquick PCR Purification Kit (Qiagen, Venlo, the Netherlands). Purified samples were loaded onto 1.5 % agarose gel and electrophoresed at 50 V for 1.5 h at 25 °C. The target DNA fragments were excised from the gel and purified with a QIAquick Gel Extraction Kit (Qiagen). The purity of DNA samples was assessed with a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) by using a High-Sensitivity DNA Kit (Agilent). DNA concentrations were determined with a Quant-iT PicoGreen dsDNA Assay Kit (Molecular Probes, Eugene, OR, USA). Molecular concentrations were calculated according to the 454 Life Sciences protocol (Roche Diagnostics). Emulsion PCR and pyrosequencing of the libraries were done according to the 454 Life Sciences protocol (Roche Diagnostics). Sequencing was performed on a 454 GS Junior sequencer (Roche Diagnostics).

Sequence analysis

To construct reference databases for AOB- and AOA-*amoA*, all publicly available *amoA* sequences longer than

450 bp (AOB-*amoA*) and 598 bp (AOA-*amoA*) were downloaded from the Web site of the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>). Unique sequences were found from among the downloaded sequences by using Mothur software (Schloss et al. 2009). Sequences including gaps and insertions were deleted from the database.

Raw 454 sequences were sorted by using MIDs, screened in quality (average quality score ≥ 25), and the priming sites trimmed by using Mothur software to obtain sequences of ≥ 449 bp for AOB-*amoA* and 425 bp for AOA-*amoA*. Unique sequences were found from among the trimmed sequences by using Mothur software. The unique sequences were checked with Mothur software by using the UCHIME algorithm (Edgar et al. 2011) for potentially chimeric sequences by using the constructed databases as references. Suspected chimeric sequences were subjected to database homology searches on NCBI. Non-chimeric sequences were aligned with the dppartree algorithm in Mafft software (Katoh and Toh 2007). Calculation of pairwise distances between the aligned sequences, and clustering of the sequences into operational taxonomic units (OTUs) by using a furthest neighbor algorithm (cutoff values: 0.05 for AOB and 0.07 for AOA) were performed with Mothur. The most common sequences in each OTU were selected as sequences representative of the OTUs. The reason for adopting cutoff values that differed between AOB and AOA was the difference in their diversity—i.e., there was diversity within one genus in the case of AOB but within and among a variety of genera in the case of AOA.

Phylogenetic assignment

The AOA- and AOB-*amoA* sequences obtained were analyzed with MEGA6 software (Tamura et al. 2013). Reference sequences of *amoA* genes were obtained from NCBI. The sequences were aligned by using the ClustalW program (Chenna et al. 2003). Each of the AOA- and AOB-*amoA* phylogenetic trees was constructed by using the maximum likelihood method with the Jukes–Cantor model and assessed by using 1000 bootstrap replicates.

Data deposition

The sequences described in this study have been deposited in the DNA Data Bank of Japan. The accession number is DRA003027.

Statistical analysis

Statistical analyses of measured data—i.e., key statistics, correlations, ANOVA, and multiple regressions—were

conducted with SAS Add-In for Microsoft Office (SAS Institute Inc., Cary, NC, USA). The Bray–Curtis dissimilarity (Bray and Curtis 1957) was applied to evaluate the dissimilarity of AOB or AOA compositions among the sampling points. This index originally quantifies the dissimilarity of flora or fauna of higher organisms among sites by using identified species and their populations. The index value ranges from 0 to 1: a larger value denotes a larger dissimilarity. We used the identified OTU as “species” and the read number of OTU as “population.” Note that comparison of dissimilarity by combining AOB and AOA was impossible because of the different OTU cutoff values—i.e., the different species boundaries—between AOB and AOA.

Results

Soil properties and AOP

Table 2 shows the soil properties and AOP at each sampling point. Soil pH in the vegetation plot was weakly alkaline, reflecting the carbonate content of the parent material (Mann et al. 1986); the carbonate content (as the difference between TC and TOC) ranged from 9.8 to 21.0 mg C g⁻¹ ds. TOC ranged from 37.9 to 66.5 mg C g⁻¹ ds and TN from 2.1 to 4.6 mg N g⁻¹ ds. TOC/TN ranged from 12.1 to 18.4. NH₄-N content was on the order of one-thousandth of TN, and the NO₃-N content was one order lower than the NH₄-N content. The soil pH of the reference plot was similar to that of the vegetation plot; however, the TOC, TN, and NH₄-N contents were significantly lower than those of the vegetation plot ($P < 0.05$).

In the *S. polaris*-moss vegetation, the mineral soil AOP at points covered by plants (from C04 to C12) ranged from 2.9 ± 1.4 to 14.1 ± 1.8 (mean \pm standard deviation) ng N g⁻¹ ds h⁻¹. AOP at the points in the vegetation plot covered by only soil crust (C01 to C03) was 2.3 ± 0.7 ng N g⁻¹ ds h⁻¹ and that at the reference plot (REF) was 1.1 ± 0.1 ng N g⁻¹ ds h⁻¹—significantly lower than AOP at the points covered by plants ($P = 0.05$) except in the case of points C05, C09, and C12 (Table 2).

The following variables were selected to assess correlations with AOP: Moss, the thickness of the moss layer; Org, the thickness of the organic layer; SWC, the soil water content; pH, the soil pH; and the contents of TOC, total organic carbon; TN, total nitrogen; NH₄-N, ammonium nitrogen; and NO₃-N, nitrate nitrogen. Moss and Org (i.e., variables easily measured in the field) were candidate variables for a simple index of AOP. The correlations among the variables are shown in Table 3. AOP was positively correlated with Moss ($P < 0.01$) and with SWC and

Table 2 AOP and properties of mineral soils

Point	AOP (ng N g ⁻¹ ds h ⁻¹)	Soil water content (% w/w)	pH (H ₂ O)	TC (mg C g ⁻¹ ds)	TOC (mg C g ⁻¹ ds)	TN (mg N g ⁻¹ ds)	TOC/TN ratio	NH ₄ -N	NO ₃ -N
<i>Salix polaris</i> –moss vegetation									
C01–03	2.3 (0.7) f	18.3 (1.5)	7.7	48.8 (0.7)	39.0 (0.4)	2.12 (0.03)	18.4 (0.5)	2.8 (0.1)	0.26 (0.02)
C04	7.8 (1.3) cde	28.7 (2.7)	7.7	62.9 (1.0)	47.8 (2.3)	3.35 (0.08)	14.3 (0.4)	4.5 (0.5)	0.68 (0.05)
C05	3.8 (2.8) ef	21.8 (2.8)	7.3	75.2 (2.4)	54.2 (2.8)	4.48 (0.15)	12.1 (1.0)	5.7 (0.1)	0.17 (0.02)
C06	9.2 (0.8) bcd	26.0 (2.4)	7.8	61.1 (2.0)	45.5 (1.9)	3.74 (0.10)	12.2 (0.5)	7.0 (0.5)	0.35 (0.01)
C07	13.8 (2.7) ab	27.8 (1.4)	7.9	48.5 (0.3)	37.9 (0.3)	2.62 (0.04)	14.5 (0.1)	4.2 (0.2)	0.14 (0.00)
C08	14.1 (1.8) a	32.7 (0.4)	7.6	81.2 (1.1)	66.5 (3.7)	4.60 (0.05)	14.5 (0.9)	7.7 (0.3)	0.62 (0.00)
C09	4.5 (1.8) def	31.9 (0.6)	7.8	54.5 (1.2)	44.0 (1.5)	3.40 (0.09)	12.9 (0.1)	3.9 (0.0)	0.18 (0.00)
C10	9.8 (1.8) abc	39.7 (1.0)	7.6	70.2 (1.4)	54.8 (3.2)	4.22 (0.08)	13.0 (1.0)	9.2 (0.7)	0.24 (0.02)
C11	8.5 (0.4) cde	32.7 (2.6)	7.8	59.8 (1.1)	45.0 (2.7)	3.71 (0.11)	12.1 (1.1)	6.8 (0.1)	0.63 (0.10)
C12	2.9 (1.4) f	19.6 (2.2)	7.5	68.9 (2.3)	54.1 (3.1)	4.01 (0.11)	13.5 (0.6)	4.7 (0.2)	0.51 (0.00)
Reference plot of bare soil									
REF	1.1 (0.1) f	15.4 (0.7)	7.6	17.9 (0.5)	14.8 (0.5)	0.92 (0.02)	16.1 (0.2)	0.52 (0.02)	0.08 (0.01)

Values in parentheses denote standard deviations ($n = 3$)

AOPs with the same letter (a–f) are not significantly different according to Tukey's multiple comparison test ($P = 0.05$)

AOP ammonia oxidation potential, ds dry soil, TC total carbon, TOC total organic carbon, TN total nitrogen, NH₄-N ammonium nitrogen, NO₃-N, nitrate nitrogen

Table 3 Correlation coefficients among variables in mineral soils

	AOP	Moss	Org	SWC	pH	TOC	TN	NH ₄ -N
Moss	0.749**							
Org	-0.147	0.214						
SWC	0.701*	0.773**	0.433					
pH	0.425	0.375	0.093	0.313				
TOC	0.467	0.542	0.154	0.546	-0.305			
TN	0.427	0.496	0.231	0.583	-0.326	0.946**		
NH ₄ -N	0.642*	0.509	0.098	0.779**	-0.068	0.800**	0.857**	
NO ₃ -N	0.326	0.486	-0.222	0.305	0.055	0.545	0.473	0.409

AOP ammonia oxidation potential, Moss moss layer thickness, Org organic layer thickness, SWC soil water content, pH soil pH, TOC total organic carbon, TN total nitrogen, NH₄-N ammonium nitrogen, NO₃-N nitrate nitrogen

* $P < 0.05$; ** $P < 0.01$

NH₄-N ($P < 0.05$). Significant correlations among the explanatory variables were found in the following combinations: (1) Moss and SWC; (2) SWC and NH₄-N; (3) TOC and TN; (4) TOC and NH₄-N; and (5) TN and NH₄-N (Table 3; all $P < 0.01$).

We then conducted a multiple regression analysis between AOP and the candidate explanatory variables. Moss and Org were chosen as the explanatory variables for the simple index model. As the candidate explanatory variables for the soil property model, NH₄-N as an indicator of substrate availability for ammonia oxidation and SWC and pH as indicators of environmental conditions affecting ammonia

oxidation were selected. The results of multiple regressions with forced entry are shown in Table 4 and Fig. 3. F -tests indicated that the simple index model was significant ($P = 0.014$), but the soil property model was not ($P = 0.057$). The normalized regression coefficients for the simple index model indicated that the effect of Moss on AOP was positive and that of Org on AOP was negative (Table 4). In contrast, all three variables in the soil property model had positive effects on AOP (Table 4). As a consequence of the multiple regressions, we can roughly estimate the AOP in mineral soil under *S. polaris*–moss vegetation by measuring the thicknesses of only the moss and the organic layers.

Table 4 Results of multiple linear regressions for AOP

	Regression 1: Simple index			Regression 2: Soil property					
	Intercept	Regression coefficient		Adjusted R^2	Intercept	Regression coefficient		Adjusted R^2	
		Moss	Org			SWC	pH		NH_4-N
Estimation	1.810	2.744	-0.783	0.574*	-88.10	0.067	11.45	1.082	0.480
Normalization	0	0.817	-0.321		0	0.109	0.430	0.586	

AOP ammonia oxidation potential, Moss moss layer thickness, Org organic layer thickness, SWC soil water content, pH soil pH, NH_4-N ammonium nitrogen

* $P < 0.05$

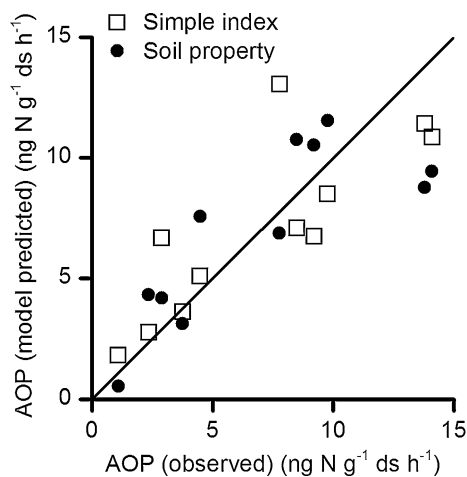


Fig. 3 Fitness of multiple regression models for ammonia oxidation potential (AOP). The simple index model is a function of moss layer thickness and organic layer thickness. The soil property model is a function of soil water content, soil pH, and ammonium nitrogen content. See Table 4 for the regression coefficients. ds dry soil

Characteristics of *amoA*

We determined the copy numbers of AOB- and AOA-*amoA* (Fig. 4). The copy numbers of AOB-*amoA* in the vegetation plot (points C06 to C08) ranged from 1.6 to $3.3 \times 10^5 \text{ g}^{-1} \text{ ds}$. That in the reference plot (point REF) was $6.4 \times 10^4 \text{ g}^{-1} \text{ ds}$ —one order smaller than in the vegetation plot. The copy numbers of AOA-*amoA* were similar regardless of the sampling point and ranged from 7.1 to $9.8 \times 10^6 \text{ g}^{-1} \text{ ds}$; this was nearly two orders larger than the copy numbers of AOB-*amoA* in the vegetation plot. Neither the copy number of AOB-*amoA* nor that of AOA-*amoA* was significantly correlated with AOP ($P = 0.05$), although these relationships were positive (Fig. 5). In addition, no significant correlations were found between the copy numbers of AOB- or AOA-*amoA* and the soil properties (data not shown).

The mean sequencing read of AOB-*amoA* was 3305 ± 1058 (standard deviation), as obtained from 12 samples ($n = 3$ for each of four points, i.e., C06, C07, C08,

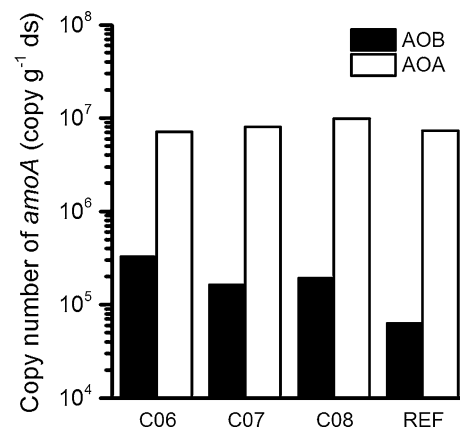


Fig. 4 Copy numbers of ammonia-oxidizing bacteria (AOB)-*amoA* and archaea (AOA)-*amoA*, as determined by quantitative PCR. ds dry soil

and REF; see Online Resource 1 for raw data). The sequencing reads were grouped into 24 OTUs at an OTU cutoff of 0.05. The cluster of AOB-*amoA* genes defined by Avrahami and Conrad (2003) and Zhang et al. (2009) was used for the phylogenetic analysis. All OTUs were affiliated exclusively with the *Nitrosospira* genus and were placed into clusters 1, 2, 3, 4, 9, and ME (Fig. 6a). The major OTUs from 1 to 6 (in total accounting for more than 93 % of the total number of reads) were grouped into cluster ME (found on Mount Everest; Zhang et al. 2009) with a high level of identity (>94.4 %) (Fig. 6a).

The mean sequencing read of AOA-*amoA* was $10,795 \pm 3546$ (standard deviation), as obtained from 12 samples (the same ones as used for AOB; see Online Resource 1 for raw data). The sequencing reads were grouped into 13 OTUs at an OTU cutoff of 0.07. The clusters and clades of AOA-*amoA* genes defined by Pester et al. (2012) and Alves et al. (2013) were used for the phylogenetic analysis. All OTUs were classified into the *Nitrososphaera* cluster and were distributed into clades A, B, and *Nitrososphaera* (Fig. 6b). The major OTUs from 1 to 5 (in total accounting for more than 95 % of the total number of reads) were grouped into clade A. At our study

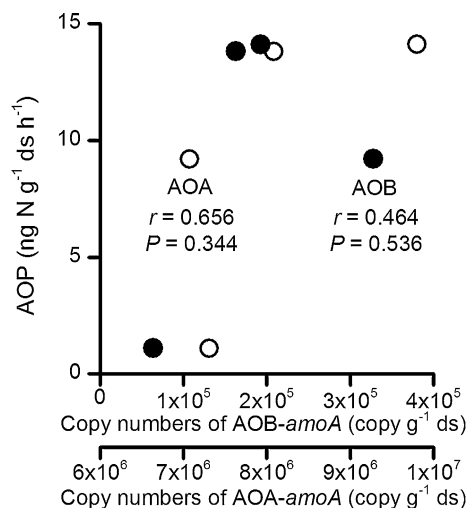


Fig. 5 Relationship between ammonia oxidation potential (AOP) and copy numbers of ammonia-oxidizing bacteria (AOB)-*amoA* and archaea (AOA)-*amoA*. *ds* dry soil

site, a number of the same sequences of AOA in the high Arctic at Svalbard that had been determined by Alves et al. (2013) were identified (Fig. 6b). However, although Alves et al. (2013) also found a *Nitrosopumilus*-like cluster of AOA, mainly in wet moss tundra soil in Svalbard, a similar AOA was not detected in our samples.

We examined the averaged relative abundances of AOB- and AOA-*amoA* OTUs (Fig. 7); OTUs with maximum abundances of less than 2 % were gathered as “others.” The following were the main features of the AOB-*amoA* composition: OTU1 was common in both the vegetation plot and the reference plot; OTUs 2, 3, 5, and 6 were dominant only in the vegetation plot; and OTU4 was dominant only in the reference plot. The Bray–Curtis dissimilarity of AOB ranged from 0.23 to 0.38 within the vegetation plot (C06, C07, and C08) and from 0.72 to 0.75 between the vegetation plot and the reference plot (C06, C07, or C08 versus REF). This result means that AOB diversity largely differed between the vegetation plot and the reference plot, even though all of the identified OTUs were classified into the same genus. For AOA-*amoA*, the sum of the relative abundances from OTU1 to 6 accounted for more than 96 % at every point (Fig. 7). The Bray–Curtis dissimilarity ranged from 0.18 to 0.40 among all points, and there was no marked difference in AOA composition between the vegetation plot and the reference plot.

Discussion

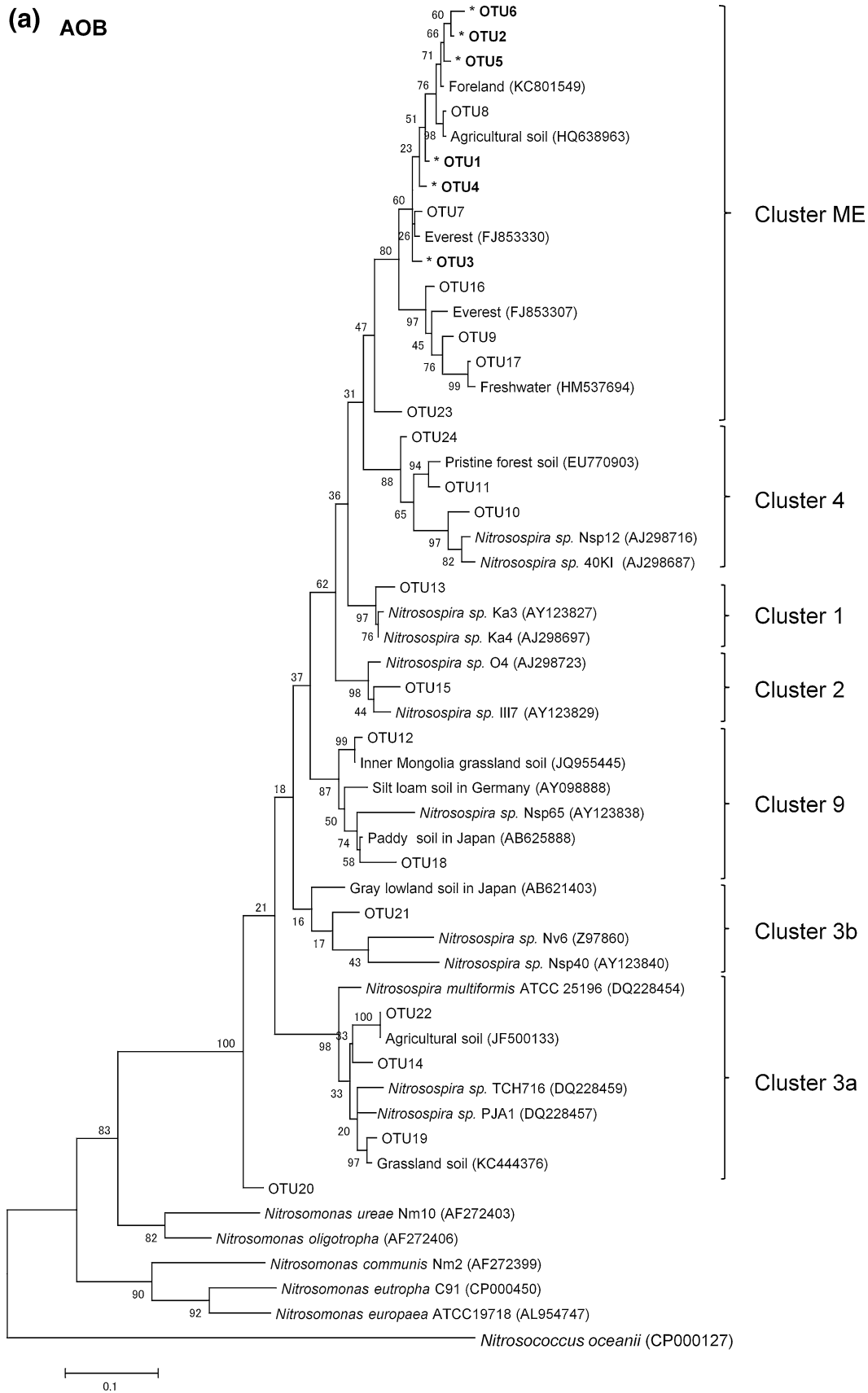
Comparison of AOP with results from early studies

Nitrification rates are subject to various conditions, such as substrate availability, temperature, pH, soil moisture,

Fig. 6 a Phylogenetic tree of ammonia-oxidizing bacteria (AOB)-*amoA*. The Jukes–Cantor model and the maximum likelihood method are used to obtain the phylogenetic tree. Bootstrap values based on 1000 replicates are shown at the nodes. Operational taxonomic units (OTUs) with asterisks denote the most abundant OTUs according to read number of each OTU (Table S1 in Online Resource 1). **b** Phylogenetic tree of ammonia-oxidizing archaea (AOA)-*amoA*. The Jukes–Cantor model and the maximum likelihood method are used to obtain the phylogenetic tree. Bootstrap values based on 1000 replicates are shown at the nodes. Operational taxonomic units (OTUs) with asterisks denote the most abundant OTUs according to read number of each OTU (Table S2 in Online Resource 1). Sequences with underlines are identified by Alves et al. (2013). Note that our phylogenetic tree has a topology different from that of Alves et al. (2013) owing to differences in the lengths of the sequences. The identity with known sequences is 100 % for OTUs 1 to 8, 11, and 12, and 99.8 % for OTUs 10 and 13

aerobic status, and, perhaps, functionally heterogeneous nitrifiers and their populations (Klotz and Stein 2011). Various approaches are available for measuring nitrification rates. It is possible to measure the in situ rate without substrate or the potential rate with the addition of sufficient substrate; the gross rate or net rate; and the ammonia oxidation (nitrite production) rate (e.g., AOP with inhibition of nitrite oxidation) or the nitrate production rate (e.g., by the ^{15}N dilution technique). Our study measured AOP, i.e., the potential nitrite production rate with sufficient substrate at constant pH and temperature. We should recognize that these differences in methods make it difficult to compare nitrification or ammonia oxidation rates among relevant studies. Even so, it should still be possible to discuss the degrees of apparent difference in AOP or nitrification rates among studies.

Before any such discussion, we need to explain our methodological basis. Our method of measuring AOP basically followed that of Belser and Mays (1980). The substrate of AMO is ammonia (not ammonium). There is pH-dependent dissociation–association equilibrium between ammonium and ammonia in which the ammonium concentration should be high enough to obtain a sufficient concentration of ammonia in the solution when the pH is low. Our conditions (i.e., the use of 2 mM ammonium) provided about 7 μM ammonia at the given temperature (10 °C) and pH (7) and were sufficient to measure AOPs. A too high ammonium concentration inhibits the activity of ammonia oxidizers that favor low ammonium concentrations (e.g., clade D of AOA at Svalbard; Alves et al. 2013). In addition, a low substrate concentration seems desirable for high-Arctic soils, because high rates of ammonium input by atmospheric deposition or fertilization, or both, are hardly expected there. Our incubation temperature of 10 °C was close to the daily maximum temperature at the study site in summer. Although some early studies adopted a high incubation temperature, such as 20 °C, we consider



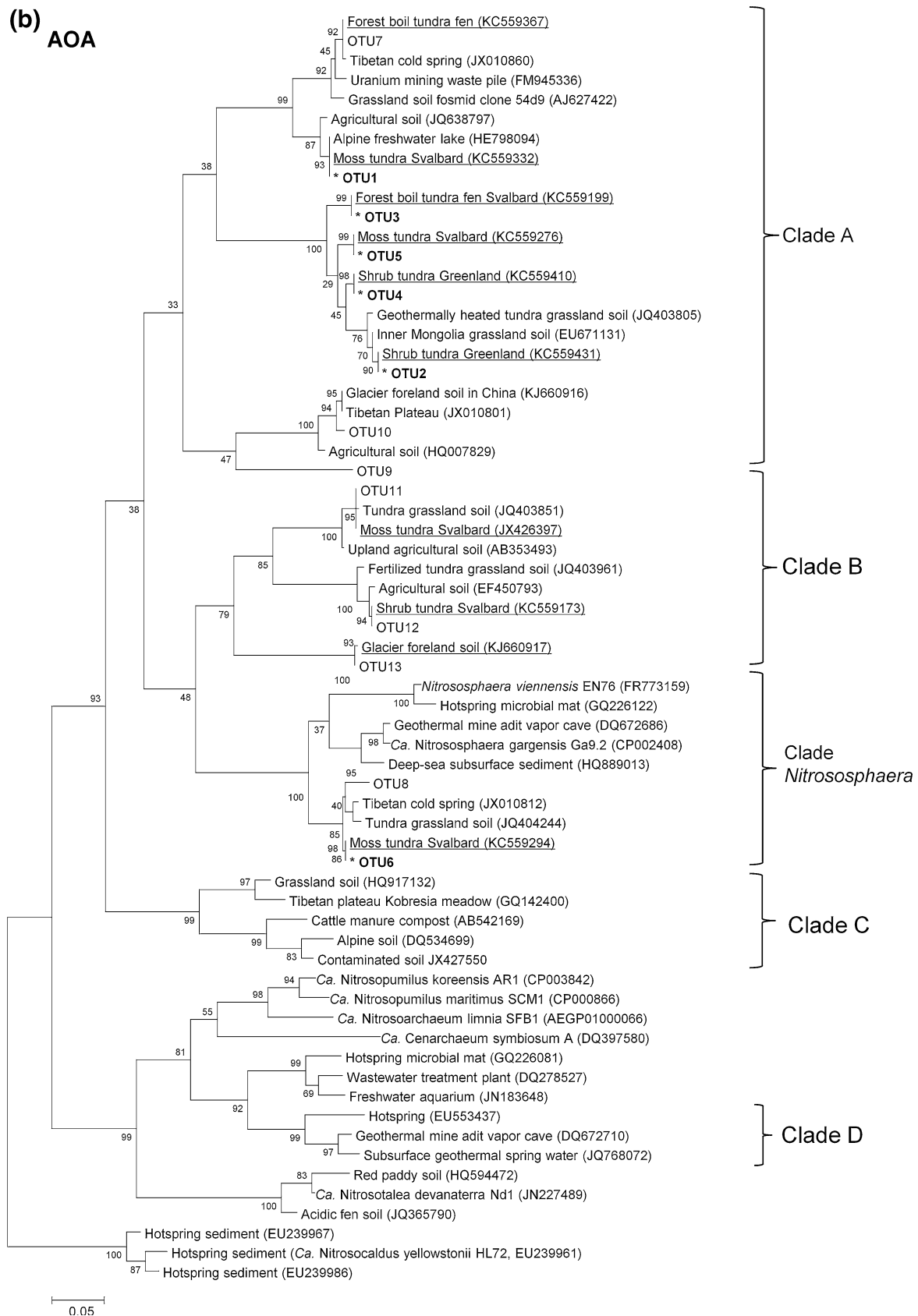


Fig. 6 continued

this too high in comparison with actual conditions in the high Arctic (at least at present).

The mineral soil AOP in the vegetation plot at our study site ranged from 2.3 to 14.1 ng N g⁻¹ ds h⁻¹ (Table 2). We compared these values with other reported values of AOPs or nitrification rates in Arctic mineral soils. Alves et al. (2013), by adding 1.7 to 2.5 mM ammonium at 15 °C, found potential gross nitrification rates ranging from about 10 to 200 ng N g⁻¹ ds h⁻¹; their rate on moss tundra on a dry mound (i.e., under vegetation similar to that at our study site) was about 20 ng N g⁻¹ ds h⁻¹. We can say roughly that the ammonia oxidation potentials of moss tundra mineral soils in the two studies were similar to each other, taking the measurement conditions into consideration (i.e., similar substrate concentrations but a higher incubation temperature in the study by Alves et al. than in our study). Banerjee and Siciliano (2012), after adding 8 mM ammonium at 20 °C, found higher AOPs than ours on Canadian Arctic tundra (range 21 to 178 ng N g⁻¹ ds h⁻¹). The tenfold difference in AOPs is ascribable not only to their higher substrate concentration and incubation temperature but possibly also to differences in both the species structure and populations of ammonia oxidizers. Sanders et al. (2010), after adding 1.5 mM ammonium at unspecified in situ temperatures, reported that some of the mineral soils in polygons in northeastern Siberia did not show AOPs, whereas others had values of 30 and 100 ng N g⁻¹ ds h⁻¹. Wild et al. (2013) gave in situ gross nitrification rates at 7 °C of about 100–110 ng N g⁻¹ ds h⁻¹ on heath tundra in Greenland and tussock tundra in northeastern Siberia and about 230 ng N g⁻¹ ds h⁻¹ on shrub tundra in northeastern Siberia. Thus, in summary, potential ammonia oxidation rates are of the order of 10 ng N g⁻¹ ds h⁻¹ on moss tundra in polar semideserts and 100 ng N g⁻¹ ds h⁻¹ under vegetation with relatively rich biomass, such as heath, tussock, and shrub tundra.

Factors controlling AOP

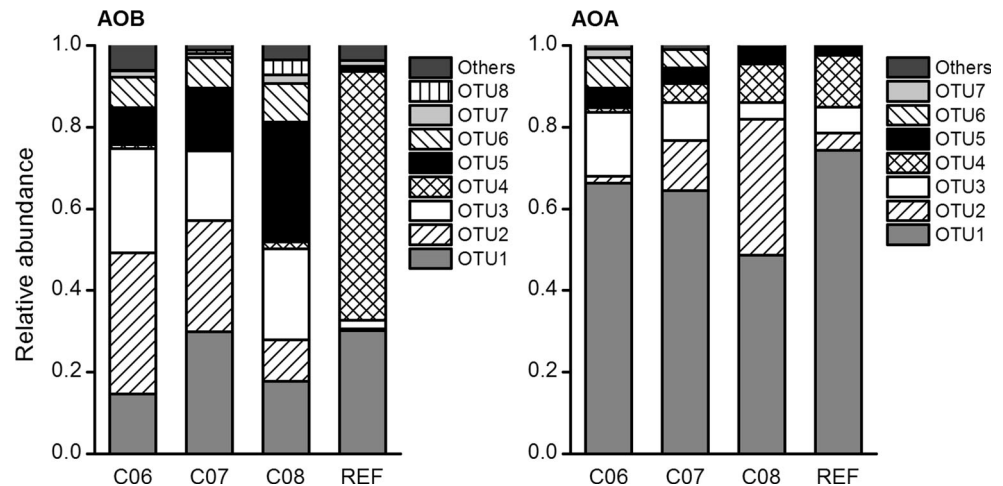
Possible factors controlling AOP are environmental factors such as substrate availability, soil pH, moisture, and temperature; and microbial factors such as population and interspecies differences in ammonia oxidation among AOB and AOA (e.g., optimum ammonium concentration and pH, and ammonia oxidation ability). Banerjee and Siciliano (2012) summarized that edaphic rather than genetic factors are important controllers of ammonia oxidation in Arctic ecosystems, whereas Alves et al. (2013) concluded that nitrification is possibly more directly dependent on nitrifier population structure and physiologic or metabolic properties than on abiotic factors. Our spatially limited data are insufficient to evaluate the conclusion of Banerjee and

Siciliano (2012). However, our data support the conclusion of Alves et al. (2013) via the following findings: no significant correlations between AOP and the copy number of AOB- or AOA-*amoA* (Fig. 5), and the identification of a number of species of the same AOA clade A in our study (Fig. 6b) as in the AOA of Alves et al. (2013). Clade A of AOA has low, or no, AOP (Alves et al. 2013); therefore, its high abundance reduces the correlation between AOP and the AOA population.

AOP is also affected by soil properties. For example, AOP is positively correlated with soil moisture content on the Canadian Arctic tundra, regardless of spatial scale (Banerjee and Siciliano 2012), and is strongly affected by pH (Nicol et al. 2008; Erguder et al. 2009). We collected our mineral soils at 12 points in a 10 × 10-m area of vegetation. This spatial scale corresponds to the medium scale (1–10 m) in the study by Banerjee and Siciliano (2012). According to their study, we expected that AOP would be correlated with soil moisture content, soil pH, TOC, and inorganic N content. In fact, our data showed that soil water content (SWC) and ammonium nitrogen content (NH₄-N), in addition to moss layer thickness (Moss), were significantly and positively correlated with AOP (Table 3). The positive effect of Moss is ascribable to the positive effect of soil moisture on AOP (Banerjee and Siciliano 2012) through the significant positive correlation between Moss and SWC (Table 3). The significantly positive correlation between SWC and NH₄-N (Table 3) also supports the positive effect of soil moisture on AOP. The positive effects of SWC, pH, and NH₄-N on AOP in the multiple regression (Table 4) affirmed current knowledge regarding tundra soils (e.g., Banerjee and Siciliano 2012). However, the multiple regression model based on these three soil properties was not statistically significant (Table 4). We can infer that this limited area with the same vegetation (i.e., *S. polaris*-moss vegetation) had small variations in these soil properties; this therefore resulted in weak contributions of these variables to the multiple regression model used to estimate AOP.

The apparent contradiction between the studies of Banerjee and Siciliano (2012) and Alves et al. (2013) regarding the importance of edaphic factors versus genetic factors in the control of AOP in high-Arctic soils is in line with our finding that the diversity of ammonia oxidation ability among ammonia oxidizers reduces the strength of the relationship between AOP and total *amoA* copy number in AOB or AOA. It is therefore desirable to extract ammonia oxidizers representing each species or clade and then quantify their ammonia oxidation ability and abundance. In assessing ammonia oxidation ability, we should elucidate quantitative relationships by controlling factors such as substrate, pH, and temperature conditions. These factors should be the subject of future study.

Fig. 7 Relative abundances of ammonia-oxidizing bacteria (AOB)-*amoA* and archaea (AOA)-*amoA* sequences. OTU operational taxonomic unit



Characteristics of the identified ammonia oxidizers

The AOB that we found in Ny-Ålesund, Svalbard, consisted exclusively of the genus *Nitrosospira* (Fig. 6a). In most cases, two genera, *Nitrosomonas* and *Nitrosospira*, are detected in various soil environments in the world, with dominance of *Nitrosospira* (Kowalchuk and Stephen 2001). *Nitrosomonas* clusters are found mainly in agricultural soils receiving large amounts of N fertilizer (Kowalchuk and Stephen 2001). Supply rates of $\text{NH}_4\text{-N}$ in Arctic soils are probably insufficient to feed the *Nitrosomonas* cluster. The genus *Nitrosospira* can be divided into clusters 0, 1, 2, 3a, 3b, 4, 9, 10, 11, and 12 on the basis of AOB-*amoA* sequences from a variety of environments (Avrahami and Conrad 2005). Molecular ecological experiments have revealed that *Nitrosospira* clusters 2, 3, and 4 are the dominant groups in soils worldwide (Kowalchuk and Stephen 2001). These three clusters were less abundant (<2 %) at our study site (Fig. 6a) than in other studies. Instead, the *Nitrosospira* cluster ME, which was identified and classified from cold alpine soils on Mount Everest (Zhang et al. 2009), accounted for more than 97 % of the total sequencing reads in our Arctic soil samples (Fig. 6a and Online Resource 1). Temperature strongly affects AOB composition, shifting the population composition within *Nitrosospira* clusters (Avrahami et al. 2003; Avrahami and Conrad 2005). Therefore, the *Nitrosospira* ME cluster perhaps adapts to low temperatures and might play an important role in ammonia oxidation in Arctic soils. Recently, a psychrotolerant *Nitrosospira* species was also isolated from Canadian lake sediment (Urakawa et al. 2015). Similar discoveries can be expected in future studies.

AOA can be categorized into five clusters: *Nitrosopumilus*, *Nitrososphaera*, *Nitrososphaera* sister cluster,

Nitrosotalea, and *Nitrosocaldus*; the first four clusters have been detected in various environments, including soils (Pester et al. 2012). In many cases, the *Nitrososphaera* cluster is dominant in soils worldwide (Pester et al. 2012; Jiang et al. 2014). The AOA at our study site consisted exclusively of the *Nitrososphaera* cluster. The identified *Nitrososphaera* OTUs could be grouped into three clades on the basis of the classification proposed by Alves et al. (2013) (Fig. 6b). They found that the ammonia oxidation activity of clade A of AOA was lower than those of clades B and C and of AOB. Examination of our soil samples showed that clade A, which included OTUs 1–5, 7, 9, and 10 (Fig. 6b), accounted for more than 96 % of the total sequence reads of AOA-*amoA*. The findings suggest that these sequences within clade A of AOA found in Svalbard have adapted well to cold climates.

We examined differences in ammonia oxidizers between the vegetation plot and the reference plot. Key differences between the two plots were that the reference plot had less substrate availability (e.g., see $\text{NH}_4\text{-N}$ in Table 2). For AOB, the *amoA* copy numbers in the vegetation plot were one order larger than that in the reference plot (Fig. 4), and the pattern of OTU dominance differed between the vegetation plot and the reference plot (Fig. 7). These findings suggest that the AOB population is generally susceptible to changes in substrate availability and that AOB are diverse with respect to their optimum substrate conditions: we found a cosmopolitan unit (OTU1), as well as several that predominated in the vegetation plot (OTUs 2, 3, 5, 6) and one that predominated in the reference plot (OTU4) (Figs. 6a, 7). Therefore, the dominant AOB species might vary in response to changes in environmental conditions. In the case of AOA, both *amoA* copy numbers and *amoA* diversity differed little between the vegetation plot and the reference plot

(Figs. 4, 7). This implied that AOA were generally ubiquitous and less affected than AOB by environmental conditions. The latter implication might be attributable to the fact that AOA—or microbes extracted as AOA—do not depend on ammonia oxidation alone (Mußmann et al. 2011; Alves et al. 2013). In addition, Alves et al. (2013) found the *Nitrosopumilus* cluster under vegetation similar to ours in Svalbard; our use of different primers might have been the reason behind the absence of the *Nitrosopumilus* cluster from our results.

Are AOB or AOA the main contributors to ammonia oxidation in high-Arctic soils? AOP values in the Canadian Arctic tundra are occasionally positively correlated with both AOB- and AOA-*amoA* copy numbers (Banerjee and Siciliano 2012), whereas neither AOB nor AOA was correlated with AOP in our study (Fig. 5). Alves et al. (2013) found that AOA was the major ammonia oxidizer in high-Arctic tundra (mainly in Svalbard): AOA were detected at almost all of their study sites (10 of 11), but AOB were found at only half of their sites (5 of 11). Two of their sites—Lon-mt2 and Lon-mt3 in Longyearbyen, Svalbard—had vegetation similar to that at our study site, i.e., moss tundra over mineral soil on a dry mound. The ratios of the copy numbers of AOA to those of AOB at their above-mentioned two sites ranged from 5 to 400. By contrast, the AOA to AOB copy number ratios at our study site ranged from 22 to 51 for the vegetation plot and 115 for the reference plot. The AOA to AOB copy number ratios at our study site were within the range of those of Alves et al. (2013), but we detected AOB at all of our sampling points. The AOB identified in our study were mainly psychrotolerant species (Fig. 6a). The AOA we identified were mainly from clade A (Fig. 6b), with low, or no, ammonia oxidation ability (Alves et al. 2013), even though they, and not AOB, accounted for the majority of the population (Fig. 4). It is, therefore, possible that the AOPs measured at our study site, i.e., in mineral soils under *S. polaris*-moss vegetation in Ny-Ålesund, Svalbard, were driven by mainly AOB.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies performed by the authors using human participants or animals.

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