Large cryoconite aggregates on a Svalbard glacier support a diverse microbial community including ammonia-oxidizing archaea

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Abstract

The aggregation of surface debris particles on melting glaciers into larger units (cryoconite) provides microenvironments for various microorganisms and metabolic processes. Here we investigate the microbial community on the surface of Aldegondabreen, a valley glacier in Svalbard which is supplied with carbon and nutrients from different sources across its surface, including colonies of seabirds. We used a combination of geochemical analysis (of surface debris, ice and meltwater), quantitative polymerase chain reactions (targeting the 16S ribosomal ribonucleic acid and amoA genes), pyrosequencing and multivariate statistical analysis to suggest possible factors driving the ecology of prokaryotic microbes on the surface of Aldegondabreen and their potential role in nitrogen cycling. The combination of high nutrient input with subsidy from the bird colonies, supraglacial meltwater flow and the presence of fine, clay-like particles supports the formation of centimetre-scale cryoconite aggregates in some areas of the glacier surface. We show that a diverse microbial community is present, dominated by the cyanobacteria, Proteobacteria, Bacteroidetes, and Actinobacteria, that are well-known in supraglacial environments. Importantly, ammonia-oxidizing archaea were detected in the aggregates for the first time on an Arctic glacier.

Keywords: glacier, cryoconite, microbial diversity, nitrogen, ammonia oxidation, Svalbard

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1. Introduction

Glaciers and ice sheets in the Arctic, including Svalbard, are melting at an accelerating rate (Kohler et al 2007, Pritchard et al 2009, Moholdt et al 2010, Nuth et al 2010, Gardner et al 2011), and receive considerable amounts of carbon (Slater et al 2002, Hansen and Nazarenko 2003, McConnell et al 2007) and nutrients (Hodson et al 2009, Samyn et al 2012, Björkman et al 2013) of various origin from the atmosphere and/or local sources. The liquid water produced during melting makes glacier surfaces habitable, and the debris and aerosols deposited on glaciers can serve
as a source of nutrients and viable microbial cells (Hodson et al 2008, Stibal et al 2012a). As a result, microorganisms grow and reproduce in the glacier surface debris, called ‘cryoconite’. Active microbes associated with cryoconite produce cohesive compounds, called extracellular polymeric substances, which cause the aggregation of cryoconite on the ice surface (Takeuchi et al 2001, Hodson et al 2010, Langford et al 2010). Larger aggregates may be expected to have a longer residence time on the ice and thus enhance surface melting by their prolonged reduction of surface albedo (Boggild et al 2010, Irvine-Fynn et al 2011). The large aggregates may also provide more microenvironments for a wider range of microorganisms and metabolic processes than smaller aggregates, since diffusion of oxygenated surface waters through them is less influential on the redox conditions found there (Hodson et al 2010, Langford et al 2010). These factors then enable cryoconite to contribute to ecological succession in the glacier forefield following the continued retreat of the glacier (Kaštovská et al 2007, Edwards et al 2013).

Most Arctic glaciers receive a substantial proportion of nitrogen by episodic atmospheric nitrogen enrichment from low-latitude sources (Kühn et al 2012), and extreme nitrogen deposition events may far exceed inputs from snow accumulation and melt following the long winter accumulation period (Hodson et al 2009). Atmospheric deposition of nitrate and ammonium on Svalbard valley glaciers has increased 65% and 20% respectively since pre-industrial times, owing to anthropogenic pollution (Kekonen et al 2005). On the other hand, seabirds breeding on land may represent another source of nutrients for Arctic terrestrial ecosystems, including glaciers, due to guano deposition in the vicinity of their colonies (Stempniewicz 2005, Ellis et al 2006, Zwolicki et al 2013). Therefore, gradients of nutrient concentrations may be formed around bird colonies (Zmudczyńska et al 2008) in the Arctic that influence snow and ice ecology in a manner not unlike that already reported in the Antarctic (Hodson 2006). However, the relationship between terrestrial ecosystems in the predominantly ice-covered landscape of Svalbard and their adjacent marine ecosystems remains poorly understood. This uncertainty restricts our understanding of key biogeochemical cycles in this sensitive part of the Arctic.

Here we investigate the microbial community on the surface of Aldegondabreen, a small valley glacier in Svalbard, in order to better understand the potential for microbial utilization of nitrogen on the glacier surface. We present a combination of geochemical analysis of the surface debris, ice and meltwater, quantitative PCR targeting the 16S rRNA and amoA genes, pyrosequencing, and multivariate statistical analysis to better understand the likely functioning of this supraglacial ecosystem, with an emphasis on its potential to contribute to nitrogen cycling.

2. Methods

2.1. Field site and sampling

Aldegondabreen is a small (7.6 km²) retreating valley glacier located in the Grønfjorden area of Spitsbergen, Svalbard, 77°58′N, 14°05′E (Navarro et al 2005). The debris on its surface (cryoconite) has three likely sources: first, debris avalanches from adjacent slopes with seabird breeding sites (mainly kittiwakes Rissa tridactyla and fulmars Fulmarus glacialis on the northern slope and little auks Alle alle on the southern slopes), second, airborne dust originating from deglaciated areas (Bullard 2013), including adjacent slopes, and third, a coal power plant in the mining settlement of Barentsburg acting as a rich local source of air borne black carbon. Significant local input of debris into the supraglacial environment in combination with variable slope of the ablation zone makes this locality an interesting case of a supraglacial ecosystem with a high spatial variation of biogeochemical characteristics on a small area (figure 1). This is supported by the results of Langford et al (2011) who found considerable mineralogical and geochemical diversity in the cryoconite aggregates from Aldegondabreen.

Samples of cryoconite and supraglacial meltwater, and shallow ice cores, were collected at ten sites on the surface of Aldegondabreen (figure 1) between 22 and 24 July 2009. The sampling sites were categorized according to the slope of transects and their hydrological connection to the mountain sides on both edges of the glacier or the up-glacier snow pack. Bulk cryoconite sediment was collected (regardless of its structure) using a sterile plastic syringe and placed in a sterile WhirlPak bag (Nasco, Fort Atkinson, WI, USA). The samples were kept on ice in the dark (cooled with whirlpak bags filled with glacial ice in an aluminum zarges box) until 26 July when they were frozen to −20°C at the laboratory at UNIS, ca 70 km away (5 h by boat). Ice cores (1 m) were taken using a hand-driven Kovacs Mark III ice corer (Kovacs Enterprises, Lebanon, NH, USA) after removing the surface layer of 5–10 cm of sediment-rich ice with an ice axe to separate the chemical signal of glacial ice from cryoconite and melted snow. Due to the fragile nature of the ice cores two sterile 2 l WhirlPak bags were filled with ice from the bottom part of the core. The remaining upper part of the ice cores was discarded. The ice samples were then melted in the bags in the dark and filtered using a Millipore filter device with a vacuum hand pump and polycarbonate filters of 47 mm diameter and 0.22 µm pore size (Millipore, Billerica, MA, USA). Samples of supraglacial stream water were collected directly into sterile 2 l WhirlPak bags and immediately filtered as above. The filtration device was pre-rinsed with sample and filtrate twice before retention of the sample as there was no access to deionized water. The filtered water samples were then separated into sterile 100 ml WhirlPak bags and kept in the cold and dark until 26 July when they were frozen to −20°C. All samples were then transported frozen in an insulated box to Innsbruck. Sterile powder-free gloves were used for sample collection and handling.

2.2. Chemical analysis

Total carbon (TC) and nitrogen (TN) in the cryoconite sediment samples were analysed using a Flash EA 1112 analyzer (Thermo Electron Corporation, Delft, the
Figure 1. (A) Aldegondabreen in Grønfjorden, the study site in Svalbard. (B) Location of the sampling sites on the surface of Aldegondabreen. Transects (A)–(B) are numbered from the lowest point upstream. In transect (B) only the point B2 was analysed. (C) Northern margin of Aldegondabreen with sampling points A3 and A4 as seen from point A2 with marked areas of bird nesting activity and occurrence of large cryoconite granules. White circles indicate the area in the slopes of Productustoppen (527 m) with nesting seabirds. (D) Large cryoconite aggregate in detail with visible cyanobacterial mat on its surface. (E) View on the glacier surface at transect (A). The scale was derived from an 3 m long avalanche probe with cm scale. The probe is visible in the lower right part of the shot. The cryoconite aggregates cover the surface without producing deep melt ponds due to prevailing effect of conductive heat flux. (F) Large cryoconite sediment aggregates at point A2.

Netherlands) in the NC Soils configuration. All samples were dried at 105°C for 24 h prior to analysis. For the determination of TP (total phosphorus), pre-weighted sediment samples were suspended in 100 ml of deionized water and 1 ml 96% H₂SO₄ was added. The samples were then stored in a drying cabinet for 12 h at 160°C. Approximately 93 ml of distilled water was added and the mixture was cooled down to room temperature. Afterwards 6 ml of 10% ascorbic acid and 6 ml 20% NaOH was added, and the solution was neutralized with Vogler solution (Vogler 1966). The absorbance of the samples at 885 nm was measured using a U-2000 spectrophotometer (Hitachi, Tokyo, Japan). The detection limit was 0.9 μg l⁻¹. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined using a TOC-VCPH/TNM-1 analyser (Shimadzu, Kyoto, Japan). DOC was measured as the carbon remaining after acidification with 2 M HCl. Detection limit for both DOC and TDN is 4 μg l⁻¹. Ion chromatography (Dionex ICS-1000, Dionex, Camberley, UK) was used for the determination of ion concentrations (NO₃⁻, NH₄⁺). The detection limits were 5.0 μg l⁻¹ for both NO₃⁻ and NH₄⁺. Due to logistical constraints, only one sample per site could be transported and analysed.

2.3. Particle size and size of aggregates

Particle size was measured using high-resolution laser diffractometry particle size analysis with a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Dried cryoconite sediment (105 °C for 24 h) was suspended in deionized water up to the lower range of optimal obscuration level. The particle size range of this method is 0.02–2000 μm. Samples were sonicated using a built-in sonicator in order to break apart particle aggregates. To assist the dispersion of particles and to avoid their aggregation after sonication, sodium hexametaphosphate (NaPO₃) was added to a concentration of 5.5 g l⁻¹ (Sperazza et al 2004). Preliminary measurements showed a minimal change in particle size distribution after sonication longer than 120 s and, therefore, a sonication time of 120 s was used for all samples. Fineness of sediment was calculated from the particle size distribution as surface area per gram of dry sediment, assuming the prevailing material of the particles being CaCO₃ for density (2.71 g cm⁻³)
estimation and a spherical particle shape, using the Malvern Mastersizer 2000 software. The approach of using sediment surface area per dry weight (fineness) was chosen as a proxy for potential ‘microbial landscape’ (Battin et al. 2007).

Aggregate size (diameter of cryoconite macroscopic aggregates, figures 1(d) and (f)) was measured from photographs using the image analysis software ImageJ 1.43u. Images were photographed with a scale in the field and for each aggregate two Feret diameters were measured: first along the longest visible dimension of the aggregate and second perpendicular to it. The mean of both measurements was used to represent aggregate diameter. Only undisturbed aggregates were measured, excluding clusters of aggregates or fragments.

2.4. Microbiological analysis

Extraction of DNA from the cryoconite samples was performed using the PowerLyzer PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. Between 0.2 and 0.9 g (wet weight) of sediment was used for each extraction, and a blank containing no sediment was extracted in parallel. The quantity of total bacteria and ammonia-oxidizing bacteria and archaea was determined by quantitative PCR (qPCR) with primers targeting the bacterial 16S rRNA gene and the bacteria- and archaea-specific amoA genes encoding the active site of ammonia monooxygenase (table 1). The qPCR was set up under DNA free conditions in a pressurized clean lab and is UV-irradiation sterilized for 3 h on a daily basis. The setup was as follows: 20 µl reactions containing 10 µl of SYBR Premix Ex Taq II (TaKaRa, Kyoto, Japan), 0.8 µl of the primers (final concentration 0.4 µM) and 1 µl of template DNA. The reaction was then carried out on a CFX96 Touch qPCR system (Bio-Rad, Hercules, CA, USA) equipped with high-resolution melt (HRM) analysis.

HRM analysis is a quantitative analysis of the melt curves of product DNA fragments, allowing for the identification of small variations in nucleic acid sequences by the controlled melting of double-stranded PCR amplicons and clustering of samples according to their similarity (Garritano et al. 2009). The cycle program was 95 ºC for 1 min followed by 50 cycles of 95 ºC for 30 s, 30 s at the respective annealing temperature (table 1), and 72 ºC for 30 s. The reaction was completed by a final 72 ºC elongation step for 6 min and followed by HRM analysis in 0.1 ºC increments from 72 to 95 ºC. All qPCR reactions were performed in triplicate. Standards of the bacterial 16S rRNA gene and bacterial and archaeal amoA were prepared as dilution series from cultures of Escherichia coli, Nitrosomonas europaea ATCC19718 derived lux-marker strain (Iizumi et al. 1998), and fosmid clone 54d9 (Treusch et al. 2005), respectively, with known cell abundances. The gene copy numbers in the highest standards were 1.66 × 10^9 µl^-1 for 16S rRNA, 3 × 10^7 for bacterial amoA, and 3 × 10^6 for archaeal amoA.

The diversity of prokaryotic microorganisms in the samples was determined by pyrosequencing. One sample from each cluster showed in the HRM analysis was selected for sequencing. Amplicons (466 bp) flanking the V3 and V4 regions of the 16S rRNA gene were amplified using the primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) followed by a second round of PCR where primers with adapters and 10 bp tags were used (Hansen et al. 2012). PCR amplification was performed using 1× AccuPrime buffer II which includes 0.2 mM dNTP’s, 0.75 U AccuPrime Taq DNA Polymerase High fidelity (Invitrogen, Carlsbad, CA, USA), 0.5 µM of each of the primers, and 1 µl of DNA sample to a total of 25 µl reaction. PCR was performed with a DNA Engine Dyad Peltier Thermal Cycler (MJ Research, Massachusetts, USA), using the following cycle conditions: 94 ºC for 2 min, followed by 30 cycles of denaturation at 94 ºC for 20 s, annealing at 56 ºC for 30 s and elongation at 68 ºC for 40 s, and then a final elongation step at 72 ºC for 5 min. The conditions of the second PCR were as the first PCR, except that the number of cycles was reduced to 15 cycles. The PCR products were run on a gel and the appropriate fragments were cut and purified using the Montage DNA Gel Extraction kit (Millipore, Bedford, MA, USA). The amplified fragments with adapters and tags were quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and mixed in approximately equal concentrations (1 × 10^7 copies µl^-1) to ensure equal representation of each sample. Samples were run on one of a two-region 454 sequencing run was performed on a GS FLX Titanium Pico TiterPlate using a GS FLX Titanium Sequencing Kit XLFR70 according to the manufacturer’s instructions (Roche Diagnostics, Indianapolis, IN, USA).

| Primer | Sequence (5′ → 3′) | Annealing T | Organism | Reference |
|--------|-------------------|-------------|----------|-----------|
| 16S rRNA | | | | |
| 341F | CCT ACG GGA GGC AGC AG | 60 ºC | Bacteria | Muyzer et al (1993) |
| 518R | ATT ACC GCG GCT GCT GGT | | | |
| amoA | | | | |
| amoA-1F | GGG GTT TCT ACT GGT GGT | 58 ºC | Ammonia-oxidizing bacteria | Rotthauwe et al (1997) |
| amoA-2R | CCC CTC KGS AAA GCC TTC TTC | | | |
| 19F | GGW GTK CCR GGR ACW GCM AC | 55 ºC | Ammonia-oxidizing archaea | Leininger et al (2006) |
| A616e48× | GCC ATC CAB CRK TAN GTC CA | | | Schauss et al (2009) |
The obtained sequencing data were analysed with the QIIME Pipeline (Caporaso et al 2010). Sequences were filtered out if they did not fulfill the following criteria: perfect match to the sequence primer of the 16S rRNA gene; length between 200 and 1000 bp; maximum 6 ambiguous bases; minimum score of 50 in the quality score window; no homopolymer longer than 6 bp; maximum number of errors in barcode 1.5. After filtering the sequences were denoised using the default option in QIIME and clustered into Operational Taxonomic Units (OTUs) using Uclust (Edgar 2010) with a 97% sequence similarity. The first cluster seed in the OTU step was used as a representative sequence for each OTU. Representative sequences were then used for taxonomic classification using RDP classifier (Wang et al 2007) with a confidence threshold of 0.8 against the Greengene core set (DeSantis et al 2006). Phylogenetic analysis was performed on selected sequences. They were aligned together with 16S rRNA gene sequences of closest relatives identified with BLAST at the National Center for Biotechnology Information using MUSCLE (Edgar 2004), and edited manually in JalView (Waterhouse et al 2009). Analysis was restricted to nucleotide positions that were unambiguously aligned in all sequences. The phylogenetic tree was constructed using the maximum likelihood method using MEGA version 5 (Tamura et al 2011). Bootstrap analysis (1000 replications) was used to provide confidence estimates for phylogenetic tree topologies. 16S rRNA gene sequences obtained in the pyrosequencing analysis have been deposited in the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under accession number SRR896648. Interactive charts visualising pyrosequencing data are available as supplementary material (stacks.iop.org/ERL/8/035044/ multimedia).

### Statistical analysis

Multivariate statistical analysis was used in order to explain the variation in the data and to test the significance of environmental effects on the chemistry and the abundance of total and ammonia-oxidizing microbes. All the quantitative data except altitude were ln(x + 1) transformed prior to analysis. The hydrological connection to the mountain sides was used as a categorical variable, based upon direct field observations. All data were standardized and centred. Detrended canonical correspondence analysis (DCCA) was used to determine the length of the gradient along the first ordination axis in order to select the appropriate method for ordination of the data. Redundancy analysis (RDA) was then used in order to assess the relationships between known environmental variables and variation in the multivariate data. 999 Monte Carlo permutations in the unrestricted mode, and manual forward selection, were used for the RDA. The results of the RDA were summarized using biplot or triplot diagrams. The relative length and position of arrows in the diagrams show the extent and direction of response of the selected dependent variables to the environmental factors. All the analyses were performed in the multivariate data analysis software Canoco 5 (ter Braak and Šmilauer 2012).

### Results

Unexpected complications with molecular analysis required many repeat trials. Since logistical restrictions imposed constraints on the transport of material, these complications meant not all samples were available in a sufficient quantity and so we are only able to present a large subset of the original samples (see figure 1(B)). Table 2 shows the physical characteristics of the sampling sites. The altitude of the sites ranged from 150 m (lower end of transect C) to 280 m a.s.l. (upper end of transect A). The slope of the glacier surface declined from south to north, being steepest (−9°) on transect C. Point B2 was fed solely from streams originating in melting ice and/or snow with no hydrological connection to the slopes above the glacier, whereas the other points (A and C transects) were hydrologically connected to adjacent mountain slopes. Sediment fineness of the samples ranged from 0.43 m² g⁻¹ to a maximum of 0.76 m² g⁻¹ (0.60 ± 0.13 m² g⁻¹). The cryoconite aggregate diameter at transect A ranged from 0.42 to 3.1 cm (1.1 ± 0.35 cm, n = 350). The aggregate size distribution is shown in figure 2. No large aggregates with distinct coating of cyanobacterial mats were found in parts of the glacier other than transect A, although small aggregates of millimetre size and irregular fragments of larger aggregates were ubiquitous.

Table 3 shows the concentrations of sediment-bound and dissolved nutrients in the samples from the surface of Aldegondabreen. The concentrations of DOC ranged from 0.40 to 2.0 mg l⁻¹ (0.96 ± 0.50 mg l⁻¹; n = 8) in supraglacial meltwater, and from 0.34 to 1.4 mg l⁻¹ (0.60 ± 0.34 mg l⁻¹) in surface ice. TDN was between 34 and 180 µg l⁻¹ (110 ± 50 µg l⁻¹) in supraglacial meltwater and between 16 and 61 µg l⁻¹ (38 ± 16 mg l⁻¹) in surface ice. The concentration

| Site | Hydrological connection to mountain side | Altitude (m a.s.l.) | Slope (deg) | Sediment fineness (m² g⁻¹) |
|------|----------------------------------------|--------------------|------------|--------------------------|
| A1   | Connected                              | 165                | 6.8        | 0.705                    |
| A2   | Connected                              | 200                | 6.8        | 0.761                    |
| A3   | Connected                              | 240                | 6.8        | 0.751                    |
| A4   | Connected                              | 280                | 6.8        | 0.633                    |
| B2   | Isolated                               | 170                | 6.6        | 0.505                    |
| C1   | Connected                              | 150                | 9.3        | 0.526                    |
| C2   | Connected                              | 200                | 9.3        | 0.433                    |
| C3   | Connected                              | 250                | 9.3        | 0.473                    |
Table 3. Nutrient concentrations in cryoconite sediment, supraglacial meltwater, and surface ice on Aldegondabreen. TC (total carbon), TN (total nitrogen), TP (total phosphorus) in mg g$^{-1}$ (mean ± sd, n = 3); DOC (dissolved organic carbon) in mg l$^{-1}$, TDN (total dissolved nitrogen), NO$_3^-$, NH$_4^+$ in µg l$^{-1}$, b.d. below detection limit; n.d. not determined.

| Site | TC (mean ± sd) | TN (mean ± sd) | TP (mean ± sd) |
|------|----------------|----------------|----------------|
| A1   | 25 ± 0.87      | 2.4 ± 0.04     | 0.78 ± 0.029   |
| A2   | 26 ± 0.40      | 2.7 ± 0.10     | 0.75 ± 0.066   |
| A3   | 26 ± 3.6       | 2.2 ± 0.16     | 0.72 ± 0.004   |
| A4   | 23 ± 0.60      | 1.9 ± 0.07     | 0.70 ± 0.045   |
| B2   | 57 ± 2.1       | 2.5 ± 0.20     | 0.77 ± 0.010   |
| C1   | 46 ± 2.5       | 2.0 ± 0.08     | 0.69 ± 0.023   |
| C2   | 17 ± 0.67      | 1.2 ± 0.04     | 0.69 ± 0.017   |
| C3   | 21 ± 1.7       | 1.5 ± 0.10     | 0.69 ± 0.009   |

Table 4. Abundances of the 16S rRNA gene and bacterial and archaeal amoA genes (gene copies g$^{-1}$) in cryoconite on Aldegondabreen (mean ± sd; n = 3). b.d. below detection limit.

| Site | 16S rRNA (×10$^4$) | Bacterial amoA (×10$^4$) | Archaeal amoA (×10$^4$) |
|------|---------------------|--------------------------|-------------------------|
| A1   | 320 ± 0.76          | 0.28 ± 0.11              | 26 ± 1.2                |
| A2   | 120 ± 1.8           | 0.01 ± 0.02              | 16 ± 5.3                |
| A3   | 230 ± 59            | 1.4 ± 0.10               | 22 ± 11                 |
| A4   | 230 ± 15            | 0.29 ± 0.074             | 25 ± 0.26               |
| B2   | 4.0 ± 0.92          | b.d.                     | b.d.                    |
| C1   | 140 ± 16            | b.d.                     | b.d.                    |
| C2   | 180 ± 11            | b.d.                     | b.d.                    |
| C3   | 30 ± 2.0            | b.d.                     | b.d.                    |

of NO$_3^-$ in supraglacial meltwater ranged from values below detection limit (points A1 and C1) to 24.0 µg l$^{-1}$ at point A4. The concentrations of NO$_3^-$ in surface ice were close to (7 µg l$^{-1}$ at B2 and C3) or below detection limit. NH$_4^+$ ranged from below detection limit to 30 µg l$^{-1}$ with a mean of 14.3 (±9.7) µg l$^{-1}$ in meltwater, and from 5 to 13 µg l$^{-1}$ (7.7 ± 3.5 µg l$^{-1}$) in surface ice (table 3). The DOC:TDN ratio in supraglacial meltwater ranged from 7.4 to 14 (10 ± 2.3; n = 8) and from 10 to 34 (19 ± 8.4) in surface ice. The mean sediment-bound C:N molar ratio in the samples was 17 ± 6.0 (n = 24), with a minimum of 11 ± 0.40 at point A2 and a maximum of 27 at B2 and C1. The mean C:P molar ratio was 108 ± 46 with two outlier maxima of 190 ± 9.6 and 170 ± 6.1 at B1 and C1, respectively. Mean N:P ranged from 3.9 ± 0.64 (C2) to 8.0 ± 0.38 (A2), with an overall average of 6.2 ± 1.3. Figure 3 shows the results of an RDA analysing the variation in the chemistry data using the hydrological connection to a bird colony (connected versus isolated), altitude, and slope. The analysis explained 48.6% of the total variation in the data, with slope (26.1%, p < 0.001) as significant factors.

Table 4 shows the results of the qPCR analysis of the 16S rRNA and amoA genes in the cryoconite samples. The abundance of the 16S rRNA gene was lowest (4.0 × 10$^4$ gene copies per g of wet sediment) at point B2 and highest on transect A (1.2–3.2 × 10$^5$ g$^{-1}$). The high precision melt curve analysis coupled to the qPCR with 16S rRNA primers clustered the samples according to their similarity into four groups (A1–A2; A3–A4; B2; C1–3; data not shown). One representative was selected from each group (A1, A4, B2 and C2) for subsequent pyrosequencing. The amoA genes were only detected in samples from transect A (table 4). The abundance of bacterial amoA in the A samples was between ~100 and 14,000 gene copies per g, while that of the archaeal amoA was one to two orders of magnitude higher, ranging from 16 to 26 × 10$^3$ g$^{-1}$. Figure 4 shows the results of an RDA analysis in which the variation in the qPCR data

Figure 2. Histogram of aggregate diameters at transect A, site A2.

Figure 3. RDA biplot visualizing the effects of physical environmental variables (dashed arrows for quantitative and filled triangles for categories) on the chemistry (solid arrows) of cryoconite holes on Aldegondabreen. Only significant factors (p < 0.01) are shown.
Figure 4. RDA triplot showing the effects of environmental variables (dashed arrows for quantitative and filled triangles for categories) on the abundance of 16S rRNA and amoA genes (solid arrows) in cryoconite holes on Aldegondabreen. Sites are marked by empty grey circles. Only significant factors \((p < 0.01)\) are shown.

Table 5. Sequencing depth and diversity and dominance indices for the selected samples of cryoconite from Aldegondabreen.

| Site | Sequences per sample |
|------|-----------------------|
| A1   | 58 171                |
| A4   | 33 334                |
| B2   | 357                   |
| C2   | 52 796                |

(both 16S rRNA and amoA) was explained using physical (mountain slope connection, slope, sediment fineness) and chemical (sediment TC, TN, TP, water DOC, \(\text{NH}_4^+\) and \(\text{NO}_3^-\)) data as predictors. The weight of sediment used for DNA extraction was used as a covariate. The analysis explained 57.8% of the total variation in the qPCR data, with sediment fineness explaining 39.9% \((p = 0.001)\) and mountain slope connection 17.9% \((p = 0.002)\).

Table 5 shows the results of the pyrosequencing analysis of the selected samples. The average sequence length after quality check was 461 bp (ranging between 186 and 522 bp). Between ∼33 000 and 58 000 sequences were obtained for all samples except B2, for which only 357 sequences were generated. The highest diversity as expressed by the Shannon index was found in sample A4, whereas sample B2 showed the highest dominance (Berger–Parker index). The prokaryotic diversity in the samples on the phylum level is shown in figure 5. Cyanobacteria were the dominant group in all samples except A4, accounting for over 60% of the community in sample A1. Proteobacteria (14–33%), Bacteroidetes (2.0–23%) and Actinobacteria (1.4–34%) were also present in high numbers in all the samples. The dominant sequences in A1 were filamentous cyanobacteria of the genera Microcoleus (17%) and Phormidium (11%) and a member of Bacteroidetes related to Sphingobacteria (5%).

4. Discussion

Aldegondabreen is an example of a small Arctic valley glacier with marked spatial variations in the physical and chemical environments across its surface. The amount of debris can be significant on the surface of Aldegondabreen and exceed 4 kg m\(^{-2}\). Due to the vicinity of the mining settlement of Barentsburg, black carbon (BC) can represent an important contribution to this debris. However, our field reconnaissance and statistical analyses (table 3) showed lateral inputs of material at different locations on the glacier and their redistribution by slope avalanching and runoff were particularly significant. However, the missing data points from the centre of the glacier undermine the statistical analysis to some extent, and so it should also be noted that Langford et al (2011) found clear differences between the mineral debris in the centre of the glacier (principally wind input) and at its sides (mainly avalanche input) using geochemical analysis. Langford et al (2011) also found significant spatial variability in organic matter composition on the glacier surface, with cryoconite near to the centre dominated by carbohydrate-rich organic matter and associated humification products, whilst cryoconite near to the glacier edge contained both a lesser quantity and a less diverse composition of organic matter.
This is consistent with our data showing the highest content of OC in cryoconite at point B2 in the centre of the glacier (table 3) and the differences therefore most likely result from the avalanching of freshly eroded rock at the glacier margins.

The size of the Aldegondabreen cryoconite aggregates (figures 1 and 2) is typically far in excess of those found on other glaciers and offers rather different habitat to the mm-scale aggregates described elsewhere (Hodson et al 2010, Takeuchi et al 2010). We therefore propose that the combination of a relatively gentle slope of the glacier surface, fine sediment derived from local shales, and input of nutrients associated both with the avalanche debris and atmospheric deposition collectively enable the development of large cryoconite aggregates that support a distinct microbial community. The microbes then further facilitate the growth of the aggregates through production of extracellular polymeric substances that have cohesive properties (Hodson et al 2010, Langford et al 2010).

The total microbial abundance in cryoconite on Aldegondabreen, determined as the abundance of the 16S rRNA genes, is consistent with previous data from other Svalbard glaciers based on microscopy counts (Stibal et al 2008a, Anesio et al 2010, Langford et al 2010). The structure of the microbial community is also similar to that of other Svalbard sites (Edwards et al 2011, Cameron et al 2012a) and dominated by photoautotrophic cyanobacteria and mostly heterotrophic Proteobacteria, Bacteroidetes, and Actinobacteria (figure 5). The difference in cyanobacterial abundance between the samples may be attributed to the fact that chloroplasts are also identified as cyanobacteria, and that a significant amount of plant residue from nearby tundra can be deposited on the glacier surface by aeolian transport. We also suggest that the high dominance of a chloroplast sequence in sample B2 was caused by the presence of chloroplast-containing plant tissue in the sample; this may also have caused a lower efficiency of molecular analysis and led to the low number of sequences retrieved from the pyrosequencing analysis (table 5).

The slope of the glacier surface is assumed to affect the development of cryoconite aggregates most via the erosive action of meltwater, which is supported by the fact that transect A contained the largest, well-developed cryoconite aggregates (figure 1(d)) even though both edges of the glacier were influenced by inputs from the mountain sides. Although the difference in slope between the side transects (A versus C) was relatively small (ca 2.5°), our RDA analysis showed a significant effect of it on the concentrations of DOC and both sediment-bound and dissolved total nitrogen (figure 3). Again, while the statistical analysis is undermined by the missing data from transect B, the importance of flowing water has been noticed before on Arctic glaciers (Edwards et al 2011, Stibal et al 2012b). In fact even smaller differences in slope were shown to control nutrient concentrations and microbial abundance and activity on the surface of the Greenland Ice Sheet (Stibal et al 2012b).

Sediment fineness, being a measure of particle surface area per sediment dry weight, is assumed to affect the formation of cryoconite aggregates simply due to the surface area available for adhesion of microbial cells, their products, or other sticky substances. This is consistent with the presence of large granules only on transect A which contained the finest sediment (table 2). The size distribution of aggregates measured at transect A shows a distribution slightly skewed towards smaller diameters with an average close to 1 cm. Further investigation of the aggregate distribution pattern is needed to test if this distribution pattern will be found consistent over larger scale and deeper insight into the cryoconite formation process will be needed for its interpretation.

The presence of seabird nesting sites on the valley walls adjacent to Aldegondabreen suggests a potential for high organic nutrient input into the system (Odasz 1994, Stempniewicz 2005, Ellis et al 2006, Zwolicki et al 2013), especially at the sides of the glacier. The measured concentrations of both sediment-bound and dissolved nitrogen in our samples were, however, similar to those on other Svalbard glaciers (Stibal et al 2008b, Hodson et al 2010, ....
the NO\textsuperscript{3−} it to nitrite (Kowalchuk and Stephen 2001), and might
be active on an Arctic glacier. This is the first evidence of ammonia-oxidizing archaea present on
Aldegondabreen, as mentioned above, enables the functioning of specialized microbial guilds such as
ammonia oxidizers in this case. Other metabolic pathways requiring stable conditions otherwise not attainable on
the glacier surface may be possible within these large aggregates and should be a focus of future research of biogeochemical
processes in glacial ecosystems.

Given the potential importance of sea birds as nutrient vectors in the present study, it is worth considering whether
the case of Aldegondabreen represents an exceptional case, or if such bird colonies near glaciers are commonplace in
Svalbard. The available data on the distribution of seabird colonies (Norsk Polar Institute, http://svalbardkartet.npolar.
no/Viewer.html?Viewer=Svalbardkartet) show at least tens of cases where bird colonies lie above glacial surfaces in
Western Svalbard. These glaciers are not in close proximity to the usually visited sites surrounding Ny-Ålesund and
Longyearbyen. Therefore, more effort needs to be directed outside of these areas if the role of marine ecosystems in the
subsidy of glacial ecosystems is to be fully appreciated.

5. Conclusions

The surface of Aldegondabreen, a small valley glacier in Svalbard, receives carbon and nutrients from multiple sources,
including bird nesting sites on the adjacent valley walls. The combination of nutrient input, slope-related supraglacial meltwater flow and the presence of fine surface debris (cryoconite) likely supports the formation of large cryoconite aggregates in some areas of the glacier surface. A diverse microbial community was found to inhabit the cryoconite on Aldegondabreen, dominated by the cyanobacteria, Proteobacteria, Bacteroidetes, and Actinobacteria, typical for supraglacial environments. Allochthonous material including chloroplasts (probably plant tissue-derived) was also identified in the molecular analysis. Importantly, ammonia-oxidizing archaea (AOA) were, for the first time on an Arctic glacier, detected in our samples using qPCR, which is consistent with this finding and suggests that cryoconite may be yet another archaea-dominated ammonia oxidation environment.

Our results suggest that the presence of ammonia oxidizers is principally controlled by the sediment character (figure 4). Therefore, we suggest that the combination of factors contributing to the formation of large cryoconite aggregates present on Aldegondabreen, as mentioned above, enables the functioning of specialized microbial guilds such as ammonia oxidizers in this case. Other metabolic pathways requiring stable conditions otherwise not attainable on the glacier surface may be possible within these large aggregates and should be a focus of future research of biogeochemical processes in glacial ecosystems.

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