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ORIGINAL PAPER

Carbon and nitrogen biogeochemical cycling potentials of supraglacial cryoconite communities

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Abstract Microorganisms have a crucial role to play in the cycling of nutrients within glacial environments. These systems are often nutrient-limited, and so biogeochemical reactions, which ensure the availability of nutrients for microbial communities, are critical for the maintenance of these systems. This study uses molecular biology to characterise the supraglacial cryoconite microbial communities that are capable of cycling carbon and nitrogen in a range of glacial environments. Organisms with the potential to photosynthesise were identified, including Cyanobacteria, Actinobacteria, Betaproteobacteria, Stramenopiles and Haptophyceae. Organisms with the potential to perform nitrification and denitrification processes were also identified and featured Betaproteobacteria, Alphaproteobacteria, Thaumarchaeota and Cyanobacteria. While it is unlikely that the chemical and physical parameters of the supraglacial environment will facilitate optimal rates of all of the nitrogen-related biogeochemical processes, the transport of these cryoconite communities to downstream locations, where more favourable conditions may prevail, will perhaps provide a valuable inoculation of microorganisms with the genetic potential to catalyse these reactions elsewhere.

Keywords Glacier · Cryoconite · Microbial diversity · Biogeochemical · Carbon · Nitrogen

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Introduction

Microbial communities have an essential role to play in the cycling of nutrients in all ecosystems (reviewed in Zehr et al. 2003). In the cryosphere, microorganisms are thought to perform biogeochemical cycles within soils (Nordin et al. 2004), snow (Jones and Deblois 1986), lakes (Canfield and Green 1985) and both in supraglacial (Hodson et al. 2005; Telling et al. 2010, 2011) and subglacial (Tranter et al. 1994; Hodson et al. 2005; Barker et al. 2006; Wynn et al. 2006, 2007; Boyd et al. 2011) environments. Supraglacial microbial communities, for example, within the snowpack or within cryoconite debris (0.1–3 mm dark, granular aggregates: Gerdel and Drouet 1960; Takeuchi et al. 2001b), are known to cycle carbon through photosynthesis and respiration pathways (Fogg 1967; Säwström et al. 2002; Mueller et al. 2005; Foreman et al. 2007; Hodson et al. 2007; Stibal and Tranter 2007; Anesio et al. 2009; Hodson et al. 2010a, b; Telling et al. 2010). They are also thought to contribute towards nitrogen cycling within the glacial environment through the catalysis of nitrification (Hodson et al. 2005; Wynn et al. 2007) and nitrogen

fixation (Telling et al. 2011). It has been suggested that the transport of organic carbon and nitrogen, associated with supraglacial microbial communities, to the subglacial environment provides a key substrate for microbial biochemical processes in the subglacial environment (Hodson et al. 2005; Wynn et al. 2007). Cryoconite holes (water filled depressions upon the glacier surface containing a layer of cryoconite debris) have been identified as being important hydrological and biological systems within glacial environments, providing refuge from the extreme conditions of the cryosphere (reviewed in MacDonell and Fitzsimons 2008). Within cryoconite holes, and in particular within cryoconite debris, diverse communities of bacterial, eukaryotic and archaeal microorganisms exist (Säwström et al. 2002; Edwards et al. 2011; Cameron et al. 2012). These communities have been found to be metabolically active during the summer (Säwström et al. 2002; Hodson et al. 2007; Stibal and Tranter 2007; Anesio et al. 2009; Hodson et al. 2010a, b; Telling et al. 2010, 2011).

Organisms found within cryoconite communities can potentially be linked into a multi-trophic web (Säwström et al. 2002). At the base of this food web, bacterial and eukaryotic autotrophic organisms exist, with the potential to fix atmospheric carbon dioxide into biologically available organic carbon, through photosynthesis. The first rate limiting step of photosynthesis is catalysed by the ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) enzyme (Ellis 1979), of which the large subunits of the predominant form (form I; Spiridonova et al. 2004; Selesi et al. 2005) are encoded by *cbbL* genes (Kusian and Bowien 1997: otherwise named as the *rbcL* gene in older nomenclature or when referring to eukaryotic organisms; Tabita 1988). *CbbL* genes have been found within green-like autotrophic bacterial groups (including plants, green algae, Cyanobacteria and representatives of some Alpha-, Beta- and Gammaproteobacteria), red-like autotrophic bacterial groups (including non-green algae and representatives of some Alpha- and Betaproteobacteria; Watson and Tabita 1997; Spiridonova et al. 2004; Selesi et al. 2005) and autotrophic eukaryotes.

In the nitrogen cycle, nitrogen gas is fixed into ammonia (NH_3) by nitrogen fixation which is catalysed by the nitrogenase enzyme (Howard and Rees 1996; Fig. 1); encoded by the nitrogen fixation (*nif*) gene (Zehr et al. 2003). Ammonia readily converts to ammonium (NH_4^+, ionised ammonia) under acidic pH conditions (Howard and Rees 1996). The collective process of nitrification is an energy-producing reaction involving the aerobic oxidation of ionised ammonia into nitrite (NO_2^-) (by ammonia oxidation) and nitrite into nitrate (NO_3^-) (by nitrite oxidation; reviewed by Bothe et al. 2007). The first step of ammonia

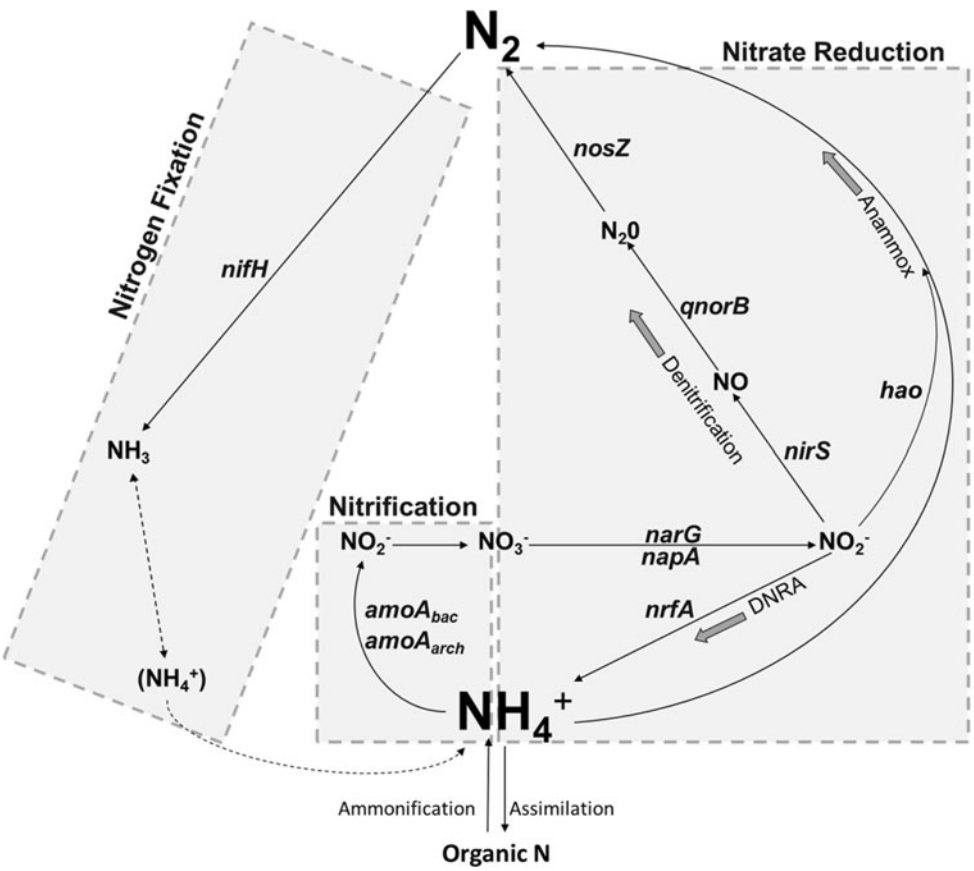

Fig. 1 Major biochemical processes of the nitrogen cycle. The functional genes that were targeted within this study are shown in lowercase italics (with the exception of the *nor* gene which is expressed as *qnor*). The specific gene locus that was targeted is described after the functional gene in uppercase and subscript italics. The *dashed arrow* depicts the non-microbially mediated transformation of ammonia to ammonium

oxidation is catalysed by the ammonia monooxygenase protein (Hollocher et al. 1981), the first subunit of which is encoded by the *amoA* gene (McTavish et al. 1993). The reduction of nitrate to nitrite is catalysed by nitrate reductase proteins that are either membrane-bound (encoded by the *nar* operon; Warnecke-Eberz and Friedrich 1993) or are located within the periplasm (encoded by the *nap* gene; Siddiqui et al. 1993). Once nitrite is formed, it can be reduced further by one of the three main anaerobic pathways: (1) the multistep reduction of nitrite to form dinitrogen gas – termed denitrification (Zumft 1997), (2) the formation of ammonium by dissimilatory nitrate reduction to ammonium (DNRA) (Knowles 1982) or (3) the coupling of ammonium oxidation to the reduction of nitrite to form dinitrogen gas by anaerobic ammonium oxidation (anammox) (Mulder et al. 1995; van de Graaf et al. 1995). In denitrification, nitrite is reduced to nitric oxide (NO) using either of two nitrite reductase proteins, NirS and NirK (encoded by the nitrite respiration genes, *nirS* and *nirK*); nitric oxide is then reduced to nitrous oxide (N_2O) using nitric oxide reductase (encoded by the nitric oxide respiration gene, *nor*) and nitrous oxide is reduced to dinitrogen gas using nitrous oxide reductase (encoded by the nitrous oxide respiration gene, *nosZ*; Zumft 1997). DNRA is catalysed by formate dehydrogenases, encoded by the *nrfA* gene (Darwin et al. 1993), and the anammox pathway is partially catalysed by hydroxylamine oxidoreductase, which is encoded by the *hao* gene (Schalk et al. 2000; Strous et al. 2006).

This study aims to establish whether genes encoding some of the key enzymes, introduced above, that catalyse the biogeochemical reactions of the carbon and nitrogen cycle are detectable in cryoconite debris. The identification of these genes within cryoconite communities establishes the genetic potential for these processes to occur and highlights the possible role of cryoconite communities in the nutrient cycling of the supraglacial environment. Specifically, genes associated with photosynthesis, nitrogen fixation, nitrification, denitrification and DNRA were examined. Furthermore, the diversity of organisms containing these genes was investigated through sequence analysis, allowing for the diversity of organisms that may be important in catalysing these biogeochemical reactions within cryoconite hole environments to be examined.

Materials and methods

Sampling sites and cryoconite hole sampling

Cryoconite was sampled from individual holes at 9 glacial locations in Northern and Southern Hemisphere Polar regions (Table 1; Fig. 2). At all locations, one hole was sampled, with the exception of the Svalbard Midtre Lovénbreen location, where four holes were sampled. Cryoconite granules were extracted using a sterile plastic pasteur pipette. Material was placed into sterile Eppendorf tubes and any excess liquid was removed. Samples were frozen

Table 1 Location and situation of cryoconite sampling sites

Location	Glacier identification	Site code	Latitude and longitude	Characteristics of location
Antarctica—Signy Island	Tuva Glacier (unofficial name)	A-S	60°41′S 45°38′W	Small, west-facing outlet glacier from Signy Island Ice Cap
Greenland—South West Ice Sheet	Russell Glacier	G-K	67°09′N 50°01′W	West facing flanks, Central-West Greenland Ice Sheet
Greenland—Kronprins Christian Land	Greenland Ice Sheet	G-Kp	79°55′N 24°06′W	North East-facing flanks, North-East Greenland Ice Sheet
Svalbard—Central Spitsbergen	Longyearbreen	S-L	78°10′N 15°30′E	Small valley glacier, Central Svalbard
Svalbard—Central Spitsbergen	Rieperbreen	S-R	78°7′N 16°4′E	Small valley glacier, Central Svalbard
Svalbard—Central Spitsbergen	Foxfonna	S-F	78°08′N 16°07′E	East facing flank of small mountain ice cap, Central Svalbard
Svalbard—North West Spitsbergen	Midtre Lovénbreen	S-M	78°53′N 12°03′E	Small valley glacier, North-West Svalbard
Svalbard—West Nordaustlandet	Vestfonna	S-V	79°48′N 18°24′E	West-facing flank of ice cap, North Svalbard
Norway—Jostedalsbreen Ice Cap	Austerdalsbreen	N-J	61°35′N 06°58′E	South-East facing outlet glacier from Jostedalsbreen Ice Cap, Central-East Norway

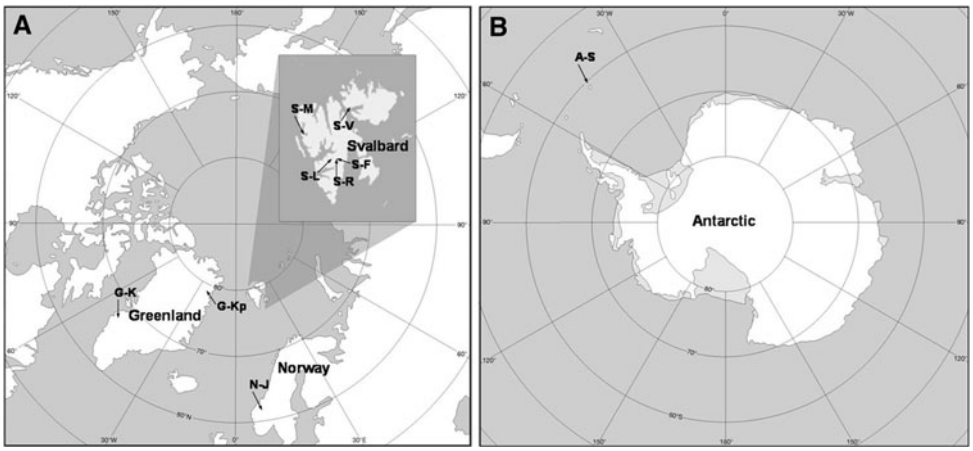

Fig. 2 Location of cryoconite sampling sites in **a** Arctic and **b** Antarctic regions. Sampling locations: Greenland: South West Ice Sheet (G-K) and Kronprins Christian Land (G-Kp), Svalbard: Longyearbreen (S-L), Rieperbreen (S-R), Foxfonna (S-F), Midtre Lovénbreen (S-M) and Vestfonna (S-V), Norway: Jostedalsbreen Ice Cap (N-J), and Antarctic Signy Island (A-S)

at −20 °C on return to the field laboratories. Material was transported back to the UK frozen and on arrival, the samples were stored at −80 °C. Table 2 shows the chemical properties of the cryoconite water that bathes the cryoconite debris. Nutrients were generally measured at sub-mg L^{-1} levels, suggesting that the cryoconite samples tended to be bathed in very dilute glacial meltwaters. The exception was Signy Island, where snowmelt is heavily enriched in atmospherically derived marine aerosol and nutrients from a local penguin colony (most notably inorganic nitrogen and phosphorous; Hodson 2006). Elsewhere, PO_4^{3-} levels are very often below the detection limit of conventional ion chromatography (ca. 0.01 mg L^{-1}). Levels of DOC are not well known at many of the study sites, but most likely lie in the range 0.1–1.0 mg L^{-1} according to studies elsewhere (e.g. Priscu and Christner 2004).

Nucleic acid extractions from cryoconite granules

Genomic DNA was extracted from cryoconite using the PowerSoil™ DNA isolation kit (Mo Bio laboratories, Cambridge, UK) in accordance with the manufacturer's instructions and using approximately 0.8 g (dry weight) of solid cryoconite material. DNA was eluted into a final volume of 30 µL of nuclease-free water (Ambion, Warrington, UK).

Polymerase chain reaction (PCR) amplification

Functional genes were amplified using polymerase chain reaction (PCR). Reactions (50 µL) comprised 0.4 µM of each forward and reverse oligonucleotide primer, 200 µM of each deoxyribonucleotide triphosphate (dNTPs), 1 mM $MgCl_2$, 1× buffer (Bioline, London, UK), 2.5 U of *Taq* polymerase (Bioline) and 1 µL of DNA. The primers and PCR cycling conditions are detailed in Table 3. PCR products were visualised by gel electrophoresis with ethidium bromide staining to ensure the correct size fragment was amplified.

Clone library construction, sequencing and analysis

PCR products were purified using the QIAquick PCR purification kit (Qiagen), ligated into the pCR2.1®-TOPO® TA cloning vector (Invitrogen, Paisley, UK) and transformed into One Shot® Chemically Competent *Escherichia coli* TOP10 F' cells (Invitrogen) in accordance with the manufacturer's instructions. Transformed cells were plated on Luria–Bertani (LB) agar containing ampicillin (50 µg mL^{-1}) and X-gal (20 µg mL^{-1}) and incubated overnight at 37 °C. White colonies were picked at random and used to inoculate 100 µL of LB broth containing ampicillin. After 2-h incubation at 37 °C, 1 µL of each culture was used in a PCR to amplify insert DNA using vector-specific primers T3 (5′-ATT AAC CCT CAC TAA AGG GA-3′) and T7 (5′-TAA TAC GAC TCA CTA TAG GG-3′) (Invitrogen). The amplified vector inserts were purified using SureClean (Bioline) and were sequenced using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with T3 and T7 primers. Sequencing was performed in an ABI 3730 Genetic Analyser. Sequences were edited manually using Chromas Pro version 1.34 (http://www.technelysium.com.au). Chimeras were identified using Mallard version 1.02 (Ashelford et al. 2006). FASTA formatted sequences were translated into protein sequences using ExPASy (Gasteiger et al. 2003), and protein sequences were compared to the GenBank database using Basic Local Alignment Search Tool for protein (BLASTp; Altschul et al. 1990). Bioedit version 7.0.5.3 (Hall 1999) was used to align the library of sequences to themselves and to the top BLAST hits using ClustalW (Thompson et al. 1994), which also presented an additional opportunity to highlight and alter any errors within the sequence reading. Mega version 4.1 (Tamura et al. 2007) was used to calculate evolutionary distances and create phylogenetic trees, using the Neighbour-Joining method (Saitou and Nei 1987) with bootstrap analysis (1,000 replicates).

Table 2 Average ± SD data of the chemistry of cryoconite holes by location (with the exception of locations 3 and 6), which are maximum concentrations from nearby ice cores, or where number of holes sampled (*n*) was one)

	Na (mg/L)	K (mg/L)	Mg (mg/L)	Ca (mg/L)	Cl (mg/L)	SO_4 (mg/L)	PO_4 (μg/L)	DO (mg/L)	TN (%)	NH_4 (μg/L)	NO_3 (μg/L)	TC (%)	TDIC (mg/L)	DOC (mg/L)
1. Antarctica Signy Island (A-S)[a]	11.5 ± 9.51	0.274 ± 0.193	0.718 ± 0.522	0.207 ± 0.167	10.2 ± 7.77	1.63 ± 1.22	8 ± 6	n.d.	0.06 ($n = 1$)	41 ± 38	66 ± 32	0.46 (n = 1)	<12.0	0.757 ± 0.571
2. Greenland South-West (G-K)[b]	0.463 ± 0.330	0.147 ± 0.115	0.029 ± 0.021	0.116 ± 0.109	0.604 ± 0.337	0.171 ± 0.142	n.d.	12.15 ± 0.42	0.10 ($n = 1$)	16 ± 17	86 ± 57	0.69 ± 0.17	12.16 ± 0.415	n.d.
3. Greenland North-East (G-Kp)[c]	<0.138	<0.007	<0.109	<0.607	<0.293	<0.528	n.d.	n.d.	n.d.	<54	<659	2.15 ± 0.91	n.d.	n.d.
4. Svalbard Central Spitsbergen (S-L)[b]	0.957 ± 0.792	0.147 ± 0.115	0.029 ± 0.021	0.571 ± 0.624	1.69 ± 2.22	0.345 ± 0.359	n.d	12.35 ± 0.500	0.21 ($n = 1$)	8 ± 17	66 ± 65	2.69 ± 0.56	0.535 ± 0.415	n.d.
5. Svalbard North-west Spitsbergen (S-M)[b]	0.468 ± 0.208	0.057 ± 0.068	0.052 ± 0.016	0.305 ± 0.063	0.349 ± 0.297	0.127 ± 0.061	4 ± 12	12.35 ± 0.500	0.25 ($n = 1$)	8 ± 13	22 ± 10	2.51 ± 0.57	0.214 ± 0.220	n.d.
6. Svalbard Vestfonna (S-V)[d]	<2.76	n.d.	n.d.	n.d.	<5.32	<1.20	n.d.	n.d.	n.d.	n.d.	<372	6.42 ± 0.58	n.d.	n.d.
7. Norway Jostedalsbreen (N-J)[e]	0.335 ± 0.072	0.219 ± 0.060	0.072 ± 0.005	1.383 ± 0.002	0.134 ± 0.000	2.41 ± 0.139	n.d.	n.d.	n.d.	n.d.	39 ± 7	n.d.	3.88 ± 0.488	n.d.

No data was available for Svalbard Foxfonna (S-F) or Rieperbreen (S-R) locations, with the exception of total carbon (1.54 % ($n = 1$) and 1.69 ± 0.59 %, respectively). However, due to the close proximity of S-F and S-R glaciers to the Svalbard Longyearbreen glacier (S-L), these S-L data have been used as a preliminary representation for data within this central Spitsbergen area

DO data include dissolved oxygen; *TN* total nitrogen; *TC* total carbon; *TDIC* total dissolved inorganic carbon; *DOC* dissolved organic carbon

n.d. no data

[a] Hodson (2006); [b] Hodson et al. (2008); [c] Indicative chemistry from ice core (Clausen et al. 2001); [d] Indicative chemistry from ice core (Matoba et al. 2002); [e] Yde unpublished data

Table 3 PCR primers used and cycling conditions

Target gene and metabolic pathway involvement of gene product	Primer pair	5′–3′ Nucleotide sequence	PCR cycling conditions	References
cbbLR	CbbLR1F	AAG GAY GAC GAG AAC ATC	Initial denaturing: 94 °C for 2 min	Selesi et al. (2005)
First rate limiting step of photosynthesis	CbbLR1R	TCG GTC GGS GTG TAG TTG AA	30 cycles of: 94 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min	Selesi et al. (2005)
			Final elongation step of 72 °C for 10 min	
rbcL	rbcL-F	GAT GAT GAR AAY ATT AAC TC	Initial denaturing: 95 °C for 3 min	Paul et al. (2000)
First rate limiting step of photosynthesis	rbcL-R	ATT TGD CCA CAG TGD ATA CCA	40 cycles of: 95 °C for 1 min, 52 °C for 1 min, 72 °C for 1 min 30 s	Paul et al. (2000)
			Final elongation step of 72 °C for 10 min	
cbbLG	RubIgF	GAY TTC ACC AAR GAY GAY GA	Initial denaturing: 95 °C for 2 min	Spiridonova et al. (2004)
First rate limiting step of photosynthesis	RubIgR	TCR AAC TTG ATY TCY TTC CA	30 cycles of: 95 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min	Spiridonova et al. (2004)
			Final elongation step of 72 °C for 10 min	
nifH	nifH-F	TGY GAY CCN AAR GCN GA	Attempted: initial denaturing: 94 °C for 5 min	Zehr and McReynold (1989)
Nitrogen fixation	nifH-R	AND GCC ATC ATY TCN CC	35 cycles of: 94 °C for 10 s to 30 s, 50 °C to 52 °C for 50 s, 72 °C for 50 s	Zehr and McReynold (1989)
			Final elongation step of 72 °C for 10 min	
amoAarcheal	amo111F	TTY TAY ACH GAY TGG GCH TGG ACA TC	Initial denaturing: 94 °C for 5 min	Treusch et al. (2005)
Ammonia oxidation	amo643R	TCC CAC TTW GAC CAR GCG GCC ATC CA	35 cycles of: 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min	Treusch et al. (2005)
			Final elongation step of 72 °C for 10 min	
amoAbacterial	amoA-1F	GGG GTT TCT ACT GGT GGT	Initial denaturing: 94 °C for 5 min	Rotthauwe et al. (1997)
Ammonia oxidation	amoA-2R	CCC CTC KGS AAA GCC TTC TTC	35 cycles of: 94 °C for 1 min, 57 °C for 1 min 30 s, 72 °C for 1 min 30 s	Rotthauwe et al. (1997)
			Final elongation step of 72 °C for 10 min	
napA	napA v66	TAY TTY YTN HSN AAR ATH ATG TAY GG	Initial denaturing: 94 °C for 5 min	Flanagana et al. (2006)
Nitrite oxidation	napA v67	DAT NGG RTG CAT YTC NGC CAT RTT	35 cycles of: 94 °C for 30 s, 49 °C for 40 s, 72 °C for 30 s	Flanagana et al. (2006)
			Final elongation step of 72 °C for 10 min	

Table 3 continued

Target gene and metabolic pathway involvement of gene product	Primer pair	5′–3′ Nucleotide sequence	PCR cycling conditions	References
narG	narG 1960F	TAY GTS GGS CAR GAR AA	Initial denaturing: 95 °C for 5 min	Philippot et al. (2002)
Nitrite oxidation	narG 2659R	TTY TCR TAC CAB GTB GC	8 Touchdown cycles of : 94 °C for 30 s, 59 °C for 45 s (dropping the temperature by 0.5 °C each cycle for 8 cycles), 72 °C for 45 s	Philippot et al. (2002)
			30 cycles of: 94 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s	
			Final elongation step of 72 °C for 7 min	
nirS	nirS1F	CCT AYT GGC CGC CRC ART	Initial denaturing: 95 °C for 2 min	Braker et al. (1998)
Nitrite reduction	nirS6R	CGT TGA ACT TRC CGG T	30 cycles of: 95 °C for 1 min, 61 °C for 1 min, 72 °C for 1 min	Braker et al. (1998)
			Final elongation step of 72 °C for 10 min	
qnorB	q-norB2F	GGN CAY CAR GGN TAY GA	Initial denaturing: 94 °C for 5 min	Braker and Tiedje (2003)
Nitric oxide reduction	q-norB5R	ACC CAN AGR TGN CAN ACC CAC CA	35 cycles of: 94 °C for 30 s, 55 °C for 40 s, 72 °C for 30 s	Braker and Tiedje (2003)
			Final elongation step of 72 °C for 10 min	
nosZ	nosZ 752F	ACC GAY GGS ACC TAY GAY GG	Initial denaturing: 94 °C for 5 min	Hunter et al. (2006)
Nitrous oxide reduction	nosZ 1773	ATR TCG ATC ARY TGN TCR TT	35 cycles of: 94 °C for 30 s, 57 °C for 1 min, 72 °C for 1 min	Scala and Kerkhof (1998)
			Final elongation step of 72 °C for 10 min	
nrfA	nrfA 2F	CAC GAC AGC AAG ACT GCC G	Attempted: initial denaturing: 94 °C for 5 min	Smith et al. (2007)
Dissimilatory nitrate reduction to ammonium	nrfA 2R	CCG GCA CTT TCG AGC CC	30 to 35 cycles of: 94 °C for 10 to 45 s, 53 °C to 62 °C for 40 s to 1 min, 72 °C for 40 s to 1 min	Smith et al. (2007)
			Final elongation step of 72 °C for 10 min	
Hao	Brod 541F	GAG CAC GTA GGT GGG TTT GT	Attempted: initial denaturing: 94 °C for 3 min	Penton et al. (2006)
Anammox	Brod 1260R	GGA TTC GCT TCA CCT CTC GG	30 cycles of: 95 °C for 45 s, 55 °C to 60 °C for 1 min, 72 °C for 1 min	Penton et al. (2006)
			Final elongation step of 72 °C for 10 min	

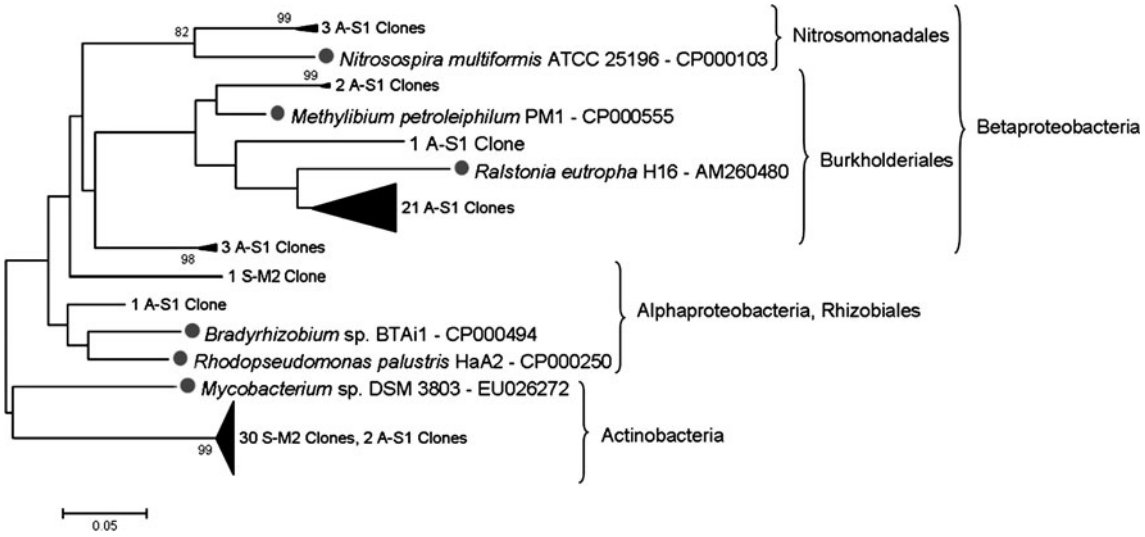

Fig. 3 Neighbour-joining phylogeny of CbbLR protein sequences, cloned from Antarctic Signy Island (A-S1) and Svalbard Midtre Lovénbreen (S-M2) locations and from closest-related GenBank database sequences. The number of clones within each collapsed tree branch is indicated. Closest-related database sequences (with accession number) are indicated by a *circle*. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 5 % sequence divergence

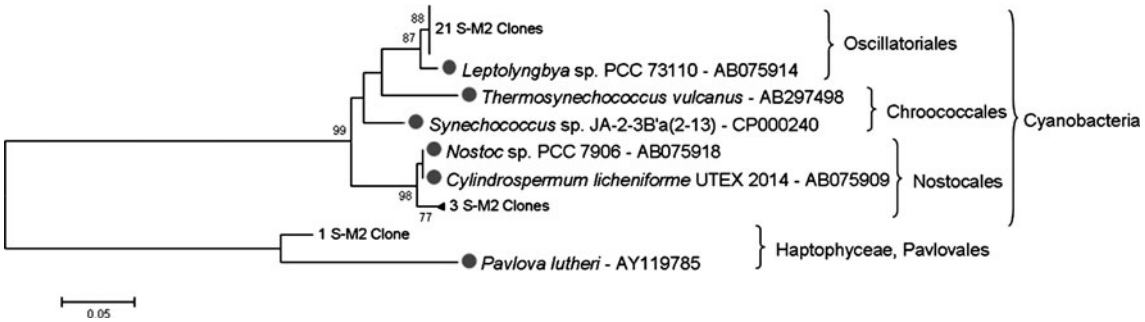

Fig. 4 Neighbour-joining phylogeny of CbbLG protein sequences, cloned from Svalbard Midtre Lovénbreen (S-M2) using RubIg oligonucleotide primers (Spiridonova et al. 2004) and from closest-related GenBank database sequences. The number of clones within each collapsed branch is indicated. Closest related database sequences (with accession number) are indicated by a *circle*. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 5 % sequence divergence

Nucleotide sequence accession numbers

Nucleotide sequences were submitted to the EMBL nucleotide sequence database. The following accession numbers were created: *cbblR*: HE774752–HE774812, *cbblG*: HE774813–HE774836, *rbcL*: HE774837–HE774905, *napA*: HE774906–HE774915, *narG*: HE774916–HE775005, *qnorB*: HE775006–HE775016, *nosZ*: HE775017–HE775086, *amoA*: HE792983–HE793010, *nirS*: HE793011–HE793030.

Results

Distribution and diversity of cbbL genes within cryoconite communities

To investigate the presence and diversity of non-green bacterial primary producers, partial *cbbLR* sequences, encoding the large subunit of form I red-like RubisCO, were amplified from all of the cryoconite communities studied (five communities were investigated from three different glacial locations), using primers designed by Selesi et al. (2005) (Table 3). When gene products, amplified from a Svalbard Midtre Lovénbreen and an Antarctic Signy Island cryoconite community, were sequenced, the phylogenetic diversity of these two communities differed (Fig. 3). The majority of the Midtre Lovénbreen gene sequences (97 %) were most closely related (74–76 % identity) to *cbbL* gene sequences from an Actinobacteria *Mycobacterium* organism (accession number: EU026272, Park et al. 2009), while only 6 % of the Signy Island sequences were most closely related to this sequence (with each clone sequence sharing 75 % identity to the database sequence). The majority of the Signy Island sequences (72 %) were most closely related (80–100 % identity) to sequences of the *cbbL* gene from Betaproteobacteria Burkholderiales, for example,

Table 4 PCR detection of functional genes from DNA isolated from cryoconite communities originating from Antarctic, Svalbard, Greenland and Norwegian locations

		Antarctic	Svalbard								Norway	Greenland	
		Signy Island	Midre Lovénbreen				Foxfonna	Longyearbreen	Rieperbreen	Vestfonna	Jostedalsbreen	Kangerlussuaq	Kronprins Christian Land
		A-S1	S-M2	S-M5	S-M6	S-M16	S-F2	S-L1	S-R1	S-V1	N-J1	G-K2	G-Kp1
Carbon Cycling	*cbbLR*	Seq	Seq	Prod	Prod						Prod		
	rublg	Prod	Seq	Prod	Prod	Prod					Prod		
	rbcL	Seq	Seq	Prod	Prod	Prod					Prod		
Nitrogen Cycling	*nifH*	X	X	X			X			X			X
	amoAarc	Seq	Multi	Multi	Multi		Multi	Multi	Multi	Multi		Multi	Multi
	amoAbac	Seq	Multi	Multi	Multi		Multi	Multi	Seq	Multi		Multi	Multi
	napA	Prod		Prod	Prod		Seq	Multi					
	narG	Seq	Prod	Seq			Seq		Seq	Seq	Prod		Prod
	nirS	Seq	Multi	Prod	Multi	Multi	Seq			Prod			
	qnorB						Seq						
	nosZ	Seq	Multi		Multi	Multi	Seq	Multi	Multi	Seq	Seq	Multi	X
	nrfA	X	X	X	X	X	X	X	X	X	X		X
	brod	X	X		X		X	X	X	X	X		X

Seq = PCR products were amplified, and clones containing these products were sequenced to identify the functional gene of interest. Prod = PCR products of the correct size were amplified using oligonucleotide primers specific to the functional gene of interest; however, no further analysis was performed on these products. Multi = PCR products were amplified using oligonucleotide primers specific to the gene of interest; however, products of multiple sizes were amplified, one of which was of the correct size. No further analysis was performed on these products. X = No products, or no products of the correct size, were amplified using oligonucleotide primers specific to the gene of interest

Ralstonia eutropha (accession number: AM260480, Pohlmann et al. 2007). Both the Signy Island library and the Midtre Lovénbreen library each contained a single clone that was most closely related to Alphaproteobacteria Rhizobiales *cbbL* sequences (82 and 81 % identity, respectively; Fig. 3).

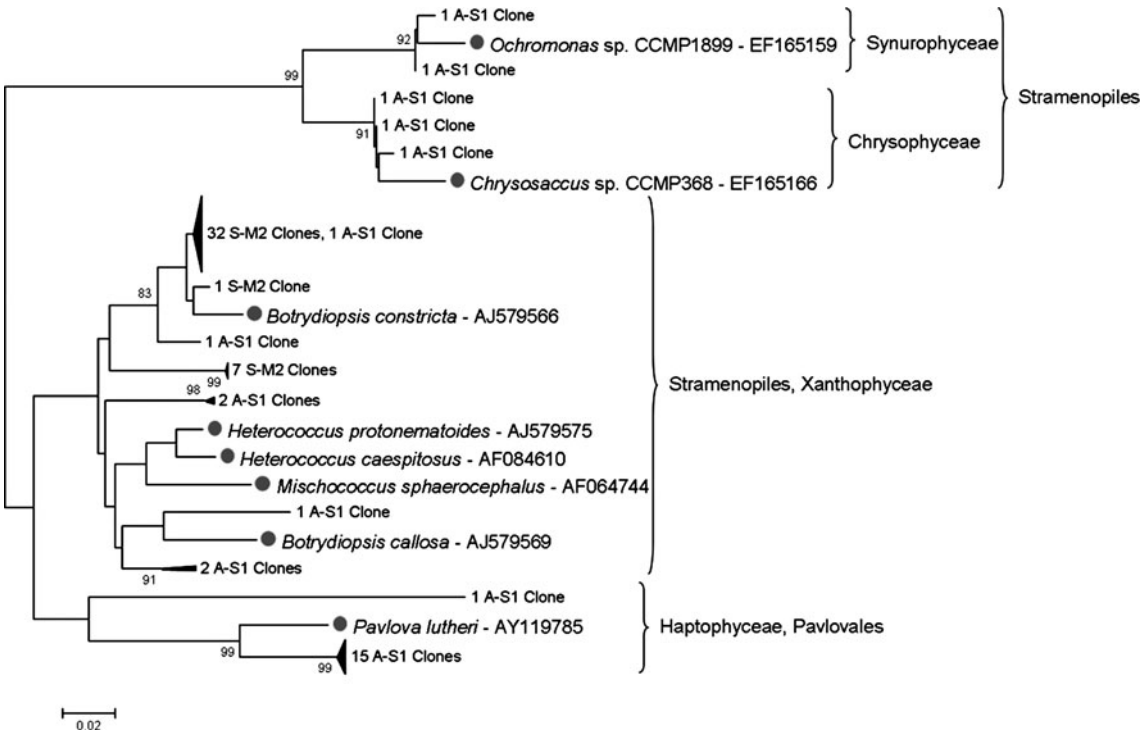

Fig. 5 Neighbour-joining phylogeny of RbcL protein sequences, cloned from Antarctic Signy Island (A-S1) and Svalbard Midtre Lovénbreen (S-M2) locations and from closest-related GenBank database sequences. The number of clones within each collapsed branch is indicated. Closest-related database sequences (with accession number) are indicated by a *circle*. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 2 % sequence divergence

Amplifications of the *cbbLG* gene, encoding the large subunit of form I green-like RubisCO, yielded PCR products of the correct size in all of the communities studied (six communities were investigated from three different glacial locations) when amplified using the RubIg primer set (Spiridonova et al. 2004; Table 3). When products from a Svalbard Midtre Lovénbreen community were sequenced, the majority of the clones (96 %) were found to be most closely related to cyanobacterial *cbbL* gene sequences (Fig. 4). 84 % of the clones were most closely related to Cyanobacteria Oscillatoriales *Leptolyngbya* sequences (e.g. accession number: AB075914, Tomitani et al. 2006, 83–86 % identity), while 12 % of the clones were most closely related to a Cyanobacteria *Nostoc* sequence (accession number: AB075918, Tomitani et al. 2006, 88–90 % identity). One clone showed 90 % identity to a eukaryotic haptophyceae *Pavlova lutheri* sequence (accession number: AY119785, Yoon et al. 2002; Fig. 4).

Primer sets, designed to amplify genes encoding the large subunit of RubisCO within eukaryotes (Wawrik et al. 2002) were used to investigate the presence and diversity of these genes within cryoconite communities. *rbcL* genes were amplified from all of the communities investigated (six communities were investigated from three different glacial locations; Table 4). Clones from an Arctic (S-M2) and an Antarctic (A-S1) community were sequenced and revealed distinct communities. Clones from the Svalbard Midtre Lovénbreen community were all closely related to Stramenopiles Xanthophyceae organisms, with the majority of the clones (82.5 %) having 98–99 % identity to *Botrydiopsis constricta rbcL* sequences (accession number: AJ579566, Negrisolo et al. 2004). Clones from the Antarctic Signy Island community contained sequences related to a greater range of taxa, including those from Stramenopiles and Haptophyceae (Fig. 5). Of the Stramenopiles identified within the A-S1 community, clones with similarities to Xanthophyceae, Synurophyceae and Chrysophyceae sequences were identified (Fig. 5).

Distribution and diversity of nitrogen cycling functional genes within cryoconite communities

Nitrogen fixation

The presence of a nitrogen fixing community within cryoconite was investigated through the identification of the *nifH* gene, one of three genes encoding the nitrogen fixating nitrogenase enzyme. Despite several attempts to optimise PCR conditions, amplification of the *nifH* gene from cryoconite communities was unsuccessful when degenerate oligonucleotide primers, designed by Zehr and McReynolds (1989), were used (six communities were investigated from five different glacial locations; Table 4).

Nitrification

To investigate the presence and diversity of a nitrifying community, bacterial and archaeal *amoA* genes, encoding ammonia monooxygenase enzymes that catalyse ammonia oxidation, were amplified from cryoconite. Batcerial- and archaeal-related PCR products were amplified from all 10 of the communities investigated (from eight different glacial locations); however, both bacterial- and archaeal-specific *amoA* gene primers produced amplicons of multiple lengths from each of the communities studied (Table 4). When PCR products from the Signy Island community (A-S1) were excised and sequenced, bacterial- and archaeal-specific *amoA* genes were confirmed to be present. Bacterial-related *amoA* genes were similarly isolated and sequenced from the Svalbard Rieperbreen community. Purification and sequencing of archaeal-specific *amoA* gene products (*amoAarc*; Treusch et al. 2005) of the correct size [around 532 base pairs (bp)] from the A-S1 community resulted in five clones, all of which were closely related (sharing 93–96 %

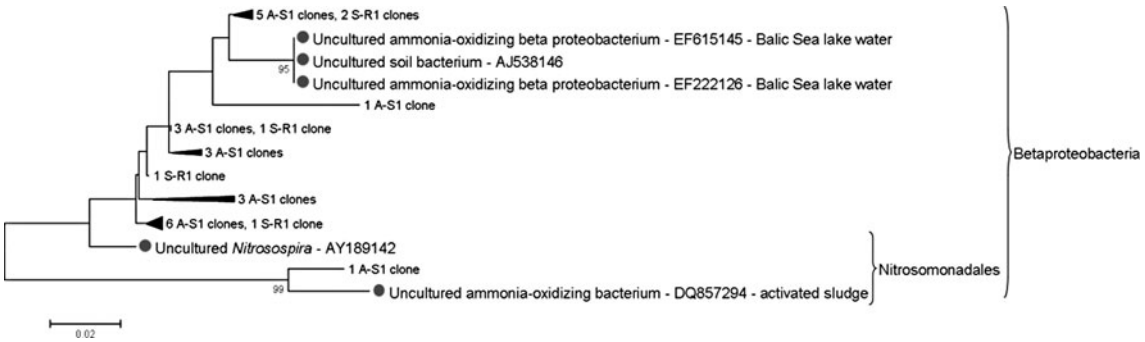

Fig. 6 Neighbour-joining phylogeny of AmoAbac protein sequences, cloned from Antarctic Signy Island (A-S1) and Svalbard Reiperbreen (S-R1) locations and closest-related GenBank database sequences. The number of clones within each collapsed branch is indicated. Closest related database sequences (with accession number) are indicated by a *circle,* and the environmental origin of uncultured clones is detailed. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 2 % sequence divergence

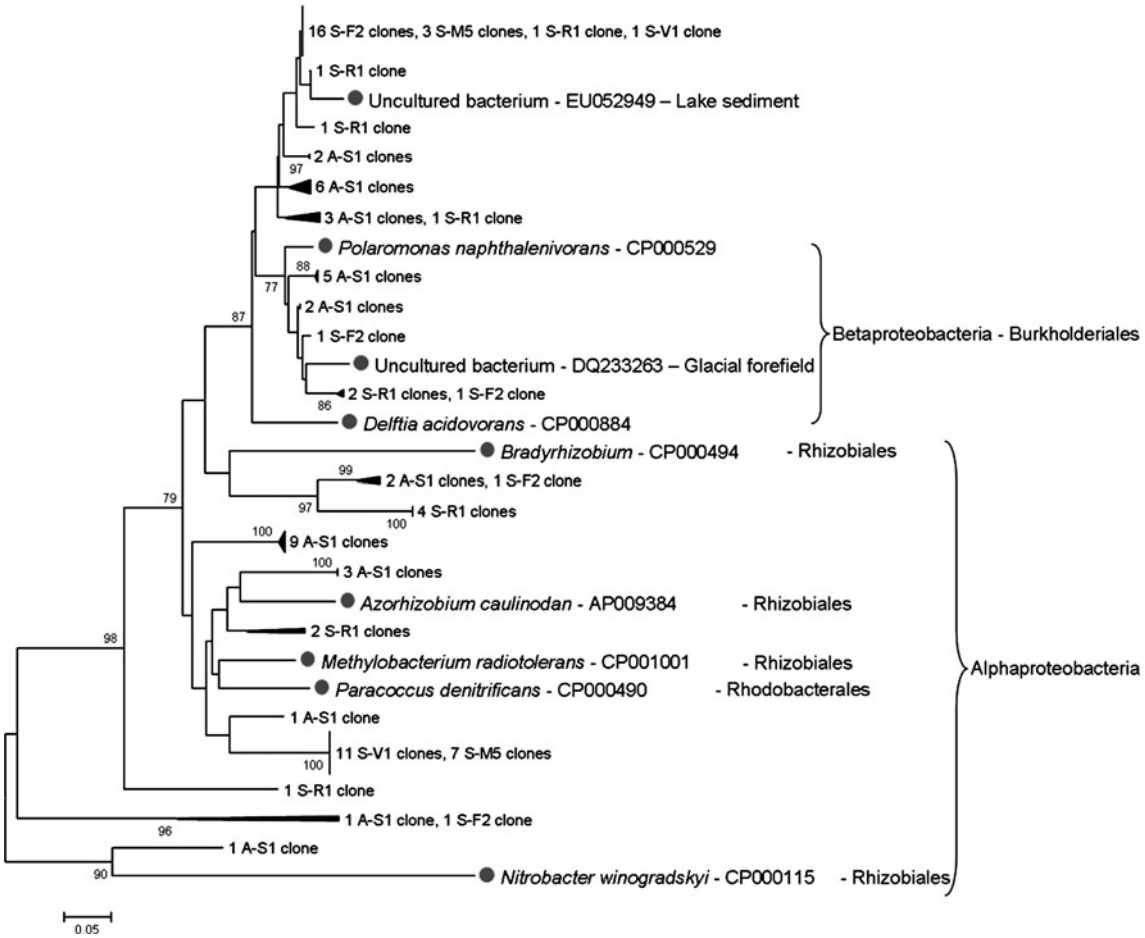

Fig. 7 Neighbour-joining phylogeny of NarG protein sequences, cloned from Antarctic Signy Island (A-S1), Svalbard Reiperbreen (S-R1), Svalbard Midtre Lovénbreen (S-M5) and Svalbard Vestfonna (S-V1) locations and closest-related GenBank database sequences. The number of clones within each collapsed branch is indicated. Closest-related database sequences (with accession number) are indicated by a *circle,* and the environmental origin of uncultured clones is detailed. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 5 % sequence divergence

identity) to *amoA* genes of uncultured Crenarchaeote (now Thaumarchaeota) clones (e.g. accession number: EU022817, Santoro et al. 2008). Amplification and sequencing of bacterial-specific *amoA* genes of the correct size (approximately 470 bp), using primers designed by Rotthauwe et al. (1997) (*amoAbac*), from an Antarctic Signy Island (A-S1) and a Svalbard Rieperbreen (S-R1) community was successfully undertaken. Sequencing of these bacterial *amoA* products revealed clones that were all closely related (92–97 % identity) to *amoA* genes from uncultured ammonia-oxidising Betaproteobacteria (e.g. accession number: EF615145, Kim et al. 2008) or from uncultured Betaproteobacteria belonging to the Nitrosomonadaceae family (e.g. accession number: AY189142, Mintie et al. 2003; Fig. 6).

Nitrate reduction

Cryoconite communities were analysed for the presence and diversity of genes encoding protein products essential for denitrification and DNRA and for the presence of 16S rRNA genes of anammox bacteria. Genes encoding periplasmic and membrane-bound nitrate reductase (*napA* and *narG*, respectively) were amplified from cryoconite to investigate the presence and diversity of communities capable of reducing nitrate to nitrite. *napA* and/or *narG* genes were amplified from all ten of the cryoconite communities investigated (from eight different glacial locations; Table 4). A clone library was made of *napA* genes from a Svalbard Foxfonna community (S-F2), in which *napA* clone sequences shared 76–84 % identity to the most closely related cultured sequences, which belonged to either Alpha-, Beta- or Gammaproteobacteria classes. Clone libraries of the *narG* gene were constructed from an Antarctic Signy Island community and from Svalbard communities, including Midtre Lovénbreen, Foxfonna, Rieperbreen and Vestfonna. When clone sequences were compared to the GenBank database, the sequences that were most closely related (89–94 % identity) were those of

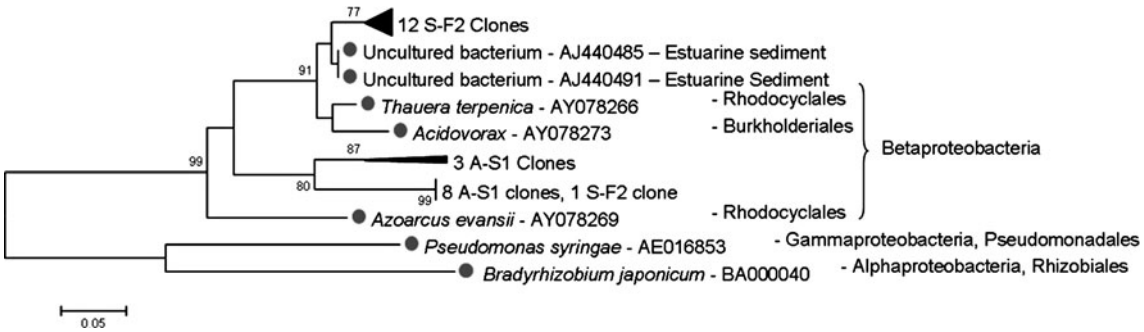

Fig. 8 Neighbour-joining phylogeny of NirS protein sequences, cloned from Antarctic Signy Island (A-S1) and Svalbard Foxfonna (S-F2) locations and closest-related GenBank database sequences. The number of clones within each collapsed branch is indicated. Closest-related database sequences (with accession number) are indicated by a *circle,* and the environmental origin of uncultured clones is detailed. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 5 % sequence divergence

narG genes belonging to unidentified or uncultured bacterial clones with no classification descriptions (e.g. accession number: EU052949, Strief et al. unpublished; Fig. 7). Lower levels of similarity (80–91 % identity) were seen between clone sequences and sequences related to those from Alphaproteobacteria, for example the Rhodobacterales *Paracoccus denitrificans* (accession number: CP000490, Copeland et al. unpublished) and the Rhizobiales *Bradyrhizobium* (accession number: CP000494, Giraud et al. 2007), as well as to Betaproteobacteria Burkholderiales, for example *Polaromonas naphthalenivorans* (accession number: CP000529, Copeland et al. unpublished; Fig. 7).

Genes associated with the reduction of nitrite were also investigated within cryoconite communities. *nirS* genes, encoding nitrite reductase proteins, which are essential for denitrification, were amplified from all seven of the communities investigated (from four different glacial locations; Table 4). However, PCR products from several of the communities contained fragments of multiple lengths, one of which was of the expected 858 bp length. PCR products from an Antarctic Signy Island (A-S1) and a Svalbard Foxfonna (S-F2) community were sequenced and were found to be most closely related (79–100 % identity) to *nirS* sequences from uncultured bacterial clones (e.g. accession number: AJ440485, Nogales et al. 2002). Cryoconite *nirS* sequences were identified with lower levels of identity (80–93 % identity) to members of four orders; Betaproteobacteria Rhodocyclales, for example *Thauera terpenica* (accession number: AY078266, Song and Ward 2006), Betaproteobacteria Burkholderiales, for example *Acidovorax* (accession number: AY078273, Song and Ward 2006), Alphaproteobacteria Rhizobiales, for example *Bradyrhizobium japonicum* (accession number: BA000040, Kaneko et al. 2002) and Gammaproteobacteria Pseudomonadales, for example *Pseudomonas syringae* (accession number: AE016853, Buell et al. 2003; Fig. 8).

The presence of *nrfA* genes within cryoconite communities, which encode nitrite reductase enzymes for DNRA, was investigated. PCR products of varying sizes were amplified from several communities when *nrfA* gene-specific primers were used. However, attempts to sequence these products were unsuccessful (eleven communities from eight glacial locations were attempted; Table 4). Molecular markers of the anammox reaction (identified using the Brod primer sequence set which targets the 16S rRNA genes of anammox-related bacteria (Penton et al. 2006) were not amplified from any of the cryoconite communities investigated within this study (nine communities from eight glacial locations were attempted; Table 4).

The nitric oxide reductase gene (*qnorB*), whose protein product is responsible for catalysing the reduction of nitric oxide to nitrous oxide, was amplified and sequenced from a Svalbard Foxfonna cryoconite community (S-F2) (Table 4). When sequences were compared to the GenBank database, similarities to the most closely related sequences ranged from 79 to 83 %. Three sequences with over 80 % identity to the cloned sequences were identified, including the Alphaproteobacteria *Xanthobacter autotrophicus* (accession number: CP000781, Copeland et al. unpublished, 81 % identity), the Deltaproteobacteria *Sorangium cellulosum* (accession number: AM746676, Schneiker et al. 2007, 81 % identity) and the Gammaproteobacteria *Actinobacillus succinogenes* (accession number: CP000746, Copeland et al. unpublished, 82 % identity). Further sequences, most closely related to uncultured bacteria clones, were additionally identified.

The nitrous oxide reductase gene (*nosZ*), whose protein product catalyses the reduction of nitrous oxide to nitrogen gas, was amplified and sequenced from four cryoconite communities (Antarctic Signy Island, Norway Jostedalsbreen, Svalbard Foxfonna and Svalbard Vestfonna). PCR products from a further six communities from four different glacial locations were amplified and were found to contain products of multiple lengths, one of which was a product of the expected length (1,021 bp; Table 4). *nosZ* genes were not amplified from the Greenland Kronprins Christian Land community (G-Kp2). When cloned

sequences were compared to sequences in GenBank, highest similarities (89–98 % identity) were found to *nosZ* sequences from uncultured organisms of unknown lineage (e.g. accession number: AM419672, Braker et al. unpublished; Fig. 9). Matches to sequences of classified organisms with over 80 % similarity to the *nosZ* clones included Betaproteobacteria Burkholderiales (e.g. *Ralstonia pickettii*, accession number: CP001069, Lucas et al. unpublished, 80 % identity), Betaproteobacteria Rhodocyclales (e.g. *Azoarcus*, accession number: AM406670, Krause et al. 2006, 80–81 % identity) and Alphaproteobacteria Rhizobiales (*Rhodopseudomonas palustris*, accession number: CP000301, Copeland et al. unpublished, 82–84 % identity; Fig. 9).

Discussion

Genes encoding the large subunit of green-like and red-like bacterial form I RubisCO and eukaryotic form I RubisCO were identified through PCR amplification within all of the cryoconite communities investigated from Antarctic, Svalbard and Norway locations. Functional genes, associated with the carbon cycling of glacial microbial communities, have not been reported previously. However, molecular and observational diversity studies of cryoconite communities have revealed the presence of photosynthetic organisms, including Cyanobacteria and Chloroplastida (Gerdel and Drouet 1960; Christner et al. 2003; Mueller and Pollard 2004; Edwards et al. 2011). Additionally, studies measuring the rates of photosynthesis, respiration and protein synthesis (Säwström et al. 2002; Foreman et al. 2007; Hodson et al. 2007; Stibal and Tranter 2007; Anesio et al. 2009; Telling et al. 2010), and the acquisition, storage and loss of carbon from cryoconite holes (Lafrenière and Sharp 2004; Stibal et al. 2008) have revealed that carbon is imported, cycled within and exported out of this ecosystem during summer. Clones related to members of the Cyanobacteria Oscillatoriales and Nostocales orders were amplified from a Svalbard Midtre Lovénbreen community (S-M2) using primer sets designed to amplify the *cbbLG* gene. Other cryoconite diversity studies have also identified these Cyanobacterial classes (Christner et al. 2003; Porazinska et al. 2004; Mueller and Pollard 2004; Edwards et al. 2011; Cameron et al. 2012). Autotrophic bacteria containing red-like type I RubisCO *cbbLR* genes were

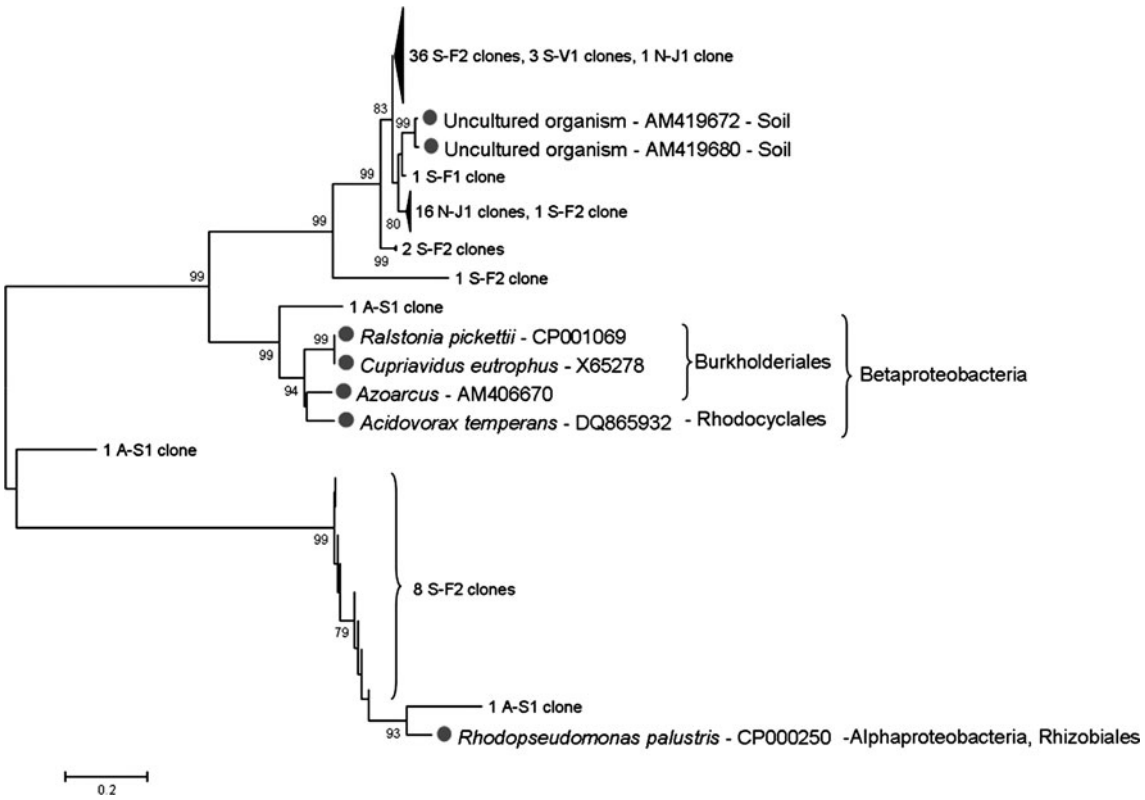

Fig. 9 Neighbour-joining phylogeny of NosZ protein sequences, cloned from Antarctic Signy Island (A-S1), Svalbard Foxfonna (S-F2), Svalbard Vestfonna (S-V1) and Norway Jostedalsbreen (N-J1) locations and closest-related GenBank database sequences. The number of clones within each collapsed branch is indicated. Closest-related database sequences (with accession number) are indicated by a *circle,* and the environmental origin of uncultured clones is detailed. Bootstrap values of >75 % are shown (from 1,000 replicates). *Scale bar* represents 20 % sequence divergence

identified within Burkholderiales, Actinobacteria and Rhizobiales taxa, which have been previously identified within the A-S1 and/or S-M2 communities via 16S rRNA gene analysis (Cameron et al. 2012). Additionally, clones related to the Betaproteobacteria Nitrosomonadales order, which have not been previously described with respect to cryoconite microbial communities, were identified within the A-S1 community.

The presence of eukaryotic autotrophs within cryoconite communities was investigated using primers designed to amplify the *rbcL* gene. Clones related to Stramenopile species, including Synurophyceae, Chrysophyceae and Xanthophyceae classes, were identified within the A-S1 and S-M2 communities, and clones related to Haptophyta Pavlovales were identified within the A-S1 community. Clones related to these organisms have been previously identified through 18S rRNA gene analysis of the same cryoconite communities (Cameron et al. 2012), and similarly, Haptophyta-related clones have been identified within an Antarctic Dry Valley cryoconite community (Christner et al. 2003).

Cyanobacteria have been noted within all cryoconite diversity studies (e.g. Gerdel and Drouet 1960; Wharton et al. 1985; Takeuchi et al. 2001a; Christner et al. 2003), and several studies have described cryoconite communities as being dominated by these organisms (Vincent et al. 2000; Säwström et al. 2002; Mueller and Pollard 2004). In addition to these Cyanobacteria, this current study has identified other organisms, such as photosynthetic Stramenopiles and Proteobacteria, which may also photosynthesize to produce biologically available carbon.

Genes encoding the nitrogenase enzyme for the catalysis of nitrogen fixation were unsuccessfully amplified from any of the cryoconite communities studied, regardless of the primer set having been designed to and having previously targeted Cyanobacterial *nifH* genes (Zehr and McReynolds 1989). Despite this, 16S rRNA gene diversity studies (Cameron et al. 2012) and functional gene studies of the *cbbLG* gene (presented here) identified the presence of Cyanobacteria species belonging to the Nostocaceae family within cryoconite originating from Antarctica, Svalbard and Greenland. Nostocaceae-related organisms contain heterocyst cells that are capable of nitrogen fixation (Tomitani et al. 2006). Furthermore, previous 16S rRNA gene diversity studies of cryoconite communities (e.g. Christner et al. 2003; Cameron et al. 2012) and this current study have revealed a diversity of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes and Archaea, a proportion of which may have the genetic potential to fix nitrogen. Further investigations using alternative *nifH* primer designs may be successful in identifying the range of organisms within these communities that can fix nitrogen. Although the presence of nitrogen fixation genes found within communities associated with supraglacial environments has not previously been published, Arctic subglacial environments (Boyd et al. 2011), Antarctic microbial mats (Jungblut and Neilan 2009) and microorganisms present within the ice cover of an Antarctic frozen lake (Olson et al. 1998) have been found to contain *nifH* genes. The glacial snowpack has been recognised as a major store and source of nutrients such as ammonium (Tranter et al. 1993; Kuhn 2001; Hodson et al. 2005). If cryoconite communities, containing *nifH* genes, are able to actively fix nitrogen (as demonstrated upon Midtre Lovénbreen; Telling et al. 2011 and upon Leverette glacier, Greenland; Telling et al. open discussion article), these supraglacial niches will contribute a further source of ammonium to downstream habitats.

Nitrification genes were amplified and sequenced from organisms related to Betaproteobacteria from both Arctic and Antarctic cryoconite communities and to archaeal Thaumarchaeota species from Antarctic cryoconite communities. The genetic potential for nitrification and measurements of nitrification process rates have not been previously studied within the supraglacial environment. However, nutrient budget studies have found that microbially mediated nitrification occurs in the region between the supraglacial snowpack and the ice margin, most likely within sedimentary environments such as cryoconite, subglacial till, lateral moraine and talus slopes (Tranter et al. 1994; Hodson et al. 2005; Wynn et al. 2007; Hodson et al. 2010a, b). Its occurrence in debris-poor habitats such as the snowpack is suspected, but nutrient addition experiments remain unequivocal (Wynn et al. 2007). Interestingly, an Arctic subglacial study (Boyd et al. 2011) identified similar communities of bacterial ammonia-oxidising *amoA* genes as have been found within this current study. In addition, these subglacial communities have been found to contain archaeal *amoA* genes (Boyd et al. 2011). The similarity of these ammonia-oxidising communities, between supraglacial and subglacial environments, is suggestive that supraglacial systems, such as cryoconite holes, act as microbial sources to the subglacial ecosystem.

Genes encoding enzymes to catalyse the four dissimilatory steps of denitrification, enabling the reduction of nitrate to dinitrogen gas, were amplified from several cryoconite communities of Arctic and Antarctic origin. The amplification and sequencing of these denitrifying functional genes revealed a diversity of Betaproteobacteria Burkholderiales, capable of catalysing each of the four stages of denitrification. Additionally, clones relating to Betaproteobacteria Rhodocyclales and Alphaproteobacteria Rhizobiales and Rhodobacterales were also identified through the amplification of two or more denitrifying functional genes. Denitrification processes have not been previously noted within supraglacial environments.

However, subglacial microbial communities have been thought to reduce or deplete surface originating nitrate stocks through denitrification (Tranter et al. 1994; Hodson et al. 2005). Stable isotope measurements of $^{15}N–NO_3$ presented by Wynn et al. (2006) also provide compelling evidence for the occurrence of this process at Midtre Lovénbreen. A Canadian Arctic subglacial *narG* gene study similarly found a high diversity of organisms capable of nitrate reduction by nitrate reductase (Boyd et al. 2011). In this subglacial community analysis by Boyd et al., many clones were featured with a high similarity to *Polaromonas naphthalenivorans*, as was similarly found within this present study.

Given the typical environmental conditions of cryoconite water (an oxidising environment with Eh values of over 300 mV; Foreman et al. 2007), the reductive process of denitrification seems an unlikely process to occur here. This is especially true when compared to the low redox environment of the subglacial environment (Eh = 90 mV; Wynn et al. 2007; Mikucki et al. 2009). However, the centre of each cryoconite granule is often a region of dark decomposing matter (Takeuchi et al. 2001a; Langford et al. 2011), which may provide an anoxic microzone for denitrification to occur. Similarly, cryoconite communities, carrying the genetic ability for denitrification, may be flushed into subglacial zones, where they may commence the denitrification process and seed the subglacial ecosystem. Interestingly, microbial communities found on the soils of a glacial forefield have been found to contain genes responsible for denitrification (Deiglmayr et al. 2006; Kandeler et al. 2006). Furthermore, within this current study, several Arctic and Antarctic *narG* sequences were most closely matched to *narG* sequences originating from a glacial forefield (accession number: DQ233263; Deiglmayr et al. 2006). Thus, it may be possible that the transportation of cryoconite either through the subglacial environment to the glacial forefield, or directly from the glacier surface during ice retreat, may contribute to the colonisation of these denitrifying communities (Porazinska et al. 2004; Foreman et al. 2007; Řehák et al. 2007; Hodson et al. 2008; Schütte et al. 2009). Similarly, the aeolian and aqueous transportation of fragments of local, and perhaps distant, niches onto the glacial surface are likely to be a major seedling factor of cryoconite communities and thus will have an influence over their functional potential (Mueller et al. 2001; Cameron et al. 2012). However, many of the sites in this current study are in close proximity to maritime, soil, lake, river and/or rock environments, and thus, the physical and chemical conditions of the supraglacial environment may be so different that they render alien populations from the surrounding environment inactive.

Genes encoding the periplasmic nitrite reductase enzyme, which catalyses the ammonification of nitrite (DNRA), were not identified by gene sequence analysis within this study. The inability to amplify *nrfA* genes raises the question of whether these systems do in fact have the genetic potential to process the direct reduction of nitrite to ammonium, or whether these genes were simply not detected due to methodological reasons (von Wintzingerode et al. 1997). DNRA processes may compete with denitrification under anaerobic conditions, influenced by nitrate concentrations and the environmental redox potential (Matheson et al. 2002; Dong et al. 2009). As with denitrification processes, DNRA reactions may be limited or absent within cryoconite holes. However, even the dispersal of small numbers of DNRA-capable organisms to other niches (with lower redox potentials) may enable these communities to thrive. Within the low nutrient abundance that is typical of glacial environments (Table 2), DNRA processes, which prevent the loss of nitrogen from the biosphere, would be advantageous over denitrification (Matheson et al. 2002). Anammox catalysing communities were not identified within cryoconite. However, the Brod primer set (Penton et al. 2006), which was used for analysis, was designed to target 16S rRNA gene sequences of the Planctomycetes phylum, which have not previously been identified within cryoconite communities. Thus, further studies relating to the detection of anammox pathways within these systems are urged in the future.

Conclusion

Genes associated with photosynthesis, nitrification and denitrification were identified within all of the Arctic and Antarctic communities investigated. Additionally, organisms with the ability to perform nitrogen fixation were identified in previous diversity studies. Sequence data from functional gene analyses revealed the potential photosynthetic importance of Cyanobacteria, Actinobacteria, Betaproteobacteria, Stramenopiles and Haptophyceae within cryoconite. Similarly, Betaproteobacteria and Thaumarchaeota organisms, containing genes for nitrification, and Alphaproteobacteria and Betaproteobacteria, carrying genes for nitrate reduction via denitrification, were identified as being potentially important for nitrogen cycling within cryoconite communities. Although the process rates of the biogeochemical reactions under investigation within this current study are unknown, the genetic potential of these communities to undertake microbially mediated carbon and nitrogen cycling is an important one. In addition, the eventual transportation of cryoconite communities to other niches within the biologically sparse glacial environment (Porazinska et al. 2004; Foreman et al. 2007; Stibal et al. 2008; Schütte et al. 2009) may provide a

valuable source of organisms with the potential to perform biogeochemical cycling processes elsewhere.

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