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Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems

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Abstract

By way of metagenomics and high-throughput pyrosequencing, we addressed the hypothesis that cyanobacterial mats in polar aquatic ecosystems maintain a nutrient-rich microenvironment via decomposition and scavenging processes. Analysis of more than 592,554 genomic deoxyribonucleic acid (DNA) reads (total of 11.5 million base pairs) showed that the ribosomal and protein-coding genes of two High Arctic ice-shelf mat communities were dominated by *Proteobacteria*, not *Cyanobacteria*, which implies a broad range of bacterial decomposition and nutrient recycling processes in addition to phototrophy. Principal component analysis of genes for light-, nitrogen-, and phosphorus-related processes provided evidence of partitioning of mat function among taxonomically different constituents of the mat consortia. Viruses were also present (notably *Alpha-*, *Beta-*, *Gammaproteobacteria* phages and cyanophages), which likely contribute to cellular lysis and recycling, as well as other Bacteria, Archaea, and microbial eukaryotes. Nitrogen-related genes were dominated by ammonium-assimilation systems, implying that the microbial mats are sites of intense mineralization, but not N-oxidation, since nitrification genes were absent. Nutrient scavenging systems were detected, including genes for transport proteins and enzymes for converting larger molecules into more readily assimilated inorganic forms (allantoin degradation, cyanate hydrolysis, exophosphatases, phosphonatases). Metagenomic profiling results underscore the rich diversity of microbial life even in extreme polar habitats, and the capability of mat consortia to retain and recycle nutrients in the benthic microenvironment.

Cyanobacteria are widely distributed in both freshwater and marine systems and represent the earliest known oxygenic photosynthesizing organisms (Battistuzzi et al. 2004; Altermann 2008). Filamentous forms of *Cyanobacteria* are the primary structuring agents in fossil and modern-day stromatolites and microbialites (Allwood et al. 2006; Kupriyanova et al. 2007) found in shallow, often tropical, marine environments (Reid et al. 2000; Sprachta et al. 2001). They are also the main structuring elements in microbial mats common in extreme habitats, such as geothermal springs, hypersaline basins, hyperoligotrophic ponds, hot and cold deserts, and polar ice shelves (Hoffmann 1999; Ward and Castenholz 2000; Gorbushina 2007). In many environments, the cyanobacterial mats achieve high biomass stocks despite nutrient-poor conditions in the overlying water column, for example, in phosphorus-deficient springs (Breitbart et al. 2008), nutrient-deficient tropical seas (Paerl et al. 2001), and ultra-oligotrophic Arctic (Bonilla et al. 2005) and Antarctic (Hawes and Schwarz 2001) lakes. This implies that cyanobacterial mat communities are capable of scavenging nutrients from the water column and sediments, and tightly recycling these nutrients within the microbial mat environment.

Microbial mat communities are complex consortia of many interdependent species, and they are therefore difficult to study using standard microbiology techniques. An alternative approach is to apply metagenomics, whereby the whole community is profiled by sequencing its total deoxyribonucleic acid (DNA) content. Analysis of the metagenome offers an avenue for identifying all taxa within communities and opens the possibility of exploring metabolic potential within an environment without cultivation (Handelsman 2004; Tyson et al. 2004). Recent application of this approach to compare microbial communities from disparate habitats suggests that there are fundamental differences in major metabolic subsystems linked to environmental factors (Dinsdale et al. 2008). Two low-latitude cyanobacterial microbialite community metagenomes are publicly available (Breitbart et al. 2008; Dinsdale et al. 2008); however, there have been no similar studies in the polar regions, where microbial mats often dominate total ecosystem biomass and productivity (Vincent 2000).

Geochemical analyses of the interstitial water in polar microbial mats have shown that these mats often contain dissolved organic as well as inorganic nutrient concentrations that are one or more orders of magnitude greater than in the overlying water (Mueller and Vincent 2006). Enrichment bioassays in a high arctic lake showed that the phytoplankton in the ultra-oligotrophic water column was highly responsive to nutrient addition, while mat communities at the bottom of the lake showed no effect in terms of biomass or photosynthetic production following nutrient addition over 2 weeks. In the present study, we hypothesized that microbial mat communities are nutrient replete

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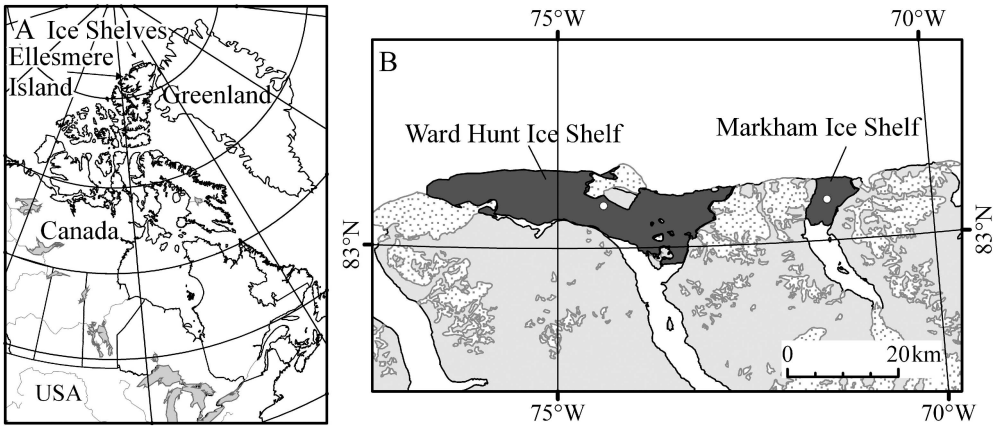

Fig. 1. Location of sampling sites along the northern coast of Ellesmere Island in High Arctic, Canada. Dark gray indicates the extent of the two ice shelves at the time of sampling (2006); the white circles indicate sites where the mats were collected.

because of decomposition and recycling processes within the mat, and they have an ability to scavenge multiple forms of organic and inorganic nutrients. We addressed this hypothesis by way of metagenomic profiling of two High Arctic cyanobacterial mat communities that achieved large standing stocks at the base of clear, nutrient-poor meltwater pools.

Methods

Study sites and sample collection—Sampling and field measurements were conducted from 12 to 14 July 2007 on the Ward Hunt Ice Shelf (WHI) and Markham Ice Shelf (MIS) along the northern coastline of Ellesmere Island in the Canadian High Arctic (Mueller et al. 2005, 2006) (Fig. 1; Table 1).

The extent of the WHI at the time of sampling was ~ 420 km^2. WHI has characteristic parallel ridges and troughs, which in summer form into long meltwater ponds (up to 15 km long, ~ 3 m deep, and 10–20 m wide). MIS was smaller (50 km^2), and in July 2007, about one third of its surface was covered with marine sediments that originated below the ice shelf. This sampling was 1 yr prior to the unusually warm summer of 2008, during which there was a 23% loss of ice-shelf habitat along the northern Ellesmere Island, including complete break-out of the Markham Ice Shelf (Vincent et al. 2009). The mat-harboring sediments occurred on raised mounds of the ice and in meltwater ponds (Mueller et al. 2005). The mats were composed of 1-cm-thick loosely cohesive aggregates (referred to as matlets by Mueller et al. 2005) that were olive-green in color and a thin (100 μm), more cohesive orange layer at the surface, as reported earlier (Vincent et al. 2004*a,b*). Microbial mat material was collected into sterile 50-mL Falcon tubes from 20-cm depth in the meltwater ponds. They were stored in the dark at 0°C to 4°C for 1 to 3 d, then frozen at −20°C, and subsequently transferred to −80°C. The pH, conductivity, and temperature of the overlying water at each mat site were determined using a portable meter (Oakton Instruments, pH/Con 10 Series). Three mats from each ice shelf were pooled to obtain sufficient DNA for metagenomic sequencing.

Table 1. Temperature, pH, and conductivity of the meltwater ponds for the three samples that were pooled for each metagenome. MIS, Markham Ice Shelf; WHI, Ward Hunt Ice Shelf.

Site	Location north	Temperature (°C)	pH	Conductivity (μS cm^{-1})
MIS 1	83°01.899′N 71°30.896′W	1.4	6.29	640
MIS 2	83°01.898′N 71°30.812′W	1.1	6.52	492
MIS 3	83°01.898′N 71°30.800′W	2.8	6.79	779
WHI 1	83°04.960′N 74°26.065′W	0.4	6.14	50.3
WHI 2	83°04.950′N 74°26.225′W	1.5	6.24	740
WHI 3	83°04.949′N 74°26.28′W	0.8	6.98	364

DNA extraction and sequencing—The mat samples were freeze-dried using a Freeze Dryer System 12 (Labconco) to avoid interference from exopolymeric substances (EPS) during subsequent steps. DNA was extracted in 800 μL of XS-buffer (1% potassium-ethyl-xanthogenate; 800 mmol L^{-1} ammonium acetate; 20 mmol L^{-1} ethylenediaminetetra-acetic acid [EDTA]; 1% sodium dodecyl sulfate [SDS]; 100 mmol L^{-1} Tris-HCl, pH 7.4; Tillet and Neilan 2000) and ground with a sterile micropestle. The mixture was incubated at 65°C for 5 h and frozen overnight at −20°C. Cell debris was removed by centrifugation at 12,000 × *g* for 10 min. An equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) was then added to the aqueous phase and centrifuged at 12,000 × *g* for 3 min. The two washing steps were repeated four times. DNA was precipitated by addition of 1 volume of isopropanol and 1/10 volume of 4 mol L^{-1} ammonium acetate overnight at −20°C. The precipitated DNA was concentrated by centrifugation at 12,000 × *g* for 10 min and washed with 70% ethanol. The extracted DNA

was resuspended in 1× Tris-EDTA (TE) buffer. Ribonucleic acid (RNA) was removed from the extracts by addition of 2 μL of ribonuclease A (RNaseA) (Roche, 10 mg mL^{-1}) and incubated for 30 min at 37°C. RNase was removed by one phenol : chloroform : isoamyl alcohol (25 : 24 : 1) step. The DNA was then precipitated, washed, and resuspended in 1× TE buffer. Three DNA extracts from individual mats taken at each sample site were pooled, and ~ 5 μg of total DNA were used for each pyrosequencing run (Margulies et al. 2005) using a 454 Sequencing System (Roche 454 Life Sciences) at the McGill University and Genome Québec Innovation Centre (Montreal, Québec, Canada). While the sequencing protocol is based on polymerase chain reaction (PCR), it avoids cloning steps and associated potential biases. Each read file produced by a pyrosequencing run is associated with one quality file, which contains the quality score for each base.

Bioinformatics and statistical analyses—Potential artificial pyrosequencing replicates were screened for using the 454 replicate filter proposed by Gomez-Alvarez et al. (2009) (http://microbiomes.msu.edu/replicates/). Short or low-quality sequences with ambiguous bases (multiple internal Ns) were not included in our analysis, and only matches > 50 nucleotides and > 65% similarity to a taxonomic group were included.

All metagenomic sequences were compared to ribosomal and protein-coding gene databases using the MetaGenome Rapid Annotation with Subsystem Technology (MG-RAST) server version 2.0 (http://metagenomics.nmpdr.org; Overbeek et al. 2005; Meyer et al. 2008). MG-RAST used Basic Local Alignment Search Tool N (BLASTN) and Basic Local Alignment Search Tool X (BLASTX) (Altschul et al. 1990) algorithms for comparisons with ribosomal genes databases and protein-coding gene databases, respectively. Taxonomic analyses in MG-RAST consisted of comparing our metagenomic sequences against the Ribosomal Database Project (RDP) (release 10; http://rdp.cme.msu.edu/index.jsp; Cole et al. 2005, 2007) for 16S ribosomal ribonucleic acid (rRNA) gene identification and against the European rRNA small subunit (SSU) database to identify 18S rRNA gene sequences. The ribosomal sequences were also compared to published 16S rRNA gene sequences originating from arctic mats in the same geographic area of our study (Bottos et al. 2008; Jungblut et al. 2010) using BLASTN, only matches ≥ 100 nucleotides and ≥ 97% similarity were considered positive (*E*-value of 1×10^{-5}). Further taxonomic information was gained using the MG-RAST server to compare our data sets against the protein-coding gene database http://www.theseed.org/wiki/index.php/Home_of_the_SEED, which is referred to as the SEED database. The two metagenomes are publicly available at this site. Results from the different taxonomic analyses were combined to calculate the final taxonomic classification increasing the number of significant hits (Meyer et al. 2008). Viruses were specifically searched for by way of BLASTN analyses against San Diego State University Center for Universal Microbial Sequencing database (SCUMS at http://scums.sdsu.edu) to identify bacteriophages and against GenBank for eukaryotic viruses: (www.ncbi.nlm.nih.gov/genomes/genlist.cgi?taxid=10239 andtype=5andname=Viruses). We used the metagenomic SEED viewer of the MG-RAST server version 2 to identify sequences matching functions of interest. The metabolic comparisons performed within MG-RAST were conducted on the subsets of sequences showing similarities to each major metabolic process within SEED. The tabular view filters option was used to narrow searches within subsystems and retrieve the number of hits for specific genes. Each sequence corresponding to a function of interest found with the SEED platform was then checked against the GenBank database using BLASTN (www.ncbi.nlm.nih.gov/Genbank/index.html) to retrieve the taxonomic origin of the given sequence. All BLAST output files were parsed by our custom scripts written in Ruby (www.ruby-lang.org/). The best hit for each sequence was automatically selected and classified as "known" if its match against the relevant database was significant (*E*-value < *E*-value cutoff, specified below) or as "unknown" when no significant hit was found in database.

To ascertain the dominant groups involved in light capture and nitrogen and phosphorus uptake pathways, we matched the protein-coding genes to their phyla of origin, or in the case of *Proteobacteria* to class, by comparing our metagenomic sequences against the SEED database using BLASTX. A matrix of the relevant detected genes pooled from both genomes was constructed and analyzed by principal component analysis (PCA) using the PAlaeontological STatistics software (PAST) (Hammer et al. 2001). These PCA ordinations were used to investigate the relationships between taxonomic groups and gene function in the mats. Additional statistical comparisons were tested using SigmaStat software.

Results

Environmental conditions at the two sites were similar; mat temperatures ranged from 0.4°C to 2.8°C. The pH of the waters overlying the mat samples was similar and near neutral, ranging from 6.14 to 6.98. Conductivity was more variable, from 50.3 to 779 μS cm^{-1} (Table 1). There were no consistent or statistically significant differences in terms of environmental variables between the two sites.

The average Guanine-Cytosine (GC) content of the two metagenomes was similar: 55.02% (SD 11.5) for MIS and 53.76% (SD 11.2) for WHI. The two pyrosequence yields were 256,849 base pairs (bp) from Markham Ice Shelf (MIS) and 335,705 bp from Ward Hunt Ice Shelf (WHI), with average lengths of 208 bp and 184 bp, respectively, giving an overall average of 194 bp and total of 11.5 million bp. The number of artificial replicates was relatively small and always < 6%, and all were removed from the analysis. Around one third of the total retained sequences had matches against the SEED Phylogenetic Profile database; 108,338 matches for the MIS samples and 127,728 matches for the WHI samples (Table 2). There were 66,123 significant hits for MIS and 78,144 for WHI against the SEED Metabolic Profile subsystems database (Overbeek et al. 2005; Meyer et al. 2008). The phylogenetic and

Table 2. Percentage of total significant hits found from combined MIS and WHI sequences when compared to databases designated in the top row. Taxonomic analysis based on rRNA genes was performed with BLASTN against RDP II and Silva databases. SEED and Swiss-Prot were used with BLASTX, indicating taxonomic placements based on protein-coding genes. *E*-value cutoff: 1×10^{-5} for all. Total of significant hits from both MIS and WHI sequences; na, not applicable.

	RDP II or Euro rRNA SSU	SEED
Total significant hits	469	236,066
Bacteria		
Cyanobacteria (%)	12.4	22.7
Proteobacteria (%)	20.1	46.7
Bacteroidetes (%)	8.7	10.7
Actinobacteria (%)	4.7	8.3
Firmicutes (%)	0.2	3.1
*Chloroflex*i (%)	1.1	0.9
Other (%)	39.5	5.5
Eukaryotes		
Metazoa (%)	4.7	0.4
Fungi (%)	5.1	0.2
Viridiplantae (%)	0	0.2
Other (%)	2.8	0.06
Archaea		
Crenarchaeota (%)	0	0.06
Euryarchaeota (%)	0	0.9
Other (%)	0	0.002
Virus		
Bacteriophage (%)	na	0.07
Other (%)	na	0.02

functional results were similar for the mats from the two ice shelves (Figs. 2, 3).

Classification of microbial communities based on protein-coding genes—Only 40% of the sequences could be phylogenetically identified to the domain level in SEED database using an *E*-value of 1×10^{-5} (Table 2), and 98% of these were Bacteria, with Eukaryota and Archaea accounting for just under 1% each. The details of taxonomic attributions varied very little between WHI and MIS; both were mostly *Proteobacteria* ($\sim$ 49%) especially the classes *Alphaproteobacteria* and *Betaproteobacteria* (Fig. 2). Among *Deltaproteobacteria*, *Desulfuromonadales*, which are sulfate reducers, were dominant. *Cyanobacteria* represented $\sim$ 18% of total bacterial coding sequences in the MIS sample and were slightly more common in the WHI sample (Fig. 2). The cyanobacterial orders represented were mostly *Nostocales*, *Chroococcales*, and *Oscillatoriales*. Archaea in general were rare, and *Euryarchaeota* were three times more common than *Crenarchaeota*. Eukaryota gene-coding sequences mostly were matched to Metazoa, Fungi, and Viridiplantae (Fig. 2; Table 2).

Taxonomic classification of the microbial communities based on ribosomal genes—Small subunit (SSU) ribosomal genes represented a low percentage ($< 1\%$) of the total sequences (Table 2). One third of the 191 MIS and nearly half of the 263 WHI 16S rRNA gene sequences to Bacteria could not be classified to any lower taxonomic level. The remainder could be matched to phyla, and the majority fell into *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Actinobacteria* (high GC content bacteria) (Table 2). Relaxing the criteria, using an *E*-value of 0.01 against the European rSSU database, suggested that 303 MIS and 338 WHI sequences were from eukaryotes, with $\sim$ 40% of those closest to Metazoa, and the remainder closest to diverse protist taxa and Viridiplantae (Table 3).

A comparison between the metagenomes obtained here and recently published results of clone libraries from WHI and MIS mats increased the taxonomic resolution of some of the pyrosequences. There were 113 hits to bacterial sequences identified from WHI and MSI mats from Bottos et al. (2008), including to sequences of the genera *Cryobacterium*, *Leptothrix*, *Sandarakinorhabdus*, *Sphingomonas*, and *Arenimonas*. Similarly, there were 40 hits to the cyanobacterial sequences from Jungblut et al. (2010). These included, in both metagenomes, three species that were previously thought to be endemic to Antarctica: *Phormidium priestley*, *Leptolyngbya frigida*, and *Leptolyngbya antarctica*.

We found 601 MIS and 655 WHI significant hits to bacteriophages (Table 3). *Alpha-*, *Beta-*, and *Gammaproteobacteria* phages were the dominant sequences, consistent with the dominant representation of their hosts in the microbial mats. Cyanobacterial phages were also abundant in both samples. Eukaryotic viruses were searched for in GenBank, and our sequences had highest similarities to a virus originally described from the green alga *Chlorella*, while 28 were most similar to the *Acanthamoeba* virus *Mimivirus*.

Functional gene analysis—The subsystem distributions for the MIS and WHI samples were similar: $\sim$ 25% of all sequences were classified into 23 functional subsystems (Fig. 3). Among the sequences that were assigned to subsystems, 15% were associated with the category Cluster Based Subsystems, which are genes or features that are relatively conserved along the chromosomes of at least four species but have not been assigned to a specific subsystem (National Microbial Pathogen Data Resource; http://www.nmpdr.org/FIG/wiki/view.cgi). Among the sequences classified within subsystems, carbohydrate metabolism sequences were the most abundant, followed by amino acids and derivatives and protein metabolism (Fig. 3).

To address the question of whether different taxonomic groups had the potential to contribute to different aspects of nutrient recycling or scavenging, we then targeted organic and inorganic nutrient subsystems for nitrogen and phosphorus. We also investigated the taxonomic origin of genes responsible for light capture in this energy-limited system. Among the latter, we identified genes involved in the photosystem subsystems PSI and PSII, proteorhodopsin synthesis, a PSII-like subsystem (bacterial photosynthesis), and phycobiliprotein synthesis.

Overall, using BLASTX against SEED, results indicated 532 MIS and 691 WHI sequences involved in nitrogen cycling

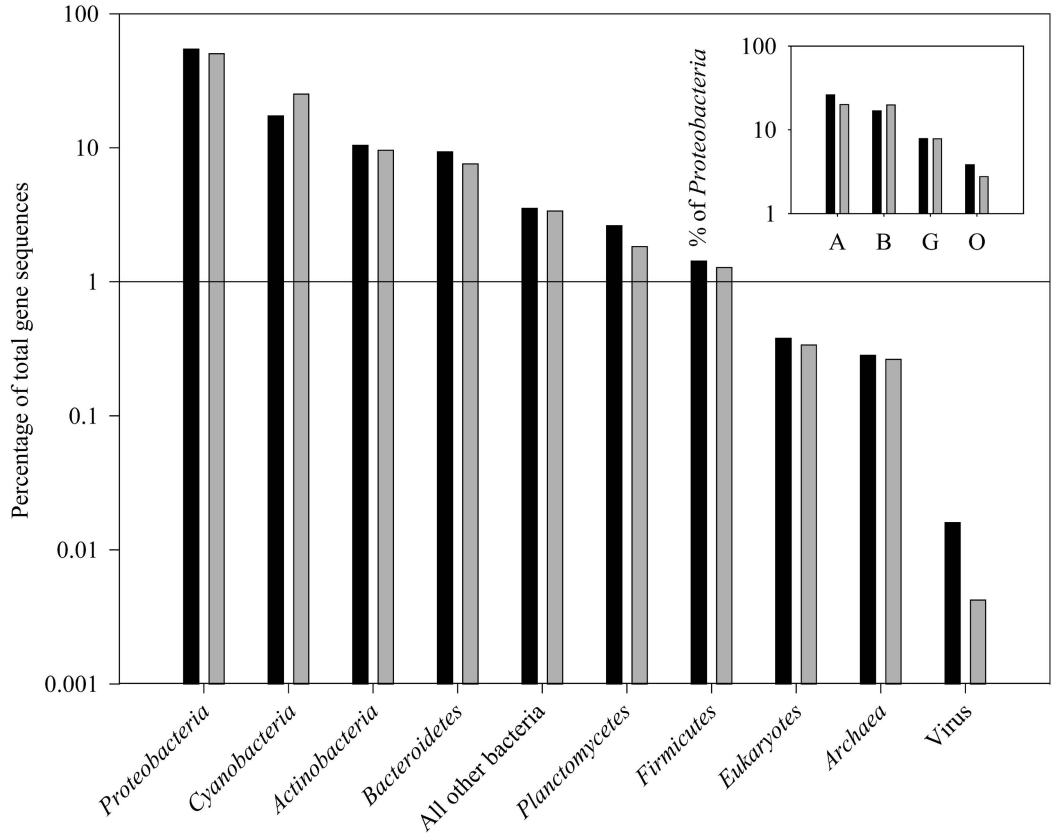

Fig. 2. Inferred taxonomic distributions from MIS (black bars) and WHI (gray bars) microbial mat metagenomes. Sequence classification is based on BLASTX similarities to SEED from protein-coding genes. Insert: contribution of the major classes of *Proteobacteria* (A = *Alphaproteobacteria*; B = *Betaproteobacteria*; G = *Gammaproteobacteria*; O = other *Proteobacteria*). Note the log scale of the *y*-axis in both graphs.

and metabolism, including the ammonia-assimilation subsystem and the nitrate and nitrite ammonification subsystem. Organic nitrogen degradation (e.g., allantoin and chitin degradation) genes accounted for 20% of all nitrogen metabolism genes, whereas 6% of these were grouped into the cyanate hydrolysis subsystem. Nitrogen fixation genes were rare, and no nitrification genes were identified.

Similarly, we identified 1048 MIS and 1281 WHI sequences associated with phosphorus uptake and metabolism, including genes coding for alkylphosphonate transport and assimilation, phosphonate metabolism, and cyanobacterial phosphorus uptake. The majority of these were specifically associated with phosphate metabolism, including genes dedicated to orthophosphate cellular transport, permease proteins PstA and PstC, the adenosine triphosphate-binding protein PstB, and the periplasmic phosphate-binding protein PstS. Other phosphate regulon gene sequences matched *phoB* and *phoR*, the two-component regulatory system of phosphate signal transduction. Phosphate transport system regulation via the Pho regulon was also identified, with genes for PhoU and PhoQ and phosphonatases (2-aminoethylphosphonate transporters, aminotransferase, phosphonoacetaldehyde hydrolase), along with exopolyphosphatase and polyphosphate kinase.

To identify the potential relationships between taxonomic groups and metabolic function, the relevant genes for light harvesting, N cycling, and P cycling were selected and analyzed by PCA. In total, 32 protein-coding genes for these three functional categories were represented from 19 major groups of Bacteria. The PCA ordination for light-related genes showed a strong separation via the first component (which accounted for 86% of the variance) between *Cyanobacteria* plus a group of uncertain affinity and all other taxonomic groups, including the *Alpha-*, *Beta-*, and *Gammaproteobacteria* (Fig. 4A). The closeness of the former two groups along the first axis suggests that the unknown group may be related to *Cyanobacteria*; however, they were well separated by the second PCA component (13%), and their alignment along the PS-II vector suggests that they play a major role in oxygenic photosynthesis, and that some of these organisms may synthesize proteorhodopsin.

PCA analysis for the nitrogen-related genes showed that genes originating from *Proteobacteria* (*Alpha-*, *Beta-*, and *Gamma-*), *Cyanobacteria*, and *Actinobacteria* were well separated along the first component axis from other taxonomic groups (Fig. 4B). These former groups all were located in the same *x*-axis direction of the gene vectors (accounting for 64% of the variance), suggesting their dominance of nitrogen-cycling processes in the mats. The second PCA component (17%) strongly differentiated *Cyanobacteria* from the *Proteobacteria* and *Actinobacteria*. Their ordination loci relative to the principal vectors

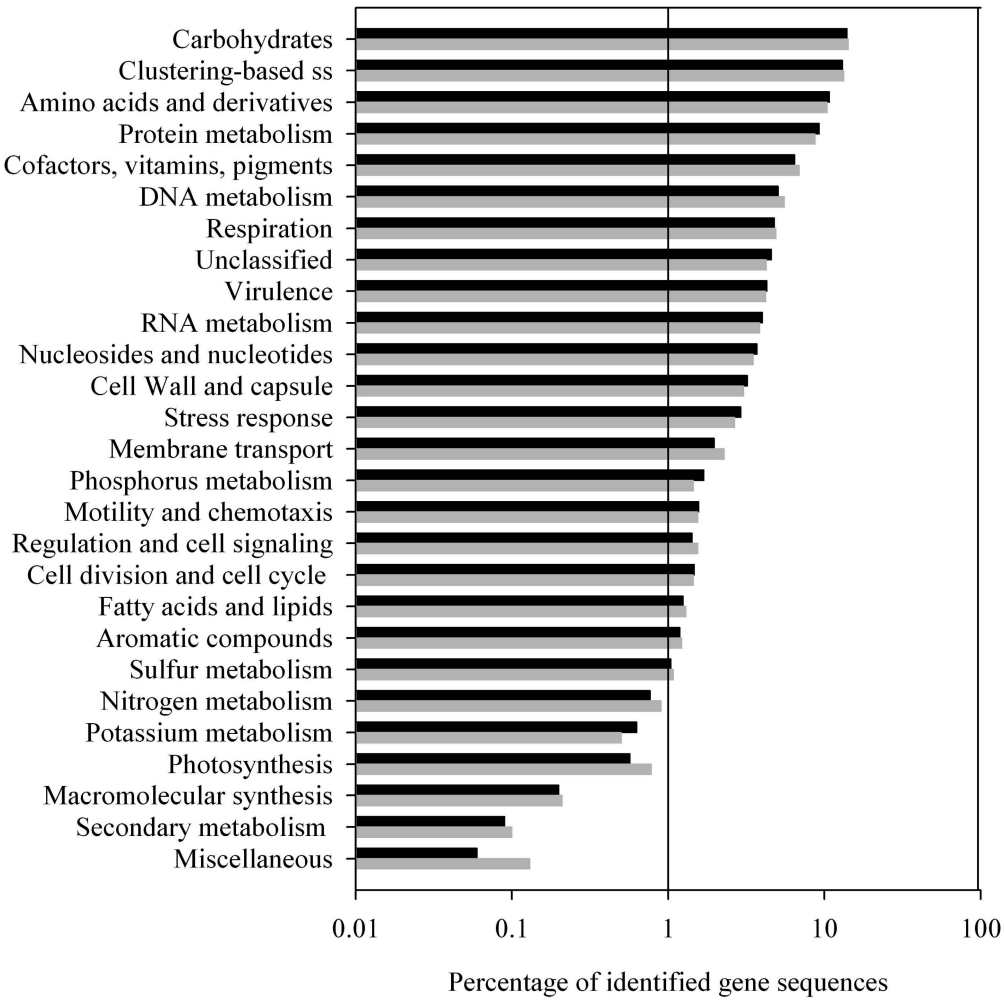

Fig. 3. Percentage of sequences assignable to functional categories for MIS (black bars) and WHI (gray bars) metagenomes. Sequence groupings in subsystem (ss) categories were performed by the MG-RAST server using the SEED database. Note the log scale of the *y*-axis.

indicated that genes originating from *Cyanobacteria* play a major role in ammonium assimilation, nitrate and nitrite reduction to ammonium, and cyanophycin metabolism. The urea and allantoin degradation genes also fell in this quadrant; however, *Gammaproteobacteria* and the unknown group also plotted in this sector (albeit with much lower first and second component scores than *Cyanobacteria*), and may also be involved in these processes. In contrast, the *Betaproteobacteria*, *Alphaproteobacteria*, and *Actinobacteria* plotted in the quadrant of the vectors for the transport of peptides, including branched chain forms, suggesting the major role of these organisms in mineralization of nitrogenous biopolymers. The cyanate hydrolysis gene vector also was located in this sector.

The PCA analysis also showed a strong separation of taxonomic groups according to phosphorus-related genes (Fig. 4C). The first PCA component (74% of the variance) separated *Proteobacteria* (*Alpha-*, *Beta-*, and *Gamma-*), *Actinobacteria*, and *Cyanobacteria* from all other taxonomic groups, suggesting their dominant role in P cycling. The second axis (11%) showed that the gene vectors for phosphate transport systems were located in the direction of *Betaproteobacteria*, as well as *Actinobacteria*, *Cyanobacteria*, and *Gammaproteobacteria*, while *Alphaproteobacteria* fell at the opposite extreme, in the direction of vectors for alkyl phosphonate utilization, exopolyphosphatase, and polyphosphate kinase, in addition to phosphate transport and regulation.

Discussion

The WHI and MIS differed in certain environmental features; for example, the MIS had higher quantities of exposed marine ice, conductivities, dissolved organic carbon, and liquid water habitats (Mueller et al. 2006). Despite these differences, our metagenomics analysis of the microbial mat communities from WHI and MIS indicated that their genetic makeup and potential were similar in terms of functional and taxonomic composition. This indicates that the major selective pressures are similar in these two ice-shelf environments and that the mats have accumulated similar taxa over time, possibly with exchange

Table 3. Sequence matches to Eukaryota obtained using the MG-RAST server against Euro rRNA SSU database (*E*-value cutoff: 0.1). Positive hits to the SCUM database for prokaryote viral sequences and GenBank for eukaryote viral sequences (*E*-value cutoff: 1×10^{-4}).

Major clade	MIS	WHI
Metazoa	1842	2162
Viridiplantae	536	607
Unknown eukaryotes	517	610
Fungi	424	521
Alveolata	257	315
Euglenozoa	186	201
Stramenopiles	120	159
Rhodophyta	102	146
Microsporidia	51	73
Acanthamoebidae	48	55
Diplomonadida	47	56
Cercozoa	45	35
Parabasalidea	39	60
Cryptophyta	28	37
Haptophyceae	26	29
Mycetozoa	13	22
Glaucocystophyceae	10	17
Entamoebidae	9	5
Polycystinea	9	22
Mesomycetozoa	9	7
Choanoflagellida	7	3
Heterolobosea	7	10
Granuloreticulosea	4	8
Lobosea	4	10
Acantharea	3	8
Corallochytrium	2	2
Ancyromonadidae	2	2
Plasmodiophorida	2	1
Psorospermium	0	1
Apusomonas	0	1
Mastigamoeba	0	1
Eukaryotic virus	261	269
Bacteriophage	601	655

between sites. Calculations based on measured production rates and standing stocks of biomass show that the mat communities are perennial and take several years to accumulate (Mueller et al. 2005). This implies that the metagenomes analyzed in the present study are integrations of multiple years of community development and do not resolve shorter temporal scales of variation such as seasonal changes.

Most studies report that the phylum *Cyanobacteria* dominates Arctic and Antarctic microbial mat assemblages (Jungblut et al. 2005; Vincent 2007), and three cyanobacterial orders were well represented in the metagenomes. Our PCA analysis indicated that photosynthetic genes tracked the same direction as the *Cyanobacteria*, consistent with this group as the dominant phototrophs. This expected result validated our approach and underscored the major role played by *Cyanobacteria* in energy acquisition for the mat consortium.

Contrary to expectation, our analyses indicated that heterotrophic bacteria, especially three classes of *Proteobacteria*, contributed more genetic material to the system than *Cyanobacteria*. Although it could be argued that the *Cyanobacteria* themselves have acquired protein-coding genes from other major taxonomic groups, our calling protocol for similarity was such that any transfers would have been very recent and with little time for any nucleotide bias selection to occur, since the gene assignments to the taxonomic groups were unambiguous. Nevertheless, the possibility of viral transfer of relatively large amounts of DNA among microorganisms cannot be discounted (Allen et al. 2006). In addition to protein-coding genes, the SSU rRNA genes of a variety of bacteria including *Actinobacteria* and *Bacteroidetes* were also important constituents of the mat metagenomes. The dominance of noncyanobacterial genotypes has also been reported in photosynthetic mats elsewhere, such as in the hypersaline waters of Guerrero Negro, Mexico (Ley et al. 2006).

While a DNA-based study can only provide information on the potential functions of different groups within the mats, our PCA analyses provided evidence of partitioning and interdependence of mat functions among taxonomically different constituents of the mats, for example, polypeptide assimilation and mineralization by *Proteobacteria* and ammonium assimilation by *Cyanobacteria*. These observations are consistent with the concept of microbial mat consortia as tightly coupled biogeochemical systems of diverse taxa living within a matrix of heterogeneous microenvironments (Paerl and Pinckney 1996; Stal 2000).

Other microbial groups contributed to a much lesser extent to the microbial mat metagenomes. Archaea are often reported from cold environments (Cavicchioli 2006), including Arctic freshwater systems where *Euryarchaeota* are generally more abundant than *Crenarchaeota* (Galand et al. 2008; Pouliot et al. 2009). However, Archaea contributed less than 1% of total identified genes in the microbial mats. Microscope observations and targeted 18S rRNA clone libraries report diverse protist communities that include diatoms, ciliates, cercozoans, and euglenoids (Vincent et al. 2000), and when using the *E*-value 0.1 against the European ribosomal small subunit (rSSU) database, we found matches to the majority of these eukaryotic groups (Table 3). Viruses are ubiquitous in aquatic ecosystems, with rapid turnover rates in the natural environment that depend upon active hosts (Short and Suttle 2005). We found the viral community of both metagenomes to contain *Proteobacteria* phages and cyanophages, reflecting the heterotrophic and phototrophic hosts in the mats. In addition, eukaryotic viral genes were present, although less abundant. Viral lysis can play a major role in microbial recycling processes via the ''viral shunt'' (Suttle 2007), and this is likely to be an important mechanism contributing to the flux of carbon, nitrogen, phosphorus, and other elements from the particulate to dissolved pools.

Proteobacteria species have diverse aerobic and anaerobic metabolic pathways (Madigan et al. 2000) and likely promote degradation and rapid recycling of organic material within the mats. We found genes implicated in both aerobic (photosynthesis, respiration) and anaerobic metabolic pathways, suggesting adaptation to a wide variety of conditions that occur seasonally or as micro-

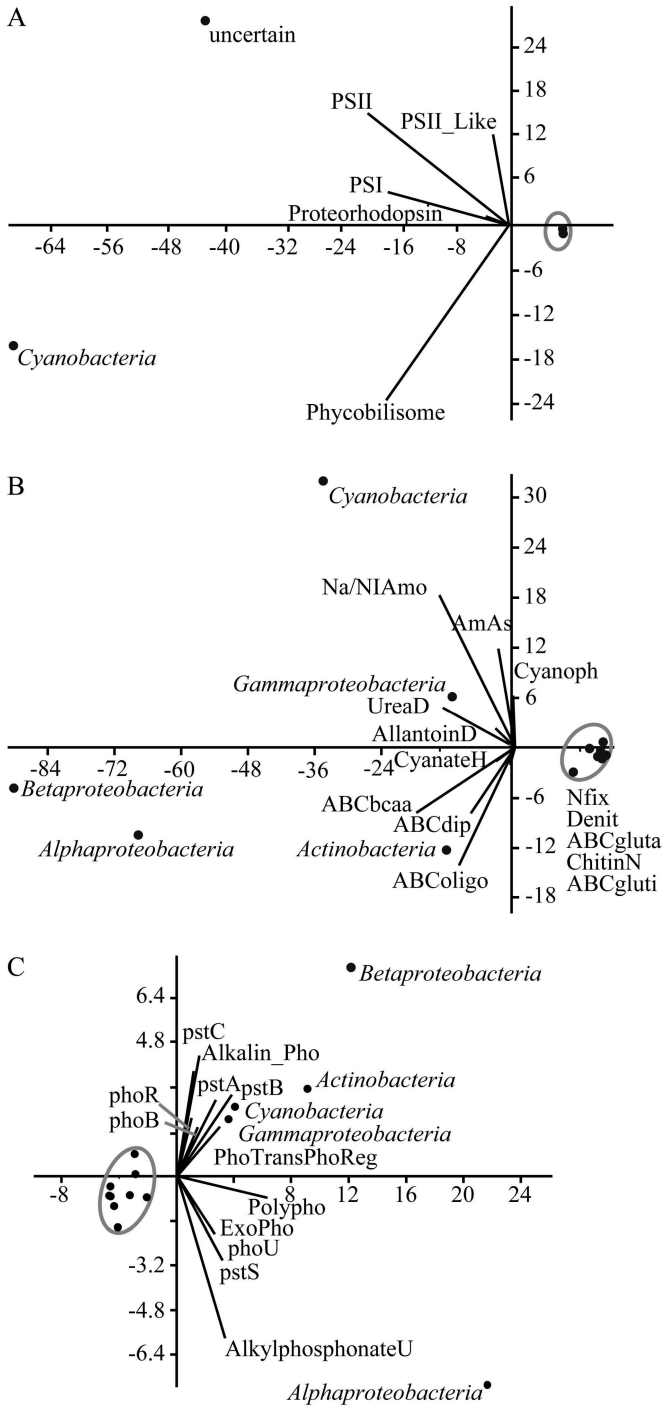

Fig. 4. Principal component analysis of gene functions from different bacterial groups. (A) Genes involved in light capture. (B) Nitrogen uptake and transformation genes. (C) Phosphorus uptake and transport genes. Horizontal and vertical axes represent component 1 and component 2, respectively. Gray circles contain all other identified taxa. Abbreviations: PSI, photosystem I; PSII, photosystem II; PSII-like, bacterial photosystem II–type photosynthetic reaction center; Nfix, nitrogen fixation; AmmAss, ammonium assimilation system; Denitrif, denitrification; AllantoinD, allantoin degradation; UreaD, urea degradation; CyanateH, cyanate hydrolysis; Na and NiAmo, nitrate and nitrite reduction to ammonia; ChitinN, chitin and N-acetylglucosamine utilization; Cyanoph, cyanophycin metabolism; ABCbcaa, ABC transporter for branched-chain amino acids; ABCdip, ABC transporter for dipeptides; ABCgluta, ABC transporter for glutamate and aspartate; ABCglut, ABC transporter for glutamine; ABColigo, ABC transporter for oligopeptides; Alkalin_Pho, alkaline phosphatase; PhoTransPhoReg, high-affinity phosphate transporter and control of PHO regulon; ExoPho, exopolyphosphatase; Polypho, polyphosphate kinase; AlkylphosphonateU, alkylphosphonate utilization; pstA, phosphate transport system permease protein pstA; pstB, phosphate transport system permease protein pstB; pstC, phosphate transport system permease protein pstC; pstS, phosphate ABC transport, periplasmic phosphate-binding protein pstS; phoU, phosphate transport regulator phoU; PhoR, phosphate regulon sensor protein phoR; phoB, phosphate regulon transcriptional regulatory protein phoB.

zones. In other environments, microbial mats may be vertically stratified, where biochemical and physical gradients (Golubic and Seong-Joo 1999; Stal 2000) are generated in part by external physical and chemical factors and in part through bacterial and eukaryotic metabolic activities such as sulfate reduction and oxidation, photosynthesis, denitrification, nitrification, nitrogen fixation, fermentation, and methanogenesis (Stal 2000), leading to segregation of the different microbes within the mat consortia (Kunin et al. 2008). In the High Arctic ice-shelf habitats, actively photosynthesizing mats are well oxygenated, and no anoxic layers have been reported during the summer months with 24-h irradiance (Mueller et al. 2005, 2006). However, photosynthesis is low or absent for much of the year during winter darkness, and in spring and fall when sun angles are low and the melt pools are covered by ice and snow. Anoxic conditions could occur, and for at least part of this time, liquid water is available, facilitating the persistence of genes for anaerobic metabolism such as archaeal methane cycling and denitrification.

Functional gene assignments and metabolic pathways of the MIS and WHI metagenomes were similar, suggesting comparable selective forces operating at the sites as a result of the same environmental conditions. In both habitats, previous studies have shown that the mats underlie nutrient-poor waters and are oases of high concentrations of organic material; dissolved inorganic and organic nutrients in the mat interstitial waters are higher than in the overlying water (Mueller and Vincent 2005; Mueller et al. 2005), consistent with the results obtained here of genomic dominance by proteobacterial decomposers in the mats. The functional gene profiling also implies degradation of diverse organic materials and transport of multiple nutrient-containing substrates.

Overall, ammonia and urea metabolism dominated the inorganic nitrogen system. Both of these are common products of microbial decomposition processes. Ammonia may also be produced via nitrogen fixation and nitrate and nitrite reduction. Neither archaeal nor bacterial nitrification genes were detected in the metagenomes. This is consistent with the relatively low concentrations of nitrate found in the mats, and it implies that ammonium is primarily recycled as a nitrogen source for phototrophs

rather than providing an energy supply for chemotrophs. The presence of genes implicated in cyanate hydrolysis and allantoin degradation, as well as a variety of genes for polypeptide transport, indicates multiple decomposition and recycling pathways.

Numerous genes that imply phosphorus deficiency and scavenging were identified, including hydrolytic enzymes such as alkaline phosphatases (Paytan and McLaughlin 2007). We identified sequences of the phosphate regulon system (*phoB* and *phoR*), which consists of transporters and hydrolytic enzymes that are inducible under phosphate starvation (Table 2). This regulon acts to control cellular responses to low extracellular phosphate concentrations and assimilation of phosphorus from the environment (Rusch et al. 2007; Breitbart et al. 2008). High proportions of sequences involved in intermediary metabolism and membrane transport (Fig. 2) were also reported for the microbialite cyanobacterial community in Cuatro Ciénegas, an ultra-oligotrophic freshwater system in Mexico, and these were thought to relate to phosphorus recycling (Breitbart et al. 2008) and P limitation. Bonilla et al. (2005) suggested that P-limitation of Arctic microbial mat communities is unlikely; however, the N to P ratios of pore water within the ice shelf mats ranges from 44 to 66 (Vincent et al. 2004*a*; Mueller et al. 2005). This is much higher than the canonical Redfield ratio of 16 (Redfield et al. 1963), and would be consistent with P imbalance. This relative surplus of nitrogen also likely explains the minimal presence of nitrogen-fixing genes. It is also consistent with the finding that the nitrogen-fixing genes did not point in the direction of *Cyanobacteria* in the PCA plot, implying a lack of nitrogen-fixation among the *Nostocales* detected in these mats. The presence of genes for phosphatases and transport of phosphonate, alkylphosphonate, and orthophosphate implies that the microbial community recycles and scavenges a wide range of P-containing compounds within the mat microenvironment, and from the overlying water column.

The diversity of life is still vastly undersampled (Head et al. 1998; Sogin et al. 2006; Rusch et al. 2007). Since characterization of organisms and proteins lags behind the acquisition of sequence data, an alternative or complementary use of such data is to compare communities not by "species" or Operational Taxonomic Units (OTUs), but to infer characteristics of communities from different environments and deduce adaptive function. Tringe et al. (2005) pioneered this approach with three disparate communities (open-ocean surface water, whale fall, and farm soil), and subsequently, Dinsdale et al. (2008) compared many more communities and categorized metabolic potential within different biomes, noting, for example, differences between freshwater and marine biomes. The present study draws attention to the characteristics of microbial mat communities as nutrient scavenging and recycling systems that have capabilities to recycle, transport, and assimilate diverse nutrient-containing substrates. These features are likely to be preeminently important in allowing the accumulation of large, nutrient-replete populations of phototrophs and heterotrophs, despite the nutrient-poor conditions of their overlying environment.

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