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Microbial Activity in Dust-Contaminated Antarctic Snow

Alison K. Thurston, Karen Foley, Shelby Rosten,
Susan Taylor, Robert B. Haehnel, and Robyn A. Barbato

September 2023

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Robert B. Haehnel, and Robyn A. Barbato

US Army Engineer Research and Development Center (ERDC)
Cold Regions Research and Engineering Laboratory (CRREL)
72 Lyme Road
Hanover, NH 03755-1290

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Abstract

During weather events, particles can accumulate on the snow near the Pegasus ice and Phoenix compacted-snow Runways at the US McMurdo Station in Antarctica. The deposited particles melt into the surface, initially forming steep-sided holes, which can widen into patches of weak and rotten snow and ice. These changes negatively impact the ice and snow runways and snow roads trafficked by vehicles. To understand the importance of microbes on this process, we examined deposited dust particles and their microbial communities in snow samples collected near the runways. Snow samples were analyzed at the Cold Regions Research and Engineering Laboratory where we performed a respiration study to measure the microbial activity during a simulated melt, isolated microorganisms, examined particle-size distribution, and performed 16S rRNA gene sequencing. We measured higher levels of carbon dioxide production from a sample containing more dust than from a sample containing less dust, a finding consistent with viable dust-associated microbial communities. Additionally, eleven microorganisms were isolated and cultured from snow samples containing dust particles. While wind patterns and satellite images suggest that the deposited particles originate from nearby Black Island, comparisons of the particle size and chemical composition were inconclusive.

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Preface

This study was conducted for Headquarters, US Army Corps of Engineers, under Program Element Number 0603119A, Project Number BO3. The technical monitor was Dr. Simone Whitecloud, US Army Engineer Research and Development Center–Cold Regions Research and Engineering Laboratory (ERDC-CRREL).

The work was performed by the Biogeochemical Sciences Branch of the Research and Engineering Division, ERDC-CRREL. At the time of publication, Mr. Nathan Lamie was branch chief; and Dr. John Weatherly was acting division chief. The deputy director of ERDC-CRREL was Dr. Ivan P. Beckman, and the director was Dr. Joseph L. Corriveau.

We would like to thank Mr. Chris Berini, Dr. George Blaisdell, Mr. Kevin Bjella, Ms. Jennifer Morikawa, and Ms. Margaret Knuth for their contributions to this body of work.

We acknowledge the use of imagery from the NASA Worldview application (https://worldview.earthdata.nasa.gov/), part of the NASA Earth Observing System Data and Information System (EOSDIS).

As this study used similar methods as previously published work, portions of Sections 2.5.3 and 2.5.4 have been modified and reprinted from Thurston et al. (2021, 2022), respectively.

COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

1 Introduction

1.1 Background

1.1.1 McMurdo Station Antarctica

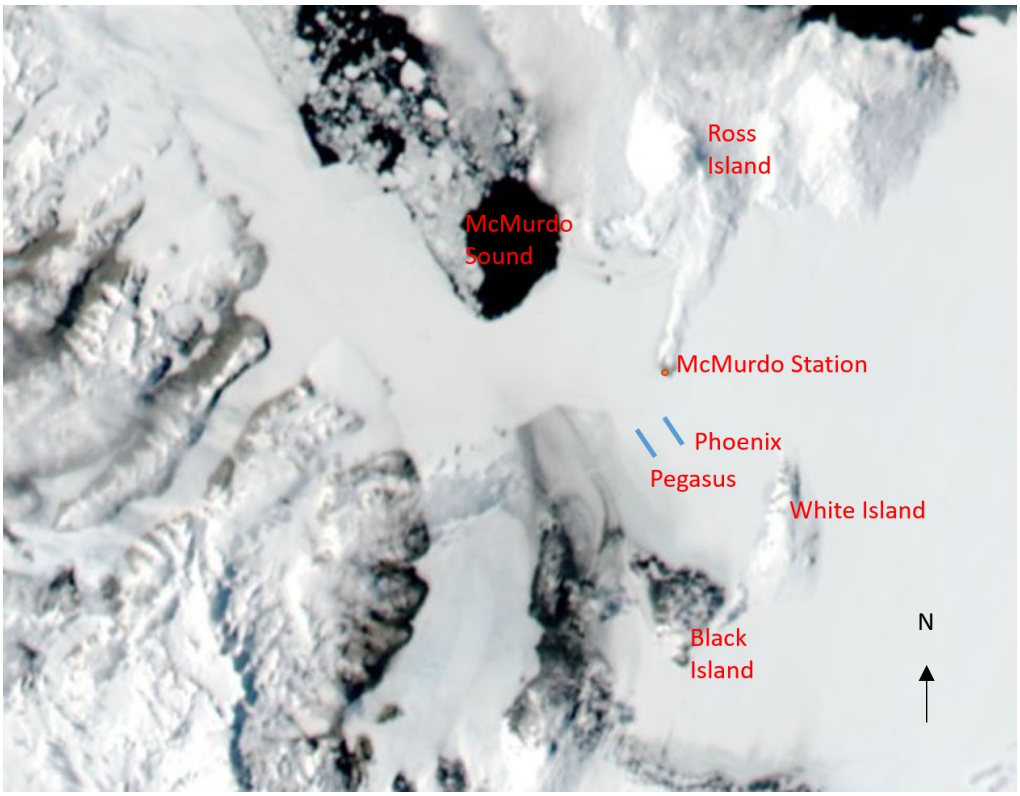
McMurdo Station (latitude –77.8500, longitude 166.6667) is the primary US station in Antarctica. McMurdo is positioned on Ross Island in the McMurdo Sound, next to the McMurdo Ice Shelf, a small lobe located in the western region of the Ross Ice Shelf. To the south of McMurdo are Black Island and White Island. The Dry Valleys are situated to the west of McMurdo (Figure 1). McMurdo Station operates all year round; however, staffing and operations increase during the summer months to support research being carried out all over the Antarctic continent. Personnel and supplies must be brought in by water or air to the station and redistributed overland or by air to South Pole and science camps. The extreme environment and complex terrain of this region present obstacles to all means of transport. In the past three decades, two primary airfields have been used for wheeled aircraft operations from October through March: the Pegasus and Phoenix Runways. While the Pegasus Runway served as the main runway for much of this time, in 2017, the aging Pegasus Runway was replaced with the Phoenix Runway, which is situated closer to McMurdo Station.

Phoenix is a white runway and was created by continually compacting snow until there was a 1 m hard base (Haehnel et al. 2019). Phoenix is rated to handle landing a C-17 Globmaster III aircraft and is a specialized runway, as snow runways are typically constructed to accommodate ski-equipped aircraft as opposed to the wheeled landing gear that the Globemaster and other large aircrafts are equipped with. After snowfall, the new snow on the runway is compacted immediately to maintain runway strength. A detailed description of the creation of the Phoenix Runway, including site selection, snow runway strength, construction, maintenance, and evaluation and validation, can be found in Haehnel et al. (2019).

Pegasus Runway operated from 1993 to 2016 (Haehnel et al. 2019). Unlike Phoenix Runway, Pegasus was a glacial ice airstrip. Pegasus was built on thick floating glacial ice, which had no net mass change throughout the year and is referred to as a "zone of superimposed ice" (Haehnel et al. 2019; Klokov and Diemand 1995). Pegasus's location was chosen in part

because of the way snow accumulates on the McMurdo Ice shelf, where the annual accumulation was zero in an area in between the accumulation zone and the ablation zone (Klokov and Diemand 1995). Although originally an ice runway, in 2000, a compacted snowcap was added to increase the albedo of the runway surface and to protect the underlying, lower albedo ice from melting during the warmest part of the year (mid-December through mid-January). As of 2016, the Pegasus airstrip had moved half of a mile from its original location due to ice flow, eventually moving into an area where mineral dust was being deposited. The particle deposition affected the stability of the snow matrix such that it was unable to support landing aircrafts in the midsummer months. The increased maintenance to manage dust and the loss of operational time due to dust deposition eventually lead to Pegasus's closure and the impetus to construct a new airstrip, Phoenix Runway.

Figure 1. Satellite image of McMurdo Station and surrounding geographic features. McMurdo Sound is located to the northwest of McMurdo Station. It is partially covered by sea ice with areas of exposed water. To the south of McMurdo Station are the runways and Black Island. To the west are the dry valleys. (Image was captured using NASA Worldview, https://worldview.earthdata.nasa.gov/.)



1.1.2 Mineral Deposition on Snow

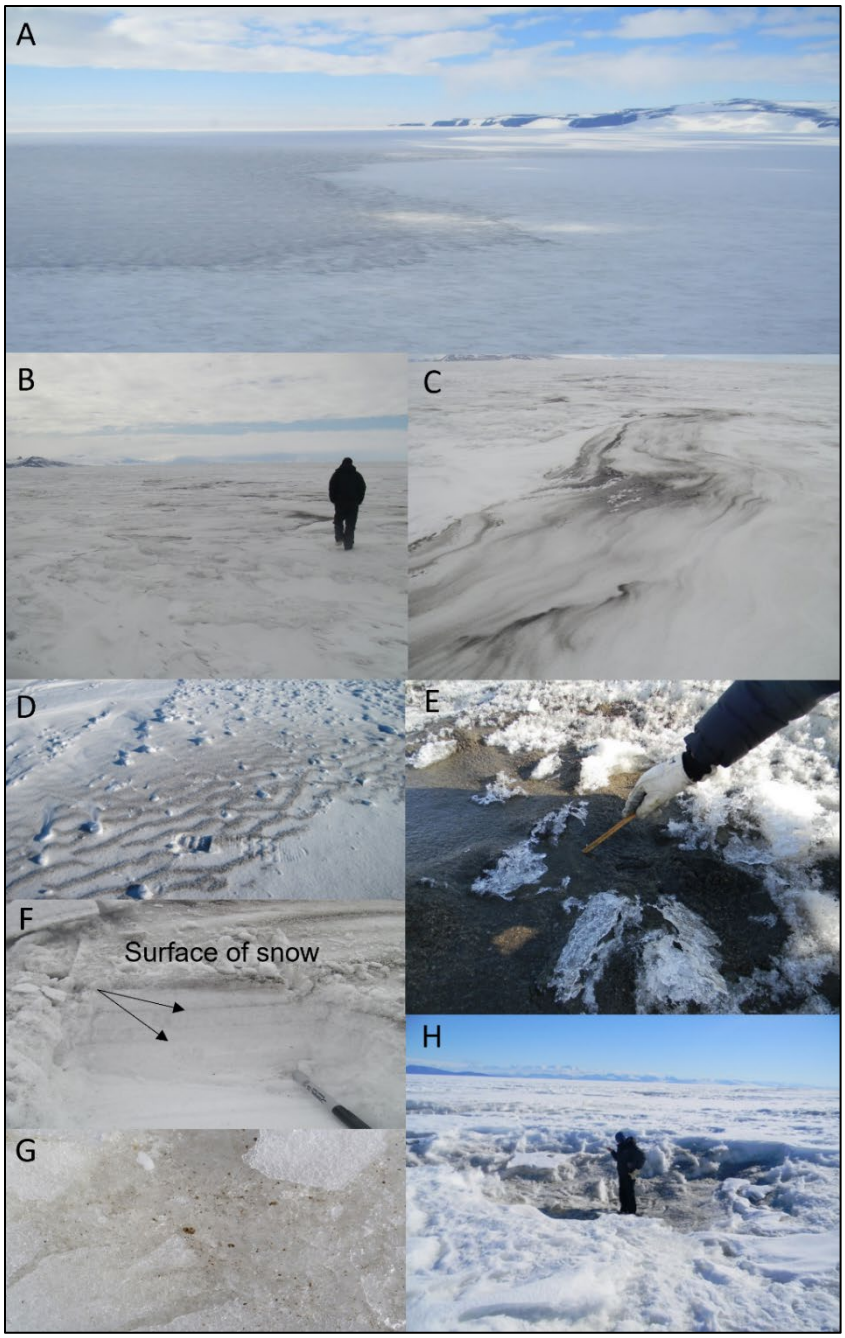
Particle deposition on snow decreases the albedo, or snow reflectivity. The decreased albedo allows for increased absorption of solar radiation, causing localized snowmelt and affecting snowpack structure (Conway et al. 1996; Skiles et al. 2018). In general, sources of particulate matter can be from geological landforms such as deserts, industrial pollution, and volcanic activity (Gili et al. 2022; Laluraj et al. 2009; Skiles et al. 2018). Transport can occur via short-distance (few meters) or long-distance (thousands of kilometers) surface-wind transport or weather events such as rain and snow. The distance of transport depends on the particle size and environmental conditions (Adebiyi et al. 2023). Haehnel et al. (2019) reported particle deposition over vast regions around the runways and the 23 km (14 mi)* road leading past Phoenix, ending at Pegasus. These particles are dark and reduce the surface albedo of the ice, thereby changing the energy balance of the runway and accelerating warming and weakening of the runway ice (Figure 2). When the particulate matter is left undisturbed, the deposited dust forms areas of uneven, dark, wet, structurally compromised snow that spreads across the landscape over time (Haehnel et al. 2019). While the exact source of the particles are unknown, weather patterns suggest that the prevailing northerly winds in the area could transport particulate material from nearby exposed rock sources such as Black Island, which is situated south of the runways (Klokov and Diemand 1995).

In addition to the mineral particulate, microorganisms are known to be associated with dust-deposited material and have impacts on snowmelt and structure. Studies on dust deposition on high alpine snowpack show that microbe-containing dust deposited during multiple weathering events affected snow-grain formation (Courville et al. 2020; Thurston et al. 2021). Most notably, cryoconite holes are water-filled holes that contain organic and inorganic (mineral) material, typically found on glaciers worldwide, including Antarctica, that affect biogeochemical cycles and surface albedo (Rozwalak et al. 2022). Fountain et al. (2004) reported that cryoconite holes in the Taylor Valley typically ranged from 5 to 145 cm diameter; however, they documented the formation of a cryoconite hole spanning 30 m in diameter and 5 m deep on the Canada Glacier in Antarctica. They suggested that wind direction and location near a particle source provide

* For a full list of the spelled-out forms of the units of measure used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

opportunity for these large cryoconite holes to form. Cyanobacteria and algae are among the organisms found in cryoconites, and the exopolysaccharide compounds mostly produced by the cyanobacteria contribute to fusing of organic material with inorganic material, forming granules (Rozwalak et al. 2022). McIntyre (1984) estimated that up to 10% of the energy contributing to cryoconite hole formation came from biological sources in narrow cryoconite holes on the Mantee Glacier in Canada. Firsthand accounts from on-site personnel at US McMurdo Station in Antarctica describe the spreading of particulate material in the area of Pegasus during the summer months (Haehnel et al. 2019). These accounts provide compelling evidence of potential significant biological activity, prompting an investigation into the biological components of the dust particulate matter.

Figure 2. During periods of high winds, particles are transported and deposited on the Pegasus ice runway at McMurdo, Antarctica: (*A*) The south edge of the contaminated area looking east-southeast toward White Island. (Image reproduced from Haehnel et al. 2019. Public domain.) (*B* and *C*) Wide distribution of particulate material. (Images by Susan Taylor.) (*D*) Windrows of deposited materials. (Image by Kevin Bjella.) (*E*) Mat of undisturbed particulate matter, which spread throughout the summer. (Image reproduced from Haehnel et al. 2019. Public domain.) (*F*) The snow surface and layers of particulate matter after snow deposition (*black arrows*). (Image by Susan Taylor.) (*G*) Particulate matter on the snow surface. (Image by ERDC-CRREL.) (*H*) The effects of particulate-matter concentration on the snow structure. (Image reproduced from Haehnel et al. 2019. Public domain.)



1.2 Objectives

The focus of this study was to examine the characteristics of particles and microorganisms deposited on and around the Pegasus Runway and to explore Black Island as a possible origin. Our objectives were the following:

1. Characterize the particles in terms of their size distribution, densities, and mineral constituents.
2. Compare the mineralogy of deposited particles with those of nearby soils (mineral substrates) to constrain their origin.
3. Determine microbial taxonomy in surface snow and perform a lab incubation to measure activity during melt.

1.3 Approach

This study compiled data together from analyses performed on three different snow sample sets. The samples were collected in 2009, 2011, and 2016 from different areas on and around the Pegasus Runway.

For snow collected in 2009, we conducted a study to analyze the chemistry of the deposited particles using light microscopy and scanning electron microscopy (SEM) in combination with energy dispersive X-ray analysis (EDAX). The samples collected in 2011 were used to examine the particle-size distribution. Additionally, as part of the analyses, we compared the collected samples to soil samples collected in 2010 at Black Island, a nearby potential source.

Using snow samples collected in 2016, we measured the activity of the microorganisms associated with the dust particles via carbon dioxide (CO_2) production during a simulated thaw. DNA sequencing aimed to identify the types of organisms present. Last, eleven of these microorganisms were isolated and cultured. These isolates are now part of ICE COLD (Innovative, Collaborative, Exploratory Cold Regions Organism Library for Discovery), a repository of cold-environmental microorganisms housed at the US Army Cold Regions Research and Engineering Laboratory (CRREL).

2 Methods

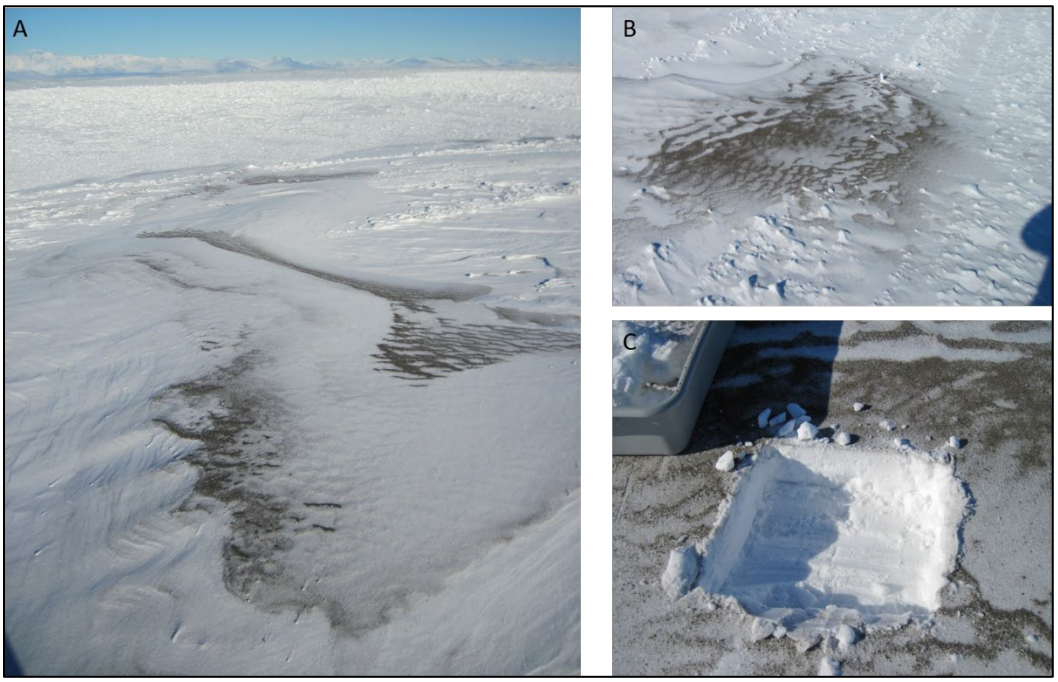
2.1 Sample Collection

The samples used in this study were collected opportunistically by volunteers and consist of three sample collections over a 7-year timespan. It is important to note, particularly for the earlier collections, that due to personnel turnover there is limited information available regarding the specific details of collection and processing of the snow samples. The presented information is what could be accurately determined from files and personal communications.

2.1.1 2009 Pegasus Runway Collection

Particles deposited on the snow near the Pegasus Runway were collected in 2009 for preliminary analysis (Robert Haehnel, pers. comm., May 2023; Figure 3). The sample collected in 2009 was used for the SEM-EDAX analysis (methods described in Section 2.3).

Figure 3. Images of dust deposited on and around the Pegasus Runway during 2009: (*A* and *B*) View of particles lying on snow dunes. (*C*) Image of clean snow under a snow dune. (Images by Kevin Bjella.)



2.1.2 2010 Black Island Soil Collection

Soil samples from Black Island were collected in January of 2010 by Jennifer Morikawa with Raytheon Polar Services Company and shipped to CRREL. In total, four samples were collected from three locations (Table 1). These were used in both the SEM-EDAX (Section 2.3) and particle-size-distribution analysis (Section 2.3).

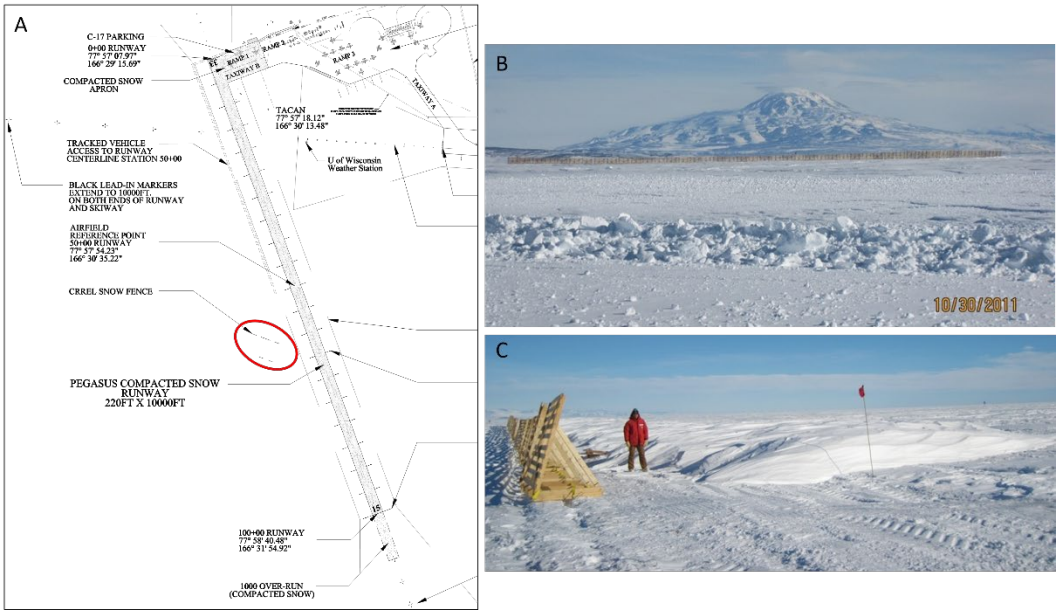
Table 1. Description of soil samples collected on Black Island, Antarctica.

Sample	Elevation (ft.)	Longitude and Latitude
Black Island 1	188	−78.132117, 166.195517
Black Island 2	349.7	−78.133717, 166.175483
Black Island 3	530.7	−78.133700, 166.148167
Black Island 4	530.7	−78.133700, 166.148167

2.1.3 2011 Pegasus Runway Collection

In 2011, snow fences were constructed in between the runway and Black Island to attempt to mitigate particle deposition (Figure 4). Snow cores were collected along the fence line after deposition events. The samples were melted and filtered to collect the deposited particles, which were shipped to CRREL for analysis (Robert Haehnel, pers. comm., September 2022). These samples were used for the particle-size-distribution analysis (Section 2.3)

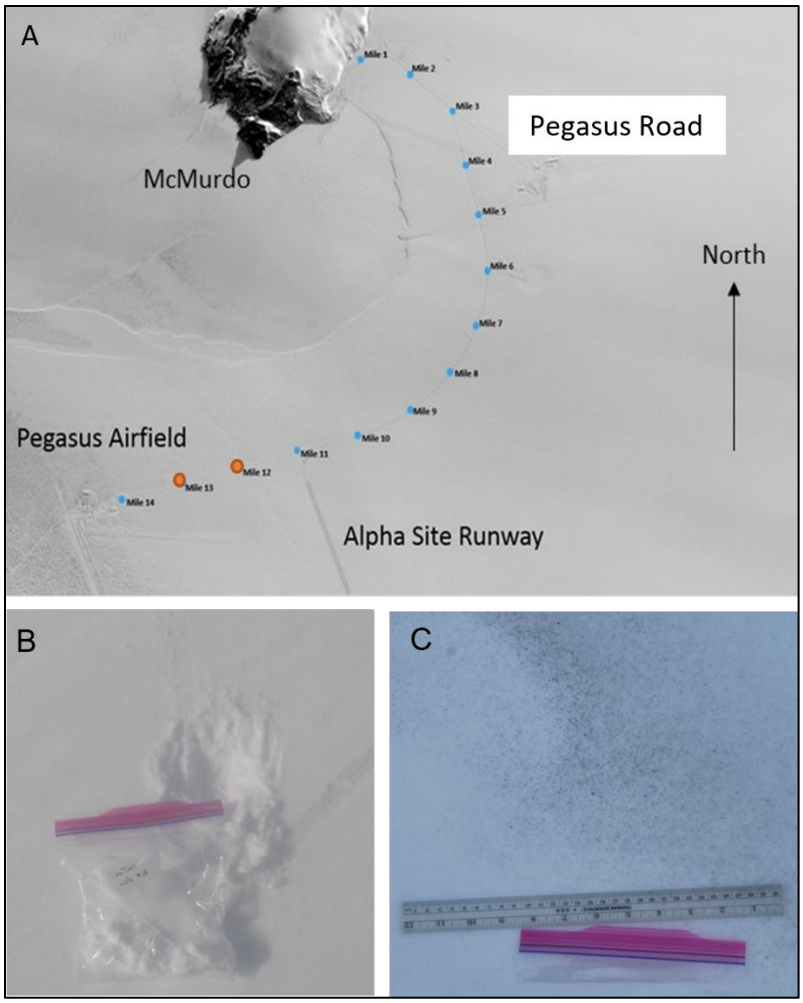
Figure 4. (*A*) National Science Foundation partial map of the area around Pegasus Runway. The *red circle* marks the location of the snow fences next to the runway. (*B*) Image of Black Island with snow fences in the foreground. (*C*) Close-up image of snow fences. (Images provided by Margaret Knuth.)



2.1.4 2016 Ice Road Collection

In December 2016, three snow samples were collected between Miles 12 and 13 (Figure 5*A*) along the ice road leading to the Pegasus Runway. Latex gloves were worn over winter gloves, and each sample was collected using a clean Ziplock bag (Susan Taylor, pers. comm., May 2022). Through visual inspection, two of the snow samples appeared to contain dark particulate matter (Snow Sample 1 and Snow Sample 3), and one of them contained less particulate matter and was denoted as "clean" snow (Snow Sample 2). Figures 5*B* and 5*C* are examples of snow containing more or less particulate matter. The snow samples were shipped frozen back to CRREL for biological analyses (Section 2.5–2.6). No particle characterizations were performed on these samples.

Figure 5. (*A*) Image of the ice road leading to Pegasus and Phoenix Runways (Alpha Site Runway). Miles are marked, and Miles 12 and 13 are marked in *orange*. (Image adapted from Haehnel et al. 2019. Public domain.) Images of snow depicting examples of (*B*) snow containing less particulate matter and (*C*) snow containing more particulate matter. (Images provided by Susan Taylor.)



2.2 Particle-Size Distribution

Samples from Pegasus (2011) and Black Island (2010) were melted and dried. Samples were preweighed prior to addition to the sieve stack for particle-size-distribution determination. Samples were added to the largest aperture sieve (75 mm openings) and shaken to allow the particulate matter to move through the sieve stack (Table 2). After completion, each sieve was weighed, and the particle-size distributions were calculated and displayed as percent finer (amount of sample that is smaller than the indicated sieve size).

Table 2. Sieve sizes and names.

Sieve	mm
3 in.	75
2 in.	50
1.5 in.	37.5
1 in.	25
0.75 in.	19
0.375 in.	9.5
No. 4	4.75
No. 10	2
No. 20	0.85
No. 40	0.425
No. 60	0.25
No. 100	0.15
No. 200	0.075

2.3 Microscopy and Energy Dispersive X-Ray Analysis

The particles collected near Pegasus Runway in 2009, as well as those collected from Black Island in 2010, were examined optically and photographed with an optical microscope. A few tens of grains were then imaged with an FEI XL-30FEG-SEM with an X-ray detector at Dartmouth College, Hanover, NH. No quantitative elemental analyses were possible as the particles presented no flat, polished surface. Alternatively, point spectra were taken to obtain qualitative information about their compositions. The particles were not carbon coated, so we used the SEM's low-vacuum mode (0.9 torr of water vapor) at a 15 keV accelerating voltage, a spot size of 5 μm^2, and a 10 mm working distance. Typical count rates were approximately 1,000 counts per second.

2.4 Cell Culture and Microbe Isolation

2.4.1 Culture and Isolation

Snow Sample 3, collected in 2016 from the ice road leading to Pegasus Runway, which visually exhibited a notable abundance of dark particulate matter, was selected for culturing the microorganisms associated with the deposited particles. The small snow sample (approximately 4 mL) was thawed at 4°C, and the resulting water and particulate solution was used to inoculate nutrient agar (NA), tryptic soy agar (TSA), and Reasoner's 2A agar (R2A) plates. NA and TSA are general microbial growth medias and

have higher nutrient levels than R2A. R2A is a low-nutrient media and is specifically used to select for slow-growing organisms in potable water. Approximately 400 µL of liquid with particulate matter was plated on agar plates (1× concentration). A tenfold dilution was made with sterile phosphate-buffered saline, and 300 µL was plated (0.1×). Plates were incubated at 25°C in an incubator (Amerex Instruments Gyromax 737) and 4°C in a fridge (Fisher Scientific, 13-968-238G). From the 0.1× plates, distinct colonies were patch plated and imaged. We attempted to isolate 22 organisms; of those, 11 organisms from the 25°C plates were successfully restreaked for isolation. Freezer stocks of each isolate were made in 15% dimethyl sulfoxide and 20% glycerol, independently. Further work with the isolates was done by reviving the isolates from the freezer stocks. Stocks were stored at −80°C until use.

2.4.2 Isolate Identification

Liquid cultures of each isolate were grown in their respective isolation media to collect cell pellets for DNA extraction. DNA was extracted using the Qiagen DNAeasy UltraClean Microbial Kit (Qiagen, 12224-250) following the manufacturer's protocol. DNA concentrations were measured on a Qubit 3.0 Fluorometer (Invitrogen, Q33216) using the dsDNA BR assay kit (Invitrogen, Q32850). Then, 1.0 µg of DNA was sent to Genewiz (Sanger sequencing, Azenta Life Sciences) for 16S rRNA gene sequencing. The Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) was utilized to compare the resulting DNA sequences against the 16S Microbial Sequences (BLASTN 2.7.1+, 20,470 sequences) for the closest taxonomic genus and species match (NCBI, n.d.; Zhang et al. 2000). The top hit is reported in the results.

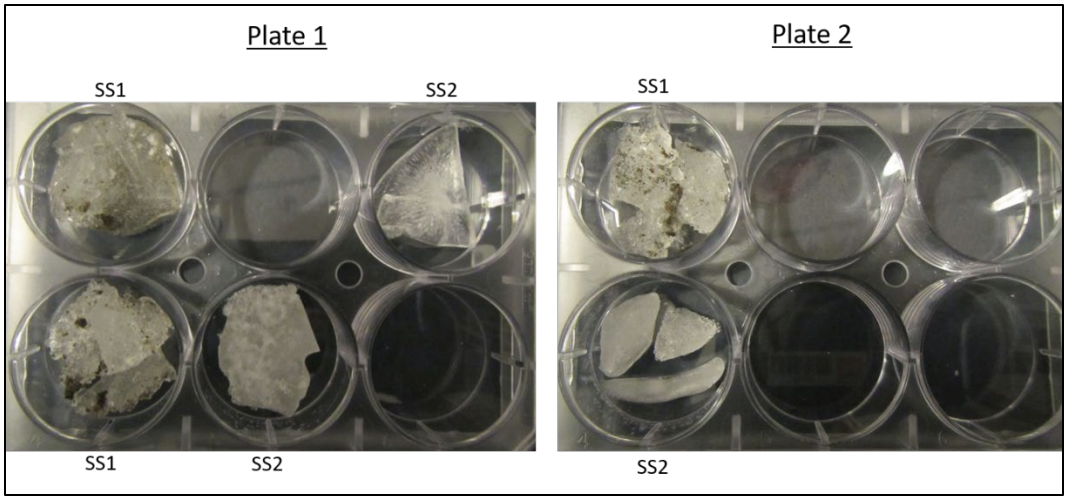
2.5 Respiration Study

2.5.1 Incubation Setup and Execution

Two snow samples collected in 2016 were selected, based on the amount of particles visually observed, to measure microbial activity during a melting event. Snow Sample 1 had visibly more dust particles than Snow Sample 2, which appeared to be almost devoid of particles. Production of CO_2 was used as a proxy to determine the microbial activity. CO_2 production was monitored using the Columbia Instruments Micro Oxymax Respirometer (CO_2 sensor, 200148-3). Samples were prepared for the respiration study using sterile technique in a −16°C cold room. Snow samples appeared to

have thawed and refroze at some point during transportation. Aluminum foil, tweezers, and glass vials were sterilized by baking at 450°C for at least 4 hours. Aluminum foil was used to protect the samples from touching any unsterile surfaces. Three replicates of each snow type were weighed in sterile glass vials and then randomly placed using sterile tweezers into two sterile six-well plates (Figure 6) (Corning, 353046). Sample plates were placed in an incubator (Sanyo Incubator, MIR153) set at −2°C at the start of the experiment. To reduce the chance of melting prior to the start of the study, the plates were placed in the incubator and attached to the instrument immediately prior to starting the experiment. The study ran for a total of 25 days. The incubator was initially set at −2°C, and temperature increased gradually until reaching 11°C. The temperature was recorded throughout the entire study (HOBO data logger, 10256833). As a control, empty wells were monitored as a baseline for CO_2 levels in the air.

Figure 6. Two six-well plates containing three replicate samples of Snow Sample 1 (SS1) and Snow Sample 2 (SS2).



2.5.2 DNA Extraction

After the respiration study, the melted snow and associated particulate matter were transferred to sterile microcentrifuge tubes for DNA extraction. To each well, 1 mL of sterile 1× phosphate-buffered saline at a pH of 7.4 was added to wash the well and collect any particulate matter that was left behind. DNA was extracted using the Qiagen DNAeasy PowerSoil DNA extraction kit (Qiagen, 47014) following the manufacturer's instructions. Following DNA extraction, DNA concentration was measured using a Qubit fluorometer with a dsDNA HS assay kit (Invitrogen, Q32851).

2.5.3 DNA Sequencing

The V1–V2 hypervariable regions of the 16S rRNA gene for bacteria and archaea was sequenced, following the manufacturer's protocols, using a Thermo Fisher Ion Torrent Personal Genome Machine sequencer at the Microbiome Analysis Center at George Mason University. Bioinformatics analysis and visualization of the 16S rRNA amplicon data were conducted using R (version 4.0.4) (R Core Team 2018; Callahan et al. 2016). BAM files converted to FastQ files were demultiplexed into individual sequencing files. Adapter removal and barcode trimming were done in R prior to running the forward reads through the *dada2* (version 1.18.0) pipeline. In *dada2*, to remove low-quality regions from the sequences, the sequences were truncated after 300 base pairs (bp); and the first 15 bp were removed, resulting in a final sequence read length of 285 bp. Sequences with an expected error rate of 2 or higher, matched known PhiX contamination, or contained ambiguous bases were removed. After dereplication, the error rates were estimated and then used to guide the construction of the amplicon sequence variant (ASV) table. Chimeric sequences were then removed using the consensus method in *dada2*. Taxonomy was assigned using Silva 138.1 prokaryotic SSU taxonomic training data (McLaren and Callahan 2021); bootstrapping was set to 50.

2.5.4 Sequence Analysis

The following methods were all performed using the R packages *phyloseq* (version 1.34.0; McMurdie and Holmes 2013) and *ggplot2* (version 3.3.3; (Wickham 2016). The sequence variant table, taxonomic assignments, and sample metadata were combined into one *phyloseq* object. Any taxa that did not possess a taxonomic designation at the phylum level were removed. Alpha diversity was calculated from the ASV counts by using the plot_richness command in *phyloseq*. To display taxonomic abundance, read counts were converted to per-sample relative abundance by dividing absolute reads counts by total reads for the sample.

2.6 Snow Sample 3 Community Analysis

To survey the microbial community from Snow sample 3, DNA was extracted from Snow Sample 3 using the Qiagen DNAeasy PowerSoil DNA extraction kit (Qiagen, 47014) following the manufacturer's instructions. The methods for DNA sequencing and analysis are described in Sections 2.5.3 and 2.5.4.

3 Results

3.1 Carbon-Rich Particles Deposited on and near Pegasus Runway

Wind patterns and satellite images suggest that the particles deposited on the Pegasus Runway likely derive from Black Island. A particle-size-distribution analysis was conducted with a sample collected at Pegasus Runway after a dust event. The Pegasus particles were compared to the four soil samples collected at Black Island. There was no gravel reported in the Pegasus sample, while every sample from Black Island had fine gravel and one contained coarse gravel (Figure 7 and Table 3). Sand composed 97% of the Pegasus sample: 24.7% medium sand and 70.5% fine sand. In contrast, the Black Island samples were composed of 50%, 56.2%, 64.8, and 69.5% sand, respectively. Overall, compared with particle-size distributions of soils from Black Island, the runway samples have a narrower size distribution and are predominantly sand-sized particles (Figures 7–9; Table 3), consistent with the material from Black Island being size sorted by wind transport. The process of wind transport leads to sorting of particle by size, whereby only small particles are lofted, carried, and deposited, leaving behind the larger particulates, like large sand particles and gravel that the wind cannot transport very far.

To examine the chemical makeup of the soil samples, we performed EDAX followed by SEM. EDAX of 2010 Black Island samples identified carbon, oxygen, sodium, magnesium, aluminum, silicon, chloride, potassium, calcium, titanium, and iron. Particles collected from nearby the Pegasus Runway in 2009 contained carbon, oxygen, magnesium, silicon, phosphorus, sulfur, and calcium (Figure 10*A* and 1010*B*). Interestingly, the collected samples from the Pegasus Runway appeared more organic in composition: relative sample counts were high for carbon and low for silicon (Figure 10*B*). In contrast, the Black Island particles' relative samples counts were high for silicon and oxygen and low for carbon, typical of mineral material (Figure 10*A*). Optical microscopy revealed that the particles collected at Pegasus tended to be whitish flattened disks (Figure 11*D*). Additionally, SEM showed that the particles from Pegasus were composed of mineral and rock fragments (whitish specs) embedded in a less-dense carbon-rich phase (dark material) (Figure 11*E* and 11*F*).

Figure 7. Particle-size-distribution curve. The analysis was conducted with four replicates from Black Island collected in 2010 (*circle*, *square*, *triangle*, and upside-down triangle) and one sample from the Pegasus Runway (*diamond*) in 2011. Each sieve is listed on the top *x*-axis and demarked by a *dotted vertical line*. The grain size in millimeters is listed on the bottom *x*-axis (log scale). See Table 2 for the exact millimeter size corresponding to each sieve.

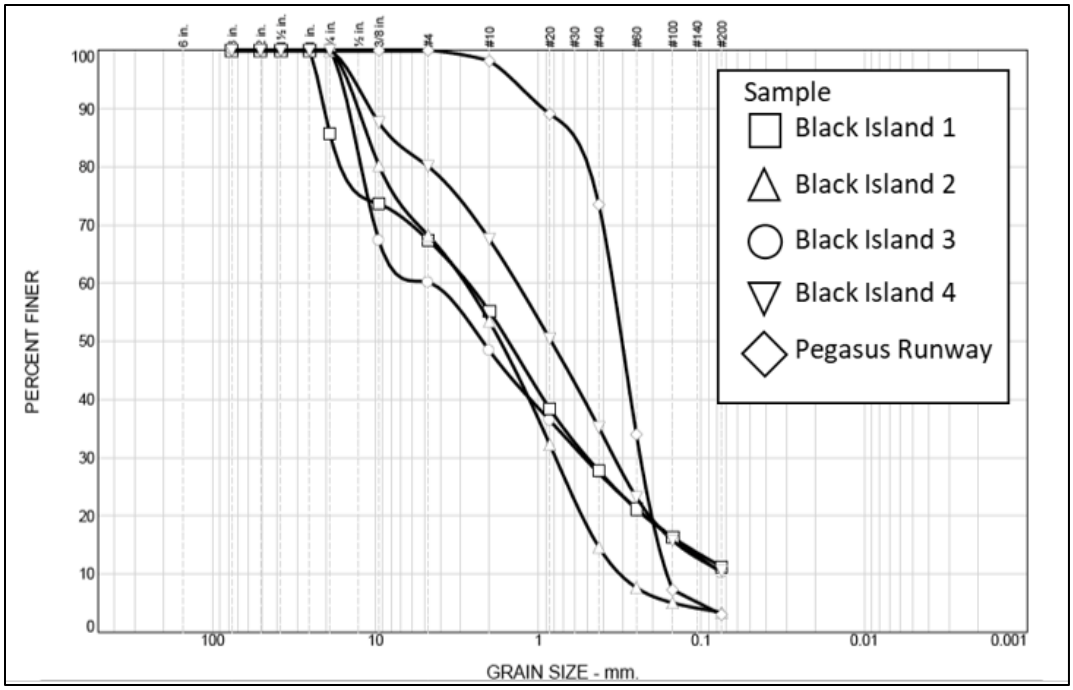


Table 3. Percent of gravel, sand, and silt and clay particles.

Sample	% > 3 in.	% Gravel		% Sand			% Fines	
		Coarse	Fine	Coarse	Medium	Fine	Silt	Clay
Black Island 1	0.0	14.3	18.3	12.3	27.4	16.5	11.2	
Black Island 2	0.0	0.0	31.8	14.8	38.8	11.2	3.4	
Black Island 3	0.0	0.0	39.9	11.7	21.1	17.2	10.1	
Black Island 4	0.0	0.0	20.0	12.5	32.4	24.6	10.5	
Pegasus Runway	0.0	0.0	0.0	1.8	24.7	70.5	3.0	

Figure 8. Microscope images of soil samples collected from Black Island in 2010.

Figure 9. Microscope images of particles collected from snow next to Pegasus Runway in 2011.

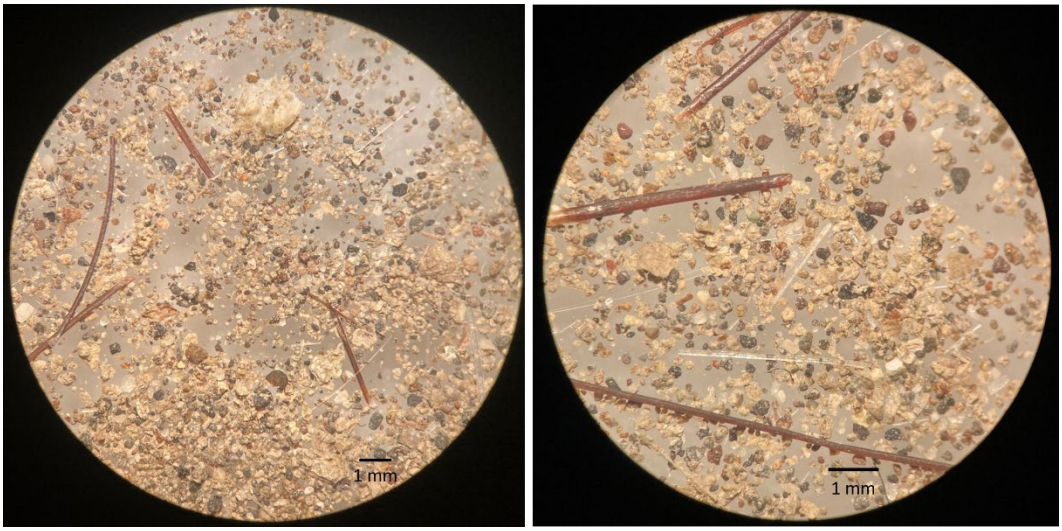


Figure 10. Energy dispersive X-ray spectroscopy (EDAX) spectra of particles from (*A*) Black Island and (*B*) Pegasus Runway.

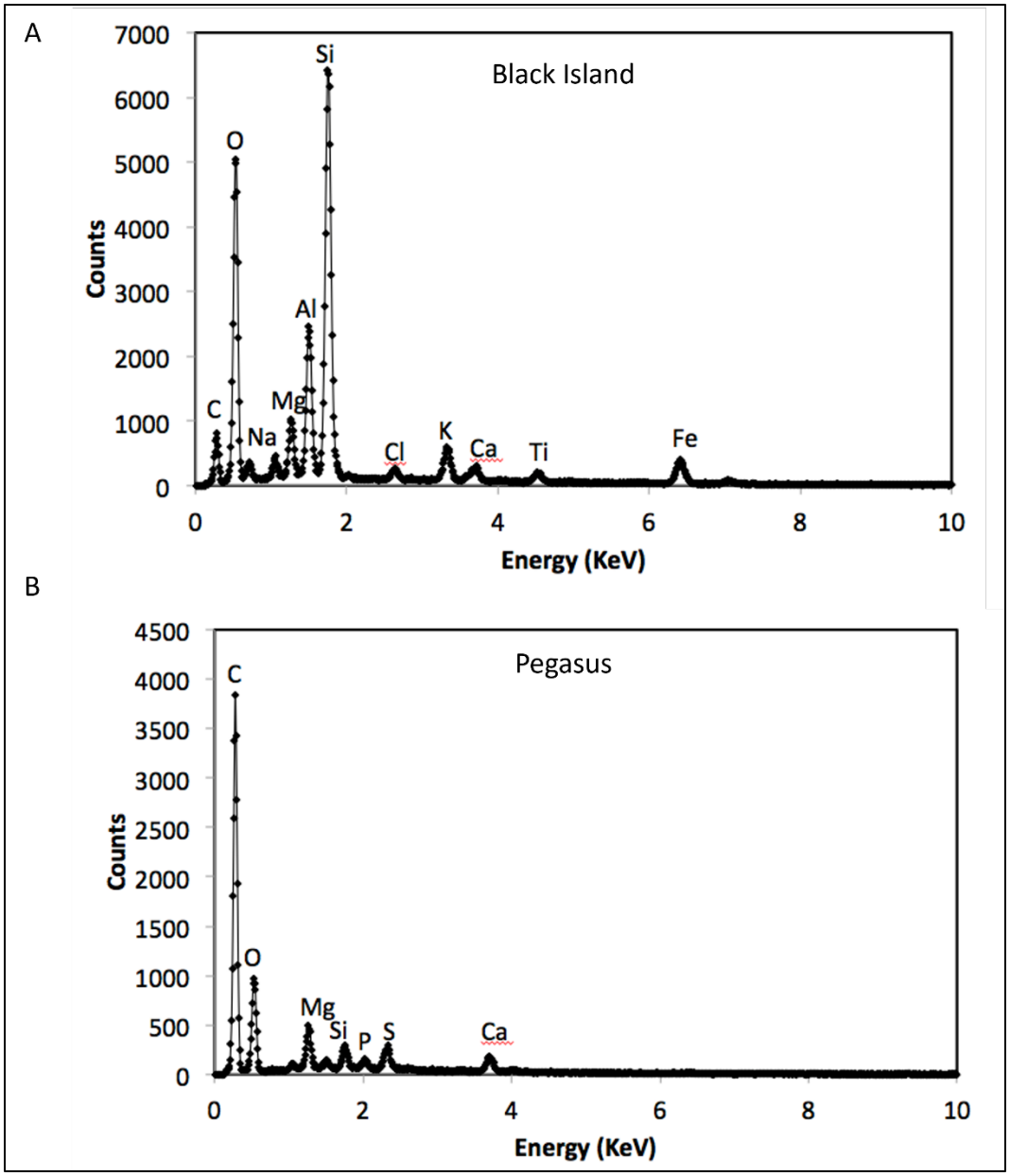
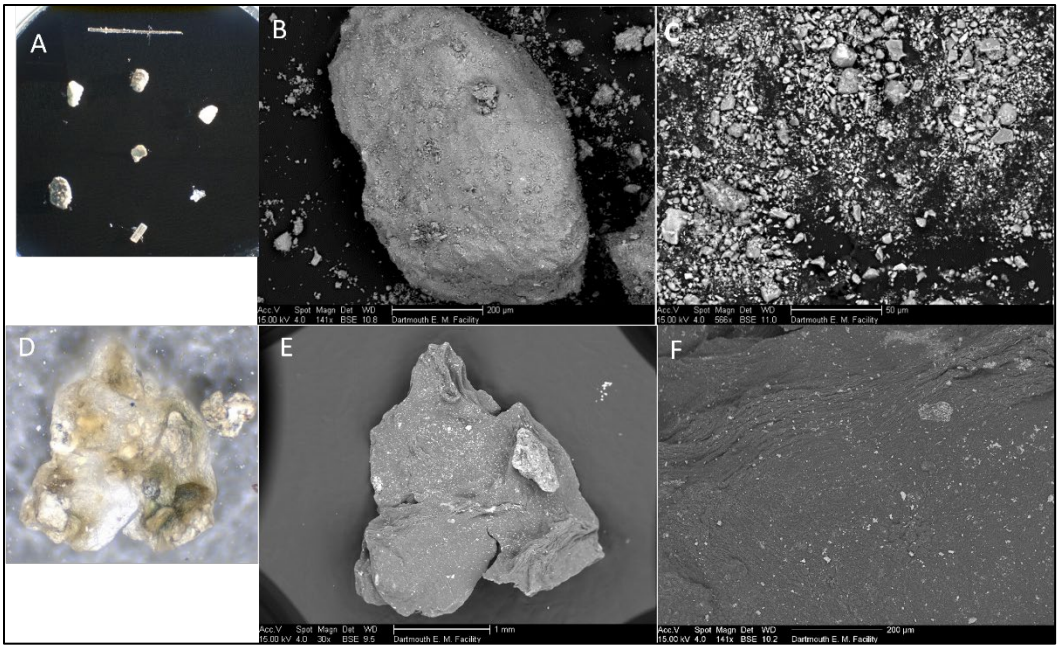


Figure 11. Optical (*A*) and scanning electron microscopy (SEM) (*B* and *C*) images of particles from Black Island collected in 2010. Optical (*D*) and SEM (*E* and *F*) images of particles from Pegasus collected in 2009.



3.2 Microorganisms Isolated from Snow Containing Particulate Matter

To investigate the biological component of the particulate matter, we attempted to culture potential microorganisms attached to dust particulates. Colonies formed within 1–3 days at 25°C but took at least 7 days on plates held at 4°C. Because of the colony morphology, we estimated that cultured microorganisms were predominantly bacterial. In general, colonies appeared damp and glossy, varied in color, and had defined margins. Many pigmented bacterial colonies were observed on the 4°C plates. We attempted to isolate 22 colonies (Figure 12, patches); however, because of varied circumstances, including the inability to isolate the organism and the loss of viability after cold storage, we were successful in isolating and preserving 11 of the isolates grown at room temperature for further study (Figure 12 and Table 4). These 11 organisms are now a part of CRREL's ICE COLD collection.

Figure 12. Microorganism growth at 25°C and 4°C from Snow Sample 3; culturing was performed on nutrient agar (NA), Reasoner's 2A agar (R2A), and tryptic soy agar (TSA). Distinct colonies from 0.1× plates were selected to obtain isolates (patched plates).

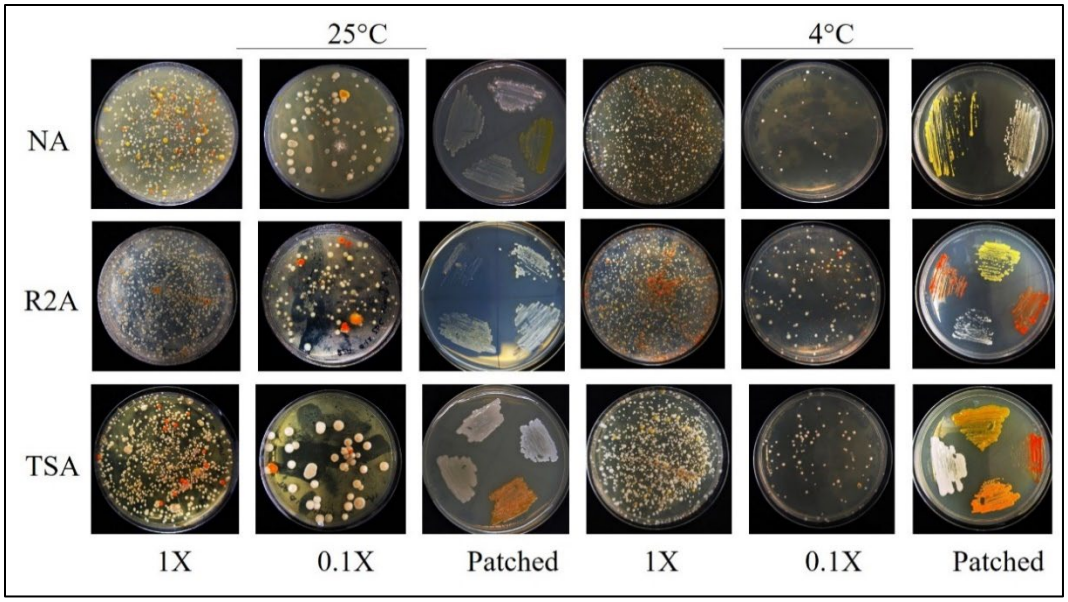


Table 4. Taxonomic classification of isolated microorganisms using the 16S rRNA sequence compared to the NCBI database.

Isolate	Best Match Genus and Species	Base Pair Coverage (% Identity)
1	*Bacillus haynesii*	1176/1179 (99.74)
2	*Psychrobacter fjordensis*	1049/1056 (99.33)
3	*Bacillus haynesii*	1155/1163 (99.31)
4	*Flavobacterium frigidarium*	1112/1119 (99.37)
5	*Psychrobacter cryohalolentis*	1132/1136 (99.64)
7	*Planococcus kocurii*	1126/1131 (99.56)
8	*Psychrobacter fjordensis*	1163/1174 (99.06)
9	*Staphylococcus warneri*	1108/1116 (99.28)
10	*Psychrobacter urativorans*	1176/1180 (99.66)
12	*Psychrobacter glaciei*	1194/1204 (99.17)
13	*Planococcus kocurii*	990/1004 (98.6)

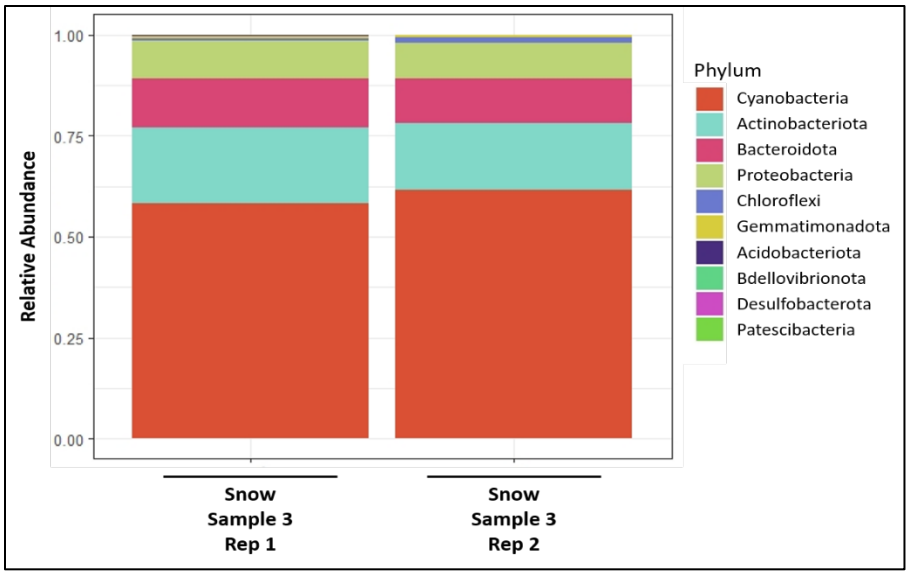
The nearest taxonomic neighbor to the isolate was determined by comparing the 16S rRNA sequence to those recorded in the NCBI 16S Microbial database. Five of the isolates best matched to species within the genus *Psychrobacter*: Isolate 2 and Isolate 8 (*Psychrobacter fjordensis*), Isolate 5 (*Psychrobacter cryohalolentis*)*, Isolate 10* (*Psychrobacter urativorans*), *and Isolate 12* (*Psychrobacter glaciei*) (Table 3). Members of genus *Psychrobacter* are able to survive and reproduce at temperatures ranging from –10°C to 37°C (Bakermans et al. 2006). Other common characteristics of *Psychrobacter* spp. are halotolerance, naturally competent, and possessing distinctive fatty acid compositions (Bakermans et al. 2006). Isolate 4 best matched (99.37% sequence identity) *Flavobacterium frigidarium,* a bacterium which was previously isolated from water around Adelaide Island in Antarctica (Humphry et al. 2001) (Table 3).

Of the isolated organisms, we had multiple matches to the same organism. Thus, we were interested in how similar the 16S rRNA genes were to each other; bacteria with less than 97% similarity of the 16S gene are considered separate species although those with less than 3% similarity do not necessarily belong to the same species (Drancourt and Raoult 2005). The 16S rRNA gene sequences were compared using NCBI BLAST (NCBI, n.d.). Isolate 1 and Isolate 3 were both identified as *Bacillus haynesii*. We compared the sequence similarity of these two organisms, and their sequences were 99% identical. Isolate 2 and Isolate 8 were both identified as *Psychrobacter fjordensis*, with 99.5% sequence similarity. Isolate 7 and Isolate 13 were both identified as *Planococcus kocurii*, with 98.5% sequence similarity.

Because many environmental microorganisms are not culturable, we assessed the microbial ecology of Snow Sample 3. DNA was extracted in duplicate from Snow Sample 3 (collected in 2016) and sent for 16S rRNA gene sequencing. Ten phyla were detected in each sample (Figure 13). Four phyla, Cyanobacteria, Actinobacteriota, Bacteriodota, and Proteobacteria, made up approximately 98% of the reads. Between 58% and 62% of the reads were identified as Cyanobacteria. Proteobacteria made up around 9% of the sample; and interestingly, Firmicutes was not detected although five of the isolated organisms matched to the phylum. Firmicutes are spore formers; and further, spore formation is a survival trait to survive challenging environments such as the cold. Firmicutes have been reported to be lysis resistant during the DNA extraction process (Junier et al. 2022). Therefore, it is possible during the DNA extraction procedure that

we were unsuccessful in lysing Firmicutes, leading to their absence in the microbial-community data.

Figure 13. Relative abundance of bacteria detected in Snow Sample 3.



3.3 Microbial Activity during an Artificial Melt

Considering that microbial communities tend to become more active as temperatures rise, we sought to determine the effect of snowmelt on microbial respiration and how it differed between snow samples with visibly higher or lower particulate matter. Snow Samples 1 (higher dust) and 2 (lower dust), collected in 2016, were incubated from −2°C to 11°C over a period of 25 days. Over time, the total accumulations of CO_2 in Snow Sample 1 (more dust particulates) were significantly higher (p-value < 0.01) than in Snow Sample 2 (Figure 14). For Snow Sample 1 and Snow Sample 2, CO_2 activity in the snow samples increased when the incubation temperature reached approximately 4°C and 9°C, respectively. The difference in CO_2 production during the incubation between our two snow samples suggests the presence of viable microorganisms associated with the particulates. Alternatively, the microorganisms could already be present in the snow and the particulate material provides a carbon source for metabolism.

At the completion of the respiration study, we examined the bacterial communities in Snow Sample 1 and Snow Sample 2. One of the Snow Sample 2 replicates did not contain enough genomic material for analysis and was excluded from further analysis. The low or undetectable DNA concentra-

tions measured in Snow Sample 2 combined with Snow Sample 2's observed lower alpha diversity when compared to Snow Sample 1 (p-value < 0.5) (Figure 15*A*) suggests low levels of microorganisms present in the sample. However, we cannot exclude that lysis-resistant microorganisms may be present and not detected by our methods.

Members of Proteobacteria and Bacteroidota appear to dominate the community after the thaw, composing over 75% of the reads in each of the Snow Sample 1 replicates; over 88% of the sample is represented with the addition of Actinobacteriota and Cyanobacteria. The disparity observed between the Snow Sample 2 replicates, with Proteobacteria and Bacteriodota composing 97% of replicate 1 and only 32% replicate 2 (Figure 15*B*), could be due to overrepresentation of sequences due to low DNA input.

DNA was not extracted prior to performing the respiration study, and so we cannot determine how the communities changed over the course of the study. Snow Sample 3 provides information about the microbial community at a different location (Figure 13), providing some line of evidence that the starting community may have been predominately cyanobacteria. However, this is inconclusive as it is not a direct comparison.

Figure 14. Total accumulation of carbon dioxide (CO_2) over the course of the artificial thaw. CO_2 respiration measurements are the average of the three replicates of Snow Sample 1, Snow Sample 2, and empty wells. The error bars represent the standard error.

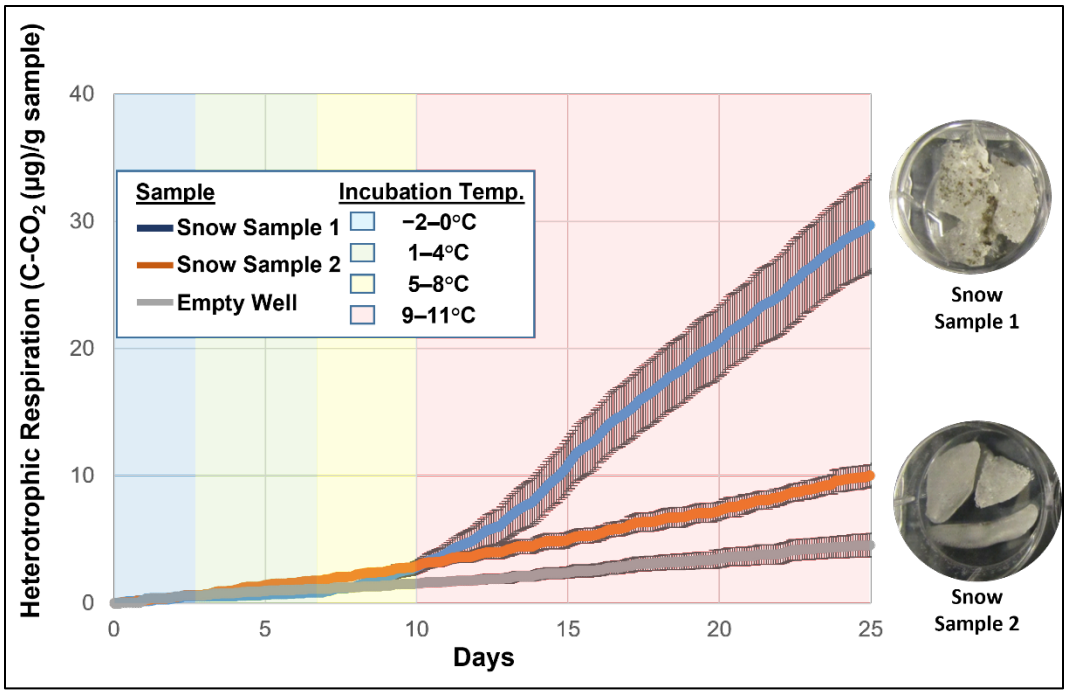
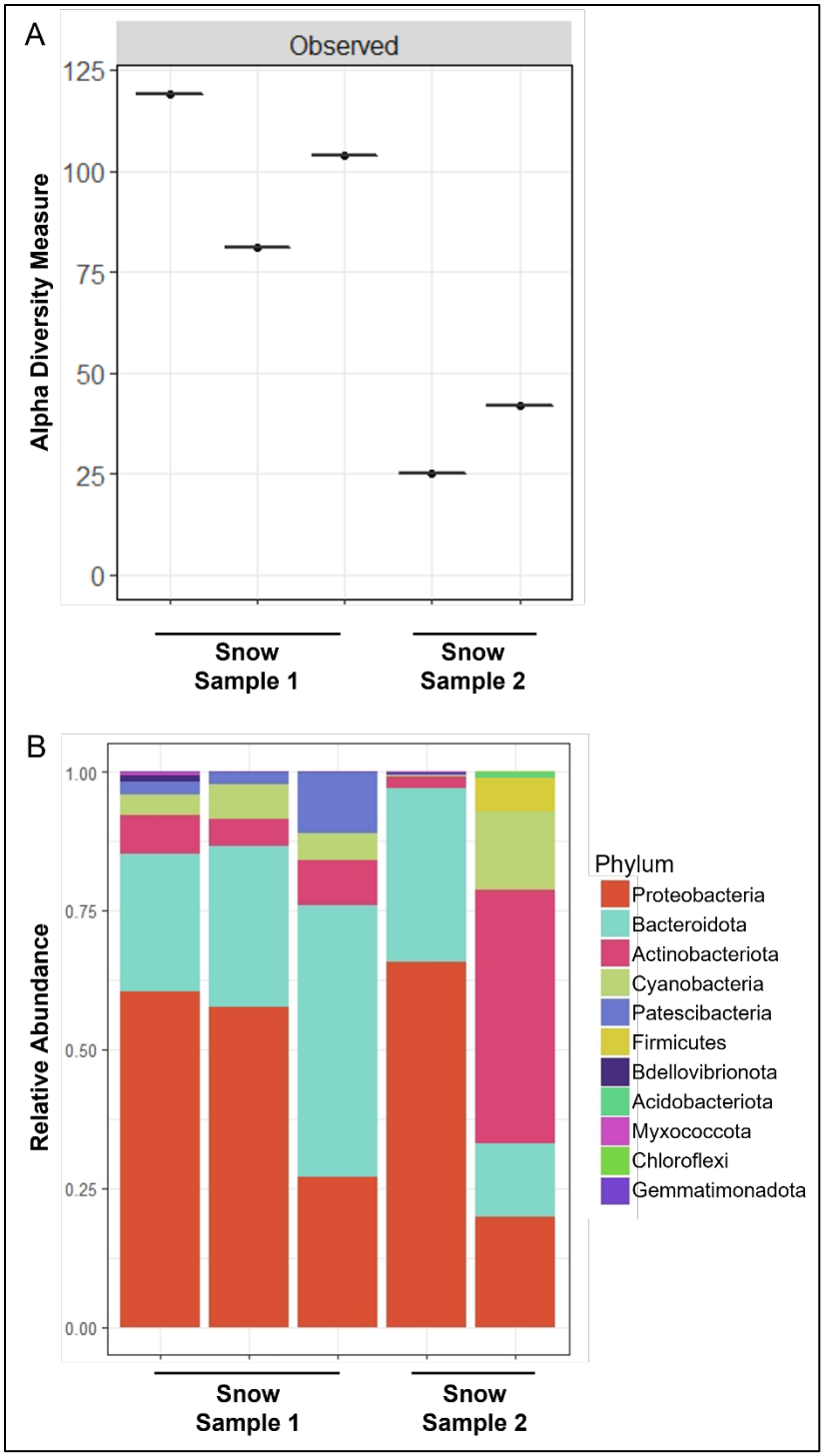


Figure 15. Figure 15. (*A*) Observed amplicon sequence variant (ASV) richness and (*B*) relative abundance of taxonomy in three Snow Sample 1 replicates and two Snow Sample 2 replicates after the artificial thaw.



4 Discussion

From the particle-size analysis and chemical composition, the wind-blown particles deposited around McMurdo Station appear to be aggregates of mineral grains and organic material. Previous studies have shown that mineral composition can disambiguate between windblown and stratosphere transport; short or longer distances; and the type of geologic origin, such as volcanic or aquatic (Bottos et al. 2014; Laluraj et al. 2009). However, with our limited number of samples and replicates, we could not determine the origin of the particulate samples. The wind direction and observations suggest that Black Island is still a likely source; however, more samples and further analysis from Black Island and runways would be necessary to conclude an origin. It has been reported that Antarctic soil communities are highly heterogeneous, varying significantly with location, climate, soil properties, and local environmental (biological) factors (Bottos et al. 2014). Including other plausible Antarctic soil locations, such as the Dry Valleys, for comparison of both the mineral composition and microbial community could help to determine the origin of the particulate material. We recognize that the samples for this study were collected at different times, so it is possible that there are different sources of the material. The particles deposited near McMurdo in 2009 appear more disk like, and the low mass of the particulates could support longer-distance aerial travel, perhaps from a farther on-continent source or from off continent.

The isolation of microorganisms related to the genus Psychrobacter indicate that these organisms are adapted for cold environments and thus are capable of proliferating on the environment of the McMurdo Ice Shelf. *Psychrobacter glaciei* sp. nov. and *Psychrobacter fjordensis* sp. nov. are capable of growth at 4°C (Zeng et al. 2015, 2016), and *Psychrobacter cryohalolentis* sp. nov. is capable of growth at –10°C (Bakermans et al. 2006). The particulate-containing snow samples contained viable microbial communities that increased activity at temperatures of less than 10°C. Temperatures can reach as high as 6°C on the McMurdo Ice Shelf during December and January (Klokov and Diemand 1995).

One interesting characteristic of the particulates is the abundance of organic material that seems to glue mineral particulates together. Perhaps this organic material is a mixture of microorganisms and extracellular polymeric substances (EPS), which are composed of polysaccharides, proteins,

and DNA; many cold-tolerant microorganisms, such as cyanobacteria, produce EPS (Rozwalak et al. 2022). Cyanobacteria was detected in all three snow samples and was dominant in Snow Sample 3. Psychrophiles also produce EPS as a cryoprotectant. For example, Marx et al. (2009) showed that *Colwellia psychrerythraea* increases exopolysaccharide production when grown at –8°C to 14°C. The above-freezing temperatures, water, and 24-hour daylight could provide an opportunity for microbial mats to form, creating the large, dark material described in Haehnel et al. (2019) later in the summer where the dust deposition remains undisturbed. Further research is necessary to elucidate the specific contributions of these microorganisms and their EPS to the cohesive nature of the particulates and their potential impacts on the snow environment.

5 Conclusion

In this study, we gained valuable insights into the biological component of particulate material present in the vicinity of the Pegasus ice and Phoenix snow-compacted runways. Through our research, we successfully isolated 11 bacteria from the samples and measured increased microbial activity in particle-laden snow during a simulated melt. However, the analysis of particle chemistry and size from a potential source yielded inconclusive results, requiring further investigation and refinement in future studies to understand the influence of particles on the snow.

As temperatures continue to rise and snowmelt becomes more frequent and intense in Antarctica, the interactions between microorganisms, particulate matter, and the changing environment could have far-reaching consequences. Understanding these complex dynamics is crucial for developing strategies to mitigate potential risks and adapt infrastructure in a manner that ensures the safety and functionality of the runway in the years to come.

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Abbreviations

ASV	Amplicon sequence variant
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
CO_2	Carbon dioxide
CRREL	Cold Regions Research and Engineering Laboratory
EDAX	Energy dispersive X-ray analysis
EPS	Extracellular polymeric substances
ICE COLD	Innovative, Collaborative, Exploratory Cold Regions Organism Library for Discovery
NA	Nutrient agar
NCBI	National Center for Biotechnology Information
R2A	Reasoner's 2A agar
SEM	Scanning electron microscopy
SS1	Snow Sample 1
SS2	Snow Sample 2
TSA	Tryptic soy agar

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6. AUTHOR(S)

Alison K. Thurston, Karen Foley, Shelby Rosten, Susan Taylor, Robert B. Haehnel, and Robyn A. Barbato

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Engineer Research and Development Center (ERDC)
Cold Regions Research and Engineering Laboratory (CRREL)
72 Lyme Road
Hanover, NH 03755-1290

8. PERFORMING ORGANIZATION REPORT NUMBER

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14. ABSTRACT

During weather events, particles can accumulate on the snow near the Pegasus ice and Phoenix compacted-snow Runways at the US McMurdo Station in Antarctica. The deposited particles melt into the surface, initially forming steep-sided holes, which can widen into patches of weak and rotten snow and ice. These changes negatively impact the ice and snow runways and snow roads trafficked by vehicles. To understand the importance of microbes on this process, we examined deposited dust particles and their microbial communities in snow samples collected near the runways. Snow samples were analyzed at the Cold Regions Research and Engineering Laboratory where we performed a respiration study to measure the microbial activity during a simulated melt, isolated microorganisms, examined particle-size distribution, and performed 16S rRNA gene sequencing. We measured higher levels of carbon dioxide production from a sample containing more dust than from a sample containing less dust, a finding consistent with viable dust-associated microbial communities. Additionally, eleven microorganisms were isolated and cultured from snow samples containing dust particles. While wind patterns and satellite images suggest that the deposited particles originate from nearby Black Island, comparisons of the particle size and chemical composition were inconclusive.

15. SUBJECT TERMS

Antarctica, Cold-adapted bacteria, Cold regions, Dust, McMurdo Station (Antarctica), Microorganisms, Runways (Aeronautics), Snow

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