ASSESSMENT OF FUNGAL AND BACTERIAL BIOAEROSOLS IN AMBIENT

AIR IN FAIRBANKS, ALASKA

By

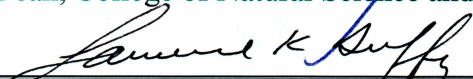Mitali S. Patil

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ASSESSMENT OF FUNGAL AND BACTERIAL BIOAEROSOLS IN AMBIENT

AIR IN FAIRBANKS, ALASKA

A

THESIS

Presented to the Faculty

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By

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Abstract

Bioaerosols are solid or liquid particles of biological origin that are suspended in the surrounding air or other gaseous environments. Bioaerosols can cause diseases, allergenic or toxicological reactions, respiratory distress, and can be potential bioterrorism threats. Studies concerning ambient bioaerosols have never focused upon central Alaska, and only one experiment has utilized the DRUM (Davis Rotating Unit for Monitoring) impactor as the collection apparatus. This study focuses on the assessment and identification of fungi and bacteria present in the ambient air collected by the DRUM impactor from March 2008 to January 2009. The samples were collected on Mylar™ and aluminum substrates (with or without apiezon coating) and subjected to DNA extraction and nested PCR using universal primers for the 16S rRNA gene in bacteria and ITS (internal transcribed spacer) region in fungi. The PCR products were used to generate a clone library, and selected clones from each sample clone library were sequenced. Sequences were taxonomically classified using BLAST for fungal identification and RDP Pipeline for bacterial identification to the genus level. Numerous species of bacteria (i.e., *Ralstonia sp., Bradyrhizobium sp., Sphingomonas sp.*) and fungi (i.e. *Fusarium sp., Cladosporium sp., Penicillium sp.*) were identified from the clone libraries, thus indicating that the DRUM impactor has potential for monitoring biological content in the air. The resulting patterns in bacteria and fungi during the course of the year indicate that the DRUM sampler may also have the potential to detect fluctuations in populations that result from meteorological conditions, seasonal cycles, and climatic conditions.

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1. Introduction

At the beginning of the 20th century, the study of aerosols, suspensions of solid or liquid particles in gas (1, 2), was a highlight of physical science because aerosols represented the smallest observable division of matter at the time. In fact, aerosols contributed to the early understanding of Brownian motion and diffusion, Millikan's measurement of electron charge, and Wilson's cloud chamber experiment for the study of ionizing radiation (2). Aerosol research then languished until the 1970s and 1980s, when environmental and health concerns arising from air pollution in the community and occupational environments became public knowledge. After the 1980s, the field expanded rapidly, promoting the development of aerosol technology, new methods for detecting and identifying aerosols. Today, aerosol technology has become an important tool for assessing how anthropogenic aerosols impact the environment, and how environmental aerosols affect society. Some common examples of aerosols created by humans or the environment are suspended soil particles, smoke, photochemical particles such as nitrates and sulfates, salts, volcanic ash, fume particles, and microbial matter. Each aerosol varies in its ability to affect vision, health, and the general quality of life of humans, and have a significant impact on ecosystems as well (1, 2).

A sub-discipline in aerosol science focuses on bioaerosols, biological particles such as bacteria, viruses, fungi, and other viable particles such as pollen, endotoxins, and peptidoglycans that are suspended in liquids or gases (1, 3, 4). Bioaerosols can be found in nearly any location, including the natural environment, the workplace, and commercial, residential, and industrial areas. Bioaerosols are present almost everywhere,

but particular bioaerosols can become potential hazards, especially in regards to agriculture, biotechnology, and non-industrial indoor environments (5). Hazards can arise from high concentrations of these particles or from exposure to unfamiliar particles, and can cause respiratory distress, microbial infections and diseases, and toxicological or allergenic reactions (1, 4, 5, 6, 7, 8). The costs of bioaerosol hazards to society are great; in fact, in the USA, bioaerosols are responsible for over 250 million episodes of respiratory infection, resulting in 75 million physician visits, 150 million days lost from work, medical care costs of 10 billion dollars, and income loss of 10 billion dollars per year (1). In addition, biological aerosols can be potential terrorist threats, such as the well-known anthrax attacks in 2001 (1, 4, 5, 9, 10).

However, despite the high costs that result from exposure to potentially hazardous biological aerosols, not much is known about these particles because this field is relatively new and has not been explored to its full potential (1). New pieces of equipment that target biological particles in the environment are being designed or modified for health studies (11), and in the recent decade, numerous ambient aerosols analyses, both indoors and outdoors, have been performed utilizing various types of samplers – HVAC (Heating, ventilation, and air conditioning) filters (12), AGI-30 (All Glass Impingers) liquid impingers (13), Surface Air Samplers (5), Swirling Aerosol Collector (5), and Biotest Reuter Centrifugal Samplers (5), to name a few. However, there is another piece of equipment that has the potential to be effective in testing for bioaerosols in the environment – the Davis Rotating Unit for Monitoring (DRUM) impactor (14). The ability to determine the identities of ambient bioaerosols using

standard aerosol sampling techniques such as the DRUM impactor (Fig. 1) may be useful in determining the bioaerosol content of particular regions and relating the bioaerosol content to the chemical composition of the non-biological aerosols collected by the instrument and the health hazards associated with the area in question.

Up until the last decade, most studies focused on the size or concentration distribution of bioaerosols rather than the actual identities of associated microbes because of poor sampling methods and low bioaerosol concentrations for genetic analyses (15). However, using the DRUM impactor to collect samples and then performing genetic analyses on the samples
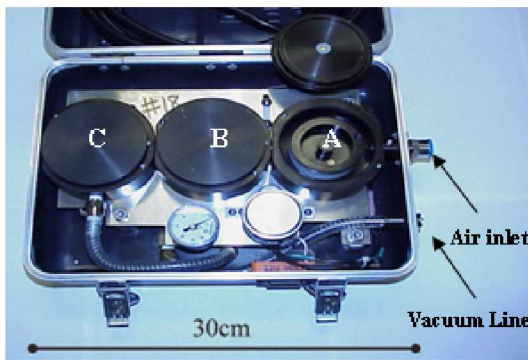
Figure 1: DRUM impactor. This is the model of DRUM impactor utilized in the experiment. From right to left, there are three stages, A, B, and C respectively. The air inlet and vacuum lines are labeled and shown with arrows pointing at the respective ports.

will help rectify this situation, and will allow the determination of the species of organisms present in the air, whether viable or nonviable (16). The DRUM impactor has been designed to reduce particle bounce and to allow for collection time determination, and has been used extensively for the collection of non-biological aerosols. If the DRUM impactor could be used for collecting bioaerosols, then not only could a representative sample of the biological community be analyzed, but the biological composition of the air could be related to the non-biological composition of the air. In addition, the time of capture of the bioaerosol content collected by the DRUM impactor could be determined, allowing for the relation of bioaerosol content to health hazards. Therefore, by validating the DRUM impactor as an effective tool for collecting a representative sample of the

bioaerosols present in an air sample, the field of atmospheric chemistry will advance significantly in determining the biological composition of the surrounding ambient air.

Detecting bioaerosols in ambient air will not only benefit the field of atmospheric chemistry, but will also have a significant impact in health matters and studies. As mentioned earlier, bioaerosols can be potential terrorist hazards, especially because they can affect large areas and many people in a short amount of time. However, monitoring the air in the targeted areas with the DRUM impactor and identifying the biological content of the collected air samples will allow for these harmful bioaerosols to be detected, or to determine whether they are rising in concentration. Monitoring for bioterrorist threats in the air in advance will allow for action to be taken against them immediately, thus preventing an outbreak of dangerous microorganisms that could potentially infect a large population of people.

Bioaerosol detection and identification studies will also greatly assist in military endeavors as well. Military personnel are constantly stationed in various areas of the world and are sent to their posts without knowing what biological hazards may be present at their stations. However, using the DRUM impactor to determine the biological composition of areas where the military will be stationed in advance will allow for the detection of potentially harmful microorganisms that the soldiers could be exposed to. Knowing the biological composition of the air in advance will allow for the military to provide soldiers with proper equipment or medication needed to combat the biological threats detected by the DRUM impactor, thus reducing the health hazards for the stationed soldiers.

Bioaerosol analyses do not have to be limited to bioterrorist threats and military personnel alone, but can also be highly useful for detection of health hazards in residential and commercial areas. For instance, using the DRUM impactor to monitor developing residential and commercial areas will help with determining whether the specified area is safe for daily activity or residence. Knowing the biological content of the ambient air in these areas will also assist in providing the public an idea of what kind of hazards are present in a particular area, especially those people who have allergies or are immunocompromised. Therefore, developing the DRUM impactor as a tool for the collection and identification of bioaerosols will be highly useful for monitoring air quality and determining potential biological threats.

This study investigates whether the DRUM impactor could be a promising tool for the collection of fungal and bacterial bioaerosols, and if samples collected by the DRUM impactor can be identified successfully using genetic tools and are indicative of patterns. This study will be looking at samples of ambient air in Fairbanks, Alaska, so we expect to see bacterial and fungal taxa that are common in outdoor air rather than human pathogens. We also expect to see a seasonal pattern for most bacteria and fungi taxa, with the highest abundances of bacteria and fungi occurring during the summer, and lowest abundances occurring during the harsh winters.

Samples were collected using the DRUM impactor during the course of a year (March 2008 – January 2009) from a port of a building on the University of Alaska, Fairbanks campus. The DNA from these samples was then extracted, amplified via a nested polymerase chain reaction (PCR) procedure to enhance detection of airborne

bacteria and fungi (13), and analyzed by gel electrophoresis to confirm the presence of DNA within the samples. Quantification of DNA was determined by purifying the pooled PCR products and analyzing the purified DNA using a spectrophotometer. Clone libraries were then constructed to taxonomically identify microbial community members in the bioaerosols and to compare seasonal variability in community structure. This study aims to reveal the microbial communities present in the ambient air as well as demonstrate the DRUM impactor as a potential tool for determining bioaerosol components in the air.

2. Methods and Materials

2.1| The DRUM impactor

A DRUM impactor is a type of cascade impactor that relies on momentum separation of particles (2). DRUM impactors utilize cylindrical impaction surfaces, drums (Fig. 2), which rotate at a constant rate under an airstream. This system allows for the

Figure 2: Drum. Side view of a cylindrical drum from a DRUM impactor.

simultaneous operation of drums in a series. The DRUM impactor model that was used in this experiment has three stages, compartments that hold a drum and a slit orifice (Fig. 3), so the sampler creates a three-drum-cascade. Each slit orifice is found in front of a drum, and determines the cutoff size for the particles collected by each drum (1, 2, 17). The slit orifice is largest for the drum closest to the air inlet, and decreases in size with each stage. Therefore, Stage A has the largest slit orifice and collects the largest particles (1.15 to 2.5 µm in aerodynamic diameter), while Stage C has the

Figure 3: DRUM impactor stage. An example of a stage, complete with a drum, the compartment, and the slit orifice (marked by arrow).

smallest slit orifice and collects the smallest particles (0.1 to 0.34 µm in aerodynamic diameter). Substrates such as Mylar, aluminum, or Teflon are taped onto the drums, so the particles are collected on the substrates, making for convenient removal and analysis of samples. All three drums operate simultaneously, so the DRUM impactor creates the 'cascade' mentioned above, and the particles impact the substrate of a particular drum according to their size.

The DRUM impactor has two ports: the air inlet and the vacuum outlet (Fig. 1). Ambient air is drawn from the surrounding environment into the DRUM impactor through use of a vacuum pump or vacuum line that is attached to the vacuum outlet. The aerosols flow in sequence through the successive stages, so the particles captured on the impaction surface of a given stage represents all particles smaller than the cutoff size of the previous stage and larger than the cutoff size of the next stage. The use of cylindrical drums instead of flat plates provides a distinct advantage despite their similar dynamics because the cylindrical shape of the DRUM allows for constant rotation. The constant rotation reduces the possibility of particle build-up on any region of the drum, thus preventing particle bounce, and allows the time of a particular sample on the drum surface to be determined (1, 2). The particular DRUM impactor model utilized in the experiment draws in ambient air at the rate of 23 liters/minute, and the drums rotate 4 mm per day, thus enabling the DRUM impactor to operate nonstop for a period of six weeks.

The DRUM impactor was created in the 1980s by the CNL (Crocker Nuclear Laboratory) Air Quality Group at University of California, Davis (UC Davis) as an efficient and portable method for collecting aerosol particles and designed to replace the prior bulky and inconvenient aerosol samplers. UC Davis determined and affirmed the accuracy and precision of the DRUM impactor in the years 1984 and 1987 respectively (18). The precision of the DRUM impactor was determined by operating four DRUM units side by side and comparing the samples obtained by each DRUM impactor. The accuracy of the DRUM impactor was confirmed by tests done both in the laboratory and

infield inter-comparisons at a very clean site near Reno, Nevada (18). By performing

these tests and obtaining satisfactory results, UC Davis validated the DRUM impactor as

a useful and effective tool for collecting aerosols such as dust, smoke, and other

nonviable particles.

2.2| Justification for using DRUM impactor to collect bioaerosols

UC Davis only tested the DRUM impactor's effectiveness in collecting nonviable

particles (18), with the exception of one project that studied the bioaerosol content of the

air in a veterinary clinic (14). This project only focused on culturing biological aerosols

collected by a DRUM impactor (14). While culturing is a useful tool, it only detects

about 1% of the organisms present in a sample (19) because not all organisms are

culturable (6). Therefore, using only culturable microbial data may lead to an

underestimate of the microbial diversity and concentrations within a sample (6, 20). This

study was indeed useful for proving that the DRUM impactor has the capability to collect

biological aerosols, but it likely did not detect the entire community composition of the

sample collected by the DRUM impactor.

DRUM impactors have the potential to be useful for collecting bioaerosols

because they can collect fine particles less than 2.5 μm in aerodynamic diameter (1, 2).

Bacteria are typically 1 μm wide and about 1-5 μm long depending on the length of the

coccus, rod, or spiral shape of the bacteria. Viruses are even smaller; the smallest viruses

have a diameter of 0.01 μm (14). Fungi are generally larger than bacteria and viruses, and

many have large fruiting bodies. However, fungi tend to release spores, resting structures

that germinate in favorable conditions, and the size of the spores are typically about 2 – 40 μm (14, 21).

The DRUM impactor for this experiment is not equipped with the appropriate slit size necessary to collect isolated viruses because it can only collect particles of 0.1 μm in aerodynamic diameter and higher. However, some viruses are bacteriophages and therefore inhabit bacteria, so when the host is collected, the virus is collected as well (14, 21). Most viruses that are not bacteriophages are transmitted through airborne and droplet transmission. In droplet transmission, the viruses are encased within a droplet, thus forming an aerosol. These viral droplets can range from 1 to 100 μm in size. Viral droplets that are smaller than 2 μm in size have a higher infectivity than the viruses themselves because of the droplet's ability to shield the internal virus from desiccation, light, and temperature (22). Therefore, while the DRUM impactor used in this experiment may not have the appropriate slit size to collect an isolated virus, the sampler has the ideal slit size range to collect viral bioaerosols and bacteriophages, fungi, and bacteria.

2.3| Bioaerosol collection

Bioaerosol samples were collected between March 2008 and January 2009 using a 3-stage DRUM impactor. A tube passing through a port in the window of the Syun-Ichi Akasofu Building laboratory

Figure 4: Collection location. Location at which samples were collected. The port through which ambient air was drawn is located three floors above the ground (marked by arrow), and the sampling location is approximately 250 ft. near boreal forests, and 30 ft. high from the pavement.

(Fig. 4), located on the campus of University of Alaska Fairbanks, in Fairbanks, Alaska, was utilized to drawn ambient air into the sampler's inlet, and the vacuum line within the laboratory maintained the pressure of the system at 13-14 mmHg. The DRUM impactor collected particles with diameters between 0.1 μm and 2.5 μm in aerodynamic diameter, and drew in air at a rate of 23 L/min, while the drums themselves rotated 4mm per day, allowing for six-week non-stop rotation.

In order to collect the bioaerosol samples, the substrates Mylar™ and aluminum were taped onto the circumference of sterilized drums in a sterile hood. The substrates were mounted onto the drums and were either inserted into the sampler directly or were coated with Apiezon-L™ to enhance the collection of bioaerosols. For most volumetric samplers, like impactors, sieve samplers, impingers, centrifugal samplers, and filter cassettes, shorter sampling times increase variability between side-by-side samples, thus lowering the representativeness of sampling (23). Therefore, substrates were changed every two weeks, a short sampling time, but long enough to allow for the low bioaerosol content of the air to be detected.

2.4| Bioaerosol DNA extractions

After each two-week sampling interval, the substrates containing the samples were removed from the drums and isolated in sterile Petri dishes to prevent contamination. The substrates were then soaked in 500 μl (0.5 mL) of sterile DNAase and RNAase-free water in sterile, nuclease-free microcentrifuge tubes for 24 hours, and were then vortexed at low speed for five minutes to remove the cells from the substrates. The substrate was then removed from the tube and the cells suspended in the water were

stored at 4 °C for no longer than 24 hours. The suspended bioaerosol samples for each of

the three stages were then combined for DNA extraction to increase the total microbial

biomass DNA extraction. DNA was extracted from the suspended cells using the DNA

Extractor SP Kit by Wako (VWR), which uses the sodium iodide extraction method to

minimize contamination by keeping the extraction reaction isolated in a single

microcentrifuge tube. Extracted DNA was stored at -20 °C (extracted DNA was not

quantified).

2.5| Bioaerosol DNA amplification & purification

For the DNA amplification, separate PCR reactions were performed for bacteria

and fungi for each month sampled. PCR reactions were performed using 0.625 µl of 20

µM primer, 1 µl of extracted DNA template (0.9 ng/µl-1.5 ng/µl), sterile DNAase-free

water, and illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare) for a total volume

of 25 µl. The primers utilized for bacteria DNA amplification were universal bacterial

16S rRNA gene primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1392R

(5'ACGGGCGGTGTGTRC-3'), and for fungi, universal fungal internal transcribe spacer

region (ITS) primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and TW13 (5'-

GGTCCGTGTTTCAAGACG-3') (ABI). Thermal cycler conditions for fungal DNA

reactions were denaturation at 95 °C for 2 minutes, followed by 25 cycles at 94 °C for 30

seconds, 56 °C for 40 seconds, and 72 °C for 3 minutes, followed by a final extension at

72 °C for ten minutes. Thermal cycler conditions for bacteria DNA reactions were

denaturation at 95 °C for 6 minutes, followed by 25 cycles at 94 °C for 1 minute, 55 °C

for one minute, and 72 °C for 1 minute and 40 seconds, followed by a final extension at

72 °C for ten minutes. 5 μl of each PCR product was then subjected to agarose gel electrophoresis using a 1% agarose gel at 100 volts for 45 minutes, and the gel was then stained with ethidium bromide for viewing purposes. However, during the first PCR test, only the positive controls run with each gel amplified, probably due to low DNA concentrations in the extracts resulting from low microbial biomass in the air on a per volume basis in relation to other commonly tested environments like water or soil (24, 25). In order to increase the yield of PCR products to enable analysis, nested PCR was performed using the previous PCR product as the template for the second PCR reaction utilizing the same primers listed before (5, 25). The nested PCR products were then pooled and purified according to the protocol included with the illustra GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare). Purified PCR products were quantified using a NanoDrop ND-1000 Spectrophotometer and the "Nucleic Acid Measurement" function on the accompanying program NanoDrop v3.1.0. Purified products were then stored at -20 °C.

2.6| Bioaerosol clone library construction

Separate clone libraries were constructed for bacterial and fungal PCR products generated from each sampling date separately for sequence analysis. Immediately prior to cloning, a 3' poly A tail was added to all purified PCR product samples by incubating (at 72 °C for 10 minutes) 1.5 μl of 50 mM $MgCl_2$, 2.5 μl of 10X PCR buffer, 0.1 μl Taq and 2.5 μl of 10mM dATP with 13.4 μl of water and PCR product combined for a final volume of 20 μl. This incubation step increases the number of clones by 25-fold, since all the purification steps result in the loss of 3'A needed for successful ligation, and thus

ensures that enough 3'A remains for the cloning ligations to be successful (63, TOPO-TA

Cloning Kit for Sequencing User Manual, Invitrogen™). Cloning was performed

following the protocol included with the TOPO-TA pCR4 vector kit (Invitrogen™).

DNA volumes ranging from 1.5 µl – 3.0 µl were utilized for the cloning reactions, and

Lysogeny broth (LB) plates contain 50 µg/mL kanamycin were used as the selective

media. Clone libraries were stored in growth blocks at -80 °C and in Granier well plates

with replicates stored at -20 °C. Clones were then stamped onto Omnitrays containing LB

Agar with 50 µg/mL kanamycin and were then sent to Macrogen, Inc. (Seoul Korea) for

plasmid purification and single extension Sanger sequencing with primers M13F (5'-

GTAAACGACGGCCAG-3') and M13R-pUC (5'-CAGGAAACAGCTATGAC-3').

2.7| Taxonomic identification of microbes in bioaerosols

Bacterial sequences obtained from Macrogen were trimmed, aligned, and

taxonomically identified using the ribosomal project II (RDP II) pipeline. Sequences with

less than 90 % confidence based on RDP's classifier were considered unclassified at that

particular taxonomic level (26). Bacteria sequences were generally identified to the genus

level.

Fungal sequences were assembled, aligned, and trimmed using CodonCode

Aligner. The low quality bases present in the sequences were then masked using the

Mask Low Quality Bases function in Microbial Pipeline, Life Science Informatics Portal

(https://biotech.inbre.alaska.edu/portal/) using 20 as the cutoff quality score. Sequences

were further trimmed with TrimSeq (http://emboss.bioinformatics.nl/cgi-

bin/emboss/trimseq) with a window size of 20 and a percent threshold of 5. Processed

sequences were then identified using BLAST, Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/). Sequences with less than 95% sequence similarity were considered unclassified at that particular taxonomic level (26). Fungi sequences were generally identified to the genus level.

2.8| Bioaerosol richness calculations, weather correlations, and PCA analyses

Once samples were successfully identified using the procedures outlined above, the genus richness, Chao1, was calculated from the data using the species richness formula outlined by Hughes et al, 2001 (27), where species richness is estimated as -

$$S_{Chao1} = S_{obs} + \frac{n_1^2}{2n_2}$$

In the equation above, S_{obs} represents the number of observed species, n_1, the number of species captured once or singletons, and n_2, the number of species captured twice. However, because the sequences were identified to the genus level, the variable 'species' was substituted with the variable 'genera' to calculate genus richness. Average Chao1 was calculated by dividing the seasonal Chao1 results by the number of months samples were collected for in each season. The dominant genera for both bacteria and fungi were then selected at both the seasonal and monthly levels. The dominant operational taxonomic units (OTU) for bacteria were those genera that comprised more than 5% of the clones per month and per season, and the dominant OTU for fungi were those genera that comprised more than 7.5% of the clones per month and per season. The dominant OTUs for bacteria and fungi per season were then compared to weather data collected by the Arctic Climate Research Center in Fairbanks, Alaska (http://climate.gi.alaska.edu/) from March 1, 2008 to January 31, 2009. Correlations between the genera, classes, phyla

and the weather data (maximum temperature, minimum temperature, and snow levels on the ground) were calculated using the Data Analysis Toolpak from Microsoft Excel (www.microsoft.com). In addition, principal components analysis was used to compare and contrast the patterns of occurrence for dominant bacteria and fungi genera, classes, and phyla to each month. Principal components analyses were calculated using R version 2.13.0 by The R Foundation for Statistical Computing (www.r-project.org/).

3. Results

<u>3.1| Community structure of bioaerosols</u>

The PCR products were analyzed by agarose gel electrophoresis, and after electrophoresis, the products were pooled, purified, and then quantified using an ND-1000 Spectrophotometer (Fig. 5A & 6A). The quantified concentrations were then graphed alongside certain weather trends, including maximum temperature, minimum temperature, snow depth (in) on the ground throughout the sampling year as recorded by the Arctic Climate Research Center in Fairbanks, Alaska (Fig. 5B & 6B). Precipitation and snowfall were excluded from this analysis due to minimal data.

The DNA concentrations of bacterial PCR products ranged from 147.6 ng/μl to 22.5 ng/μl, with the concentrations peaking in March (130.3 ng/μl) and July (147.6 ng/μl), and dropped considerably in the months of December (22.5 ng/μl) and January (32.4 ng/μl) (Fig. 5A). The intensity of the bands and the concentrations of bacterial DNA on a monthly basis seem to correspond to the weather (Fig. 5B). The bacterial DNA concentrations and band intensity start out high in early spring, when the snow begins to melt and the temperatures begin to rise. However, it is from June through August that we see a sharp increase in DNA concentration in relation to rising temperatures of summer. From September to December, the DNA concentrations begin to drop again as the temperature decreases. January seems to have a relatively high concentration of DNA considering the sharp drop in the temperature. However, it mostly seems that the band intensities in the gels and the bacterial concentrations of DNA correspond closely to weather.
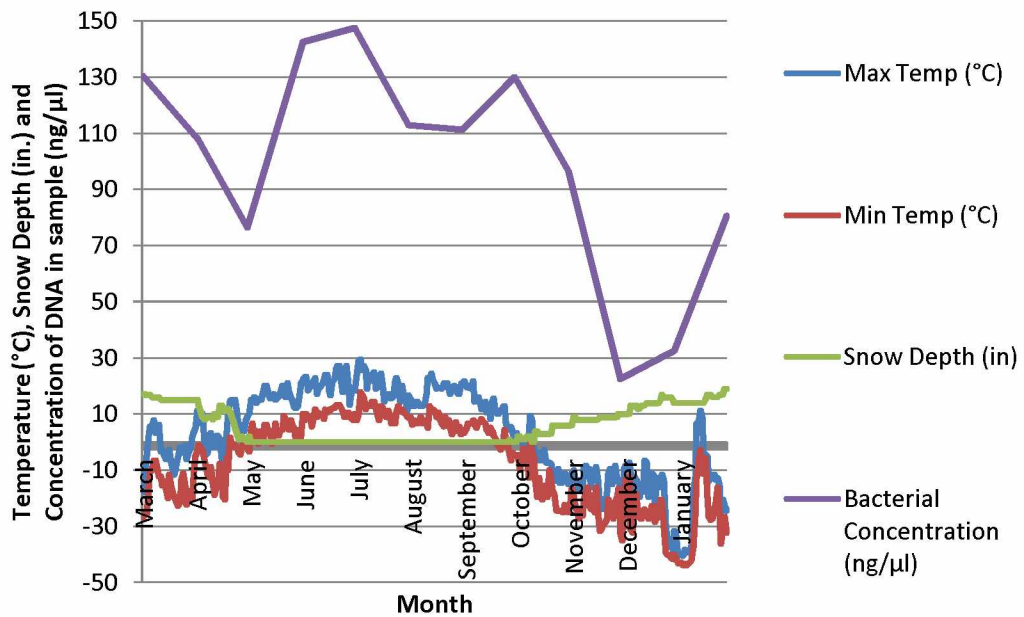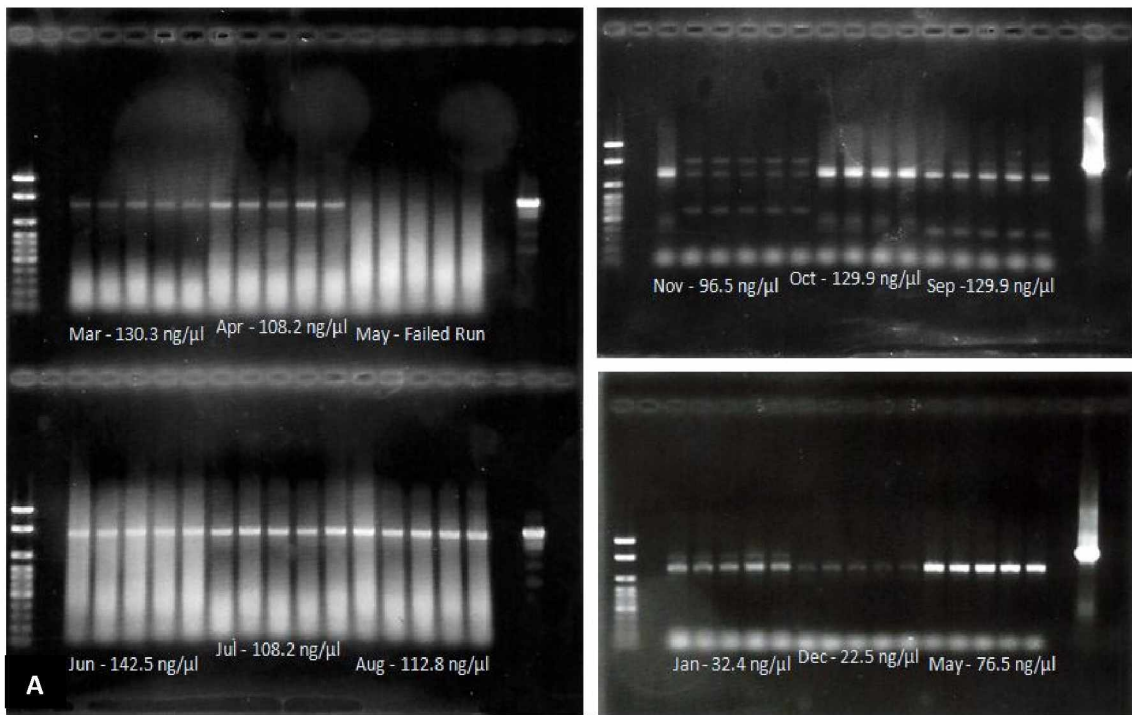
Figure 5: Bacterial gel electrophoresis and DNA concentrations of PCR products in relation to weather trends. (A) Gel images: Gel electrophoresis images for five PCR trials for each month. The concentration of DNA for each month is specified under each month's five trials. A ladder is located on the left of each gel image, and a negative and positive control on the right of each gel image. (B) Weather trends: Comparison of bacterial DNA concentrations to temperature maxima and minima, and snow depth from March 2008 to January 2009.
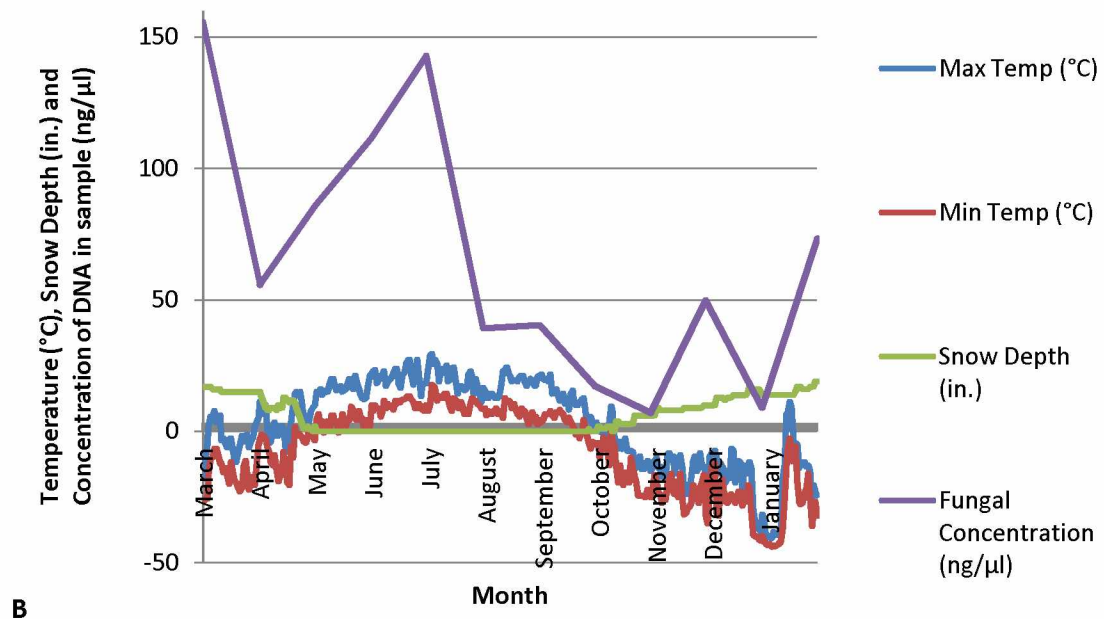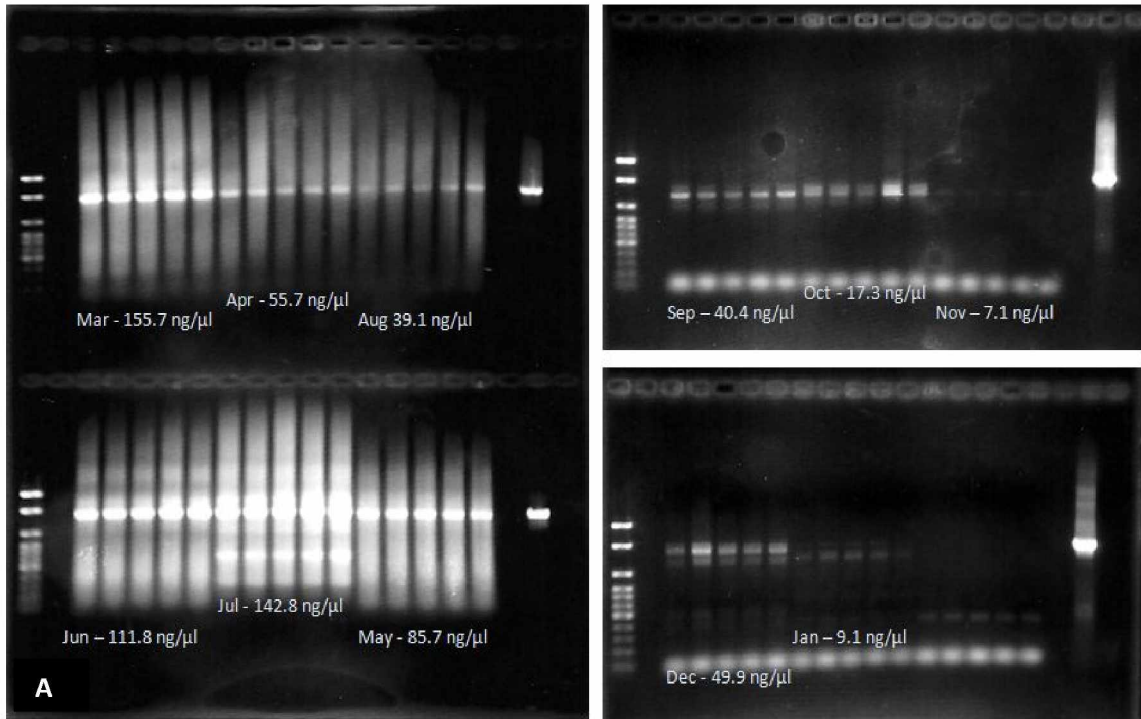
Figure 6: Fungal gel electrophoresis and DNA concentrations of PCR products in relation to weather trends. (A) Gel images: Gel electrophoresis images for five PCR trials for each month. The concentration of DNA for each month is specified under each month's five trials. A ladder is located on the left of each gel image, and a negative and positive control on the right of each gel image. (B) Weather trends: Comparison of fungal DNA concentrations to temperature maxima and minima, and snow depth from March 2008 to January 2009.

Full-length 16S rRNA gene sequences were obtained from 1278 bacterial clones generated from the PCR products. 96-192 bacterial clones were sequenced from each library, although the number of clones successfully identified to the genus level varied from month to month (Table 1). Eighty-six sequences didn't meet the 90% confidence level in RDP classifier at the phylum level, which would amount 6.3% of the total sequences if included with the original 1278 successfully identified sequences. A majority of the sequences came from the phylum *Bacteroidetes.* In addition, 27 chloroplast sequences and 11 archaeal sequences from the phylum *Euryarchaeota* were identified. The 1278 bacterial sequences were clustered into 56 genus-level OTUs based on $\geq$90% confidence classification by RDP Pipeline to a particular genus (Fig 7). Of these 56 OTUs, 13 OTUs were singletons and 13 OTUs were doubletons, occurring only once or twice respectively throughout the sampling year. The monthly genus-level richness as determined by Chao1 was variable, although the OTU count ranged from 16-28 OTUs during months of March to September, and then from 9-17 OTUs during the months from October to January. However, the seasonal genus-level richness determined by Chao1 indicated that during the spring and fall, the OTU count was 23 OTUs and 27 OTUs respectively, during the summer, the OTUs count almost doubled to about 52 OTUs, and during the winter, the OTUs count dropped to 20 OTUs (Table 1). The average seasonal genus-level richness indicated that the OTU count was about 9 and 10 OTUs in fall and winter respectively, about 8 OTUs in spring, and 17 OTUs in summer (Table 1).

The DNA concentrations of fungal PCR products ranged from 155.7 ng/μl to 7.1

ng/μl, with the concentrations peaking in March (155.7 ng/μl), July (142.8 ng/μl), and

December (40.4 ng/μl), and dropped considerably in the months of November (7.1 ng/μl)

and January (9.1 ng/μl) (Fig. 6A). The intensity of the bands and the concentrations of

DNA in fungi on a monthly basis seemed to strongly correspond to the weather (Fig. 6B).

In March, fungal DNA concentrations increase sharply alongside the beginning of the

rise in temperature. From May to August, the intensity in the bands and the

concentrations of DNA rise considerably as the temperature increases. From August to

November, the DNA concentrations and band intensities drop in response to the dropping

temperatures. In December, the DNA concentration rises once again. However, the sharp

drop in temperatures in January also results in a sharp drop in the fungal DNA

concentrations.

Full-length ITS sequences were obtained from 1,540 fungal clones generated

from the fungal PCR products. Once again, 96-192 clones were sequenced from each

library, although the number of clones successfully sequenced to the genus level varies

from month to month (Table 2). In total, fungal sequences were clustered into 79 OTUs

based on ≥95% sequence similarity and a uniform BLAST database match to a particular

genus (Fig 8). Of these 79 OTUs, 4 OTUs were singletons, and 8 OTUs were doubletons.

The monthly genus-level richness as determined by Chao1 was once again variable,

although the general trend seemed to indicate that during the months from May to

October, the OTU count increased over spring and early summer, peaked at July, and

then declined from August onwards (from 13 to 22 to 15 OTUs respectively). The OTU

Figure 7: Bacterial community structure over time at genus level. Bacterial diversity at the genus level as indicated by 16S rRNA gene clone library from March 2008 to January 2009.

count then dropped lower during the colder winter months of December and January,

dropping down to about 9 OTUs, although November had only 5 OTUs. However, the

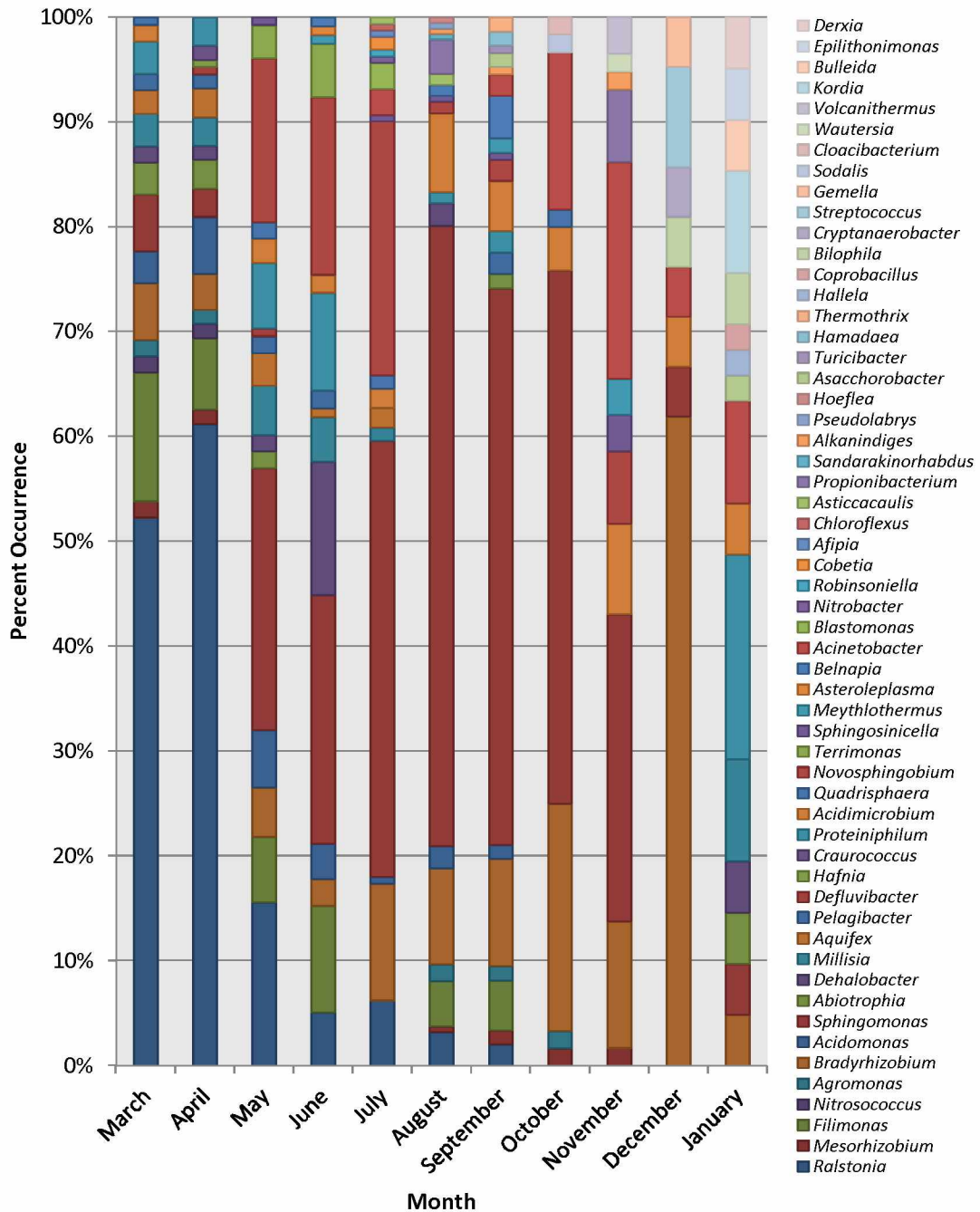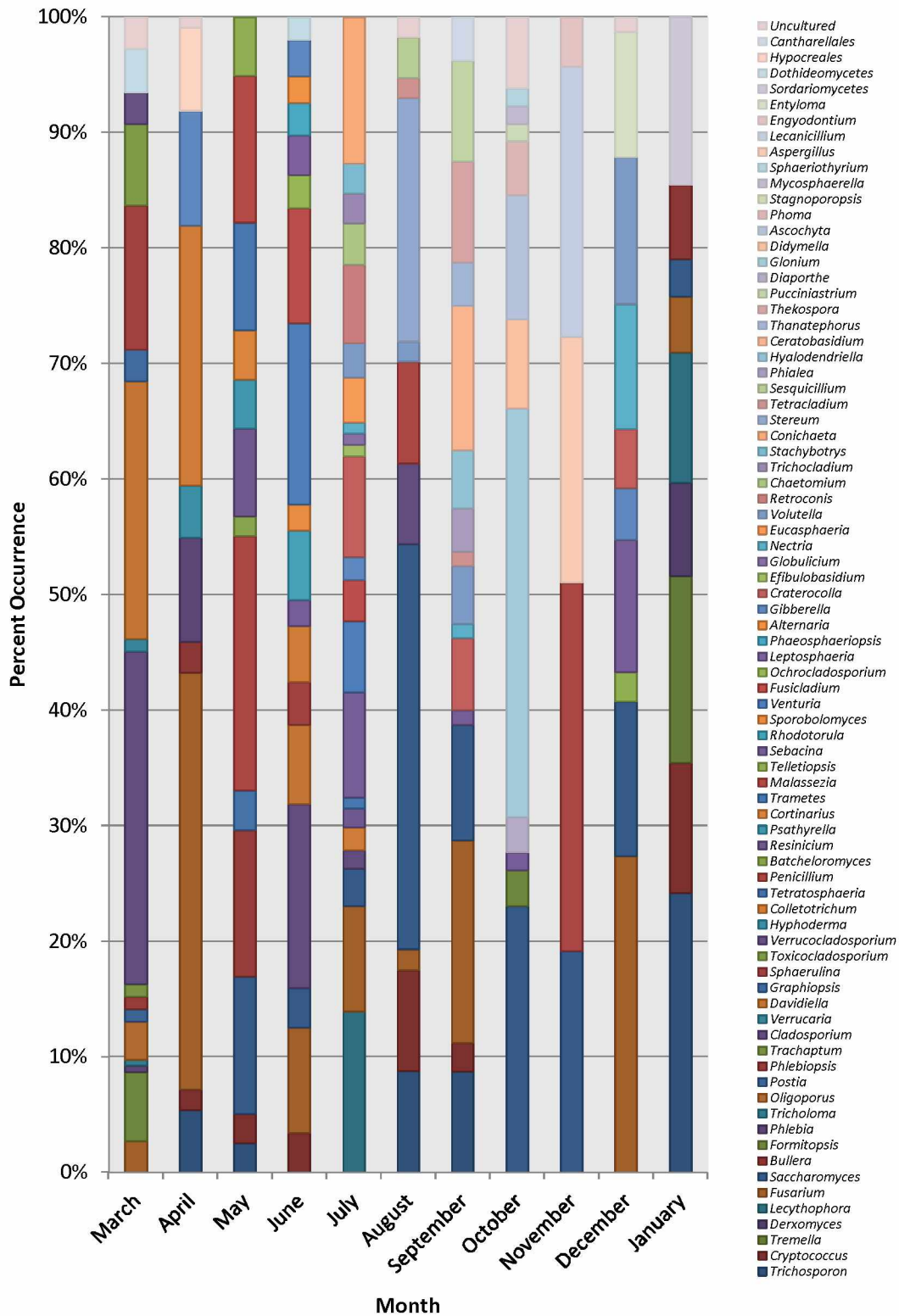**Figure 8: Fungal community structure over time at genus level.** Fungal diversity at the genus level as indicated by ITS DNA clone library from March 2008 to January 2009.

Table 1: General features of bacterial clone libraries. Bacteria 16S rRNA clones sequenced, OTU count, singleton and doubleton count, and Chao1 calculations on both a monthly and seasonal basis. The overall calculations are recorded in the last portion of the table. *Average Chao1 was calculated using the amount of months sampled per season (2 for winter, and 3 for the other seasons).

Sample	Clones Sequenced	OTUs	Singletons	Doubletons	Chao1
March	130	16	1	7	16.07
April	147	17	2	6	17.33
May	128	17	2	4	17.50
June	118	16	4	2	20.00
July	161	16	7	2	28.25
August	186	19	6	4	23.50
September	147	21	3	8	21.56
October	120	9	0	4	9.00
November	58	12	3	3	13.50
December	42	8	0	6	8.00
January	41	16	3	9	16.50

Sample	Clones Sequenced	OTUs	Singletons	Doubletons	Chao1	Average Chao1*
Spring	405	22	2	2	23.00	7.67
Summer	465	34	12	4	52.00	17.33
Fall	325	27	2	10	27.20	9.07
Winter	83	19	3	7	19.64	9.82

Sample	Clones Sequenced	OTUs	Singletons	Doubletons	Chao1
Overall	1278	6	13	13	62.50

Table 2: General features of fungal clone libraries. Fungi ITS clones sequenced OTU count, singleton and doubleton count and Chao1 calculations on both a monthly and seasonal basis. The overall calculations are recorded in the last portion of the table.

Sample	Clones Sequenced	OTUs	Singletons	Doubletons	Chao1
March	184	16	2	4	16.50
April	111	9	0	1	9.00
May	118	13	0	1	13.00
June	351	18	0	0	18.00
July	308	22	0	0	22.00
August	57	10	4	0	10.00
September	80	15	3	1	19.50
October	65	11	4	2	15.00
November	47	5	0	1	5.00
December	157	9	0	0	9.00
January	62	9	0	1	9.00

Sample	Clones Sequenced	OTUs	Singletons	Doubletons	Chao1	Average Chao1*
Spring	413	33	1	4	33.13	11.04
Summer	716	37	1	1	37.50	12.50
Fall	192	29	5	5	31.50	10.50
Winter	219	16	0	0	16.00	8.00

Sample	Clones Sequenced	OTUs	Singletons	Doubletons	Chao1
Overall	1540	79	4	8	80.00

seasonal genus-level richness determined by Chao1 showed a clear pattern, with the genus-level richness ranging from 31-33 OTUs during the spring and fall, hitting the peak at 38 OTUs during the summer, and then dropping down to 16 OTUs during the winter (Table 2). The average seasonal genus-level richness indicated that the OTU count was about 11 OTUs for both spring and fall, about 13 OTUs for summer, and 8 OTUs for winter (Table 2). However, due to incomplete sampling (there is no data for the month of February) the seasonal OTU counts may not be accurate.

3.2| Microbial diversity of bioaerosols

Fungi: A total of 79 OTUs were identified at the genus level by $\geq$95% similarity with named BLAST database sequences. An additional 17 sequences, 1.1% of 1540 successfully identified sequences were listed as uncultured fungal specimens by the database search (Fig 8). Of the 1540 sequences, 1172 (76.1%) were of ascomycetous affinity, and 315 (22.8%) of basidiomycetous affinity (Fig. 9). The 1540 successfully identified sequences were classified into 79 OTUs, 36 (45.5%) of which occurred $\geq$7.5% in at least one month throughout the sampling year (Fig 10A), and 11 (23.9%) of which occurred $\geq$7.5% in at least one season throughout the sampling year (Fig 10B). The majority of the samples came from the *Dothideomycetes* and *Sordariomycetes* classes, both of which belong to the phylum *Ascomycota* (Fig 9). The most prominent OTUs in the *Sordariomycetes* class were the genera *Fusarium*, *Nectria*, and *Volutella*, while the most prominent OTUs in the *Dothideomycetes* class were the genera *Davidiella*, *Glonium*, *Venturia*, and *Cladosporium* (Fig. 8, 10A, 10B). In the phylum *Basidiomycota*, there were three prominent classes that contributed to the majority of the *Basidiomycetes*

occurrence throughout the year, the classes *Exobasidiomycetes, Tremellomycetes,* and

Agaricomycetes (Fig 9). The most prominent OTU in the *Exobasidiomycetes* was the

genus *Entyloma,* in the *Tremellomycetes, Trichosporon,* and in the *Agaricomycetes,*

Sebacina (Fig. 8, 10A, 10B).

   Bacteria: A total of 56 OTUs were identified to the genus level to $\geq$90%

confidence level by RDP classifier (Fig. 7). Of the 1278 successfully identified sequences

that comprised the 56 OTU, 993 sequences were from the phylum *Proteobacteria*

(77.7%), 120 sequences were from *Bacteroidetes* (9.4%), 54 sequences were from

Firmicutes (4.2%), 92 sequences were from *Actinobacteria* (7.2%), and 15 sequences

were from *Aquificae* (1.1%). The remaining phyla successfully identified in the clone

library comprised $\leq$1% of the clones during the sampling year (Fig. 11).

   Eighty-six sequences run through RDP classifier could not be identified above

90% confidence level in RDP classifier. 41.9% of these sequences were from

Bacteroidetes, 20.9% from *Aquificae,* and 16.3% from *Proteobacteria,* and 10.5% from

Firmicutes. In addition, 27 sequences were identified as chloroplasts from

Cyanobacteria, and 11 sequences were identified as *Euarchaeota* from archaea.

   The 1278 sequences were classified into 56 OTU, 15 of which occurred $\geq$5% in at

least one month throughout the sampling year (Fig 12A), and 8 of which occurred $\geq$5% in

at least one season throughout the sampling year (Fig. 12B). The majority of the samples

came from the classes of *Alphaproteobacteria* and *Betaproteobacteria,* although the

classes *Sphingobacteria, Acidimicrobidae,* and *Bacteroidia* were also prominent

throughout the sampling year (Fig. 12). The most prominent OTUs in the
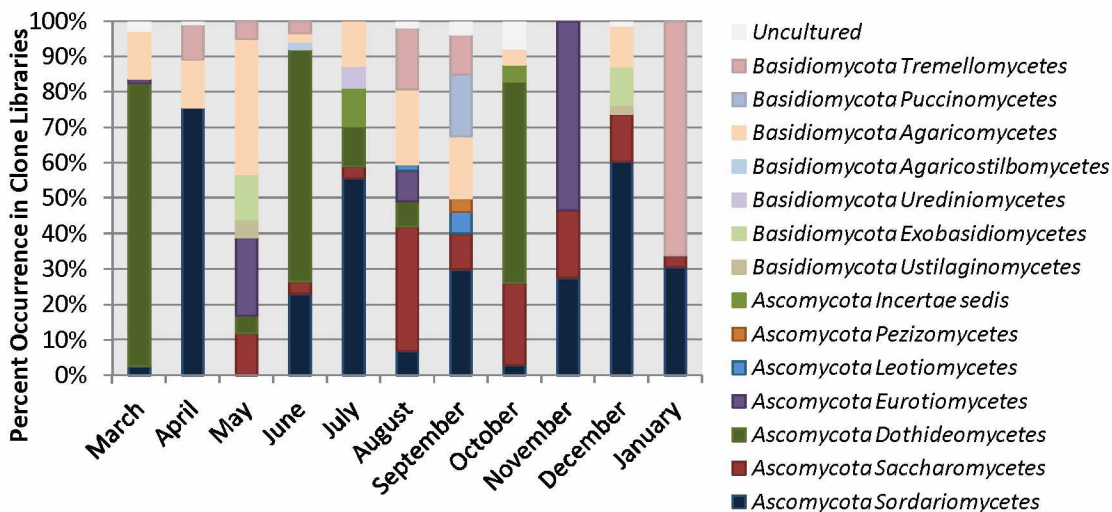
Figure 9: Fungal community structure at the phylum and class levels. Fungal diversity at the phylum and class levels as indicated by ITS clone library from March 2008 to January 2009. *Ascomycota* is marked by darker colors, and *Basidiomycota*, lighter colors.
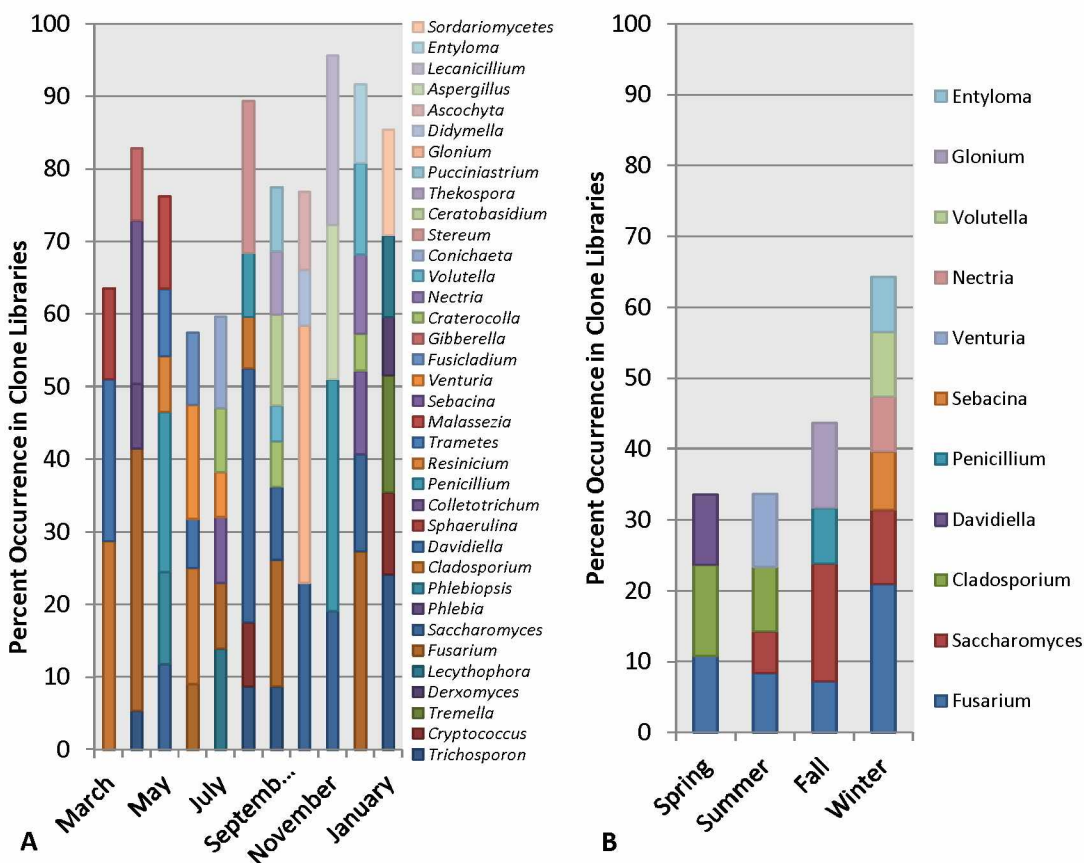
Figure 10: Dominant fungal OTUs. Dominant fungi OTUs (comprising ≥7.5% of cloned sequences) from March 2008 to January 2009. **(A) By month.** Dominant OTUs for fungi by month (≥7.5 % of monthly clone libraries) **(B) By season.** Dominant OTUs for fungi by season (≥7.5 % of the seasonal pool of libraries).
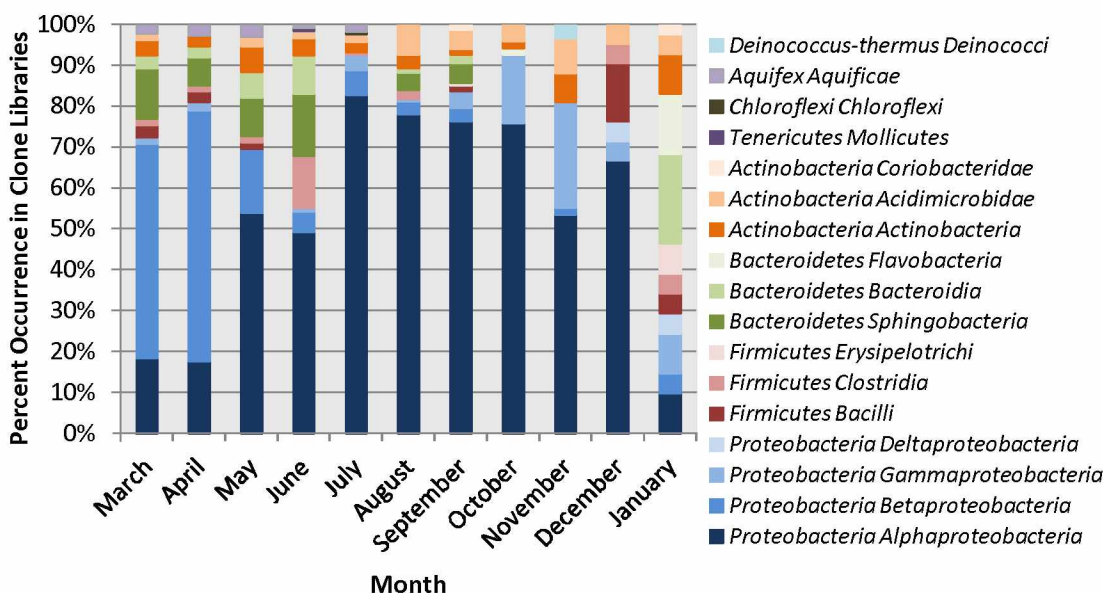
Figure11: Bacterial community structure at the phylum and class levels. Bacterial diversity at the phylum and class levels as indicated by 16S rDNA clone libraries from March 2008 to January 2009. Each phylum is shown in hues of the same color.
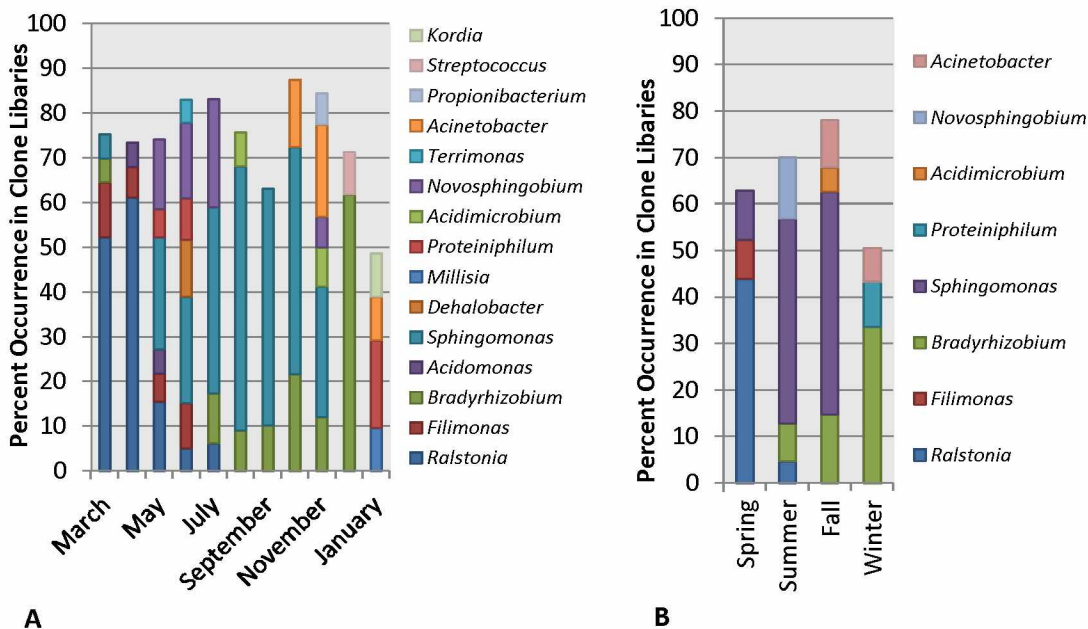
Figure 12: Dominant bacterial OTUs. Dominant bacterial OTUs (comprising ≥5.0 % of cloned sequences) from March 2008 to January 2009. **(A) By month.** Dominant OTUs for bacteria by month (≥5.0 % of monthly clone libraries) **(B) By season.** Dominant OTUs for bacteria by season (≥5.0 % of the seasonal pool of libraries).

Alphaproteobacteria class were the genera *Acinetobacter*, *Novosphingobium*,

Sphingomonas, and *Bradyrhizobium*, and in the *Betaproteobacteria* class, the genus

Ralstonia. The most prominent OTUs from the *Sphingobacteria* class was the genus

Filimonas, from the *Acidimicrobidae* class, *Acidimicrobium*, and from the *Bacteroidia*

class, *Proteiniphilum* (Fig.7, 12A, 12B).

3.3| Microbial communities in relation to weather trends

The seasonal dominant OTUs from both the bacterial and fungal clone libraries

were selected for statistical analysis to the weather variables listed above and to one

another. Bacterial and fungal communities were also analyzed in relation to these

variables at the phylum and class levels using a correlation matrix.

Fungi: The genera *Fusarium, Saccharomyces, Cladosporium, Davidiella,*

Venturia, Cryptococcus, Sebacina, and *Gibberella* were graphed alongside the maxima,

minima, and snow accumulation on the ground throughout the year (Fig. 13A and 13B).

Fusarium and *Gibberella* peak more often at colder temperatures and higher snow levels

than the remaining genera. Meanwhile, *Saccharomyces* and *Venturia* occur at the highest

ratio during the months of July and May respectively. *Davidiella* also peaks during May,

although its highest occurrence ratio occurs in March. *Cladosporium* occurs in high ratios

at March, May, and July, *Cryptococcus*, at August and January, and *Sebacina*, at July and

December (Fig. 13A). When comparing the above trends to the fungal DNA

concentrations determined for each month (Fig. 6), *Cladosporium* has a high peak in

March, when the fungal DNA concentration is 155.7 ng/μl, *Saccharomyces* in July (142.8

ng/μl), and *Fusarium* in December (40.4 ng/μl). The fact that these three fungal genera

occur in higher relative abundance when the concentrations of DNA are higher suggests

that these organisms may be very abundant during particular months.

When looking at correlation values (Table 3), there are no strong correlations between the different genera and the weather factors (Table 3A), although there is a very weak correlation between *Saccharomyces* and snow on the ground (-0.54). There is a high correlation between occurrences of *Cladosporium* and *Davidiella* (0.92), and very weak correlations between *Cladosporium* and *Venturia* (0.48), and *Gibberella* and *Fusarium* (0.50). Comparison of classes of fungi to the weather variables and one another (Table 3B) also revealed some correlations, although once again, the weather variables were only weakly correlated to the classes. The absolute values of the highest correlation coefficients between the weather variables and the classes ranged from 0.41-054. However, once again, there were some strong correlations between classes. *Ustilaginomycetes* and *Exobasidiomycetes* were strongly correlated (0.96), as were *Leotiomycetes* to *Pezizomycetes* and *Puccinomycetes* (0.97). The two phyla of fungi classified from the clone libraries were also compared to the weather variables (Table 3C), but neither of the phyla correlated significantly to the weather variables.

Bacteria: The genera *Ralstonia, Filimonas, Bradyrhizobium, Sphingomonas, Novosphingobium, Acidomonas, Dehalobacter, Millisia, Aquifex, Proteiniphilum, Acidimicrobium,* and *Acinetobacter* were graphed alongside the maxima, minima, and snow accumulation on the ground throughout the year (Fig. 14A and 14B). *Proteiniphilum* had a peak occurring at a high ratio in August and lower ratios in the former and latter months, but it also had an unusual peak in December. *Sphingomonas* also had a peak in August with lower relative abundance in the previous spring and following mid-winter months. *Acinetobacter* was not a major component of the

community throughout the year save for the months of November and January. *Acidimicrobium* remained a fairly stable proportion of the community throughout the year. *Aquifex, Novosphingobium, Acidomonas,* and *Millisia* increased in occurrence in April and peaked in May, but steadily dropped down to low occurrences at August and diminished completely thereafter. *Ralstonia* and *Filimonas* peaked in April, but slowly diminished during mid-fall, around October (Fig. 14A). When comparing the above trends to the bacterial DNA concentrations determined for each month (Fig. 5), *Ralstonia* occurred in high relative abundance during March and April, and *Sphingomonas* occurred in high relative abundance from May to November. The fact that *Ralstonia* and *Sphingomonas* occur in higher relative abundance when the concentrations of DNA are higher suggests that these organisms may be very abundant during particular months. However, it is important to note that these relative abundances do not represent absolute quantification of these organisms, since total abundances of bacteria and fungi fluctuated over the seasons (Fig. 5, 6).

When examining the correlation coefficients (Table 4), we see one strong correlation between weather and genus (Table 4A), *Sphingomonas* and snow depth (-0.79). There are also strong correlations between various genera – *Ralstonia* and *Filimonas* (0.72), *Aquifex* (0.79), and *Acidimicrobium* (-0.73); *Millisia* and *Proteiniphilum* (0.95); *Filimonas* and *Acinetobacter* (-0.74), *Acidomonas* (0.82), *Aquifex* (0.79), and *Acidimicrobium* (-0.73); *Acidomonas* and *Aquifex* (0.88), *Acidimicrobium* (-0.72), and *Acinetobacter* (-0.71); and *Aquifex* and *Acidimicrobium* (-0.85). Comparison of classes of bacteria to the weather variables and one another (Table 4B) also revealed
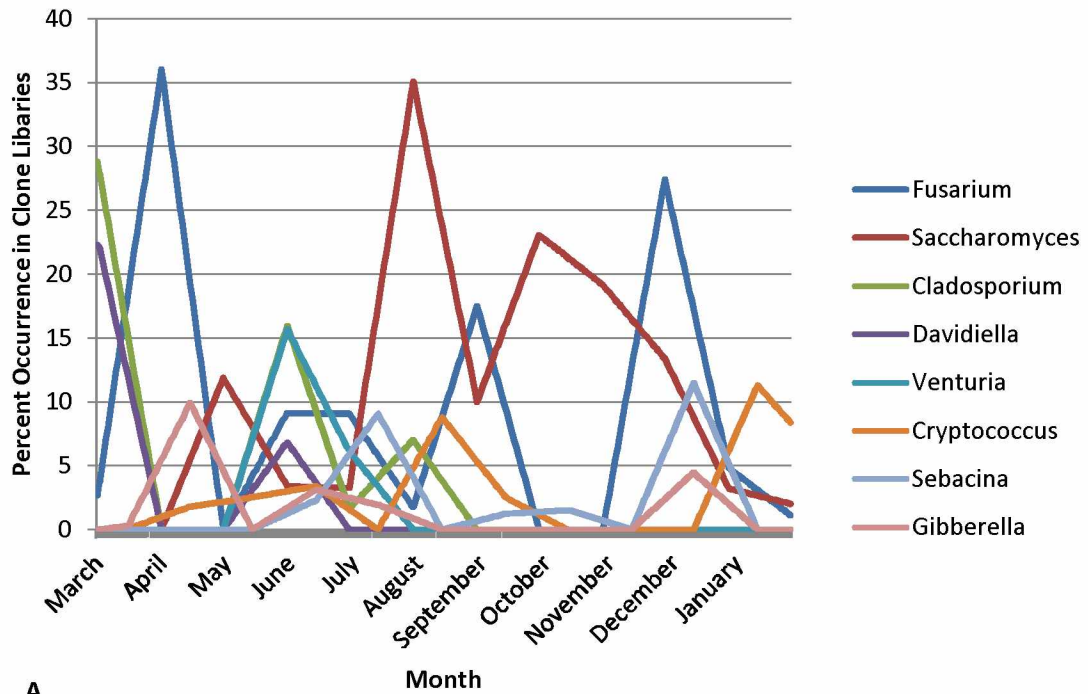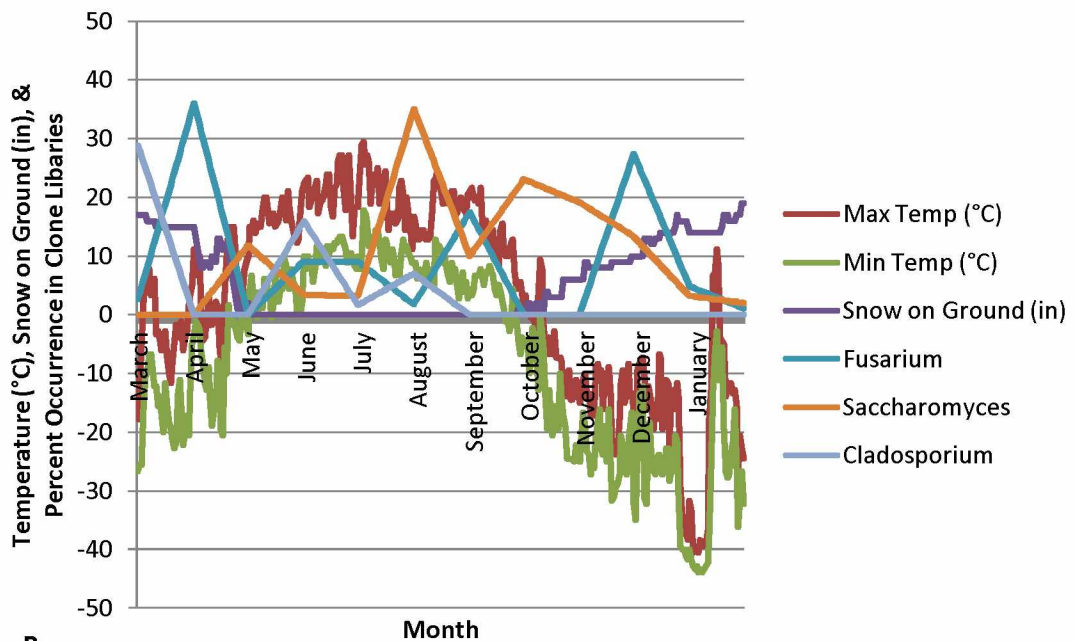
Figure 13: Annual trends in dominant fungal genera. (A) Percent occurrence. Comparison of the percent occurrence of dominant fungi genera from March 2008 to January 2009 **(B) Weather trends.** Comparison of the percent occurrence of three fungi genera (those with the strongest correlations to weather data compared to other genera, see **Table 3**), temperature maxima and minima, and snow accumulation on the ground from March 2008 to January 2009.

Table 3: Correlation analyses between fungal taxa and weather conditions. Correlation matrices are shown for (A) dominant genera, (B) classes, and (C) phyla in relation to fungal taxa, maximum and minimum temperatures, and snow depth from March 2008 to January 2009. Correlations that ranged from 0.45 - 0.70 were classified as very weak (highlighted in green) and correlations that ranged from 0.70 – 1.00 were classified as strong (highlighted in yellow).

A	Max Temp (°C)	Min Temp (°C)	Snow Depth (in)	Fusarium	Saccharomyces	Cladosporium	Davidiella	Venturia	Cryptococcus	Sebacina	Gibberella
Fusarium	-0.08	-0.14	0.35	1.00							
Saccharomyces	0.18	0.26	-0.54	-0.39	1.00						
Cladosporium	0.28	0.24	0.02	-0.02	-0.31	1.00					
Davidiella	0.05	-0.01	0.27	0.08	-0.45	0.92	1.00				
Venturia	0.49	0.48	-0.45	-0.12	-0.30	0.48	0.23	1.00			
Cryptococcus	0.01	0.03	0.04	-0.27	-0.06	-0.13	-0.23	-0.02	1.00		
Sebacina	-0.09	-0.05	0.02	0.07	0.13	-0.17	-0.26	0.04	-0.20	1.00	
Gibberella	0.01	-0.02	0.18	0.50	-0.37	-0.15	-0.13	0.04	-0.23	0.15	1.00

B	Max Temp (°C)	Min Temp (°C)	Snow Depth (in)	Trem	Sord	Sacc	Agar	Doth	Euro	Usti	Exobasid	Ured	Agaricos	Leot	Pezi	Pucc
Tremellomycetes	-0.51	-0.49	0.52	1.00												
Sordariomycetes	-0.17	-0.18	0.20	0.04	1.00											
Saccharomycetes	0.18	0.26	-0.54	-0.25	-0.27	1.00										
Agaricomycetes	0.53	0.50	-0.34	-0.28	-0.24	0.06	1.00									
Dothideomycetes	0.23	0.22	-0.10	-0.27	-0.50	-0.26	-0.15	1.00								
Eurotiomycetes	0.27	0.26	-0.13	0.17	-0.37	-0.11	0.14	0.24	1.00							
Ustilaginomycetes	-0.02	-0.05	-0.02	-0.15	0.02	-0.09	0.63	-0.26	-0.20	1.00						
Exobasidiomycetes	-0.14	-0.17	0.07	-0.14	0.18	-0.06	0.49	-0.33	-0.37	0.96	1.00					
Urediniomycetes	0.45	0.46	-0.36	-0.20	0.37	-0.11	0.00	0.02	0.10	-0.23	-0.24	1.00				
Agaricostilbomycetes	0.38	0.36	-0.36	-0.21	-0.06	-0.29	0.02	0.55	0.10	0.06	0.00	0.13	1.00			
Leotiomycetes	0.41	0.42	-0.43	-0.02	-0.17	0.39	0.23	-0.22	0.12	-0.27	-0.29	-0.11	-0.19	1.00		
Pezizomycetes	0.34	0.33	-0.36	-0.03	-0.13	0.23	0.16	-0.16	0.10	-0.23	-0.24	-0.16	-0.16	0.97	1.00	
Puccinomycetes	0.34	0.33	-0.36	-0.03	-0.13	0.23	0.16	-0.17	0.10	-0.23	-0.24	-0.16	-0.16	0.97	1.00	1.00

C	Max Temp (°C)	Min Temp (°C)	Snow Depth (in)	Ascomycota	Basidiomycota
Ascomycota	0.12	0.12	-0.20	1.00	
Basidiomycota	-0.13	-0.13	0.22	-0.99	1.00
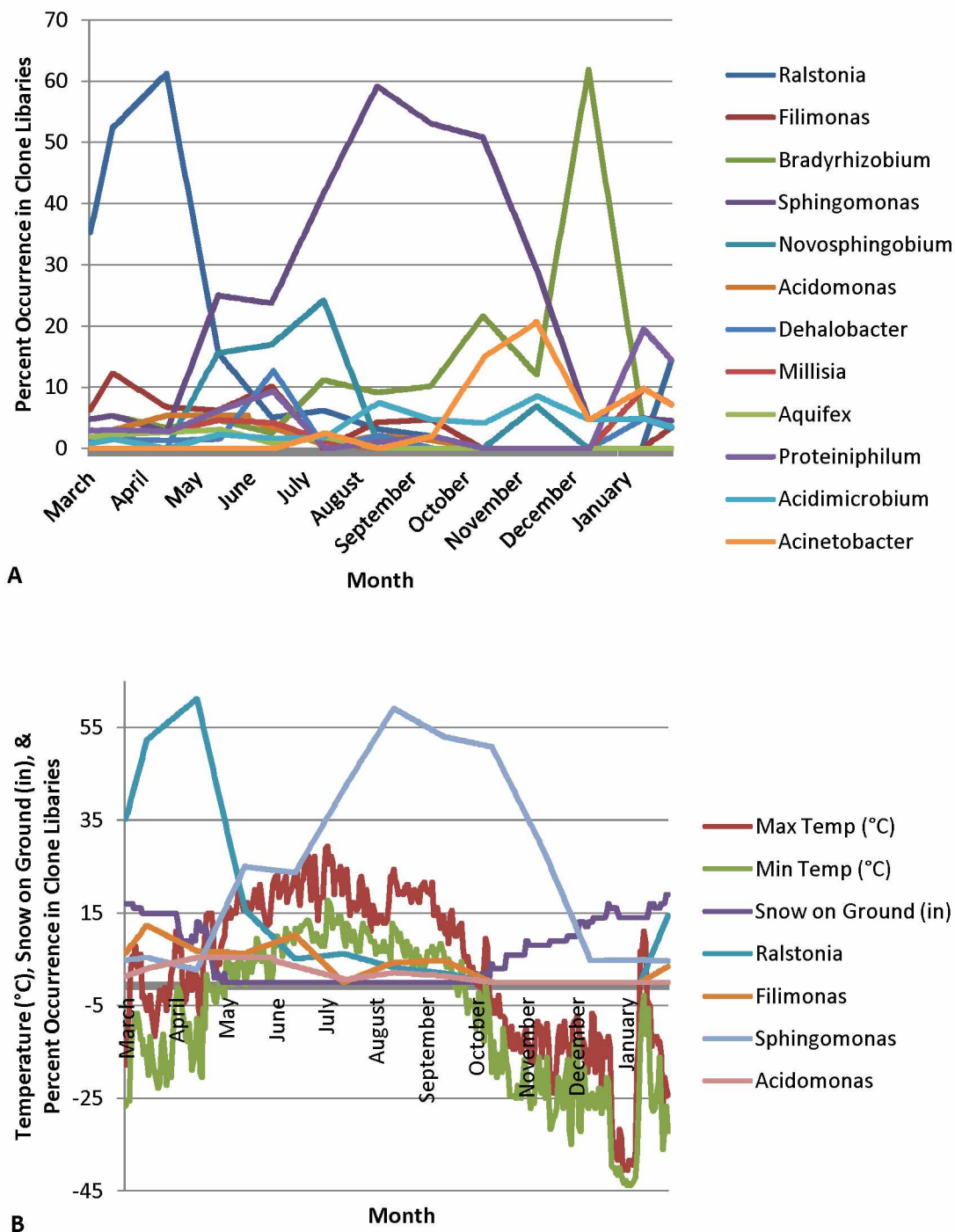
Figure 14: Annual trends in dominant bacterial genera. (A) Percent occurrence. Comparison of the percent occurrence of dominant bacteria genera from March 2008 to January 2009 **(B) Weather trends.** Comparison of the percent occurrence of four bacteria genera (those with the strongest correlations to weather data compared to other genera, see **Table 4**), temperature maxima and minima, and snow accumulation on the ground from March 2008 to January 2009.

Table 4: Correlation analyses between bacterial taxa and weather conditions. Correlation matrices are shown for (A) dominant genera, (B) classes, and (C) phyla in relation to other bacterial taxa, maximum and minimum temperatures, and snow depth from March 2008 to January 2009. Correlations that ranged from 0.45 - 0.70 were classified as very weak (highlighted in green) and correlations that ranged from 0.70 – 1.00 were classified as strong (highlighted in yellow).

A

	Max Temp	Min Temp	Snow Depth	Ral	Fil	Brad	Sphin	Novo	Acido	Dehal	Mill	Aqui	Prot	Acidi	Acine
Ralstonia	0.06	-0.01	0.34	1.00											
Filimonas	0.46	0.40	-0.08	0.72	1.00										
Bradyrhizobium	-0.52	-0.50	0.31	-0.44	-0.64	1.00									
Sphingomonas	0.50	0.57	-0.79	-0.54	-0.24	-0.11	1.00								
Novosphingobium	0.61	0.63	-0.61	-0.17	0.16	-0.32	0.21	1.00							
Acidomonas	0.51	0.46	-0.27	0.68	0.82	-0.59	-0.22	0.32	1.00						
Dehalobacter	0.33	0.32	-0.17	-0.04	0.45	-0.39	-0.19	0.52	0.34	1.00					
Millisia	-0.15	-0.18	0.38	0.24	0.27	-0.37	-0.65	0.08	0.27	0.56	1.00				
Aquifex	0.38	0.33	-0.07	0.79	0.73	-0.54	-0.39	0.38	0.88	0.18	0.32	1.00			
Proteiniphilum	-0.18	-0.20	0.33	0.00	0.12	-0.28	-0.50	0.01	0.08	0.61	0.95	0.04	1.00		
Acidimicrobium	-0.33	-0.28	-0.03	-0.73	-0.72	0.48	0.45	-0.33	-0.72	-0.39	-0.45	-0.85	-0.23	1.00	
Acinetobacter	-0.62	-0.59	0.24	-0.49	-0.74	0.44	0.08	-0.31	-0.71	-0.37	-0.25	-0.64	-0.13	0.67	1.00

B

	Max Temp	Min Temp	Snow Depth	Beta	Alph	Sphin	Gamm	Baci	Clos	Actin	Aqui	Bact	Acidi	Moll	Chlor	Corio	Erysi	Delt	Flav	Dein
Betaproteobacteria	-0.09	-0.17	0.56	1.00																
Alphaproteobacteria	0.61	0.66	-0.87	-0.67	1.00															
Sphingobacteria	0.42	0.36	-0.09	0.52	-0.27	1.00														
Gammaproteobacteria	-0.48	-0.44	0.08	-0.40	0.11	-0.68	1.00													
Bacilli	-0.63	-0.65	0.63	-0.01	-0.35	-0.34	0.05	1.00												
Clostridia	0.02	0.00	0.02	-0.12	-0.24	0.49	-0.37	0.19	1.00											
Actinobacteria	-0.51	-0.52	0.46	0.07	-0.67	0.05	0.15	0.06	0.29	1.00										
Aquificae	0.24	0.17	0.14	0.78	-0.40	0.70	-0.59	-0.21	0.08	0.10	1.00									
Bacteroidia	-0.44	-0.45	0.50	0.07	-0.70	0.13	0.28	0.53	-0.17	0.81	0.03	1.00								
Acidimicrobidae	-0.25	-0.18	-0.17	-0.70	0.43	-0.71	0.66	0.11	-0.32	0.01	-0.85	-0.20	1.00							
Mollicutes	0.38	0.36	-0.36	-0.13	0.05	0.65	-0.31	-0.27	0.85	0.05	0.17	0.18	-0.37	1.00						
Chloroflexi	0.44	0.46	-0.36	-0.16	0.40	-0.10	-0.22	-0.30	0.02	-0.21	0.16	-0.21	-0.20	0.13	1.00					
Coriobacteridae	-0.40	-0.39	0.32	-0.22	-0.31	-0.33	0.07	0.31	0.06	0.42	-0.43	0.68	0.23	-0.23	-0.23	1.00				
Erysipelotrichi	-0.59	-0.58	0.51	-0.13	-0.52	-0.33	0.10	0.42	0.22	0.65	-0.31	0.84	0.14	-0.17	-0.17	0.90	1.00			
Deltaproteobacteria	-0.69	-0.68	0.55	-0.24	-0.35	-0.48	0.16	0.88	0.28	0.31	-0.41	0.53	0.26	-0.21	-0.21	0.60	0.75	1.00		
Flavobacteria	-0.62	-0.60	0.51	-0.14	-0.52	-0.36	0.16	0.41	0.21	0.66	-0.31	0.83	0.14	-0.18	-0.18	0.86	0.99	0.74	1.00	
Deinococci	-0.34	-0.33	0.02	-0.26	0.11	-0.41	0.83	-0.03	-0.28	0.17	-0.34	-0.33	0.60	-0.16	-0.16	-0.23	-0.17	-0.02	-0.14	1.00

C

	Max Temp	Min Temp	Snow Depth	Prot	Bacter	Firm	Actino	Aquifi	Teneri	Deino	Chloro
Proteobacteria	0.50	0.52	-0.55	1.00							
Bacteroidetes	-0.30	-0.33	0.46	-0.94	1.00						
Firmicutes	-0.55	-0.57	0.55	-0.80	0.63	1.00					
Actinobacteria	-0.56	-0.52	0.25	-0.53	0.35	0.32	1.00				
Aquificae	0.28	0.22	0.07	0.07	0.13	-0.22	-0.61	1.00			
Tenericutes	0.38	0.36	-0.36	-0.25	0.32	0.19	-0.25	0.19	1.00		
Deinococcus	-0.34	-0.33	0.02	0.23	-0.42	-0.21	0.44	-0.31	-0.16	1.00	
Chloroflexi	0.45	0.46	-0.36	0.29	-0.24	-0.23	-0.30	0.18	0.13	-0.16	1.00

some correlations. *Alphaproteobacteria* was strongly correlated to snow accumulation (-0.87), and numerous classes prove to be highly correlated to one another, the most prominent correlations being between *Erysipelotrichi* and *Flavobacteria* (0.99), *Erysipelotrichi* and *Coriobacteria* (0.90), *Actinobacteria* and *Bacteroida* (-0.85), and *Bacilli* and *Deltaproteobacteria* (0.88). The phyla of bacteria classified from the clone libraries were also compared to one another (Table 4C) to reveal strong correlations between *Proteobacteria* and *Bacteroidetes* (-0.94), *Proteobacteria* and *Firmicutes* (-0.80).

3.4| Monthly microbial composition comparison

Principal components analyses (PCA) were calculated to compare and contrast the monthly patterns of occurrence for dominant genera, classes, and phyla (Fig. 15 & 16).

Fungi: Any fungal genera that had an occurrence ≥7.5% throughout the sampling year (total clones, not individual libraries) were utilized for PCA (Fig. 15A). According to the PCA results, September, December, and April had similar fungal compositions, with the relative abundance of *Fusarium*, *Colletotrichum*, *Volutella*, *Gibberella*, and *Trichosporon* contributing most to the similarities in composition between these months. October, August, and May had similar compositions as well, with the relative abundance of *Glonium* and *Stereum* acting as contributors to these three months. January and July are also share similar fungal community composition and structure. However, the months of March, June, and November are not clustered with any other month. *Cladosporium* and *Davidiella* are associated with March, *Venturia* with June, and *Penicillium*, *Lecanicillium*, and *Aspergillus* with November. The PC1 and PC2 axes covered 50 % of
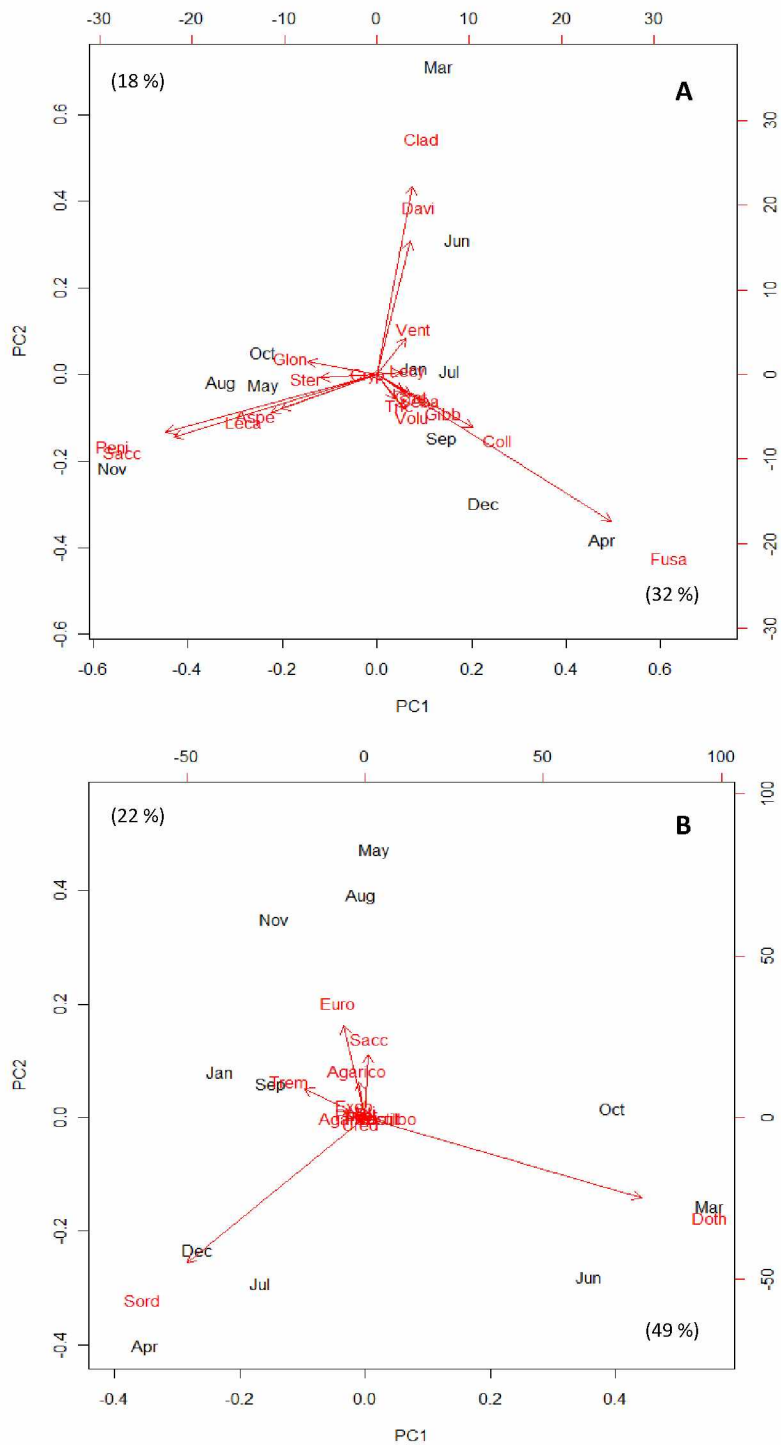
Figure 15: Principal components analyses (PCA) of monthly fungal community structure and composition. Analyses were conducted with samples from March 2008 to January 2009 at different taxonomical levels of dominant sequences in the clone libraries (A) Genera, (B) Classes, and (C) Phyla. Proportion variance covered by the components PC1 and PC2 are located at the top left corner (PC2) and bottom right corner (PC1).
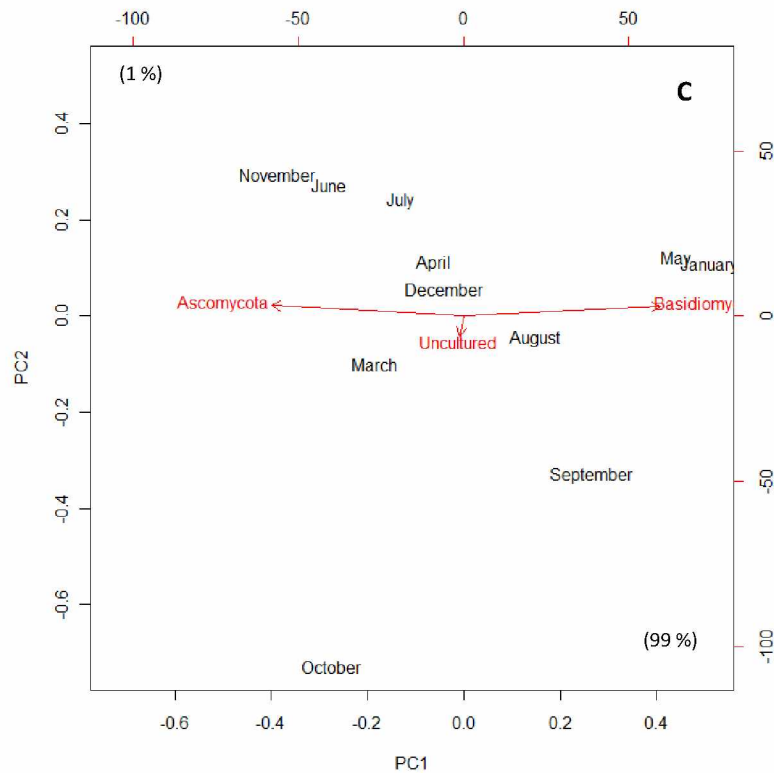
Figure 15 (continued): Principal components analyses (PCA) of monthly fungal community structure and composition. Analyses were conducted with samples from March 2008 to January 2009 at different taxonomical levels of dominant sequences in the clone libraries (A) Genera, (B) Classes, and (C) Phyla. Proportion variance covered by the components PC1 and PC2 are located at the top left corner (PC2) and bottom right corner (PC1).

the total variance during the year, each axis covering 32% and 18% of the variance respectively (Fig. 15A). Looking at the fungal class composition of each month (Fig. 15B), May, August, and November form one cluster, highly influenced by the occurrence of the classes *Eurotiomycetes* and *Saccharomycetes*. January and September form a second cluster, influenced by the occurrence of *Tremellomycetes*. December, July, and April form a third cluster due to the occurrence of *Sordariomycetes*, and October, March, and June, due to *Dothideomycetes*. The PC1 and PC2 axes covered71% of the total variance, each axis covering 49% and 22% of the variance respectively (Fig. 15B).Lastly, the fungal community composition and structure for each month was analyzed at the

phylum level (Fig. 15C). November and June form a distinctive cluster, as do April and December, and July lingers between these two clusters. May and January form another cluster and are influenced by increased Basidiomycetous composition. March, August, October, and September do not form distinct clusters. The PC1 and PC2 axes covered 100% of the total variance during the year, each axis covering 99% and 1% of the variance respectively (Fig. 15C)

Bacteria: Any bacterial genera that had an occurrence ≥5% throughout the sampling year (total clones, not individual libraries) were utilized for PCA (Fig. 16A). According to the PCA results, the months July, August, and September had similar community compositions and structure with *Sphingomonas* being a major contributor to the bacterial diversity during those months. May and June also form a distinctive cluster near *Acidomonas* and *Filimonas*, March and April form a cluster near *Ralstonia*, and November and October form another cluster near *Acinetobacter* and *Acidimicrobium*. The months of December and January don't form a cluster, but both months are strongly impacted by *Bradyrhizobium*. The PC1 and PC2 axes covered 79 % of the total variance during the year, each axis covering 52% and 27% of the variance respectively (Fig. 16A).

The bacterial class composition of each month (Fig. 16B) revealed three main clusters. One cluster, March and April, is influenced by *Betaproteobacteria*, while another cluster, July, August, and September, is associated with *Alphaproteobacteria*. January, on the other hand, is distant from the other clusters. The PC1 and PC2 axes covered 90 % of the total variance during the year, each axis covering 72% and 18% of the variance respectively (Fig. 16B). Lastly, the bacterial phylum-level community

Figure 16: Principal components analyses (PCA) of monthly bacterial community structure and composition. Analyses were conducted with samples from March 2008 to January 2009 at different taxonomical levels of dominant sequences in the clone libraries (A) Genera, (B) Classes, and (C) Phyla. Proportion variance covered by the components PC1 and PC2 are located at the top left corner (PC2) and bottom right corner (PC1).
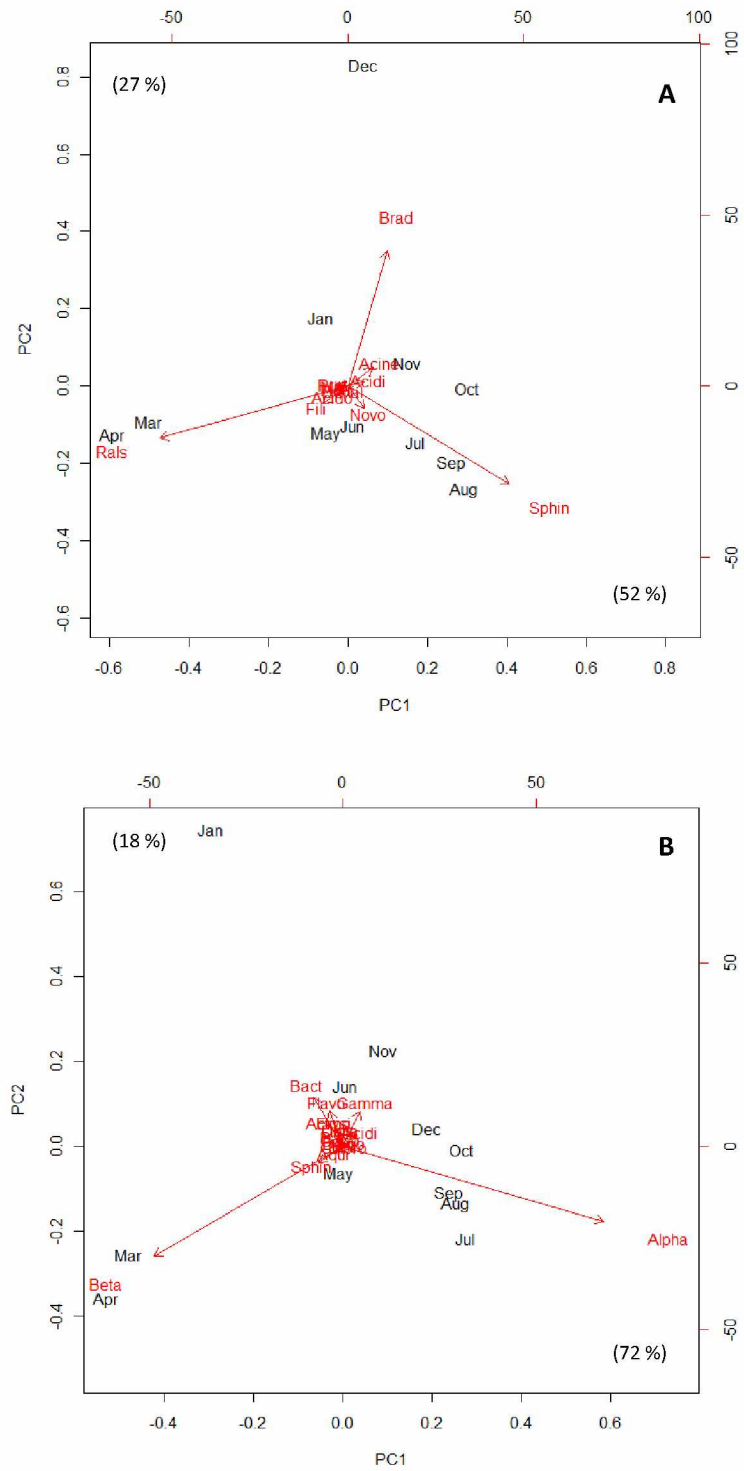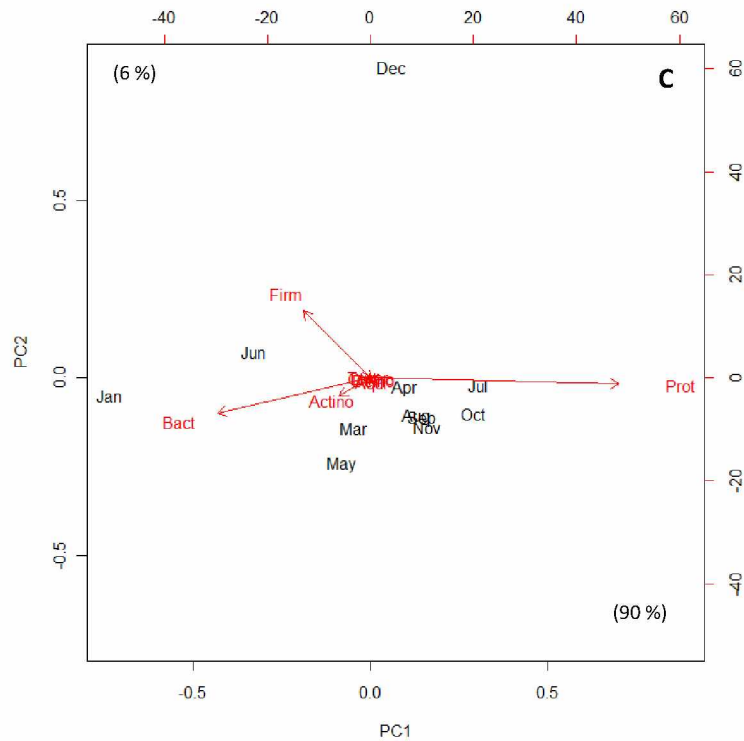
Figure 16 (continued): Principal components analyses (PCA) of monthly bacterial community structure and composition. Analyses were conducted with samples from March 2008 to January 2009 at different taxonomical levels of dominant sequences in the clone libraries (A) Genera, (B) Classes, and (C) Phyla. Proportion variance covered by the components PC1 and PC2 are located at the top left corner (PC2) and bottom right corner (PC1).

composition and structure of each month was analyzed (Fig. 16C). January appears to be influenced by *Bacteroidetes*. December is located far away from all the other months and appears to be impacted by ratios of *Firmicutes* and *Proteobacteria*. The PC1 and PC2 axes covered 96 % of the total variance during the year, each axis covering 90% and 6% of the variance respectively (Fig. 16C).

4. Discussion

High levels of $PM_{2.5}$ (mass concentration of particles with an aerodynamic diameter smaller than 2.5µm) are associated with numerous human health hazards, including respiratory diseases, cardiopulmonary diseases, lung cancer, and even mortality (28). The ≤ 2.5 µm diameter aerosols can include cells and/or spores of various fungal and bacterial species (29), and humans and animals also act as reservoirs and dispersers for microbial matter that can fall under this category (1). While numerous studies have focused on indoor bioaerosols, studies of the ambient outdoor atmospheric bioaerosol content are rather sparse (24, 30, 31, 32). In addition, studies often neglect to identify the biological aerosols and instead concentrate their research on the size and concentration distribution of the aerosols (e.g. 15). This may be because the atmosphere is a rather hostile environment for bioaerosols: ultraviolet (UV) light has the potential to kill 99.9% of all organisms (33); there is a distinct lack of nutrients in the air, which results in short-term life-spans for bioaerosols (12); and microorganisms don't typically colonize the air (31). These factors may explain why concentrations of bioaerosols in the air are low (15); However, not all UV rays have the same potential to kill organisms (33), and while there is a lack of nutrients in the air, some bioaerosols can be dispersed through the air from the soil and vegetation, forming aerosols with dust particles, water droplets, or soil particles that contain vital nutrients (31, 34). Thus, there is definitely merit in looking at the microbial content in the air.

Save for one study done by Miller & Cahill in 2000 (14) that studied the bioaerosol content of the air in a veterinary clinic via cultures, DRUM impactors have

never been used to analyze biological aerosols (14). The results presented support the

idea that DRUM impactors could serve as useful tools for collecting biological aerosols.

However, this study also goes a step further to show that DRUM impactors can collect a

wide variety of fungal and bacterial bioaerosols as well as demonstrating patterns within

these bioaerosols after the samples undergo PCR, cloning, and sequencing. The

revelation that the DRUM impactor samples can successfully be used in PCR and cloning

for identification of bioaerosols is potentially incredibly useful, especially since a greater

diversity of microbial flora is detectable using direct molecular methods such as PCR and

DNA sequencing compared to traditional cultivation of bioaerosols (30). The use of the

DRUM impactor for collecting bioaerosols for molecular identification and monitoring of

fluctuations in bioaerosol concentrations could be valuable for determining potential

bioterrorism threats and health hazards to the public or military personnel.

Using the DRUM impactor as a collection apparatus, we were able to successfully

identify 79 fungal genera from 1540 clone sequences, and 56 bacterial genera from 1278

bacterial genera (Fig. 7 & 8). The DNA concentrations of the PCR products used to the

construct our clone libraries indicated that bacteria were highly abundant from March to

November, while fungi had a variable abundance, occurring in higher abundance during

the months of March, July, and December (Fig. 5 & 6). Many of the genera were variable

in their occurrence, having been detected occasionally in only one or two months, which

seem to be common in other studies (30). This sporadic occurrence of particular genera

could partially result of the factors described above, especially the short-term lifespan of

bioaerosols in the ambient air (12). However, a more likely explanation is that the

microbial composition of air is highly variable (29), which is why using the DRUM impactor to detect trends may assist in determining when certain microbes are expected to occur and how concerning these microbes may be to humans and the ecosystem.

While the monthly fungal OTU richness calculations were variable throughout the sampling year, the seasonal trends were toward highest fungal richness during the summer, slightly reduced richness during spring and fall, and the lowest richness during winter. Looking at the average fungal richness values (which takes into account that winter had only two sampling months, while the other seasons had three sampling months), the trend is the same, but with less disparity between the seasons (Table 2). The fungal DNA concentrations displayed a more variable pattern than the OTU richness calculations, ranging from 55.5 ng/µl-155.7 ng/µl in the spring, 39.1 ng/µl-142 ng/µl in the summer, 17.1 ng/µl-40.4 ng/µl in fall, and 7.1 ng/µl-49.9 ng/µl in the winter, indicating that the lowest DNA concentration range were detected in fall, and the highest DNA concentration range were detected in spring (Fig. 5). Therefore, the OTU richness estimates and the DNA concentrations for fungi don't entirely align, indicating that in fungi, OTU richness may not entirely depend upon DNA concentrations (or fungal genera concentrations) alone.

The bacterial OTU richness estimates followed the same trend as fungal richness. However, the average bacterial richness values indicated that spring had the lowest bacterial richness. Fall and winter had slightly higher bacterial richness, but not that different from the average Chao1 value of spring, and summer had much higher bacterial richness compared to all seasons (Table 1). The bacterial DNA concentrations too, had a

clear pattern, unlike fungal concentrations, ranging from 76.5 ng/μl-130.3 ng/μl in spring,

108.1 ng/μl-142.5 ng/μl in summer, 96.5 ng/μl-129.9 ng/μl in fall, and 22.5 ng/μl-32.4

ng/μl in winter, indicating that the lowest DNA concentrations were detected in winter,

and the highest, in summer (Fig. 6). Therefore, the OTU richness estimates and the DNA

concentrations for bacteria aligned for the summer, spring, and fall, but the winter

bacterial concentrations were drastically lower than that of spring and fall despite having

similar OTU richness in comparison to the two seasons. This finding indicates that OTU

richness in bacteria may depend more heavily upon DNA concentrations or bacterial

genera concentrations than in fungi.

Temperature and water availability impact the growth and release of microbes,

especially fungal spores that need water for stimulated release (21, 35, 36). Studies also

indicate that viability decreases when relative humidity decreases and the temperatures

and solar radiation increases (37). Considering that the climate of Fairbanks, Alaska is

very dry despite snowfall from fall through spring (especially since the low temperatures

prevent the air from holding moisture), with the relative humidity ranging from about

45% - 80% from November to April. Therefore, the OTU richness seems consistent with

Fairbanks climate and weather patterns. The Fairbanks summers are warm and

punctuated by rainfalls that can easily contribute to the dispersal of spores in the air (21),

which could account for the high OTU richness in summer. Fall and spring have lower

temperatures, so the OTU richness during those seasons is less pronounced. During the

winter months, the temperatures are much lower, thus reducing the humidity, and there is

an increase in snow cover. In addition, due to the snowfall, the natural sources for

microbial growth, such as soil and vegetation, are covered with snow, which helps prevent the microbes from entering the air (8, 30, 39). This could partially account for the low microbial richness of bioaerosols during the winter, but it seems that the factors impacting microbial richness have a greater impact on bacterial richness compared to fungal richness.

Looking at the overall diversity of the fungal clone libraries, the results indicated that *Ascomycota* dominated overall diversity throughout the year, comprising 76.1% of the overall diversity, while *Basidiomycota* only comprised 22.8% of the overall diversity (Fig. 7). In most studies, *Ascomycota* is the more dominant phylum among the fungi, while the others occur in smaller percentages, so this particular study is in agreement with other previous studies (12, 38). *Basidiomycetes* favor dry release over active wet discharge, the favored method of dispersal for *Ascomycetes* (39), so perhaps both snowfall and rainfall allow for higher dispersal of *Ascomycetes* throughout the year. In fact, *Basidiomycetes* are most prominent during May, August, September, and January (Fig. 9), months during which the rainfall and snowfall in Fairbanks are considerably less, allowing for a more *Basidiomycetes*-favorable dry climate. In addition, the opposing methods of discharge might explain the anti-correlation of *Ascomycetes* to *Basidiomycetes* in the PCA (Fig. 15C).

Within the *Basidiomycetes*, most of the diversity was explained by the classes *Agaricomycetes* and *Tremellomycetes* (Fig. 7). While other studies (one which took place in a residential area in Austin, Texas, and the other taking place on a campus in Mainz, Germany) also indicate that *Agaricomycetes* comprise the majority of the *Basidiomycetes*

in their results (12, 39), our study also indicated that *Tremellomycetes* were also a major

contributor to the *Basidiomycetes* present in our clone libraries. *Sebacina, Trichosporon,*

and *Entyloma* were the most prominent OTU among the *Basidiomycetes* (Fig. 6, 8A, 8B),

but these three genera were not mentioned in other studies as prominent genera in fungal

communities (12, 30, 39). Once again, this disparity within the fungal communities could

be partially attributed to ecological conditions. Within the *Ascomycetes*, most of the

diversity was explained by the classes *Sordariomycetes* and *Dothideomycetes*, which is

also in agreement with previously executed studies (12, 40). The most prominent OTU in

the class *Sordariomycetes* are *Fusarium, Volutella,* and *Nectria,* while the most

prominent OTU in the class *Dothideomycetes* were *Davidiella, Glonium, Venturia,* and

Cladosporium (Fig. 6, 8A, 8B). While a few of the studies listed *Fusarium* as a major

component of the clone libraries (12, 36, 37, 40, 41), and almost all the studies listed

Cladosporium as the major contributor to fungal diversity (12, 30, 32, 36, 37, 40, 42, 43,

44, 45), the other fungal genera listed above were not reported as major components of

the clone libraries constructed by the other studies. In fact, most of the other studies,

which took place in urban areas like Timisoara, Romania; Austin, Texas; Nantou,

Taiwan; Benin City, Nigeria; northeastern France; Boulder, Colorado; La Plata; Lublin,

Poland and rural areas in Paris, Idaho, Portugal listed the genera *Cladosporium,*

Penicillium, Aspergillus, and *Alternaria* as the major components of their fungal clone

libraries (12, 30, 32, 37, 40, 41, 42, 43, 44, 45, 46). However, in our particular study,

while *Cladosporium* was a major contributor to the fungal diversity in our clone libraries,

Fusarium and *Saccharomyces* were much more prominent throughout the year, the

genera *Penicillium* and *Aspergillus* did not occur at high percentages during the sampling period, and the genus *Alternaria* was not detected. This may be partially attributed to the fact that *Alternaria* concentrations are typically orders of magnitude higher in hot and dry inland regions in comparison to cold temperate climates (47). In addition, *Cladosporium* is soil borne and airborne, while *Alternaria* is largely soil borne (48), which could potentially impact the concentrations of *Alternaria* present in the air, especially in temperate climates.

Looking at the overall diversity of the bacteria clone libraries, the results indicated that the gram-negative phylum, *Proteobacteria* was the most abundant phylum in our study (77.7%), while the phyla *Bacteroidetes* (9.4%), *Actinobacteria* (7.2%), *Firmicutes* (4.2%) and *Aquificae* (1.1%) all had relative abundances below 10% throughout the sampling year. Eighty-six sequences didn't meet the 90% confidence level in RDP classifier at the phylum level, and a majority of the sequences came from the phylum *Bacteroidetes*. However, these sequences would have comprised only 6.3 % of the sequence total, so they could potentially have influenced the *Bacteroidetes* abundance percentage slightly, although *Proteobacteria* would still remain the most abundant phylum in our study. Chloroplast (plant cell organelles) sequences were also identified in 16S rRNA clone libraries, since chloroplasts were originally bacteria that were taken in by plant cells as endosymbionts (29), and have retained their bacterial 16S rRNA genes, so are therefore marginally detectable by bacterial primers. Eleven archaeal sequences were also identified, all from the phylum *Euryarchaeota*, which is a phylum comprised mostly of methanogens and halophilic archaea (29). Archaea generally survive in harsher

climates (29), and most of the identified archaea sequences were found in the month of November, where the abundance of many organisms dropped significantly due to harsher weather conditions. Therefore, perhaps the change in weather allowed for an increased presence of archaea in the air.

The bacterial community composition of bioaerosols detected in this study differ from those reported win many other studies. The dominant bacterial phyla in bioaerosols seem to differ between sampling locations. For instance, in German urban and rural areas and in French rural areas, *Proteobacteria* was the dominant phylum, in an urban campus in Boulder, Colorado, *Flavobacteria* and *Bacteroidetes* were dominant, and in urban areas in Texas, *Actinobacteria, Bacteroidetes*, and *Firmicutes* were dominant phyla (24). Germany and France are the closest to the latitude of Fairbanks among the studies mentioned above, so perhaps colder climatic conditions and associated ecosystems present in temperate climates (France and Germany) and subarctic climates (Fairbanks, Alaska) are more favorable for higher abundances of *Proteobacteria*. However, the three most prominent phyla in this study are the same three prominent phyla in another bioaerosol study conducted in Austin, Texas (where the summers are hot and humid and the winters are mild) by Noris et al. (12), so the support for the latitude hypothesis is inconclusive. In addition, Boulder, Colorado is dry, just like Fairbanks, but has mild winters and coniferous forests, and has different prominent phyla compared to Germany, France, and Austin, Texas, so climate alone cannot explain the differences in bacterial profiles for different regions.

Another pertinent fact is that in soil, about 50% of the bacteria are gram-negative, and in marine waters 90% are gram-negative (24), so perhaps the soil and marine waters are major contributors to the *Proteobacteria* present in Fairbanks. However, Fairbanks, due to its location in interior Alaska, has a very low maritime influence. Warm air does travel from the southern latitudes in the summertime (which may explain the highly increased bacterial genus richness during summer), but because of the topographic features surrounding Fairbanks and the high pressure fronts during winter, the winds are usually light overall in Fairbanks, and therefore may not be a major contributor to the fungal and bacterial profile in Fairbanks. However, the light winds in Fairbanks and the seasonal wildfires near Fairbanks may contribute to the fungal and bacterial profile in the air, especially since wildfires disperse both solid and liquid particles in the air, especially acidic compounds, organics, soil particles, and metals (49). Therefore, it seems likely that soil dwelling fungi and bacteria are dispersed throughout the air in summer due to the annual wildfires near Fairbanks, Alaska.

While some of the bacterial genera frequently detected in this study have also been reported to be abundant in other studies of bioaerosols [*Ralstonia* (12, 37, 50), *Acinetobacter* (37, 46), *Novosphingobium* (37), *Sphingomonas* (24, 50, 51, 52), *and Bradyrhizobium* (37)] the genera *Acidimicrobium*, *Filimonas*, and *Proteiniphilum* have not been listed in these studies as a particularly abundant genera. In addition, other genera such as *Pseudomonas* (12, 24, 46, 50), *Micrococcus* (37, 53), and *Staphylococcus* and *Streptococcus* (12, 37, 46) have been listed as highly abundant genera in other studies that targeted an array of rural, urban, and maritime environments (and temperate climates

such as France and Poland), even though our study showed no traces of these particular genera. Therefore, while the Fairbanks fungal and bacterial profiles for the sampling year may be similar to previous studies in certain aspects, there are definitely some disparities between this and other similar studies conducted elsewhere.

We also constructed correlation matrices to examine correlations between the dominant OTUs of fungi and bacteria and temperature and snowfall accumulation (Table 3, Table 4). The fungal correlation matrix revealed no strong correlations between different genera and the weather factors of snowfall accumulation and temperature maxima and minima. There is a strong correlation between *Cladosporium* and *Davidiella* (0.92), indicating that *Cladosporium* and *Davidiella* almost always occur contemporaneously during the sampling year. However, this correlation can be explained by the fact that these two genera are from the same family, *Davidiellaceae* (see Appendix Fig. A), and that species of *Cladosporium* anamorphs (fungi at the asexual reproductive stage) readily form *Davidiella* teleomorphs (fungi at the sexual reproductive stage) in culture (54, 55). There were no strong correlations between fungal classes and the weather factors as well, although there were strong correlations between classes (Table 3B). *Ustilaginomycetes* and *Exobasidiomycetes* were strongly correlated (0.96), as were *Leotiomycetes* to *Pezizomycetes* and *Puccinomycetes* (0.97), which indicates that in this particular sampling year, these samples occurred together in similarly fluctuating abundance almost every month. At the phylum level, *Ascomycota* and *Basidiomycota* had weak correlations with the weather factors (Table 3C). Therefore, snowfall accumulation and temperature may have a limited role in determining the occurrence of fungi.

However, perhaps additional weather factors could be impacting the fungal profile throughout the year. Factors such as light intensity, relative humidity, and wind speed can also play a prominent role in bioaerosol dispersal, transfer, and transport (35).

Some bacterial taxa showed apparent relationships to weather factors (Fig. 14). There was one strong correlation between a genus and a weather factor, which (Table 4A) was between *Sphingomonas* and snow depth (-0.84). This indicates that as the snowfall accumulates on the ground, the occurrence of *Sphingomonas* decreases, and once the snow begins to melt, the occurrence of *Sphingomonas* increases. At the class level *Alphaproteobacteria* was strongly correlated to snow accumulation (-0.87), but this strong correlation is largely due to the fact that *Sphingomonas* belongs to the *Alphaproteobacteria* class (Table 4B). Because *Proteobacteria* are most prominent in soil and marine environments (24), the snow cover on the soil and soil freezing may prevent high abundances of *Proteobacteria* during the winter, but as the snow melts and the soil thaws, the *Proteobacteria* in the soil are uncovered and can easily be dispersed into the air. There were correlations between different genera as well (Table 4A). However, unlike the fungi, these genera are not in the same family. In fact, with the exception of the correlation of *Acidomonas* to *Acinetobacter*, all of these correlations represent genera from different phyla being correlated to one another, an indicator that different bacteria can have similar ecologies and habitats.

The phyla of bacteria classified from the clone libraries were compared to one another (Table 4C) to reveal strong correlations between *Proteobacteria* and *Bacteroidetes* (-0.94) and *Proteobacteria* and *Firmicutes* (-0.80). An inverse correlation

was observed in some cases, such that when *Proteobacteria* concentrations rose, the concentrations of *Bacteroidetes* and *Firmicutes* decreased, and vice versa. Therefore, while the bacteria, save for the genera of *Sphingomonas* and the class *Alphaproteobacteria*, do not seem to be impacted by the tested weather factors, the correlation matrices indicate that certain phyla of bacteria are negatively correlated to one another.

The PCA for fungi at the genus, class, and phylum level (Fig. 15) showed that at all three levels, the months April and December were always in the same cluster, as were the months of August and May, perhaps due to the similarity in weather conditions for between each set of months. However, overall, the PCA for fungi was indicative of a variable pattern to fungal abundance and highlighted that in particular months, particular fungi are of high abundance. The PCA for bacteria at the genus, class, and phylum level (Fig. 16) had a more seasonal pattern. At the genus and class level, the months mostly cluster according to season, with different genera and classes being prominent for different clusters. Therefore, while fungal patterns are variable, the bacterial patterns in community composition and structure follow a seasonal pattern. This fact that this variability in seasonal microbial community structure was detectable using our methods suggests that this approach would be useful for detecting fluctuations in the quantity of bacteria and fungi in bioaerosols from the typical levels when monitoring for potential bioterrorism threats.

In addition to local microbial sources of bioaerosols, wind trajectories might impact the abundance of particular microbes at certain times. Wind direction and

magnitude probably play an influential role in the microbial community composition, especially if wind trajectories can transport microbes from one nation to another. In future studies, the HYSPLIT (Hybrid Single-Particle Langrangian Integrated Trajectory) model (Draxler RR & Rolph GD) might help to determine whether particular microbial bioaerosols originated from locations other than Fairbanks, Alaska, and could help in determining the origin of dispersal.

This study of the fungal and bacterial community composition and structure in ambient air detected no high level pathogens or known bioterror agents during the sampling year of March 2008-January 2009 in Fairbanks, Alaska (Fig. 6, Fig. 5), but did detect some organisms that can pose as a considerable threat to health in humans and animals. Looking at fungi, genera such as *Alternaria, Penicillium, Aspergillus*, and *Cladosporium* (three of which did appear in the fungal profile of Fairbanks) have particular species strongly associated with respiratory disease, especially asthma, and are classified as type 1 allergens, allergens that bind to Immunoglobulin E (3, 8, 56, 57, 58, 59). Certain *Cladosporium* species can produce volatile organic compounds, which can have long-term health effects on humans (60). *Saccharomyces,* commonly known as yeast, is implicated in the spoilage of sugar-rich foods, and can also cause hypersensitivity in cases of extended exposure (61). Fungi also produce $\beta(1\rightarrow3)$-glucans, glucose polymers of variable molecular weight and degree of branching, and several studies suggest that these polymers play a role in bioaerosol-induced inflammatory responses and respiratory symptoms (3).

Certain species of fungi can also produce mycotoxins, and while very little is known about exposure to mycotoxins and respiratory health effects, certain mycotoxins, like aflatoxin produced by *Aspergillus*, can be potent carcinogens (3, 57). Fortunately, *Aspergillus* occurred rarely during our sampling period. Other fungal species, such as *Penicillium*, produce toxins that have yet to be implicated as extremely harmful to human health (60). However, *Fusarium*, a prominent genus in the Fairbanks fungal profile, is also known to produce toxins under certain environmental conditions, and these toxins are known for poisoning human beings and animals (56), especially toxins such as trichothecenes, zearalenones, and fumonisins (60, 62). In fact, trichothecenes from certain species of *Fusarium,* such as *Fusarium sporotrichoides* and *Fusarium oxysporum*, can be weaponized (62, 63). Since the DRUM impactor was able to detect common outdoor *Fusarium* species, the DRUM impactor most likely has the potential to detect those *Fusarium* species that can be used as potential biological weapons.

The bacterial bioaerosols in ambient air can also affect air quality, since certain cellular components of bacteria are also considered as potential health concerns. Bacteria cell wall components such as endotoxins, which are present in gram-negative bacteria, and peptidoglycans, which are more prevalent in gram-positive bacteria, are pro-inflammatory agents that can induce respiratory symptoms (4, 7, 56, 64). Subjects exposed to endotoxin inhalation have been known to experience fever, shivering, malaise, neutrophilic airway inflammation, athralgia, leukocytosis, dry cough, dyspnea, chest lightness, and bronchial obstruction (4, 7, 56, 64). Therefore, the bacterial bioaerosol profile for Fairbanks will also be useful in reducing bioaerosol-induced symptoms,

especially for those who are immunocompromised or suffer from allergies, by allowing the public to know about potential health hazards in advance.

Looking at the dominant genera of bacteria that occurred during the sampling period in Fairbanks, Alaska, there are two genera in particular that could have adverse health effects. *Sphingomonas* has been implicated in nosocomial infections that are treatable with antibiotics (65). *Acinetobacter* too, has been implicated as a key source of infection in debilitated and immunocompromised patients in hospitals (66). However, unlike *Sphingomonas*, certain strains of *Acinetobacter*, *Acinetobacter baumannii* in particular, has been categorized as a highly-resistant and virulent bacteria species (67, 68). While the DRUM impactor collected common outdoor species of *Acinetobacter* in this experiment, the ability of the DRUM impactor to collect these common species may be indicative of its potential to capture more harmful strains of *Acinetobacter* in a different environment.

Looking at the health effects described above, using the DRUM impactor to detect the fungal and bacterial profile of Fairbanks (or any other region) might assist in reducing any bioaerosol-induced respiratory problems, especially allergies and asthma. In addition, knowing the concentrations of fungi and bacteria and their fluctuations could prevent any potential outbreaks that could result from rising concentrations of bacteria. Therefore, knowing the fungal and bacterial profile of a region would be useful in reducing potential threats that could result from microbial bioaerosols.

Many of the prominent fungal species in Fairbanks have very little to no known impact on human health, but are ecologically important. Species of *Fusarium* and

Venturia often act as plant pathogens (60, 69). *Penicillium* is often found in soil samples and is associated with plant decay (60). *Nectria* is a commonly known saprophyte and parasite, and targets many plants common to Fairbanks, Alaska, such as aspen, birch, and polar (69). *Glonium* is found growing on the barks of trees and shrubs, and certain species of *Entyloma* are said to cause smut, a disease that attacks the plants reproductive system. However, some species of fungi, like *Sebacina*, are beneficial to the plants. *Sebacina* forms ectomycorrhizal symbiotic relationships with forest trees, orchids (70), and ericoid plants such as blueberry and cranberry shrubs (71).

Many of the prominent genera of bacteria also include species with ecologically important functions. *Ralstonia* causes bacterial wilt and brown rot in many species of plants (72). *Acinetobacter* residing in soil contribute to the mineralization of organic compounds (66). *Acidimicrobium* is a ferrous iron oxidizer (73), and *Bradyrhizobium* is an essential nitrogen-fixating organism, taking atmospheric nitrogen and fixing it into ammonia and ammonium (74). However, most of the prominent genera in Fairbanks are known for their species with biodegradation properties. *Sphingomonas* is known to degrade chlorinated phenols, dibenzofurans, insecticides, herbicides, and PAHs, or polycyclic aromatic hydrocarbons (75). *Novosphingobium* is also a known degrader of aromatic compounds (76). *Filimonas* are from the phylum *Bacteroidetes*, a phylum known for being able to degrade various biopolymers (77). Certain species of *Proteiniphilum* have been used for bioaugmention experiments to improve the reduction of PAHs in particular locations (78). Therefore, knowledge about the fungal and bacterial

profile of Fairbanks can also be useful in determining whether certain genera of bacteria can assist in degradation processes of anthropogenic compounds.

As seen above, most of the bacteria and fungi present in the ambient outdoor air in Fairbanks, Alaska represent common environmental taxa rather than those with known human health impacts. However, particular genera, especially *Fusarium* in fungi and *Acinetobacter* in bacteria, include species that are considerable threats to human health, and therefore may be worthy of monitoring in bioaerosols. Using the DRUM impactor for collecting bioaerosols can not only allow for the detection of these potentially hazardous bioaerosols, but can also allow for the monitoring of fluctuations in their abundance patterns. Therefore, the DRUM impactor can not only be used for collecting bioaerosols, but can also be used to determine fluctuations in the abundance of organisms that are potential health hazards, thus allowing the public to take precautions against these potential hazards.

This study demonstrates the viability of using culture-independent methods to identify microbial bioaerosols collected using a DRUM impactor, but is subject to the fundamental limitations and shortcomings of these methods. While cloning and Sanger sequencing are effective tools, they may not be able to accurately reflect all the diversity present in the atmosphere due to cost limitations. Next generation sequencing technology, such as pyrosequencing, could enable greater sequencing depth for lower cost. Therefore, other methods should be utilized alongside PCR and cloning. Some specific tools that could be useful in this scenario are pyrosequencing. Another potential limitation is PCR bias, but touchdown PCR, which relies on incremental annealing temperature decreasing

in progressive cycles, may help mitigate the bias. The higher annealing temperatures give greater specificity for primer binding, while the lower temperatures permit more efficient amplification of the products formed during the initial cycles (79). Another option along this line that would be useful to try is to perform multiple PCR reactions with different annealing temperatures to see if a specific annealing temperature is more effective and amplifying ambient bioaerosols than the others. If there is no difference in amplification, than the multiple PCR reactions can be pooled together for higher DNA abundance and concentrations. Perhaps in this manner, the concentration of DNA obtained from bioaerosols can be increased, thus reducing the need for nested PCR and allowing for other concentration-dependent techniques to be more effective.

The fact that there is no quantitative measure of the viable fungi and bacteria collected in this study also needs to be taken into account. PCR reactions amplify both live and dead cell DNA, and therefore do not account for viability. This matter can be rectified by performing reverse-transcriptase PCR (80), which first requires the extraction of RNA and then, during the PCR reactions, the RNA is reverse transcribed to DNA. Since the production of RNA is a sign that the organism is still alive, this procedure could provide an idea about the viable microbial profile. However, RNA is unstable and difficult to extract (80), and the concentrations of RNA present in the already low concentrations of bioaerosols in the air might be difficult to quantify.

The approach taken in this study allowed for qualitative comparisons of community structure and composition, but was only semi-quantitative. However, using an aerosol generator could provide an opportunity for quantitative comparisons of

bioaerosols. First, to ensure that lab contamination is not incorporated into the study (and to serve as a control), the endogenous blank located at the very top and bottom of the drum substrates can be tested for bioaerosols. We can then use an aerosol generator to create a general curve to compare with collected bioaerosols in order to validate the quantitative sampling capability of the DRUM impactor. Aerosol generators have the capability to monodisperse or polydisperse solid or liquid aerosols ranging from 0.003 μm to 200 μm, and can even electrostatically charge dispersed particles using a radioactive source. Using an aerosol generator, predetermined concentrations of bacteria and fungi can be aerosolized, and then a DRUM impactor can be used to collect the aerosolized microbes. The microbes can then be quantified using quantitative PCR, and then a collection curve comparing the qPCR results to the predetermined concentrations can be made for quantifying the bioaerosols present in the sample. However, because there are a vast amount of bacteria and fungi with varying shapes and sizes, using only specific species of bacteria and fungi in the bioaerosol generator might not accurately reflect the collection of all bacteria and fungi, and may impact the generated curve.

5. Conclusion

This study was successfully able to use the DRUM impactor as a collection apparatus for bioaerosols. The collected bioaerosols were of sufficient quantity and quality to enable DNA-based taxonomic identification and community characterization. Bioaerosol community analyses did reveal particular patterns of occurrence for certain bacterial and fungal taxa, and while some were seasonal, most of the occurrences were variable, with some taxa occurring in high abundances in summer, some taxa occurring in high abundances in winter, and others having bimodal patterns. Therefore, the DRUM impactor was demonstrated as an effective tool for collecting bioaerosols for taxonomic analyses. While the DRUM impactor captured mostly environmental bacteria and fungi commonly found in outdoor air, it was also able to capture genera that contain potentially harmful species, thus indicating that the DRUM impactor may have potential to collect pathogenic species in different environments.

Using the DRUM impactor to collect and identify fungi and bacteria is the first step in a new direction, and perhaps with time and alternative methods, the DRUM impactor can also be utilized to collect viruses and archaea present in the air (81). By validating the DRUM impactor as an effective tool for collecting a representative sample of the bioaerosols present in an air sample, this study and future studies could enhance the field of atmospheric chemistry by determining the biological composition of the surrounding ambient air. Validation of the DRUM impactor can also allow for monitoring of allergens, fluctuations in pathogenic microbes, bioterrorism agent detection, and general health sweeps of public areas, thus allowing improved health and

air quality. Therefore, validating the DRUM impactor as an effective tool for collecting

bioaerosols could be highly useful and is worthy of continued investigation in the future.

6. Acknowledgements

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8. Appendix
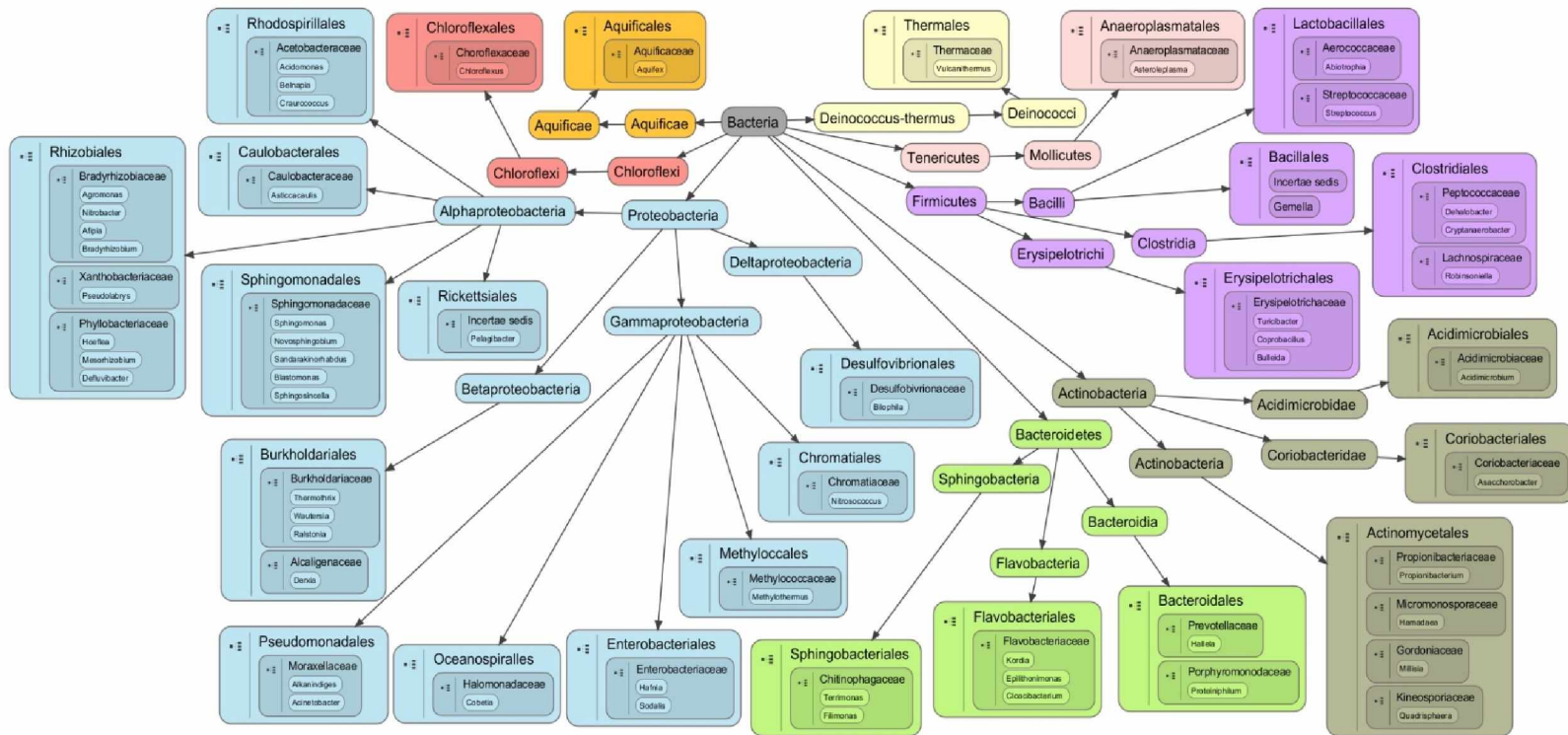
Figure A: Bacterial Phylogeny. Phylogenetic tree depicting all genera of bacteria determined by sequencing of clones from bacteria clone libraries. Tree constructed using VUE v.3.1.1 based at Tufts University (vue.tufts.edu).
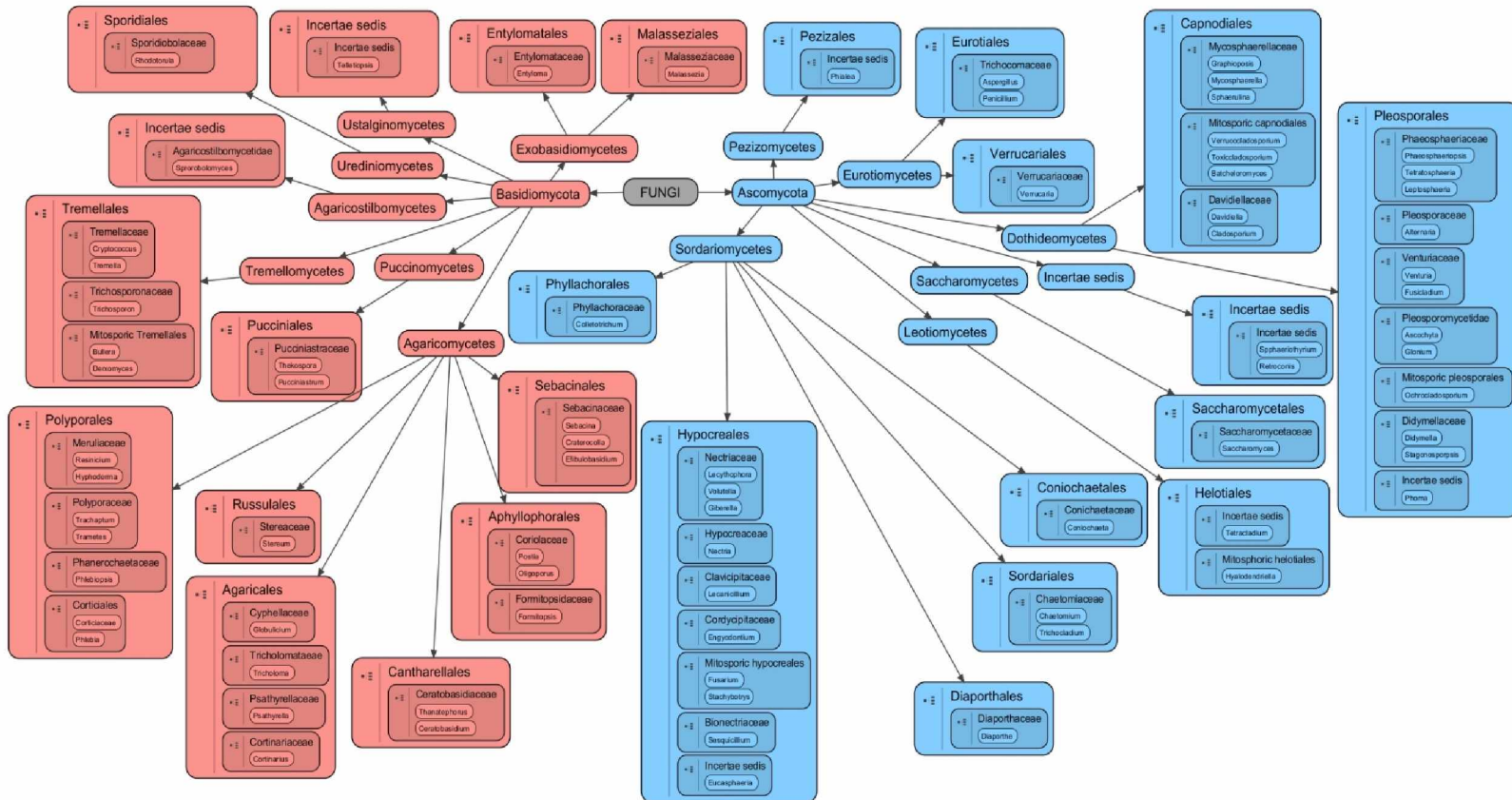
Figure B: Fungal phylogeny. Phylogenetic tree depicting all genera of fungi determined by sequencing of clones from fungi clone libraries. Tree constructed using VUE v.3.1.1 based at Tufts University (vue.tufts.edu).