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RESEARCH ARTICLE

A METAGENOMIC ASSESSMENT OF BACTERIAL CONTAMINATION OF DUST EVENTS IN SENEGAL

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Abstract

Previous work in the Caribbean and West Africa have shown that air samples taken during dust events contain microorganisms (bacteria, fungi, viruses), including human pathogens that can cause many respiratory diseases. To better understand the potential downstream effect of bacteria dust on human health and public ecosystems, it is important to characterize the source population. In this study, we aimed to explore the bacterial populations of African dust samples collected between 2013-2017. The dust samples were collected using the spatula method, then the hypervariable regions (V3 and V4) of the 16S rRNA gene were amplified using PCR followed byMiSeq Illumina sequencing. Analysis of the sequencing data were performed using MG-RAST. At the phylum level, the proportions of *Actinobacteria* (22%), *Firmicutes* (20%), *Proteobacteria* (19%), and *Bacteroidetes* (13%) were respectively predominant in all dust samples. At the genus level, *Bacillus* (16%), *Pseudomonas* (10%), *Nocardiodes* and *Exiguobacterium* (5%) are the most dominated genera in African dust samples collected in this study. The study showed that molecular characterization of dust microbial population remains a very efficient method, also applicable to the search for viruses and fungi in this type of sample. It is important to note that the majority of microorganisms identified in this study can cause respiratory diseases.

Introduction:

Every year, millions of tons of dust are aerosolized off the coast of sub Saharan Africa **(Staff 2020)**. The Sahara Desert is the most significant dust emission source in the world with long-range transport to the Caribbean, the southeastern United States, Europe, and into West Africa **(Engelstaedter, Tegen et al. 2006, Weinzierl, Ansmann et al. 2017).**

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The Sahel's precipitation regimes are divided into a dry season that spans from October through May and a wet season between June and September. Saharan dust outbreaks are generated throughout the year (wet and dry seasons in West Africa), with the Sahara acting as the largest source of dust in the world, producing some 400–700 × 106 t of dust per year **(Goudie 2001)**. This dust is transported downstream to Europe, the Caribbean, and the United States. However, large quantities of Saharan dust are transported into West Africa, especially during the dry season **(Marone 2020).**

Aeolian dust particles are thought to be carriers of microbes, abundance and community composition of microbes transported have been reported with their possible impacts on public health and ecosystems.Major aeolian dust events arise from the Sahara and Sahel deserts (African dust), Australian deserts (Australian dust), and the Taklamakan Desert, Gobi Desert and Loess Plateau (Asian dust) **(Yamaguchi, Baba et al. 2016).**

Desert dust is not like builder's sand; it contains many biological particles and allergens. A recent study in Miami found increased hospital visits for people with chronic obstructive pulmonary disease during Saharan dust events. Other studies have found problems for asthmatics, too. In June 2020, authorities in Cuba asked islanders to wear face masks and urged vulnerable people to stay indoors as a massive Saharan dust cloud travelled westwards. Alerting systems are being established across Spain and Portugal but there is need for more research into the health impacts of desert dust **(Fuller 2021)**. As the effects of climate change continue to impact our health, it is important to stay informed about the air quality in our communities **(Staff 2020).**

Dust storms in Africa have been linked to meningitis outbreaks in sub-Saharan Africa **(Agier 2013, Jusot, Neill et al. 2017)** and the coral reef decline in the Caribbean. They are also known to correlate with increased hospital emergency visits due to asthma exacerbations and other respiratory and cardiovascular complications **(Mallone, Stafoggia et al. 2011, Tam, Wong et al. 2012, Lee, Kim et al. 2013, Meo, Al-Kheraiji et al. 2013).**

Moreover, bioaerosols may have a significant influence on human health and the spread of plant diseases. Airborne microorganisms including bacteria, fungi, and viruses can have infectious, allergenic, or toxic effects on living organisms, causing diseases or allergies in humans, agricultural crops, livestock, and ecosystems, including coral reefs **(Hayedeh, Katsuhiko et al. 2018).** The dust-event-driven dispersal of bioaerosols is strongly correlated with allergen burdens and. the long-distance aerial dispersal of pathogens by the wind can spread plant diseases and human diseases, such as Kawasaki disease) **(Hayedeh, Katsuhiko et al. 2018)**.

The main colonizers found in dust particles belong to six different phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Cyanobacteria*. However, the relative abundance and load of these phyla, and especially the bacterial composition at the genera level, differs significantly between the different compartmentsof body sites **(Marsland, Trompette et al. 2015).**

The few studies investigating the roles of dust storm microbiota in disease outbreaks found correlation, but did not explore clinical evidence for causal effects. Although these studies provide a link between sandstorms and disease outbreaks, they were constrained in terms of number and scope **(Wang, Dai et al. 2016).**

In Dakar, particulate monitoring carried out by the Air Quality Management Center (CGQA) highlights increased exceedances of regulatory values. The daily limit value for PM_{10} in particular (260µg / m^3 not to exceed more than 35 days / year) is exceeded every year. For $PM_{2.5}$ fine particles, the Senegalese quality objective (120 µg / m^3), which also corresponds to the recommendations of the World Health Organization (WHO), is largely exceeded throughout the region to the detriment of the 3 million Dakar residents. This phenomenon is explained by the increase in the level of emissions of atmospheric particles in the air and anthropogenic releases (industries, transport, domestic heating) coupled with stable weather conditions (favoring the accumulation of pollutants**) (Diop and Mbow 2013).**

Beyond the desert dust, the air of Dakar is polluted allyearround. The particle count is 2.5 times above what the WHO recommends. If Senegal is one of the few countries in Africa to measure air quality, prevention is no longer enough to protect the inhabitants of Dakar **(Margot Chevance and Berthé 2018).**

The lack of data on the monitoring of air pollution and the low consideration of air quality in the transport and industry sectors led to the Government of Senegal to set up a laboratory to monitor atmospheric emissions. Some

regions in Dakar face pollution levels abovethe limits defined by NS-05-062. The pollutants concerned are particulate matter (PM_{10} and $PM_{2.5}$). The measurements obtained by the laboratory are processed to inform people continuously about pollution levels. They also provide the state with reports on abatement strategies. The results can be used to correlate with epidemiological data, in this case for respiratory and cardiovascular diseases **(Sénégal 2018)**. The majority of these bacteria are still neither identified nor characterizedbecause they remain recalcitrant to in vitro culture on culture media that have been proposed to them. These limits imposed by the in vitro culture have allowed the development of approaches that are free of them. The arrival of metagenomicsin the 1990s restricted the study of bacteria to their DNA directly extracted from the environment **(Faugier 2010)**. Ribosomal RNA (rRNA) sequences, either 16S rRNA for Archaea, bacteria and chloroplasts or 18S rRNA for eukaryotes are the routinely accepted standards for identifying the members of mixed microbial communities and defining the overall microbial diversity in the environment. Next-generation sequencing has opened up approaches to analyse microbial community in great details **(Thompson, Sanders et al. 2017)**. Although entire 16S rRNA gene sequences would provide the best resolution for taxonomic characterization, technological limitations on the length of sequence reads currently limit this approach to sections of the gene. Universal primers that bind to interspersed conserved regions, target-specific variable regions within the SSU rRNA (Small subunit ribosomal ribonucleic acid) genes that provide phylogenetic information on microbial taxa. The choice of 'optimal' variable region(s) is an ongoing debate that depends both on the sequencing technology at hand, the research question **(Choi, Bachy et al. 2017).**

The identification of respiratory pathogens is important toaddress public health concerns in resource-limited countries. The high level of povertyin West Africa, coupled with rapid population growthis a recipe to higher exposure to respiratory pathogens. In many cases, vulnerable populations (poor, young, elderly, disabled and sick) are more at risk of respiratory infections. Therefore, identifying potential respiratory pathogens in dust events can improve treatment and health outcomes.

The aims of this study areto identify bacteria associated with dust collected from Cheikh Anta Diop University from 2013 to 2017, using genomic techniques andMetaGenome Rapid Annotation with Subsystem Technology (MG-RAST) metagenomic analysis and see their impact on human health.

Materiel and Methods:

Samples collection:

The aerosol samples were collected between 2013 and 2017 from the roof of the Laboratory of Physics of the Atmosphere and Ocean Simeon FONGANG of Dakar Polytechnic School (ESP) in Cheikh Anta Diop University (UCAD), using a sterile spatula that allowed scraping and sampling during dust events. Dust samples were collected in certified, non-pyrogenic, non-cytotoxic, 20 ml Falcon Tubes with medical grade packaging that ensures good sterility.

Table 1: Dust samples collected.

Sampling dates	Sampling area
30/07/2013 (Summer) (DS 01)	Fann, Dakar
06/11/2013 (Winter) (DS 14)	Fann, Dakar
05/12/2013 (Autumn) (DS 02)	Fann, Dakar
10/03/2014 (Winter) (DS 12)	Fann, Dakar
24/06/2014 (Spring) (DS 07)	Fann, Dakar
30/01/2016 (Winter) (DS 03)	Fann, Dakar
22/08/2017 (Summer) (DS 06)	Fann, Dakar
21/12/2017 (Autumn) (DS 05)	Fann, Dakar

Determining the Occurrence of Dust Events in Dakar Senegal for Sampling:

We determine whether dust was present in Dakar, Senegal on the day of collection, through used satellite visible satellite images, visibility reports from the airport, aerosol optical depth satellite or ground-based measurements in Mbour, Senegal, and observed particulate matter (PM) concentrations measured in Dakar, Senegal, by the Air Quality Management Centre, from the Directorate for the Environment and Classified Establishments (Ministry of Environment and Sustainable Development**) (Diokhane, Jenkins et al. 2016).** We also used the Hybrid Single Particle Lagrangian Integrated Trajectory Model HYSPLIT model **(Stein et al., 2015)** to identify the potential origin of dust using 5-day back trajectories with the endpoint being Dakar, Senegal. Back Trajectories are based on Global

Data Assimilation System (GDAS) with a resolution of 1° × 1°, and we assume isentropic vertical motions, which are appropriate for desert locations where precipitation is not likely. This assumption has been used by Drame et al. (2011) to follow Saharan dust that was found above Dakar, Senegal, in July 2010. The sampling occurred throughout the year with eight samples. For the spatula method, a sufficient amount of dust (visible) had to accumulate on the glass surface before the sample was taken and hence, additional dust events may have occurred prior to the sampling. The sampling using the suspended aerosol sampler occurred on days when dust would have been present from visible satellite images although, during the summer season, dust is located above the monsoon layer with small particles gravitationally settling over Dakar, Senegal.

Total Nucleic acid extraction:

Extraction of total nucleic acid from dust sampleswas done using a modified QIAamp Fast DNA Stool Mini Kit procedure. The dust sample undergoes a lysate preparation process and includes mechanical disruption (bead beating), removal of inhibitors, purification and elution of DNA and RNA using spin columns. Extrinsic controls PhHV (Phocine Herpesvirus) and MS2 are added to each sample during the lysate preparation to evaluate extraction and amplification efficiency. The extracted total nucleic acid (TNA) is then stored at-80°C before downstream analysis. To rule out contamination during the extraction process, a blank is also processed through the complete protocol each day extractions are performed.

Library Preparation:

16S libraries were made following Illumina's protocol for 16S metagenomics library preparation using V3-V4 primers described elswhere. Blank extraction, PCR controls were included.

The gene-specific sequences used in this protocol target the 16S V3 and V4 region **(Klindworth, Pruesse et al. 2013)** as the most promising bacterial primer pair.

Illumina adapter overhang nucleotide sequences are added to the gene-specific sequences. The full-length primer sequences, using standard IUPAC nucleotide nomenclature, to follow the protocol targeting this region are:

16S Amplicon PCR Forward Primer:
5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
16S Amplicon PCR Reverse Primer:
5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC
For an initial MiSeq sequencing run, the PCR1 amplification was carried out with the following reaction mixture. The following program was used for amplification: 95°C for 3 min for initial melting; 25 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; 72°C for 5 min.

One µl of the PCR product were run on a Bioanalyzer DNA 1000 chip to verify the size and all samples were cleaned, quantified with the QubitFluorometer dsDNA HS Assay Kit and normalized (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

The Index PCR (PCR2) attaches dual indices and Illumina sequencing adapters using the Nextera XT Index Kit. The following program was used for amplification: 95°C for 3 min; 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; 72°C for 5 min.

Products were again cleaned with the Agencourt AMPure XP PCR purification kit (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. DNA concentration and fragment size were measured on a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and Agilent Bioanalyzer (Santa Clara, CA, USA), respectively.

For later HiSeq sequencing runs, the library preparation procedure was identical to the MiSeq protocol with 25 and 8 cycles for PCR1 and PCR2, respectively **(Meola, Lazzaro et al. 2015).**

Illumina sequencing:

In preparation for cluster generation and sequencing, pooled libraries are denatured with NaOH, diluted with hybridization buffer, and then heat denatured before MiSeq sequencing. Each run must include a minimum of 10% PhiX to serve as an internal control for low-diversity libraries **(Klindworth, Pruesse et al. 2013)**. Illumina recommends using MiSeq v3 reagent kits for improved run metrics. After samples are loaded, the MiSeq system

provides on-instrument secondary analysis using the MiSeq Reporter software (MSR). MSR provides several options for analyzing MiSeq sequencing data. For this demonstrated 16S protocol, select the Metagenomics workflow by following this 16S Metagenomics protocol, the Metagenomics workflow classifies organisms from your V3 and V4 amplicon using a database of 16S rRNA data **(Klindworth, Pruesse et al. 2013)**.

Metagenomic analysis:
The first step in the RNA-Seq workflow is to take the FASTQ files received from the sequencing facility and assess the quality of the sequence reads.

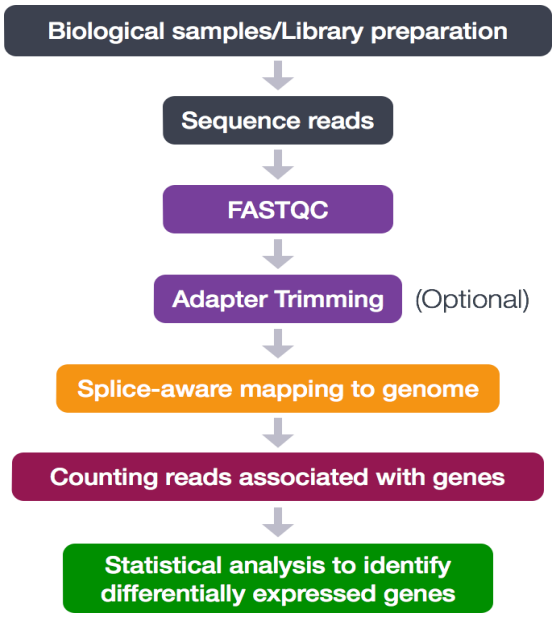


Figure 1: Steps before uploading sequences to MG-RAST.

The Illumina sequencing platforms generate between several tens to hundreds of millions of reads, enabling deep profiling of a large number of samples during a single PE run at a fraction of the cost of a study. The output of more than 100,000 reads per sample is suggested and sufficient for microbiota investigations **(Illumina 2013).**

The resultant contigs and singletons were uploaded to MG-RAST **(Meyer, Paarmann et al. 2008)**, where additional removal of artificial replicates and filtering for *H. sapiens* sequences was performed. All samples were also uploaded to MG-RAST. While MG-RAST analysis is suited well for reads of the same length, it does not provide a way to calculate the phylogenetic distribution in metagenomic samples having mixed sequences of different lengths and coverage. Therefore, after MG-RAST BLAST search, all resulting files were downloaded from the server and analyzed independently. Phylogenetic annotation of each metagenome was performed based on 16S rRNA gene fragments and sequence reads. Ribosomal RNA sequences for each metagenome after clustering (97% identity) were downloaded from MG-RAST server and classified by SILVA Aligner (with the minimum identity with query sequence 0.85), and RDP classifier (with the minimum confidence score of 0.8) **(Quast, Pruesse et al. 2013).** SILVA Aligner was utilized as the primary source of classification, while RDP classification was assigned to the sequence only when not classifiable by SILVA **(Kiseleva, Garushyants et al. 2015)**.

To perform phylogenetic annotation on all metagenomic sequences and calculate the phylogenetic rank abundances, results of BLAT search against RefSeq database was utilized. To assign taxonomy to the individual sequences the lowest common ancestor (LCA) approach was used. Nearest neighbors of the particular sequence were determined as hits with alignment length of at least 50bp, and with a maximum permitted difference of 10% from the maximum identity, but with identity not less than 60%, and BLAT e-value of at least 10^{-5}. These selected hits were used for LCA, and the common part of taxonomy for nearest neighbors was assigned to the metagenomic sequence. Abundance of each phylogenetic rank was calculated as the number of shotgun reads assigned to the particular

phylogenetic rank, where abundance for a contig is the number of reads included in this contig. All taxonomic ranges were assigned according to MG-RAST Taxonomy **(Sayers, Barrett et al. 2009, Letunic and Bork 2011).**

Results and Discussion:

Eight dust samples were analysed in this study. This was because 8 samples collected hadsufficient amount of dust required for Nucleic acid extraction. All the samples analyzed during our study were collected in the same place (Fann) and on different dates according to the forecasts of the dust events.

Characterization of bacterial community at the phylum level:

Through the diversity analysis, we confirmed that the airborne microbial environment was affected by transported dust particles, which contain various microorganisms, during dust events. We classified the sequences obtained from Illumina sequencing data according using the SILVA database. A total of 19 phyla were classified and predominant phyla are presented in Fig 1.

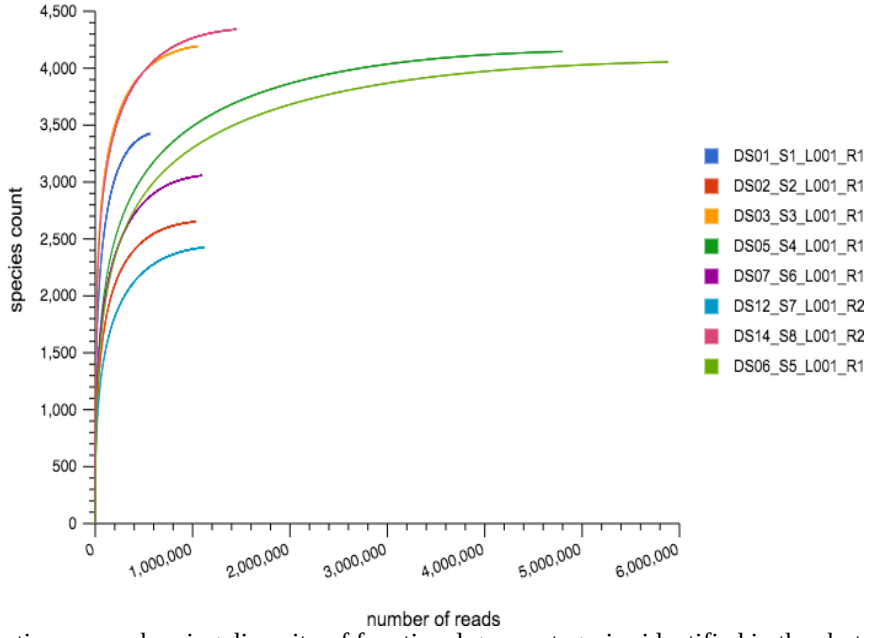
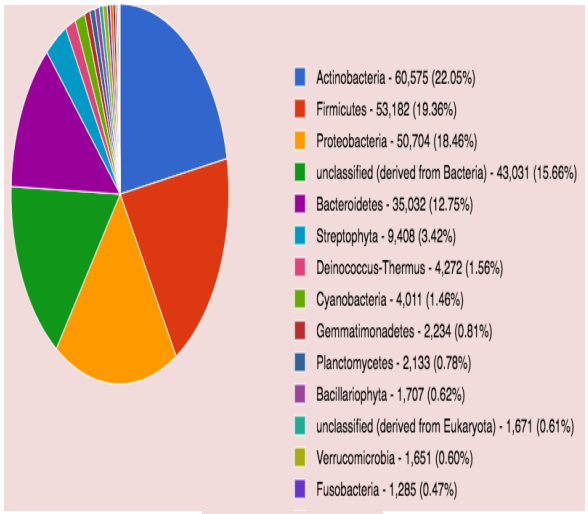


Figure 1: Rarefaction curve showing diversity of functional gene categories identified in the shotgun metagenome data sets (MG-RAST Analysis). Each curve represents an individual metagenome.

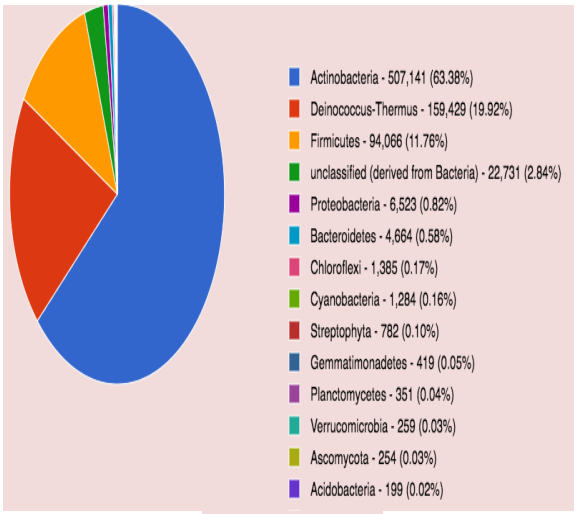
Bacteria-related metagenome sequences were parsed from the shotgun metagenomes and functionally annotated to SEED subsystems **(Overbeek, Begley et al. 2005)** in MG-RAST **(Meyer, Paarmann et al. 2008).** Functional gene categories were identified across the metagenomes and ranged from 2400 to 4300 within individual metagenomes (Fig.1).

Differences in functional diversity were not observed between metagenomes sequenced with similar coverage, suggesting that the atmosphere did not affect the functional diversity of the bacterial populations.

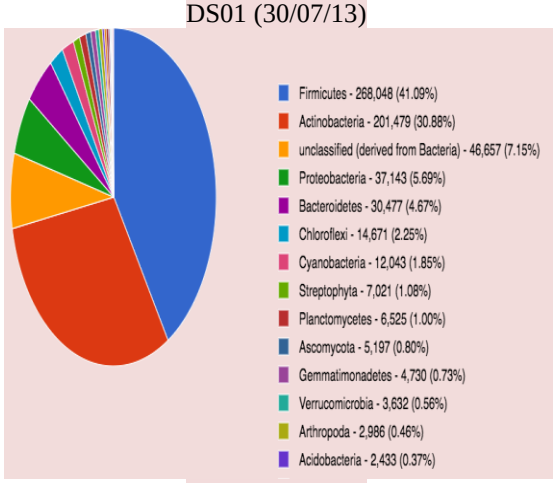
With the exception of metagenomes DS05 and DS06, the rarefaction curves don't approach an asymptote, suggesting that increased sampling would have greatly increased the number of functional categories identified in the bacteria-related metagenome sequences (Fig. 1). Therefore, the shotgun metagenomes appear to have sampled the functional diversity of the biocrust bacteria adequately for statistical comparisons.



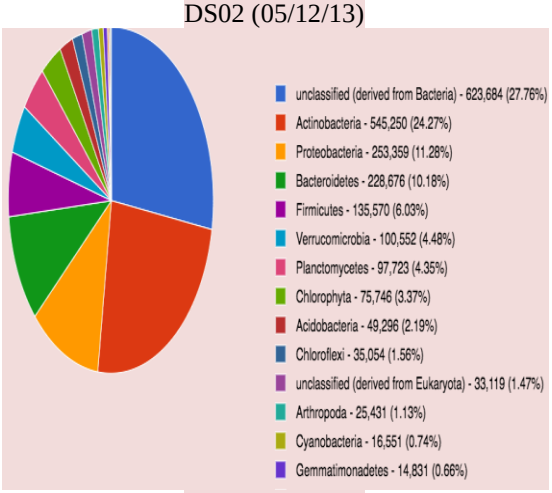
DS01 (30/07/13)



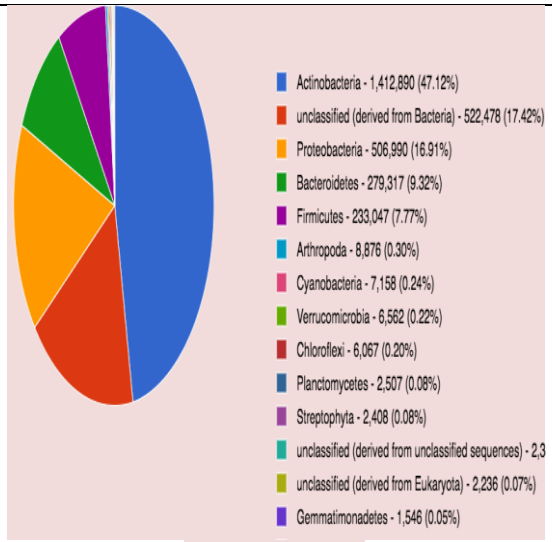
DS02 (05/12/13)



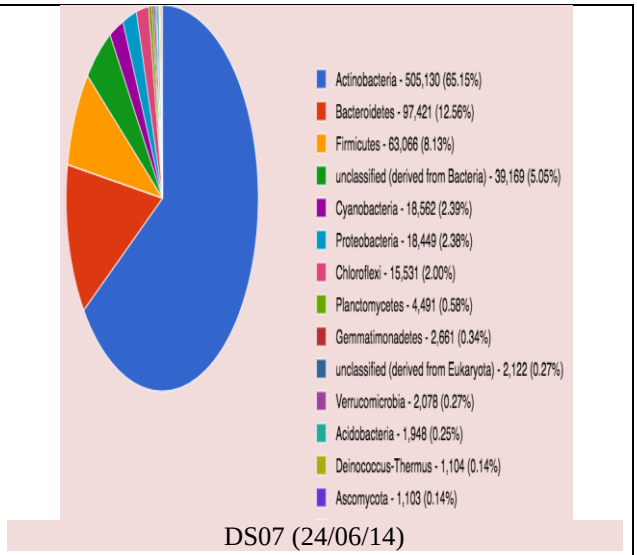
DS03 (30/01/16)



DS05 (21/12/17)



DS06 (22/08/17)



DS07 (24/06/14)

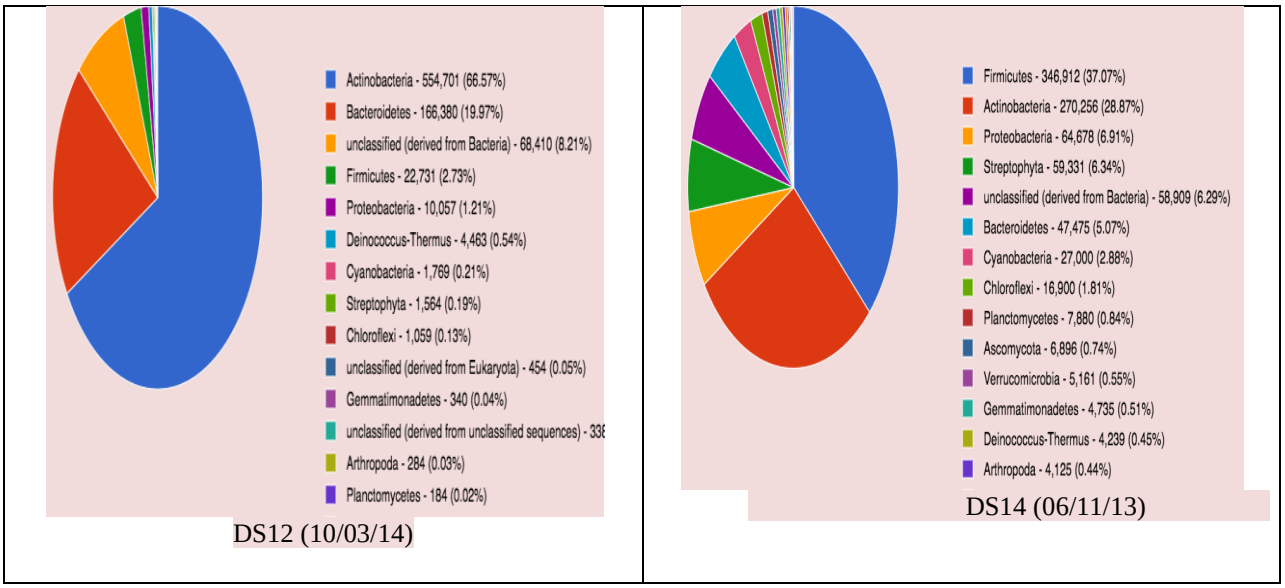


Figure 2:- Bacteria abundance (%) at phylum level for each sample Each image illustrates the distribution of Phylum for each sample. Results was obtained with MG-RAST.

Our results show that the proportion of *Actinobacteria*was predominant (5 samples out of 8 samples) with 22.05% for DS01, 63.38% for DS02, 47.12% for DS06, 65.15% for DS07 and 66.57% for DS12. But *Firmicutes* dominated DS03 and DS14 with 41.09% and 37.07% respectively. Unclassified bacteria are predominant in DS05 sample with 27.07%. The proportions of each phylum differ across samples. Overall, we have the same phylum composition in almost all samples.

As expected, the airborne bacterial community structure showed significant differences among the dust samples, which was consistent with previous reports that airbornebacterial concentration and community composition were shaped by meteorological factors **(Franzetti, Gandolfi et al. 2011, Yamamoto, Bibby et al. 2012, Qi, Shao et al. 2014, Gao, Yan et al. 2016, Du, Du et al. 2017)**. Notably, our study also revealed that the effects of these events on airborne bacteria were slighter than the effects of temperature and exhibited differences between fall and winter, as reported elsewhere **(Li, Yang et al. 2018)**.

Pollution is a global risk factor impacting an estimated 9 million premature deaths worldwide with ambient air pollution contributing **(Landrigan, Fuller et al. 2018).**Several studies have linked winter season dust conditions to meningitis, which is common in the dry season (January–April). Hyper-endemic meningitis cases and occasionally epidemics occur during this period **(Agier 2013, Martigny and Chiapello 2013)**.

As shown in Figure 2, although *Actinobacteria*, *Proteobacteria* and *Firmicutes* were predominant phyla in all samples, their relative abundances showed obvious seasonal changes. As the temperature decreased over time, from November to January the relative abundance of *Actinobacteria* gradually decreased, too **(Tang 2018)**. However, *Firmicutes* and *Actinobacteria*, gradually increased, because of the seasonal distinction of predominant bacteria, the characteristics of bacterial changes at distinct stages thatrequired to be examined in other haze studies.

Comparative analysis of the bacterial community composition revealed that the ubiquitous bacterial phyla in the samples were *Actinobacteria, Firmicutes, Proteobacteria* and *Bacteroidetes*(Fig. 2), which are typically the most abundant phyla in the atmospheric environment of the Gobi Desert **(Maki, Kurosaki et al. 2016)**. Of these phyla, *Actinobacteria, Firmicutes, Proteobacteria* and *Bacteroidetes* remained the dominant phyla in all the samples (Fig. 3)**(Tang 2018).**

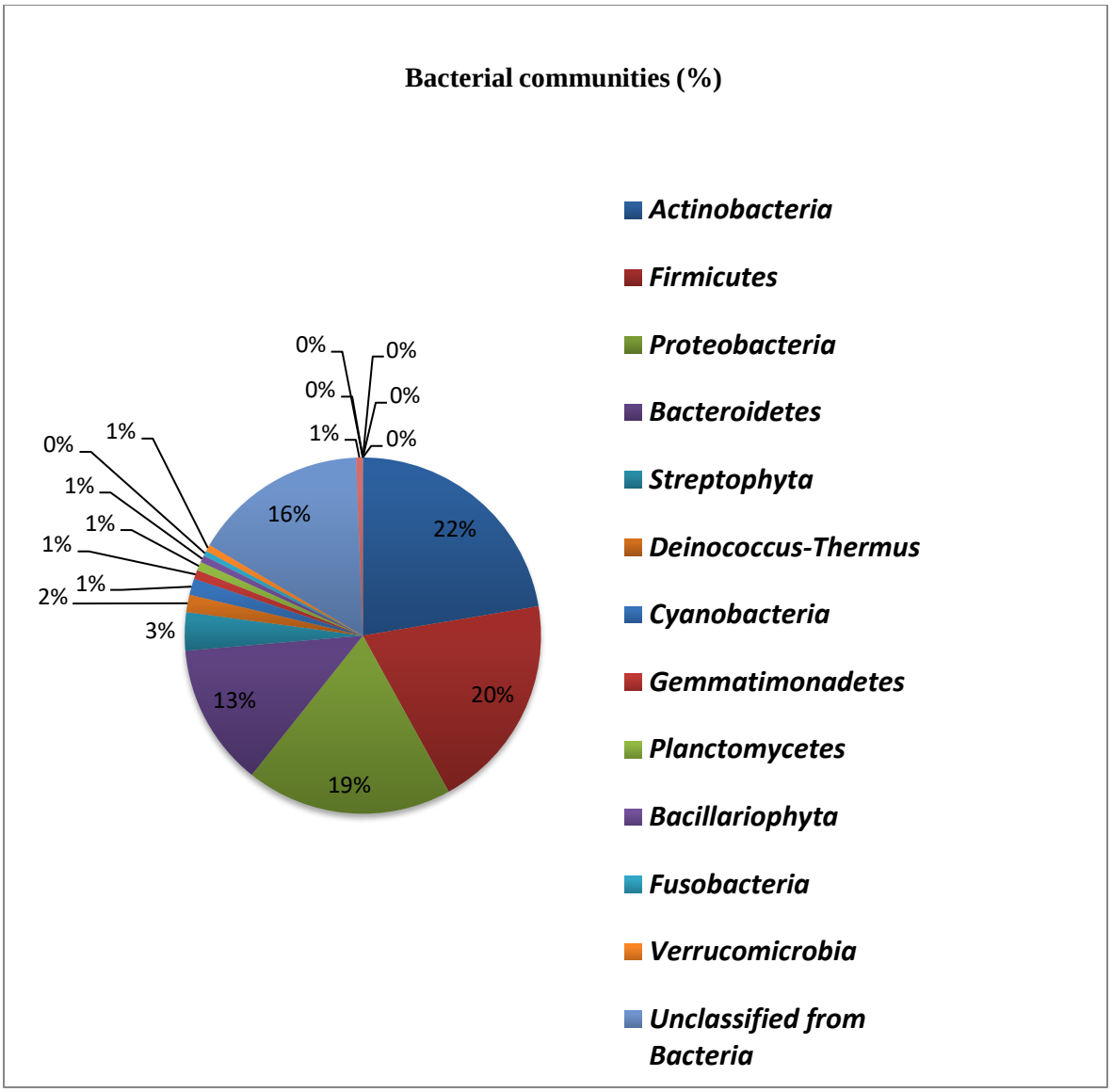


Figure 3:- Global bacterial communities (%) at the phylum level in the Soudano-sahelian dust events. Each color represents a Phylum. Results obtained with MG-RAST.

All 16S rDNA clone libraries were diverse and included sequences commonly found in marine and soil ecosystems. Phylogenetic analysis of partial rDNA revealed that sequences clustered with the following phyla: *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Cyanobacteria*. Sporiferous bacteria such as *Firmicutes* were frequently encountered in all clone libraries containing representatives from Mali in West Africa, a known source region for dust storms **(Paraskevi, Manolis et al. 2008).**

The ambient bacterial community composition differed from that found in dust storms. In these samples, the most abundant phyla were: *Proteobacteria*, *Firmicutes*, and *Actinobacteria. Actinobacteria* are highly abundant in arid and desert soils **(Gat, Mazar et al. 2017)**.Research on dustborne microorganisms have shown that the number of cultivable bacteria and fungi in the atmosphere significantly increases when African dust storms occur. It has been reported that the proportion of dustborne microorganisms are dominated by bacteria rather than fungi in samples taken in the US Virgin Islands and Bamako. Species belonging to the *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and other genera were identified. A systematic study linking dustborne microorganisms and soil microbial communities in dust source regions could lead to a better understanding of microbial ecology related to the global movement of dust particles **(Kenzaka, Sueyoshi et al. 2010).**

In addition to the desert microbiome, sandstorms carry large quantities of the airborne microbiota encountered along their intermediate path. By some estimate, a cubic meter of air contains hundreds of thousands of microorganisms **(Burrows, Elbert et al. 2009)**, with a diversity of taxa similar to that found in soil **(Franzetti, Gandolfi et al. 2011)**. The majority of these microbes originate from local sources, that is, soil, aquatic environments, plants, and anthropogenic pollution **(Maron 2005, Brodie 2007)**. Some of the more dominant bacterial taxa in air include *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Cyanobacteria* **(Burrows, Elbert et al. 2009, Lee, Park et al. 2017)**.Owing to the small cell size and ability to form spores of *Actinobacteria*, they might be expected to be dispersed with rain and dust clouds. Newton and colleagues suggested that the aerial dispersal cloud explain their ubiquitous occurrence in ecosystems **(Peter, Hortnagl et al. 2014).** In addition, Yamaguchi et al. (2012) showed that the phylum *Actinobacteria* was largely present in the Asian dust event samples as well as in several source soils of Asian dust, including the Gobi desert and the Taklamakan desert.

Owing to the small cell size and ability to form spores of*Actinobacteria* **(Warneckeet al., 2005)**, they might be expected to be effectively dispersed with rain and dustclouds. Newton et al. (2011) suggested thaaerial dispersal could explain their ubiquitous occurrencein lake ecosystems.

Characterization of bacterial community at the genus level:
We characterized the bacterial community at the genus level in the collected samples. For the analysis, we classified the MG-RAST data at the genus level and confirmed that a total of 52 genera were present. During the dust events, the genus *Bacillus* whichbelong to phylum *Firmicutes* was predominantly present at the proportion of 16%, and genera *Pseudomonas*, *Nocardiodes*, *Exiguobacterium*, *Deinococcus*, and *Clostridium* and *Hymenobacter* were also present (Fig. 4). This figure shows the abundance of bacterial community (%) at genus level for all samples.

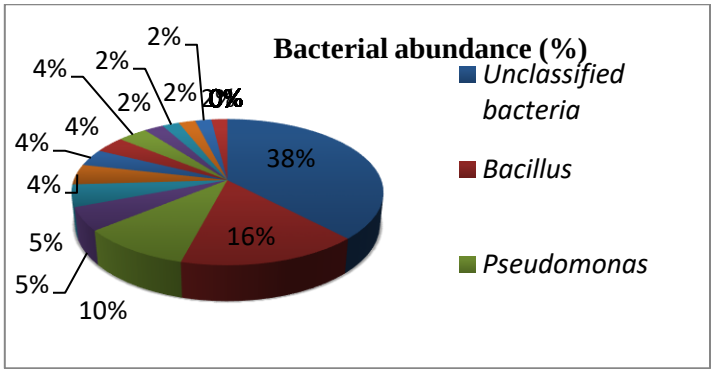
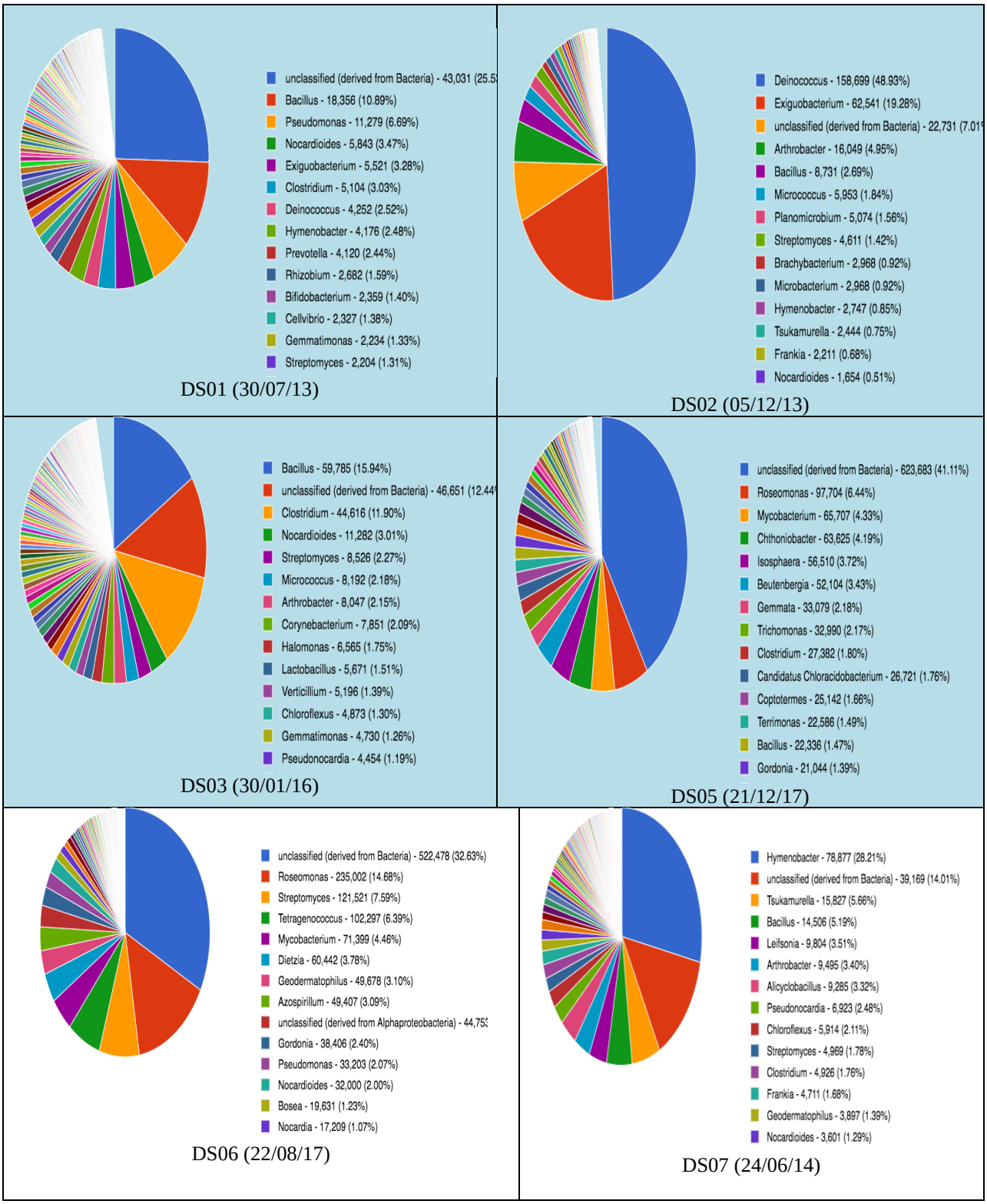


Figure 4: Bacterial abundance (%) at the genus level in all dust event samples. Each color represents a genus. Results obtained with MG-RAST

A preliminary study has demonstrated that the structure and species composition of microbial communities associated with episodic dust transport from the Sahara Desert varied significantly depending onperiods of transport. Even for dust transport events occurring within one month from each other, the dominant phylotypes and candidates for colonization were different **(Chuvochina, Alekhina et al. 2011).**

Spore-forming Bacilli of terrestrial origin have also been detected in the upper atmosphere, and different *Bacillus* taxa were retrieved mainly in dust-influenced samples **(Peter et al., 2014).**

The majority of the bacteria identified were found to be Gram positive (96%), and many are spore-formers. Bacterial genus represented in our samples contains individuals typically found in soil, the marine environment, and on human skin. *Bacillus sp*., which are prevalent in soil and capable of forming spores to survive aerial transport, constitute 16% of the bacterial isolates identified. This result is consistent with the Barbados microbial data in which most of the bacteria isolated were *Bacillus sp*. **(Kellogg, Griffin et al. 2004).**


unclassified (derived from Bacteria) - 43,031 (25.5
Bacillus - 18,356 (10.89%)
Pseudomonas - 11,279 (6.69%)
Nocardioides - 5,843 (3.47%)
Exiguobacterium - 5,521 (3.28%)
Clostridium - 5,104 (3.03%)
Deinococcus - 4,252 (2.52%)
Hymenobacter - 4,176 (2.48%)
Prevotella - 4,120 (2.44%)
Rhizobium - 2,682 (1.59%)
Bifidobacterium - 2,359 (1.40%)
Cellvibrio - 2,327 (1.38%)
Gemmatimonas - 2,234 (1.33%)
Streptomyces - 2,204 (1.31%)
DS01 (30/07/13)
Deinococcus - 158,699 (48.93%)
Exiguobacterium - 62,541 (19.28%)
unclassified (derived from Bacteria) - 22,731 (7.01
Arthrobacter - 16,049 (4.95%)
Bacillus - 8,731 (2.69%)
Micrococcus - 5,953 (1.84%)
Planomicrobium - 5,074 (1.56%)
Streptomyces - 4,611 (1.42%)
Brachybacterium - 2,968 (0.92%)
Microbacterium - 2,968 (0.92%)
Hymenobacter - 2,747 (0.85%)
Tsukamurella - 2,444 (0.75%)
Frankia - 2,211 (0.68%)
Nocardioides - 1,654 (0.51%)
DS02 (05/12/13)
Bacillus - 59,785 (15.94%)
unclassified (derived from Bacteria) - 46,651 (12.44
Clostridium - 44,616 (11.90%)
Nocardioides - 11,282 (3.01%)
Streptomyces - 8,526 (2.27%)
Micrococcus - 8,192 (2.18%)
Arthrobacter - 8,047 (2.15%)
Corynebacterium - 7,851 (2.09%)
Halomonas - 6,565 (1.75%)
Lactobacillus - 5,671 (1.51%)
Verticillium - 5,196 (1.39%)
Chloroflexus - 4,873 (1.30%)
Gemmatimonas - 4,730 (1.26%)
Pseudonocardia - 4,454 (1.19%)
DS03 (30/01/16)
unclassified (derived from Bacteria) - 623,683 (41.11%)
Roseomonas - 97,704 (6.44%)
Mycobacterium - 65,707 (4.33%)
Chthoniobacter - 63,625 (4.19%)
Isosphaera - 56,510 (3.72%)
Beutenbergia - 52,104 (3.43%)
Gemmata - 33,079 (2.18%)
Trichomonas - 32,990 (2.17%)
Clostridium - 27,382 (1.80%)
Candidatus Chloracidobacterium - 26,721 (1.76%)
Coptotermes - 25,142 (1.66%)
Terrimonas - 22,586 (1.49%)
Bacillus - 22,336 (1.47%)
Gordonia - 21,044 (1.39%)
DS05 (21/12/17)
unclassified (derived from Bacteria) - 522,478 (32.63%)
Roseomonas - 235,002 (14.68%)
Streptomyces - 121,521 (7.59%)
Tetragenococcus - 102,297 (6.39%)
Mycobacterium - 71,399 (4.46%)
Dietzia - 60,442 (3.78%)
Geodermatophilus - 49,678 (3.10%)
Azospirillum - 49,407 (3.09%)
unclassified (derived from Alphaproteobacteria) - 44,75
Gordonia - 38,406 (2.40%)
Pseudomonas - 33,203 (2.07%)
Nocardioides - 32,000 (2.00%)
Bosea - 19,631 (1.23%)
Nocardia - 17,209 (1.07%)
DS06 (22/08/17)
Hymenobacter - 78,877 (28.21%)
unclassified (derived from Bacteria) - 39,169 (14.01%)
Tsukamurella - 15,827 (5.66%)
Bacillus - 14,506 (5.19%)
Leifsonia - 9,804 (3.51%)
Arthrobacter - 9,495 (3.40%)
Alicyclobacillus - 9,285 (3.32%)
Pseudonocardia - 6,923 (2.48%)
Chloroflexus - 5,914 (2.11%)
Streptomyces - 4,969 (1.78%)
Clostridium - 4,926 (1.76%)
Frankia - 4,711 (1.68%)
Geodermatophilus - 3,897 (1.39%)
Nocardioides - 3,601 (1.29%)
DS07 (24/06/14)

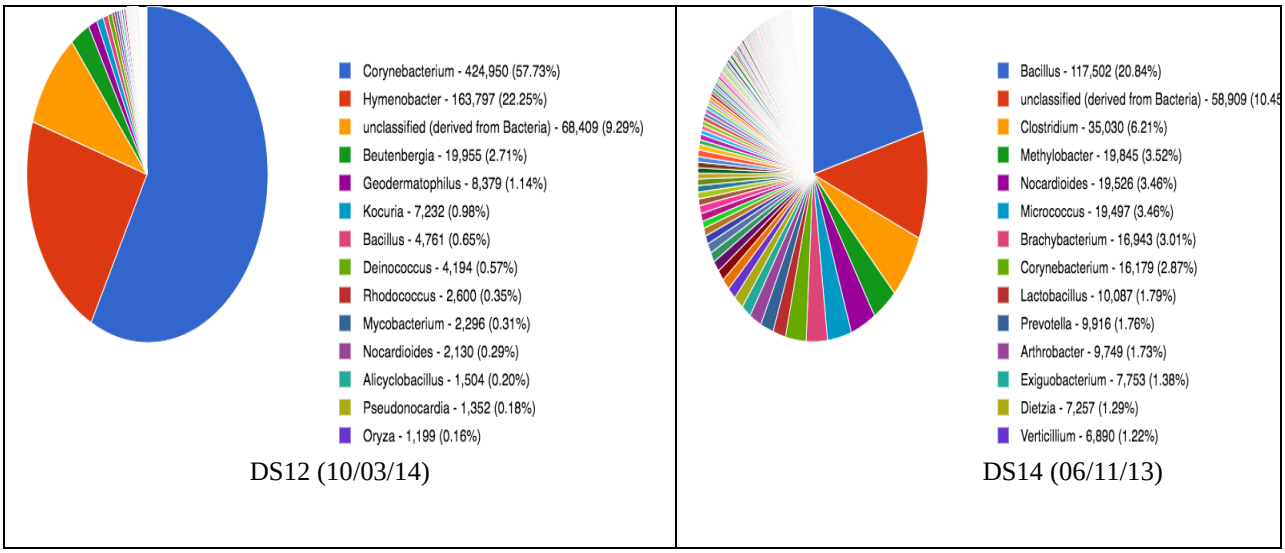


Figure 5: Bacteria abundance (%) at the genus level for each sample Each image illustrates the distribution of Genus for each sample. Results obtained with MG-RAST.

Unlike the phylum distribution (Fig. 3), we can observe a great variety of genus. For each sample, overall we have a different dominant genus. For the sample collected at 30/07/13, unclassified bacteria are predominant with 25.53%, followed by *Bacillus*, *Pseudomonas* and *Nocardiodes*.

Deinococcus belonging to *Deinococcus-Thermus* phylumdominate the sample collected at 05/12/13 with 48.93% followed by *Exiguobacterium*, *Arthrobacter* and *Bacillus*. The dominant genus identified in DS01, DS03 and DS14 samples is *Bacillus (Firmicutes* phylum) respectively with 10.89%, 15.94% and 20.84%. Genus *Roseomonas* (*Proteobacteria* phylum) is predominant in two samples (DS05 and DS06) with respectively 6.44% and 14.68%. *Corynebacterium* (*Actinobacteria* phylum) and *Hymenobacter* (*Bacteroidetes* phylum) are the most frequent genus in DS07 and DS12.

Bacteria in dust appeared to be dominated by Gram-positive microbes, as represented by the phylum *Firmicutes* (the majority of which are Gram-positive), as well as *Actinobacteria* (entirely composed of Gram-positive microbes) (Fig.3). Taxonomic identification at the genus levels (Fig.5) revealed that most of the *Firmicutes* dominating dust samples originate from the taxonomic class *Bacilli* **(Hanson, Zhou et al. 2016).**

These results reveal that genera *Bacillus* belonging to the phylum *Firmicutes* was a major component of the bacterial community transported with dust particles in South Korea during the December 2014 Asian dust event. In particular, this genus have been known to find arid or semi-arid regions, suggesting that the feature of habitat may contribute a relatively high proportion of the genera in the Asian dust samples **(Cha, Srinivasan et al. 2017).**

Similarly, analysis of airborne bacterial communities in the free troposphere at high altitude (500–3.000 m above ground level) over the Noto Peninsula in Japan during dust storms showed a high diversity of bacteria, dominated by natural-sand/terrestrial-associated taxa including endospore forming *Bacillus* members **(Hara 2015, Maki, Kurosaki et al. 2017)** isolated a number of UV tolerant endospore forming cultivable bacteria in air samples collected over the East China Sea during Asian dust events, demonstrating that dust storms carry viable bacteria **(Behzad, Gojobori et al. 2015).**

Desert microbiota has increased the abundance of genes involved in osmoregulation and dormancy, which likely contribute to their survival in hostile desert soil **(Fierer, Lauber et al. 2012).**

Microorganisms play significant roles in soil ecosystems, from their vital symbiotic interactions with plants, to their major roles in maintenance of biological soil crust, biodegradation of organic matters, and biogeochemical cycling of nutrients **(Schulz 2013).** Microbial diversity in soil is dependent on its pH, nutrient, and moisture contents. Some variations exist in the diversity of taxa that dwell in different deserts, with cold arctic deserts bearing lower

microbial diversity compared with hot deserts **(Fierer, Lauber et al. 2012).**

Some of the major bacterial taxa that frequently dwell in desert soil with high relative abundance include *Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes,* and*Cyanobacteria* **(Fierer, Lauber et al. 2012, An, Couteau et al. 2013).**

Dominant phyla found in soils, as determined by their prevalence in sequence libraries, include the ***Proteobacteria***, ***Acidobacteria***, and ***Actinobacteria***. Although this community composition is determined by a number of factors, including pH, temperature, elemental composition, and nutrient and moisture content, members of other phyla also occur less frequently **(Griffin 2007)**.

Human health may also be adversely affected by inhalation of viable microorganisms. Exposure to airborne microbes and biologically derived particulate matter can trigger allergic responses in humans.

In addition to the risk associated with exposure to airborne pathogens, exposure-response studies have shown that individuals exposed to non-pathogenic airborne microbes are at a higher risk of developing symptoms of disease than those who are not **(Kellogg, Griffin et al. 2004)**.

A primary concern with the dispersal of microorganisms via sandstorms is the potential impact on human health. A few studies have found correlations between DSM and disease outbreaks, but no major attempts were made to establish cause and effect relationships. The annual meningitis outbreaks in the Sahel region of Africa were linked to the occurrence of seasonal changes including high temperatures, dry air, and increased dust activities from African deserts **(Agier 2013, Jusot, Neill et al. 2017).**

Microbiota in airway samples from asthmatic children contained more frequently *Proteobacteria* than those from healthy controls. In contrast, *Bacterioidetes* were more common in controls than in asthmatics **(Hauptmann and Schaible 2016)**.Exposure to high concentrations of actinobacteria can cause allergic alveolitis.

Other respiratory disorders have also been reported, and although measured concentrations are low, indoor exposure is still a mixture of many different agents that may have synergistic effects.

In vitro and in vivo studies have shown that *Actinobacteria* are highly immunoactive and are therefore potentially responsible for respiratory and other disorders **(Naegele 2015)**. This next figure shows the abundance of species in the dust samples.

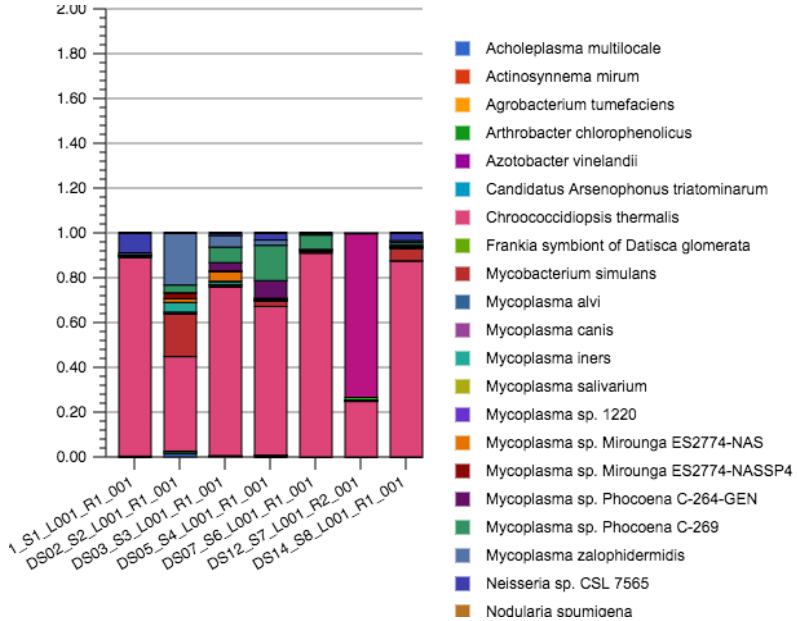


Figure 6: Abundance of species in dust events samples Each image illustrates the distribution of species for each sample. Results obtained with MG-RAST.

We can see that the most abundant species in all samples (Fig. 6) is *Chroococcidiopsis thermalis* (*Cyanobacteria* phylum) with a percentage of 91.63% during DS07 dust event. But *Azotobacter vinelandii* (*Proteobacteria* phylum) is predominant in DS12 dust event with 76% of sequences. *Neisseria sp*. CSL 7565 (*Proteobacteria* phylum) is found in DS01, DS02, DS03 and DS05 dust event while *Mycoplasma sp. Phocoena* C-269 (*Firmicutes* phylum) is almost in all samples excepted in DS01 et DS14 samples.

The high concentration of dust impacting the Caribbean may pose a significant public health threat, particularly as it pertains to respiratory disease. It is known that exposure to desert silica can result in the development of silicosis **(Griffin, Garrison et al. 2001)**.

The bacterial endotoxin LPS can also directly impact human health via inhalation. LPS is a cell wall component of Gram-negative bacteria (*Proteobacteria* Phylum) and is commonly found in organic dust. Human and animal inhalation trials have shown increases in neutrophils and lymphocytes with a reduction in alveolar macrophage phagocytosis. Short-term exposure can result in fever and reduced airflow. Long-term exposure can result in the development of lung diseases such as asthma, bronchitis, and irreversible airflow obstruction **(Griffin 2007).**

Dust storms are believed to promote infection via dust particles causing abrasions of the nasopharyngeal mucosa upon inhalation **(Molesworth, Cuevas et al. 2002).**

It is obvious from the few dust-borne microbiology studies that have identified pathogenic bacteria that there is some degree of human health risk associated with exposure to airborne desert dust. As in most outbreaks of disease, it is often the young, old, and immunocompromised who are within the higher levels of risk **(Griffin 2007).**

The occurrence of asthma and allergic diseases has increased in the past few decades and remains a major health issue for children. Asthma is a complex disease caused by the combined effects of many genetic factors interacting with environmental factors. Accumulating evidence suggests that exposure to environmental microbiota has a protective effect against childhood asthma **(Chiu 2017).** The diversity of microbial species, rather than exposure to one particular microbial component, is particularly protective. However, exposure to different types of bacteria may have different effects in allergic diseases. In this study, members of the phylum *Proteobacteria*, including the genera *Haemophilus, Neisseria*, and *Moraxella*, were found numerically to be more abundant in the airways of children with rhinitis than in the healthy controls. Clinically, these bacteria are the most common pathogens in acute and chronic sinusitis and otitis media, which are usually complicated by poorly controlled symptoms of allergic rhinitis **(Chiu 2017)**

Neisseria species have been identified to be abundant in asthmatics and related to the degree of eosinophilic inflammation. In this study, *Neisseria spp*.were found to be more abundant in children with rhinitis but not asthma without association with allergen sensitization **(Chiu 2017)**. Atypical bacterial infections from *Mycoplasma* have also been linked to chronic asthma and potential asthma exacerbations **(Newcomb and Peebles 2009).**

Allergens play an important role in the development of asthma and allergic rhinitis. Some studies have shown that the majority of the skin prick test positive patients are less than 10 years old. Rhinitis was the most common symptom in positive skin prick test persons whether associated or not with asthma and or conjunctivitis. The main contribution is that *Mycoplasma spp*. is frequently isolated in patients with allergic rhinitis without asthma **(Muñoz-Zurita, Paz Martínez et al. 2014).**

Allergic rhinitis is the most common type of chronic rhinitis, affecting 10% to 20% of the population, and evidence suggests that the prevalence of the disorder is increasing. Severe allergic rhinitis has been associated with significant impairments in quality of life, sleep and work performance. In this study we found that 36% of patients with allergic rhinitis were positive for *Mycoplasma spp* **(Muñoz-Zurita, Paz Martínez et al. 2014)**.

With an estimated number of 10-100 bacteria per 1,000 human cells, the lower respiratory tract is one of the least-populated surfaces of the human body. Similar to the intestine, the two predominant phyla detected in the airways are *Firmicutes* and *Bacteroidetes*, whereas *Actinobacteria, Proteobacteria*, and *Fusobacteria* are minor constituents of the local microbiota. The "core microbiota" of healthy individuals consists mainly of *Pseudomonas, Streptococcus, Prevotella, Fusobacteria, Veillonella*, *Haemophilus, Neisseria,* and *Porphyromonas* **(Marsland, Trompette et al. 2015).**

Recent research has made it evident that a variety of chronic lung disorders, including asthma, COPD, and cystic fibrosis, are strongly linked to a dysbiotic airway microbiota. This is usually the result of a loss in bacterial diversity due to the outgrowth of certain pathogenic bacteria. The airway microbiota of patients with chronic lung disorders presents a disease-specific phenotype. In contrast to healthy individuals, those with asthma or COPD demonstrate an overrepresentation of *Proteobacteria* (in particular *Haemophilus*, *Moraxella*, and *Neisseria spp*.) and *Firmicutes* (*Lactobacillus spp*.), whereas the proportion of *Bacteroidetes* (specifically, *Prevotella spp*.) is significantly decreased **(Sze, Gyurak et al. 2012).**

An outgrowth of pathogenic bacteria, mainly of the *Proteobacteria* phylum, can only be seen in patients with COPD infected with rhinovirus, which could explain their predisposition to secondary bacterial infections**(Marsland, Trompette et al. 2015)**.

Conclusion:

The objective of this study was to identify the bacteria present in wind and dust blowing during dust events in Dakar with a potential impact on human health. Thus, 19 phyla and 52 genera were isolated and the majority of them could cause respiratory diseases. Microbial populations transported by dust storms have the potential to affect ecosystems and public health. Genomic techniques confirm that microorganisms present in suspended dust are viable, and reveal high percentage of microorganisms can be identified and analyzed using metagenomic tools. It should be noted that several phyla found in the studies ofMarsland, Trompette et al. (2015) such *as Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria* and*Cyanobacteria*are identified in our study. These bacteria are potentially responsible for respiratory infections. However, many demanding fungi as well as all viruses, could be detected with these genomic methods. The continuation of this study will focus on the use of direct DNA extraction techniques in order to access the remaining microorganisms (fungi and viruses) present in African dust events.

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