

Supporting Information

The Microbiome of Size-Fractionated Airborne Particles from the Sahara Region

Rebecca A. Stern^a, Nagissa Mahmoudi^b, Caroline O. Buckee^c, Amina T. Schartup^{d,e}, Petros Koutrakis^d, Stephen T. Ferguson^d, Jack Mikhail Wolfson^d, Steven C. Wofsy^f, Bruce C. Daube^f, Elsie M. Sunderland^{a,d}

^aHarvard John A. Paulson School of Engineering and Applied Science, Harvard University, Cambridge, MA, USA 02138

^bDepartment of Earth and Planetary Sciences, McGill University, Montreal, QC, H3A 0E8

^cCenter for Communicable Disease Dynamics, Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA 02115

^dDepartment of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, 02115

^eScripps Institution of Oceanography, La Jolla, CA 92037

^fDepartment of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138

Number of pages: 16

Number of figures: 4

Number of tables: 5

Table of Contents

| | |
|---|-----|
| Table S1. Average meteorological conditions..... | S3 |
| Table S2. Samples tested for species with known pathogenic strains using PCR..... | S4 |
| Table S3. Primer set sequences for PCR of potential pathogens..... | S5 |
| Figure S1. Rarefaction curves of operational taxonomic units (OTUs)..... | S6 |
| Table S4. Taxonomic assignment for OTUs that differed significantly across sizes..... | S7 |
| Additional Text: Difference in bacterial diversity across particle sizes..... | S8 |
| Figure S2. Community analysis using PCoA..... | S9 |
| Table S6. Taxa with features for survival in harsh atmospheric conditions..... | S10 |
| Figure S3. Wind direction, wind speed, and HYSPLIT trajectories for sampling day 1..... | S11 |
| Figure S4. Wind rose plots of instantaneous wind direction and velocity..... | S12 |

Table S1. Average meteorological conditions on sampling days at the collection site in Bamako, Mali (Latitude 12.332 N, Longitude -7.578 W, 389 m above sea level).

| Date | Sampling Day | Wind speed (m/s) | Relative humidity (%) | Majority wind direction^a | Air temperature (°C) | Barometric pressure (mm Hg) |
|---------------|---------------------|-------------------------|------------------------------|--|-----------------------------|------------------------------------|
| Feb. 11, 2018 | 1 | 1.51 | 6.1 | NNE | 30.3 | 29.87 |
| Feb. 13, 2018 | 2 | 1.23 | 6.5 | NNE | 33.5 | 29.86 |
| Feb. 14, 2018 | 3 | 0.99 | 4.8 | NE | 35.1 | 29.84 |
| Feb. 15, 2018 | 4 | 1.41 | 2.6 | NNE | 36.0 | 29.87 |
| Feb. 16, 2018 | 5 | 1.41 | 2.0 | ENE | 37.1 | 29.89 |
| Feb. 17, 2018 | 6 | 1.26 | 4.2 | NNE | 37.3 | 29.83 |
| Feb. 18, 2018 | 7 | 1.25 | 4.0 | NNE | 39.1 | 29.78 |
| Feb. 19, 2018 | 8 | 1.14 | 10.3 | NE | 38.2 | 29.75 |
| Feb. 20, 2018 | 9 | 1.58 | 9.4 | NNE | 33.0 | 29.79 |

^a Wind direction is expressed as majority wind direction, calculated by categorizing the instantaneous wind velocity into cardinal wind directions and taking the majority from each sampling day.

Table S2. Samples tested for species with known pathogenic strains using PCR.

| Pathogen target | Sample Tested | Particle Size (μm) | + / - |
|-----------------------------------|----------------------|---|--------------|
| <i>Bacillus cereus</i> | D2.C.F | <0.5 | + |
| | D3.C.F | <0.5 | + |
| | D7.C.P1 | 2.5-1.0 | - |
| <i>Escherichia coli</i> | D3.C.F | <0.5 | + |
| | D3.C.P1 | 2.5-1.0 | - |
| | D9.C.P0.5 | 1.0-0.5 | + |
| <i>Fusobacterium nucleatum</i> | D6.C.P1 | 2.5-1.0 | + |
| | D3.C.P0.5 | 1.0-0.5 | - |
| | D9.C.F | <0.5 | - |
| | D4.C.P2.5 | 10.0-2.5 | + |
| <i>Streptococcus pneumoniae</i> | D3.C.P0.5 | 1.0-0.5 | - |
| <i>Staphylococcus epidermidis</i> | D9.C.P2.5 | 10.0-2.5 | - |
| | D8.C.P1 | 2.5-1.0 | - |
| | D5.C.P0.5 | 1.0-0.5 | - |
| | D4.C.P1 | 2.5-1.0 | - |
| <i>Pseudomonas aeruginosa</i> | D3.C.P2.5 | 10.0-2.5 | + |

Table S3. Primer set sequences for PCR of potential pathogens.

| <i>Species</i> | <i>Primer sequence</i> |
|-----------------------------------|--|
| <i>Escherichia coli</i> | EcoliuidA_FPrimer: CGGAAGCAACGCGTAAACTC EcoliuidA_RPrimer: TGAGCGTCGCAGAACATTACA EcoliuidA_Probe: /56-FAM/CGCGTCCGATCACCTGCGTC/3BHQ_1/ |
| <i>Pseudomonas aeruginosa</i> | pseuF: ACTTTAAGTTGGGAGGAAGGG pseuR: ACACAGGAAATTCCACCACCC pseuProbe: Fam-ACAGAATAAGCACCGGCTAAC-BHQ |
| <i>Streptococcus pneumoniae</i> | Sp-lytAF: ACG CAA TCT AGC AGA TGA AGC A Sp-lytAR: TCG TGC GTT TTA ATT CCA GCT Sp-lytAP: /56-FAM/CCGAAAACGCTTGATACAGGGAG/3BHQ_1/ |
| <i>Staphylococcus epidermidis</i> | StaphepiF: ACTGGTTACCCTGGTGACAAACCA StaphEpiR: ACTGGAGATCCAGAGTTTCCACCT staphepiProbe: /56-FAM/AGCCACAATGTGGGAAAGTGTAGGT/3BHQ_1/ |
| <i>Bacillus cereus</i> | bacCereusF: CTGTAGCGAATCGTACGTATC bacCereusR: TACTGCTCCAGCCACATTAC bacCereusP: /56-FAM/GGAGCTGTACAACCTTGCCA/3BHQ_1/ |
| <i>Fusobacterium nucleatum</i> | FnucleatF-R-P: proprietary |

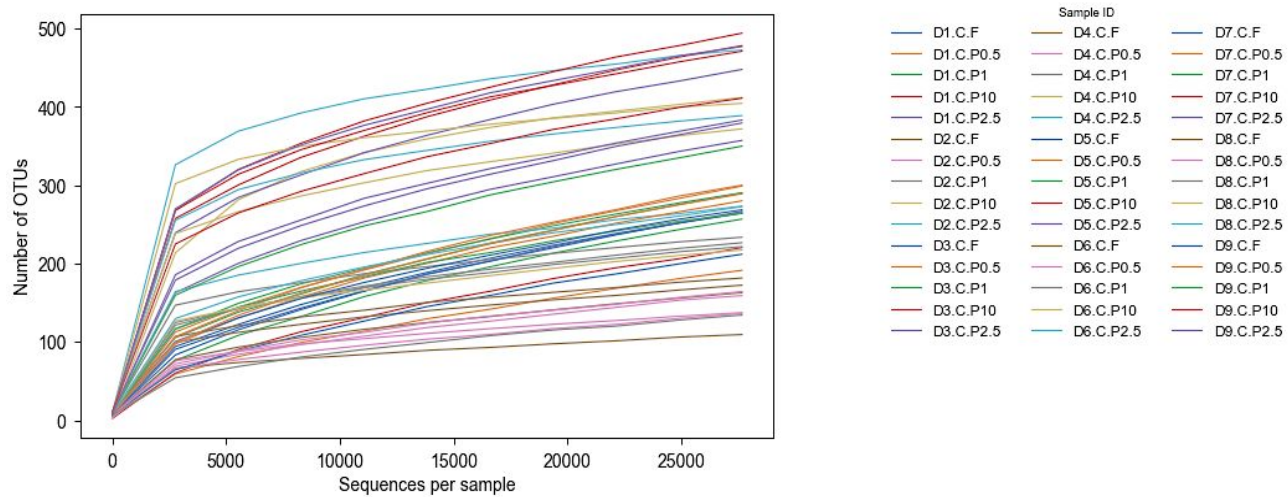


Figure S1. Rarefaction curves of operational taxonomic units (OTUs) clustered at 99% sequence identity across all samples.

Table S4. Taxonomic assignment for OTUs that differed significantly across the various particle sizes and the particle size fraction with greatest abundance.

| Taxonomic Assignment | Particle size preference (µm) |
|--|-------------------------------|
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Geodermatophilaceae; g__Geodermatophilus; s__obscurus | >10.0 |
| p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Aerococcaceae; g__; s__ | 10.0-2.5 |
| p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__ | >10.0 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Intrasporangiaceae | 10.0-2.5 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Intrasporangiaceae | 10.0-2.5 |
| p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__ | >10.0 |
| p__Firmicutes; c__Bacilli; o__Bacillales; f__Planococcaceae | 2.5-1.0 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Corynebacteriaceae; g__Corynebacterium; s__ | 2.5-1.0 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Geodermatophilaceae; g__Geodermatophilus; s__obscurus | >10.0 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardiodaceae; g__; s__ | 10.0-2.5 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Corynebacteriaceae; g__Corynebacterium; s__ | 2.5-1.0 |
| p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae; g__Balneimonas; s__ | 10.0-2.5 |
| p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Moraxellaceae; g__Psychrobacter; s__pulmonis | >10.0 |
| p__Actinobacteria; c__Actinobacteria; o__Actinomycetales | >10.0 |

Additional Text: Difference in bacterial diversity across particle sizes

Additional details on genera detected and corresponding potential sources are presented in Additional Text Table 1. At the genus level, *Geodermatophilus*, a soil-associated genus, was more abundant in the largest (>10 µm) size fraction (Kruskal-Wallis test, FDR $p < 0.05$). Prior work found strains of *Geodermatophilus* in 1-2 mm sand from the Saharan desert,¹ consistent with detection in this study preferentially on the largest particle sizes. The *Ruminococcaceae* family exhibited a preference for the largest particle size fraction (Kruskal-Wallis, FDR $p < 0.05$). This family was previously identified as an indicator for bovine fecal contamination.² The family *Dermatophilaceae*, which are common on animal and human skin, were most abundant in the largest two size fractions. Our sampling location was 11 km downwind of a cattle market, which could be a source of large, locally generated particles with distinct microbial composition.

One taxonomic class (Thermomicrobia) out of the 62 classes detected statistically differed among the five atmospheric particle sizes (Kruskal-Wallis test, FDR $p < 0.05$), with greatest abundance in the particle size range of 10.0-2.5 µm. This class has been found in a wide range of soil types, typically isolated from human activity.³ Thermomicrobia were previously found in the desert soil of the Sahara⁴, indicating the potential for local sources with larger particles that did not yet fall out due to gravitational settling.

Additional Text Table 1. Genera detected in the samples and corresponding potential sources.

| Soil | Skin | Stool | Compost | Wastewater Treatment | Land Application Biosolids |
|-----------------------|--------------------------|------------------------------|--------------------------|----------------------|----------------------------|
| <i>Bradyrhizobium</i> | <i>Propionibacterium</i> | <i>Bacteroides</i> | <i>Saccharopolyspora</i> | <i>Arcobacter</i> | <i>Clostridium</i> |
| <i>Mesorhizobium</i> | <i>Staphylococcus</i> | <i>Faecalibacterium</i> | | | |
| | <i>Corynebacterium</i> | <i>Oscillospira</i> | | | |
| | <i>Streptococcus</i> | <i>Roseburia</i> | | | |
| | <i>Rothia</i> | <i>Coprococcus</i> | | | |
| | <i>Micrococcus</i> | <i>Ruminococcus</i> | | | |
| | <i>Anaerococcus</i> | <i>Parabacteroides</i> | | | |
| | <i>Brevibacterium</i> | <i>Phascolarctobacterium</i> | | | |
| | | <i>Sutterella</i> | | | |
| | | <i>Blautia</i> | | | |

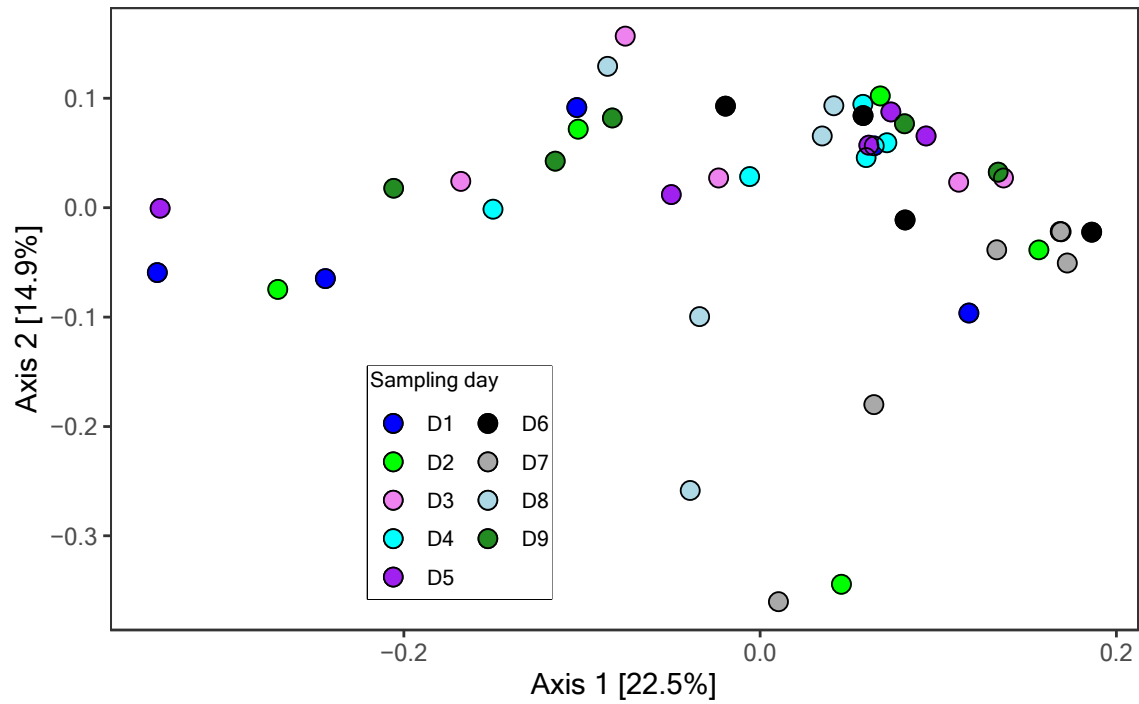


Figure S2. Community analysis using pairwise, weighted UniFrac distances visualized on a principal coordinates analysis (PCoA) plot with the percent of variation explained by each axis noted in brackets. Samples were grouped by sampling day.

Table S6. Taxa detected with features for survival in harsh atmospheric conditions.

| Taxa detected in the samples | Relevant trait |
|---|---|
| Burkholderiales, Pseudomonadales, Flavobacteriales | Common in outdoor air ⁵ |
| Bacillaceae | Forms endospores |
| Gemmatimonadetes, Thermus, Chloroflexi, <i>Psychrobacter</i> , Myxococcales | Commonly found in extreme environments |
| Gemmatomonadetes, <i>Deinococcus</i> | Associated with hyper-arid environments; bioindicators for Saharan dust events ⁶⁻⁸ |
| Gemmatomonadetes | UV protection during aerial transport due to carotenoid pigmentation ^{9,10} |
| <i>Methylobacterium</i> , <i>Rubrobacter</i> | Possess structures to resist environmental stresses ^{6,11} |
| <i>Arthrobacter</i> , <i>Methylobacterium</i> | Dessication-resistant ⁶ |
| <i>Bacillus</i> , <i>Kocuria</i> , <i>Micrococcus</i> | Detected in culture-based air samples in Mali ¹² |
| Bacteroidetes | Preference for desert soils |
| <i>Cytophagaceae</i> (<i>Hymenobacter</i>), <i>Flavobacteriaceae</i> | Pigmented and psychrotolerant; previously found in Saharan dust ¹³ |
| <i>Nocardioides</i> , <i>Sporichthya</i> , <i>Beijerinckiaceae</i> , <i>Hyphomicrobiaceae</i> , <i>Acetobacteraceae</i> , <i>Skermanella</i> , <i>Rhodocyclaceae</i> , <i>Rhodospirillaceae</i> , <i>Sphingomonadaceae</i> | Motile spores |
| <i>Mesorhizobium</i> | Motile by symbiosis with plant roots |
| <i>Patulibacter</i> , <i>Rhodobacteraceae</i> , <i>Modestobacter</i> | Psychrotolerance aids survival in Sahara at night ⁶ |
| <i>Rubrobacteraceae</i> , <i>Streptosporangiaceae</i> , <i>Pseudonocardia</i> , <i>Rubellimicrobium</i> , <i>Streptomyces</i> | Heat tolerant and thermophilic ^{6,14} |
| <i>Rubrobacter</i> , <i>Hymenobacter</i> , <i>Methylobacterium</i> | Gamma radiation-resistant ^{6,14} |
| <i>Bacillus</i> , <i>Paenibacillus</i> , <i>Arthrobacter</i> , <i>Cellulomonas</i> , <i>Janthinobacterium</i> , <i>Modestobacter</i> , <i>Pseudomonas</i> , <i>Sphingomonas</i> | UV resistant ¹⁵ |
| <i>Nocardioides</i> | Halophilic |
| <i>Bacillaceae</i> , <i>Paenibacillaceae</i> , <i>Bacillus</i> | Spore-forming |
| <i>Geodermatophilaceae</i> , <i>Pseudonocardiaceae</i> , <i>Rhodocyclaceae</i> , <i>Rubrobacteraceae</i> | oligotrophic |
| <i>Frankia</i> , <i>Beijerinckiaceae</i> , <i>Bradyrhizobiaceae</i> , <i>Rhizobium</i> , <i>Rhizobiaceae</i> , <i>Mesorhizobium</i> , <i>Azospirillum</i> , <i>Rhodospirillaceae</i> , <i>Frankia</i> , <i>Oxalobacteraceae</i> | Nitrogen-fixing |
| <i>Methylobacterium</i> | convert nitrogen gas to ammonia and feed on methanol |
| <i>Hyphomicrobiaceae</i> | phototrophic |
| <i>Rhodobacteraceae</i> , <i>Rhodocyclaceae</i> , <i>Rhodospirillaceae</i> , <i>Sphingomonas</i> (aerobic) | Photoheterotrophic |
| <i>Cellulosimicrobium</i> , <i>Actinobacteria</i> | biodegrade cellulose or lignin |
| <i>Cytophagaceae</i> | Capable of degrading plant material |
| <i>Clostridiaceae</i> , <i>Fusobacteriaceae</i> , <i>Lactobacillaceae</i> , <i>Oxalobacteraceae</i> , <i>Rhodospirillaceae</i> | Strict anaerobes |
| <i>Enterobacteriaceae</i> , <i>Myxococcaceae</i> , <i>Rhodospirillaceae</i> | Facultative anaerobes |
| Methanobacteriales | thermophilic anaerobic methanogens |

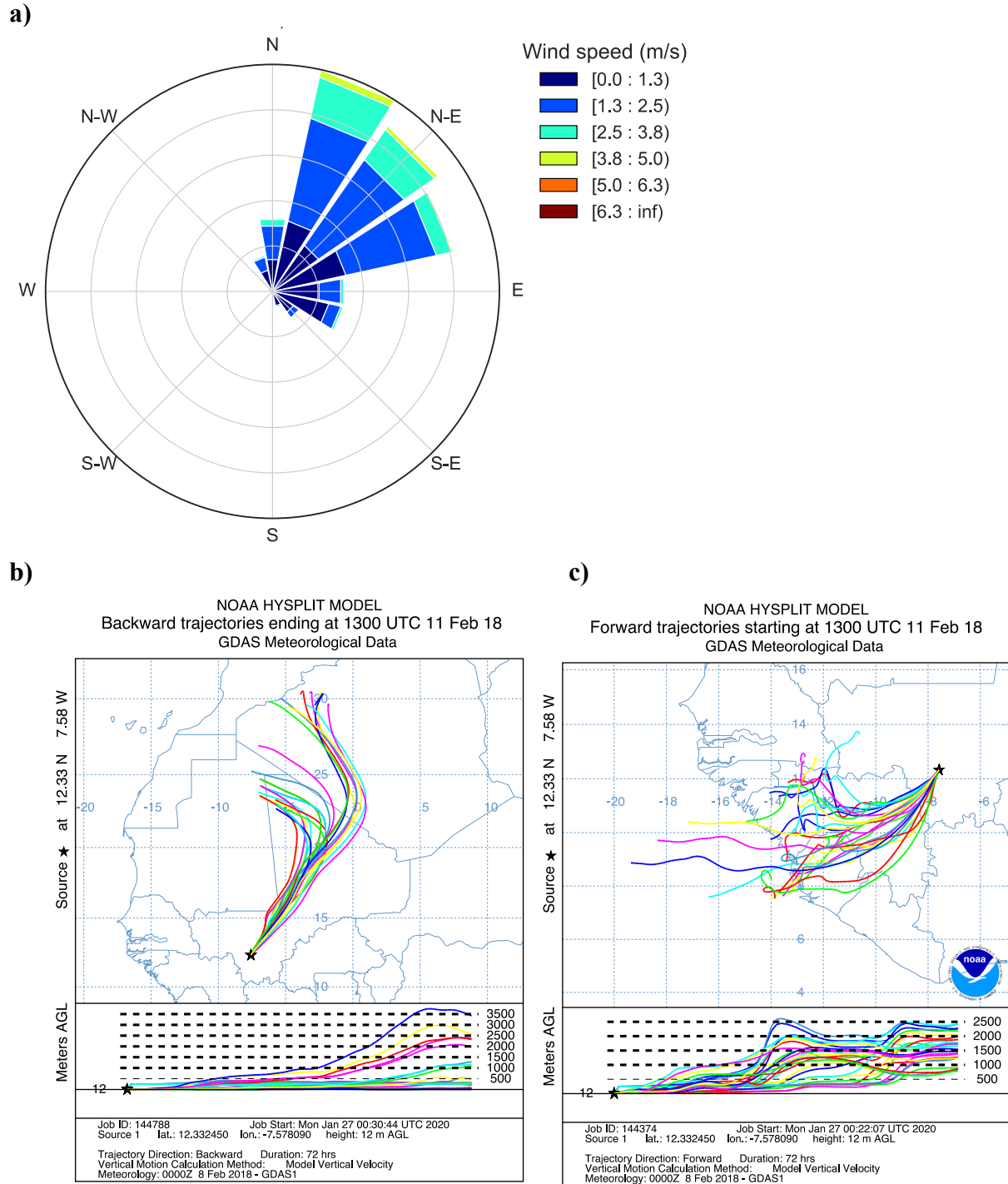


Figure S3. (a) Wind direction and wind speed for sampling day 1 (Feb. 11, 2018); (b) HYSPLIT 72-hour backward trajectories from the sampling site for sampling day 1; HYSPLIT 72-hour forward trajectories from the sampling site for sampling day 1. All other sampling days exhibited wind patterns and trajectories similar to sampling day 1 (Figure S4).

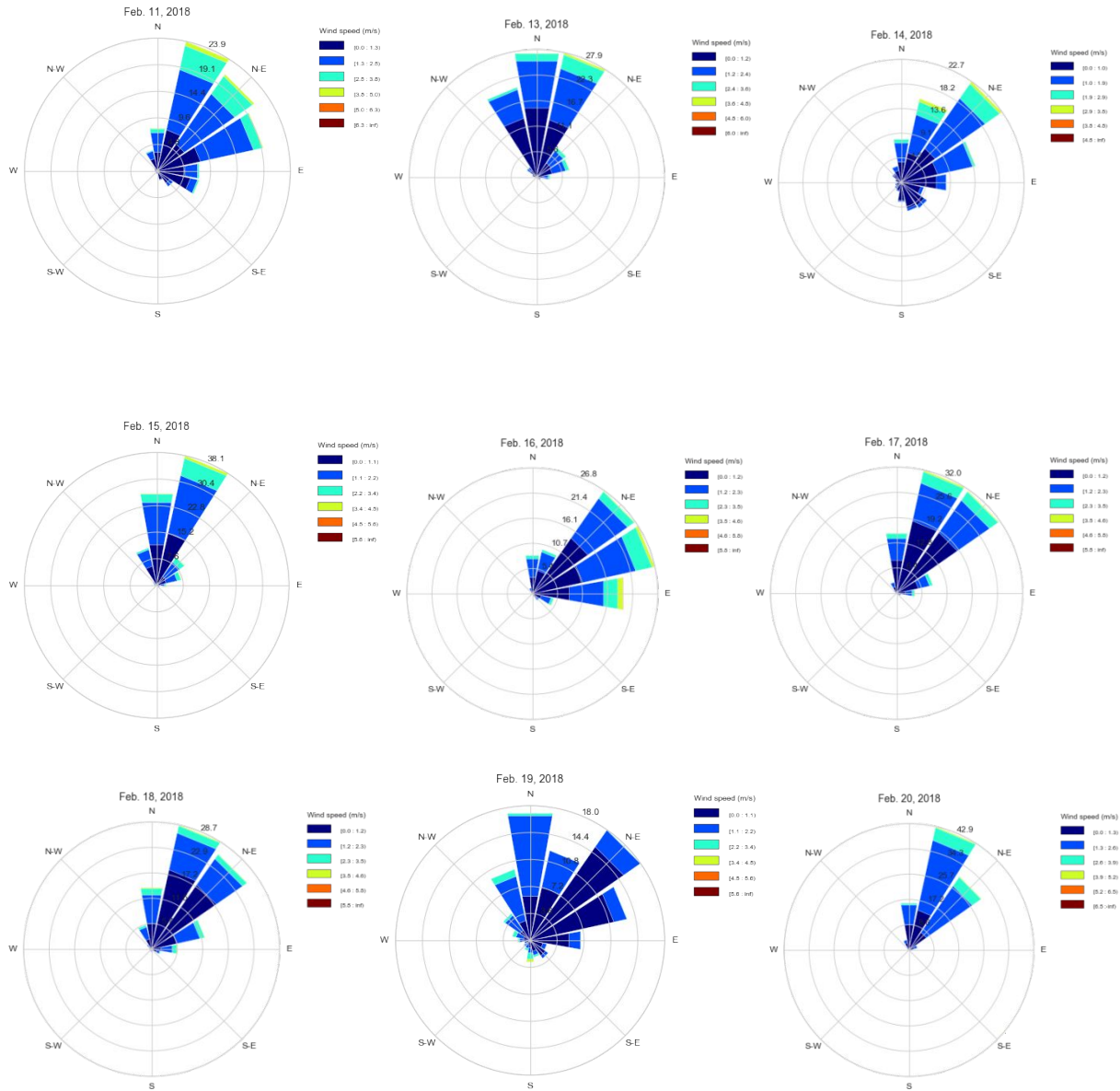


Figure S4. Wind rose plots of instantaneous wind direction and velocity (m s^{-1}) during each of the sampling days reveal the winds were predominantly from the northeast.

References

- (1) del Carmen Montero-Calasanz, M.; Göker, M.; Pötter, G.; Rohde, M.; Spröer, C.; Schumann, P.; Klenk, H. P. Geodermatophilus Africanus Sp. Nov., a Halotolerant Actinomycete Isolated from Saharan Desert Sand. *Antonie Van Leeuwenhoek* **2013**, *104* (2), 207–216.
- (2) Bowers, R. M.; Clements, N.; Emerson, J. B.; Wiedinmyer, C.; Hannigan, M. P.; Fierer, N. Seasonal Variability in Bacterial and Fungal Diversity of the Near-Surface Atmosphere. *Environ. Sci. Technol.* **2013**, *47* (21), 12097–12106.
<https://doi.org/10.1021/es402970s>.
- (3) Houghton, K.; Morgan, X.; Lagutin, K.; MacKenzie, A.; Vyssotskii, M.; Mitchell, K.; McDonald, I.; Morgan, H.; Power, J.; Moreau, J.; Hanssen, E.; Stott, M. Thermorudis Pharmacophila Sp. Nov., a Novel Member of the Class Thermomicrobia Isolated from Geothermal Soil, and Emended Descriptions of Thermomicrobium Roseum, Thermomicrobium Carboxidum, Thermorudis Peleae and Sphaerobacter Thermophil. *Int. J. Syst. Evol. Microbiol.* **2015**, *65* (12), 4479–87.
- (4) A. Belov, A.; S. Cheptsov, V.; A. Vorobyova, E. Soil Bacterial Communities of Sahara and Gibson Deserts: Physiological and Taxonomical Characteristics. *AIMS Microbiol.* **2018**, *4* (4), 685–710. <https://doi.org/10.3934/microbiol.2018.4.685>.
- (5) Prussin, A. J.; Marr, L. C. Sources of Airborne Microorganisms in the Built Environment. *Microbiome* **2015**, *3*, 78. <https://doi.org/10.1186/s40168-015-0144-z>.
- (6) Favet, J.; Lapanje, A.; Giongo, A.; Kennedy, S.; Aung, Y. Y.; Cattaneo, A.; Davis-Richardson, A. G.; Brown, C. T.; Kort, R.; Brumsack, H. J.; Schnetger, B.; Chappell, A.; Kroijenga, J.; Beck, A.; Schwibbert, K.; Mohamed, A. H.; Kirchner, T.; De Quadros, P.

- D.; Triplett, E. W.; Broughton, W. J.; Gorbushina, A. A. Microbial Hitchhikers on Intercontinental Dust: Catching a Lift in Chad. *ISME J.* **2013**, *7* (4), 850–867.
<https://doi.org/10.1038/ismej.2012.152>.
- (7) Meola, M.; Lazzaro, A.; Zeyer, J. Bacterial Composition and Survival on Sahara Dust Particles Transported to the European Alps. *Front. Microbiol.* **2015**, *6* (DEC), 1–17.
<https://doi.org/10.3389/fmicb.2015.01454>.
- (8) Chuvochina, M. S.; Alekhina, I. A.; Normand, P.; Petit, J. R.; Bulat, S. A. Three Events of Saharan Dust Deposition on the Mont Blanc Glacier Associated with Different Snow-Colonizing Bacterial Phylotypes. *Microbiology* **2011**, *80*, 125–131.
- (9) Tong, Y. Y.; Lighthart, B. Solar Radiation Is Shown to Select for Pigmented Bacteria in the Ambient Outdoor Atmosphere. *Photochem. Photobiol.* **1997**, *65*, 103–106.
- (10) Hanada, S.; Sekiguchi, Y. The Phylum Gemmatimonadetes. In *The Prokaryotes*; Rosenberg, E., DeLong, E., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Berlin, 2014; pp 677–681.
- (11) Griffin, D. W. Atmospheric Movement of Microorganisms in Clouds of Desert Dust and Implications for Human Health. *Clin. Microbiol. Rev.* **2007**, *20* (3), 459–477.
<https://doi.org/10.1128/CMR.00039-06>.
- (12) Kellogg, C. A.; Griffin, D. W.; Garrison, V. H.; Peak, K. K.; Royall, N.; Smith, R. R.; Shinn, E. A. Characterization of Aerosolized Bacteria and Fungi from Desert Dust Events in Mali, West Africa. *Aerobiologia (Bologna)*. **2004**, *20* (2), 99–110.
<https://doi.org/10.1023/B:AERO.0000032947.88335.bb>.
- (13) Fierer, N.; Leff, J. W.; Adams, B. J.; Nielsen, U. N.; Bates, S. T.; Lauber, C. L. Cross-Biome Metagenomic Analyses of Soil Microbial Communities and Their Functional

- Attributes. *Proc. Natl. Acad. Sci.* **2012**, *109*, 21390–21395.
- (14) Giongo, A.; Favet, J.; Lapanje, A.; Gano, K. A.; Kennedy, S.; Davis-Richardson, A. G.; Brown, C.; Beck, A.; Farmerie, W. G.; Cattaneo, A.; Crabb, D. B.; Aung, Y. Y.; Kort, R.; Brumsack, H. J.; Schnetger, B.; Broughton, W. J.; Gorbushina, A. A.; Triplett, E. W. Microbial Hitchhikers on Intercontinental Dust: High-Throughput Sequencing to Catalogue Microbes in Small Sand Samples. *Aerobiologia (Bologna)*. **2013**, *29* (1), 71–84. <https://doi.org/10.1007/s10453-012-9264-0>.
- (15) Toepfer, I.; Favet, J.; Schulte, A.; Schmölling, M.; Butte, W.; Triplett, E. W.; Broughton, W. J.; Gorbushina, A. A. Pathogens as Potential Hitchhikers on Intercontinental Dust. *Aerobiologia (Bologna)*. **2012**, *28* (2), 221–231. <https://doi.org/10.1007/s10453-011-9230-2>.