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Article

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

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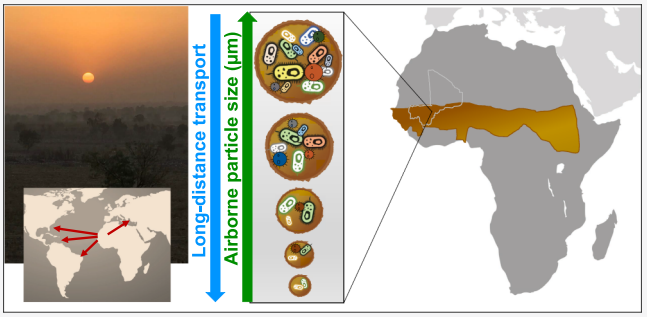
ABSTRACT: Diverse airborne microbes affect human health and biodiversity, and the Sahara region of West Africa is a globally important source region for atmospheric dust. We collected size-fractionated (>10, 10−2.5, 2.5−1.0, 1.0−0.5, and <0.5 μm) atmospheric particles in Mali, West Africa and conducted the first cultivation-independent study of airborne microbes in this region using 16S rRNA gene sequencing. Abundant and diverse microbes were detected in all particle size fractions at levels higher than those previously hypothesized for desert regions. Average daily abundance was 1.94×10^5 16S rRNA copies/m^3. Daily patterns in abundance for particles <0.5 μm differed significantly from other size fractions likely because they form mainly in the atmosphere and have limited surface resuspension. Particles >10 μm contained the greatest fraction of daily abundance (51−62%) and had significantly greater diversity than smaller particles. Greater bacterial abundance of particles >2.5 μm that are bigger than the average bacterium suggests that most airborne bacteria are present as aggregates or attached to particles rather than as free-floating cells. Particles >10 μm have very short atmospheric lifetimes and thus tend to have more localized origins. We confirmed the presence of several potential pathogens using polymerase chain reaction that are candidates for viability and strain testing in future studies. These species were detected on all particle sizes tested, including particles <2.5 μm that are expected to undergo long-range transport. Overall, our results suggest that the composition and sources of airborne microbes can be better discriminated by collecting size-fractionated samples.



INTRODUCTION

Atmospheric dust is known to harbor diverse microbial communities.[1,2] When transported over long distances, airborne microbes can affect human health, agriculture, and ecosystem function thousands of kilometers away from source regions.[3−7] The Sahara Desert is the largest global dust source, particularly during the dry Harmattan winds season between late November and mid-March.[8] Frequency of dust events and total dust emissions have increased in the Sahara because of desertification and changes in land management practices since the 1960s.[8] Large quantities (10^9 tonnes per year) of atmospheric dust particles have been transported from the Sahara to the Mediterranean, Europe, and the Americas, among other regions.[9,10] Average wintertime atmospheric dust concentrations in Saharan countries such as Mali (>300 μg m^{-3}) are much greater than the global average (21 μg m^{-3}).[11,12] However, little is known about the composition and diversity of airborne microbes in the Sahara region. The only prior study of airborne microbes in Mali used culture-based methods and open-face collection with no size-selective inlet.[13] While it laid the foundation for future work, additional work using non-culture-based detection methods is needed to fully capture the composition and diversity of these microbial communities.[14]

Most prior work on airborne microbes has focused on a single size fraction of dust, using one bulk sample collected on a filter or in an impingement medium.[13,15] Only a few studies have examined variability in the airborne microbial composition across particle size fractions,[16−19] and no size-fractionated data are presently available for the Sahara region. Such potential differences are important to understand because smaller atmospheric particles have greater propensity for long-range transport and also penetrate deeper into the lungs following inhalation, increasing risks to human health.[1,20]

Here, we investigate the influence of particle size on airborne bacterial abundance and diversity in Mali, West Africa during the Harmattan winds season under ambient (non-dust storm)

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conditions. We use these data to assess microbial transport propensity, which is inversely related to particle size. We use direct next-generation molecular sequencing of different sized dust samples obtained without microbial culture to characterize microbial abundance (defined as 16S rRNA gene copy number per m^3) and diversity (defined as the number of observed species and Faith's phylogenetic diversity) in the Sahara region. Our analysis provides better understanding of the composition, pathogenic potential, and transport propensity of microbes associated with atmospheric dust in a globally important source region.

METHODS

Atmospheric Particle Sampling. We collected integrated daily atmospheric particle samples continuously between the hours of 9:00 and 19:00 over nine consecutive days between February 11−20, 2018 in Bamako, Mali. Sampling was intended to capture the time period of greatest human activity and exposure. Particle samples reflect both local and background sources. Samples were collected using a compact cascade impactor (CCI) that separated atmospheric particles into into five size fractions (>10.0, 10.0−2.5, 2.5−1.0, 1.0−0.5, and < 0.5 μm), for a total of 45 individually analyzed samples. The CCI was designed and built by the Environmental Chemistry Laboratory at the Harvard T.H. Chan School of Public Health.[21]

No dust storms occurred on any of the sampling days. The duration of sampling averaged 7.7 h per day, and the mean total volume of air sampled was 13.8 m^3 per day. All measures of microbial abundance were normalized by the volume of air collected because we were unable to obtain particle counts. The collection device was supported on a 1.52 m tripod, resulting in an inlet height 2 m above the Mali Météo building rooftop, which was 12 m above ground level (Table S1). The collection site was located in a nonresidential area 2 km away from Bamako International Airport to minimize the influence of commercial and residential vehicle traffic. The Météo building was the tallest structure within a 1 km radius without obstructions.

Inertial impactors with flat impaction substrates have been widely used as size-selective inlets, collecting size-fractionated particles in ambient air.[22] These impactors remove larger particles, collecting the smaller particles on a downstream filter. However, when there are high particle concentrations, flat collection substrates allow some of the larger particles to bounce off because of their relatively high momentum and collect (along with the smaller particles) on the downstream filter. To overcome this limitation, an impactor was developed using porous polyurethane foam (PUF) as a substrate.[23] This substrate prevents bounce off and has a higher loading capacity for the larger particles than flat substrates. Impactors with this substrate have been used successfully for sampling in desert regions during high dust conditions.[24] This study used a cascade impactor with PUF for the largest four particle size fractions.[21] The last stage was a 47 mm diameter back-up filter. This last stage of the impactor has close to 100% collection efficiency for particles with aerodynamic diameters <0.5 μm. Although the use of two different substrates (PUF and Teflon membrane filters) could introduce bias in percent recovery, we found no evidence for this in our data.

A constant flow rate of 30 L min^{-1} was maintained throughout sample collection. Field and laboratory blanks were collected and processed simultaneously with the samples. We chose not to sterilize the PUF and filters to avoid damaging the substrates. None of the laboratory blanks contained amplifiable DNA, confirming the reliability of our approach. Face masks and gloves were worn during instrument calibration, setup, monitoring, dismantling, and sample handling to prevent contamination. All equipment was sanitized with sodium hypochlorite, ethanol, and UV irradiation between sample collection.[25]

At the end of each collection day, samples were processed in a laminar flow hood. PUF substrates and filters were individually submerged in a sterile solution of phosphate buffered saline and formalin in sterile storage tubes to satisfy international transport requirements. The phosphate buffered saline−formalin solution was made by buffering formaldehyde (37%) with sodium borate to a neutral pH and diluting with molecular-grade phosphate buffered saline to 2% final formaldehyde concentration. Storage tubes were immediately sealed with Parafilm tape and put into individual Whirl-Pak (Whirl-Pak, Nasco, USA) bags. All samples and field blanks were stored at 4 °C and transported by air to Harvard University (Cambridge, MA, USA). We compiled meteorological data from an Airmar WX200 weather station at the same time and location during sample collection (Airmar Technology Corp., Milford, NH). Average meteorological conditions are summarized in Table S1. We used the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT,[26] Version 4) to reconstruct 72 h backward and forward trajectories and confirm the majority wind direction and origins of long-range air masses.

DNA Extraction, Sequencing, and Postprocessing of Reads. We used 16S ribosomal RNA gene amplicon sequencing to characterize the composition and diversity of airborne microbial communities across the five different particle size fractions. Genomic DNA was extracted from particle samples using the PowerSoil DNA Isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's protocol, with modifications to account for the substrate materials. Each PUF substrate was cut into three pieces and extracted separately by adding it directly to the power bead mixture. Filter substrates were added directly to the power bead mixture. Substrate storage liquid was centrifuged, and the pellet was added to the power bead mixture. The V4 hypervariable region of the 16S rRNA gene in both bacteria and archaea was amplified and sequenced in duplicate for each sample using the primer pair 515F (GTGYCAGCMGCCGCGGTAA)[27] and 806R (GGACTACNVGGGTWTCTAAT).[28] These duplicates were employed as stochastic replications of the polymerase chain reaction (PCR) step to reduce potential bias. The 16S rRNA genes were amplified in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. PCR amplicons were then purified using calibrated Ampure XP beads (Beckman Coulter, USA) and pooled for sequencing. Sequencing was performed using an Illumina MiSeq platform (San Diego, CA, USA) following the manufacturer's guidelines. DNA extraction and PCR amplification were performed on controls as well as negative lab controls using no template. DNA extraction kit and lab controls on the substrates did not amplify or sequence, indicating low risk for contamination. DNA extraction, amplification, and sequencing of all samples were carried out at Molecular Research LP (Shallowater, TX, USA).

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Table 1. Daily Bacterial Abundance[a] on Different Particle Sizes

	particle size (micrometers)						
day[b]	<0.5	1.0-0.5	2.5−1.0	10.0−2.5	>10.0	total	air vol. (m^3)
1	4.34	6.11	7.77	10.2	30.4	58.8	10.6
	(1.32)	(0.491)	(1.80)	(4.21)	(6.40)		
percent	7%	10%	13%	17%	52%	100%	
2	3.98	20.5	25.6	203	279	532	13.0
	(0.836)	(20.8)	(13.3)	(42.8)	(11.9)		
percent	1%	4%	5%	38%	52%	100%	
3	3.74	7.57	23.4	40.2	97.8	172	9.24
	(0.876)	(1.27)	(2.88)	(14.0)	(12.4)		
percent	2%	4%	14%	23%	57%	100%	
4	4.45	9.35	13.8	20.1	63.0	111	15.0
	(0.840)	(1.53)	(1.03)	(5.79)	(4.02)		
percent	4%	8%	12%	18%	57%	100%	
5	2.54	7.44	7.89	10.7	46.1	74.7	13.3
	(0.702)	(0.834)	(2.02)	(3.98)	(22.2)		
percent	3%	10%	11%	14%	62%	100%	
6	8.18	9.31	20.1	132	192	361	16.8
	(2.26)	(2.05)	(4.62)	(21.1)	(7.07)		
percent	2%	3%	6%	37%	53%	100%	
7	17.1	19.9	12.5	83.4	154	287	16.4
	(22.7)	(22.0)	(2.42)	(21.0)	(42.6)		
percent	6%	7%	4%	29%	54%	100%	
8	6.45	6.61	6.06	21.3	51.7	92.1	15.3
	(1.68)	(0.469)	(0.863)	(7.95)	(29.1)		
percent	7%	7%	7%	23%	56%	100%	
9	2.01	3.17	8.35	16.4	31.2	61.1	15.1
	(0.158)	(1.57)	(2.91)	(7.09)	(4.25)		
percent	3%	5%	14%	27%	51%	100%	

[a]Abundance is shown as 10^{-3} 16S rRNA copies/m^3, with standard deviation in parentheses and percent abundance on each particle size shown as a percent of the daily total 16S rRNA copies/m^3. [b]Day refers to consecutive sampling days in February 2018 in Bamako, Mali, West Africa.

Sequence data were processed and analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline, Version 1.9.[29] Quality filtering and processing of paired-end reads were performed following Mahmoudi et al.[30] The initial QIIME workflow involved preprocessing of the data by removing primers, demultiplexing, quality filtering to discard sequences with a quality score of <19, and removing chimeric sequences via USEARCH.[31] Sequences were then clustered into operational taxonomic units (OTUs) at 99% identity. Taxonomy was assigned based on the Greengenes (v13_8)[32] database using the ribosomal database project (RDP) classifier with an 80% confidence threshold.[33−35] Sequences present in <0.005% of the total data set were removed to limit the effect of spurious OTUs on analysis.[36] Analyses were performed after rarefying the samples to the same sequencing depth (27,680 sequences) using QIIME, R v.3.5.1,[37] Phyloseq,[38] and VEGAN.[39]

Determining Bacterial Abundance via Quantitative Polymerase Chain Reaction (qPCR). Bacterial abundance was evaluated using qPCR amplification of the 16S rRNA gene using the bacteria-specific primer pair Bac2F (CCATGAAGTCGGAATCGCTAG) and Bac2R (GCTTGACGGGCGGTGT) in triplicate.[40] The qPCR mixture contained the following: 1 μM forward primer, 1 μM reverse primer, 500 nM Probe (BHQ; Integrated DNA Technologies, Inc., Coralville, IA), 1X TaqMan Universal PCR Master Mix (Applied Biosystems, Waltham, MA, USA), and 1.0 μL of template DNA. Bacterial 16S rRNA copy numbers were amplified and quantified with the StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). qPCR results were analyzed using StepOne Software Version 2.2.2.

Assessing the Presence of Potential Pathogens Using PCR. We identified the presence of species that are potential pathogens using PCR. Fifteen samples were tested for six different pathogens chosen based on qualitative evaluation of three properties: high absolute abundance in the samples at the genus level, detection in previous studies of airborne microbes, and degree of pathogenicity for humans. These included *Bacillus cereus* (n = 3 samples), *Escherichia coli* (n = 3 samples), *Fusobacterium nucleatum* (n = 4 samples), *Streptococcus pneumoniae* (n = 1 sample), *Staphylococcus epidermidis* (n = 4 samples), and *Pseudomonas aeruginosa* (n = 1 sample) (Table S2). Six different primer sets corresponding to the six species were used to perform PCR (Table S3). For each of the six species tested, 2 μL of the template DNA was used to perform PCR using TaqMan Universal PCR Master Mix (Applied Biosystems) or PowerUp SYBR Green Master Mix (Applied Biosystems) in a StepOnePlus Real-Time PCR System (Applied Biosystems). PCR was also performed using 1 μL of the PCR product after 10 cycles of PCR. PCR was carried out with an initial holding stage of 50 °C for 2 min followed by 95 °C for 10 min. The cycling stage consisted of 40 cycles of 95 °C for 15 s followed by 60 °C for 1 min. All PCR analyses were carried out at Molecular Research LP (Shallowater, TX, USA). Additional analyses are required to confirm the viability and strain of potential pathogens identified using PCR. This was not possible because samples had to be sterilized to satisfy international export

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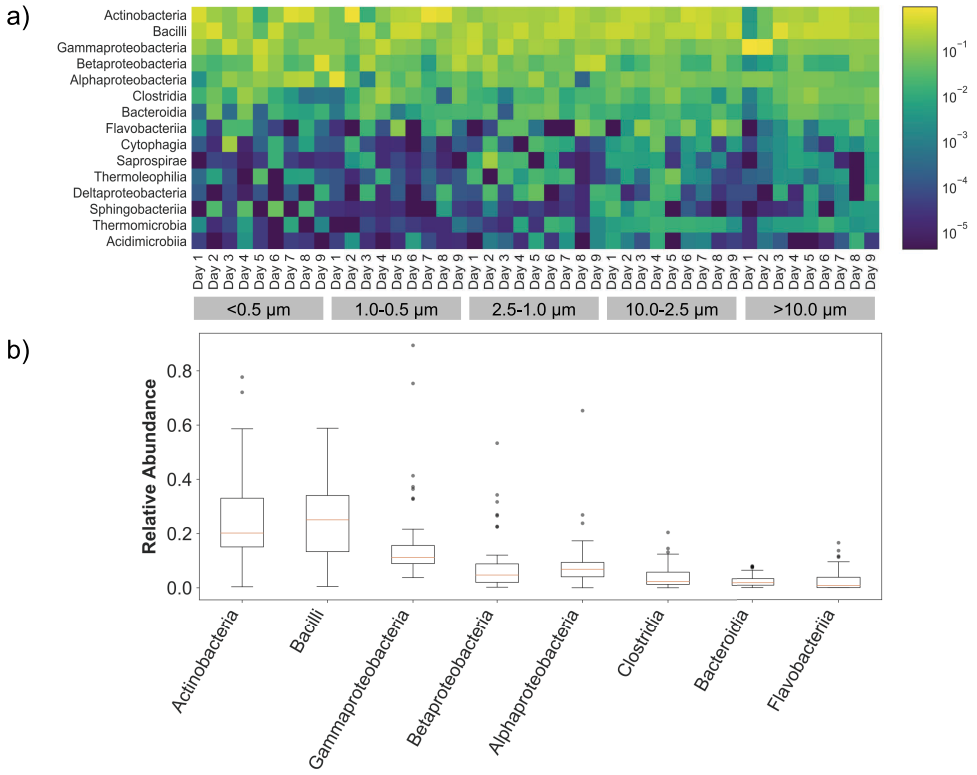

Figure 1. Dominant taxonomic composition of the microbial community detected on atmospheric particles from Mali, West Africa. Panel (a) shows the relative abundance (log scale) of classes present in at least 80% of all dust samples. The x-axis shows individual samples grouped by the particle size from the smallest to largest fractions. Panel (b) shows the relative abundance of total OTUs represented by different microbial classes.

requirements, precluding culture-based analyses and mRNA-based viability tests.

Statistical Analyses. Alpha diversity, or within-sample diversity, was evaluated using two metrics: Faith's phylogenetic diversity and number of observed species. Faith's phylogenetic diversity is a measure of diversity that accounts for the evolutionary relationships among OTUs in a community, and the number of observed species represents the number of unique OTUs in a sample as a measure of species richness. Nonparametric analysis of variance on ranks (Kruskal–Wallis) followed by Dunn's multiple comparison test was used to evaluate statistical differences in: (1) microbial abundance (defined as 16S copy number per m^3), grouped by the sampling day or by particle size fraction; (2) alpha diversity, grouped by the sampling day or by particle size fraction; and (3) relative abundance of individual OTUs, grouped by the sampling day or by particle size fraction. Beta diversity, or between-sample diversity, was evaluated using the weighted UniFrac distance and then visualized using principal coordinate analysis (PCoA) in R.[41]

RESULTS AND DISCUSSION

Abundant and Diverse Microbes Are Present in All Size Fractions of Saharan Dust. Prior work has hypothesized that remote desert regions will have a relatively low airborne microbial abundance compared to temperate and tropical regions.[42] By contrast, we found that bacterial abundance in the desert region of Mali sampled here (mean daily abundance 1.94×10^5 copies/m^3) was greater than that previously reported for more urban regions and temperate latitudes. For example, summed abundance across all particle sizes in this study (Table 1) exceeds airborne microbial concentrations reported for Beijing, China in the summer (4.7×10^4 copies/m^3)[43] and during Asian dust events (average 9.4×10^4 copies/m^3).[44]

To assess airborne bacterial diversity, our analysis of 16S rRNA gene amplicon sequences recovered a total of 3,111,322 high-quality sequences with an average read length of 252 base pairs. The sequences clustered into a total of 1572 OTUs and 514 unique species. Rarefaction curves approached a saturation plateau (Figure S1), and Good's coverage, an indicator of sampling completeness, ranged from 91 to 99%, indicating that the sequencing depth was sufficient to capture the diversity of each sample.[45] The one prior study from Mali, West Africa[13] identified a total of 94 bacterial isolates, which are sequences of bacteria identified using culturing.

Similar Bacterial Composition of Near-Field and Far-Field Saharan Dust. Similarity in the composition of near-field and far-field Saharan dust suggests that some of the airborne community structure is retained during long-distance transport. For phyla, the top three most abundant ones observed in this study were Proteobacteria (34% mean relative abundance), Firmicutes (29%), and Actinobacteria (26%). These same three phyla were detected in the one previous culture-based study in Mali.[13] Saharan dust samples collected in Europe also contained Proteobacteria as the most abundant phylum.[46−48] Firmicutes have previously been associated with long-distance dust events that originated in the Sahara.[46,49,50]

At the class level, Actinobacteria, Bacilli, and Gammaproteobacteria were the most abundant across all samples collected in this study (Figure 1b). Other important classes included Betaproteobacteria, Alphaproteobacteria, Clostridia, Bacteroidia, and Flavobacteria (Figure 1). Alphaproteobacteria, Actinobacteria, Betaproteobacteria, and Gammaproteobacteria were detected in Saharan dust intrusions in Spain (Figure 1).[51]

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Figure 2. Spearman coefficient correlation matrix for abundance (AB, 16S rRNA copies/m^3) and observed species (OS, observed OTUs) across the different particle size fractions (>10.0, 10.0−2.5, 2.5−1.0, 1.0−0.5, and <0.5 μm) on all days. Total abundance (AB Total) was calculated as the sum of the abundance across all particle sizes on each day. Average observed species (OS Average) was calculated as the average of all size fractions on each day. The color shows the strength of the positive association. Significant correlations are denoted by asterisks (*p < 0.05; **p < 0.005; and ***p < 0.0005).

Furthermore, Actinobacteria and Betaproteobacteria were abundant in samples of Saharan dust collected in the European Alps.[46]

At the family level, Betaproteobacteria families *Oxalobacteraceae* and *Comamonadaceae* were detected in this study and also abundant in Saharan dust collected in Spain.[52] Previous studies of air masses passing over the Sahara and collected in the Mediterranean indicate the presence of *Gemmatomonadetes* and *Chloroflexi*, which we also detected in this study.[53] *Gemmatomonadetes* are associated with soils from hyper-arid environments characterized by low biomass, including the Sahara Desert,[54−56] and have been proposed as "bioindicators" for Saharan dust events.[46]

Similar Patterns of Variability in Microbial Abundance Across Most Particle Size Classes. Total (summed) daily abundance varied by approximately an order of magnitude across the 9 sampling days from a minimum of 5.88 × 10^4 16S rRNA copies/m^3 on day 1 to a maximum of 5.32 × 10^5 16S rRNA copies/m^3 on day 2 (Table 1). The >10.0 μm size class contained the greatest fraction (51−62%) of total abundance across sampling days (Table 1). The smallest two size fractions of dust (<0.5 μm and 1.0−0.5 μm) each contained less than 11% of the total abundance.

We tested whether variability in microbial abundance followed the same daily patterns across particle size classes. Results showed that variability in daily abundance within each dust size fraction was well correlated with other particle size fractions, with the exception of the smallest size fraction (Figure 2, Spearman's ρ > 0.71, p < 0.05). The smallest size fraction (<0.5 μm) was not significantly correlated (Spearman's ρ = −0.2 − 0.47, p > 0.05) with all the other sizes. These results indicate that variability in microbial abundance across days is generally reflected by all size fractions of dust except those <0.5 μm. Most airborne particles less than 1 μm in diameter are formed in situ in the atmosphere rather than from surface resuspension because they are difficult to generate mechanically due to their large surface area to volume ratios.[57] Limited surface resuspension as a source of particles <1 μm could explain why microbial abundance is significantly lower for these particles compared to those >1 μm in diameter.

Abundance and Diversity Are Correlated for Larger Particles. We investigated whether large daily fluctuations in microbial abundance were correlated with variability in species diversity assessed by the number of unique OTUs detected on all particle size fractions. For particles of 0.5−10 μm diameter, abundance and diversity were not significantly correlated (Figure 2, Spearman's ρ = −0.23 − 0.45, and p > 0.05). For >10 μm-diameter particles, abundance and observed OTUs were significantly correlated (Spearman's ρ = 0.68 and p < 0.05). Particles >10 μm have an extremely short lifetime against sedimentation.[57] Thus, they are likely derived from local sources compared to more diffuse sources for smaller particles. Such relatively large particles are not easily lofted by wind suggesting that they originate from local human activities such as agriculture.

When aggregated across all sampling days, we found statistically greater abundance (Figure 3, panel A) and diversity (Figure 3, panels B and C) in particles >2.5 μm compared to those <2.5 μm (Figure 3). Particles >10 μm had significantly greater abundance and diversity than the 2.5−10 μm size fraction (Figure 3). These results suggest that larger particles may contain species that differ from the other particle sizes.

Unique Taxa Detected on Larger Particles. PCoA using the weighted UniFrac distance shows some clustering in the

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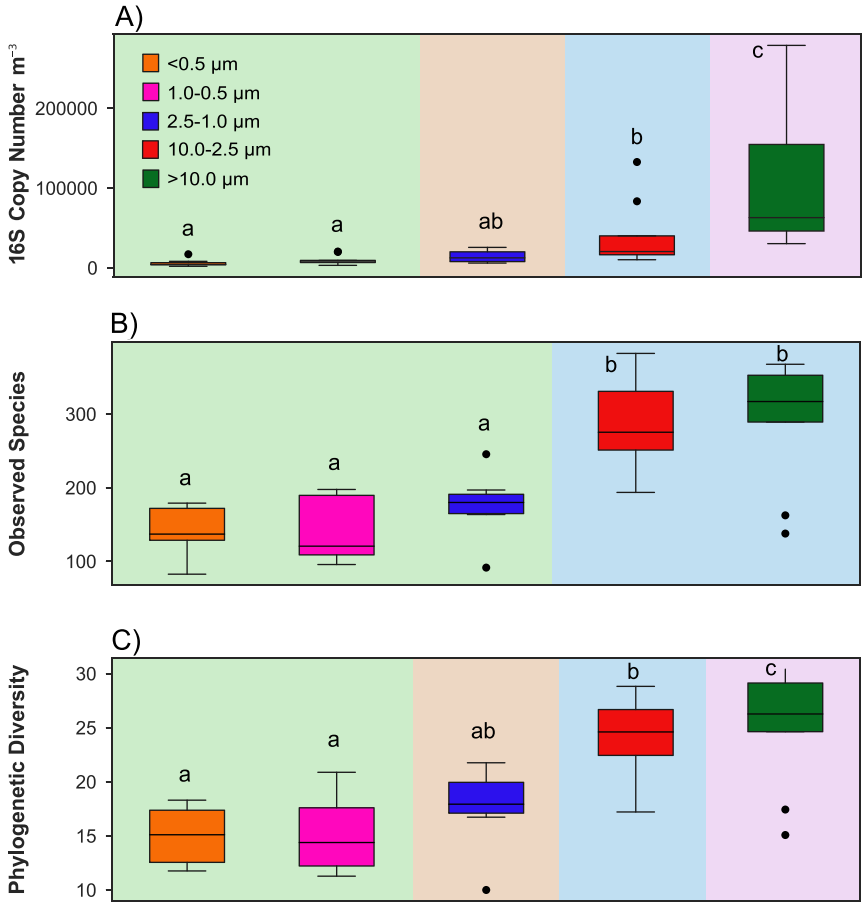

Figure 3. Effect of the particle size on airborne microbial communities: (A) microbial abundance (16S rRNA copies/m^3 as estimated by qPCR) and averaged over each size fraction; alpha diversity as measured by (B) number of observed species and (C) Faith's phylogenetic diversity. Letters above boxes indicate similarities among groups (different letters indicate the statistically significant difference, Dunn's test, $p < 0.05$; and same letters = no difference).

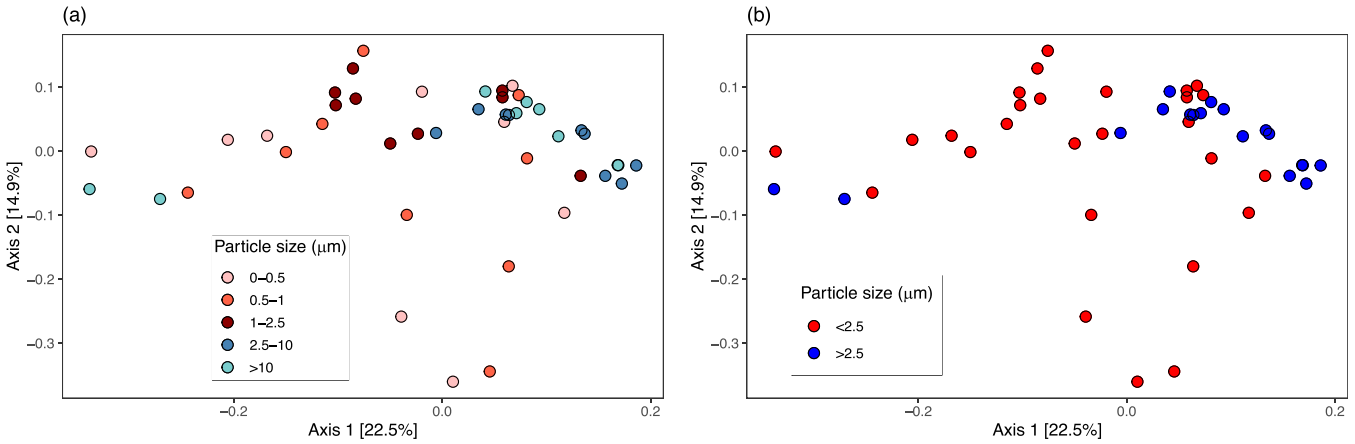

Figure 4. Community analysis using pairwise, weighted UniFrac distances visualized on a PCoA plot with the percent of variation explained by each axis noted in brackets. Samples were grouped by (a) five particle size bins and (b) smaller (<2.5 μm) and larger (>2.5 μm) particle size bins. UniFrac is a metric for beta diversity (between-sample diversity) that measures the unique fraction of the phylogenetic distance between the taxa of two different samples.

taxonomic composition among larger particles but substantial scatter among the smaller size classes of particles (Figure 4a). These results are easier to visualize when samples are grouped by particles <2.5 μm and those >2.5 μm, with more clustering evident in the >2.5 μm size fractions (Figure 4b). This suggests that there may be some unique bacteria on larger particles that are not present on smaller ones, although two exceptions are present with a substantially different taxonomic composition on days 1 and 2 (Figure S2). Particles >2.5 μm likely cluster more closely together because they originate from similar local sources, while smaller particles cluster less because they are more likely to be from a varied mixture of sources.[58]

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Large particles contained the greatest number of OTUs, and small particles contained the fewest (Figure 3). Because particles <2.5 μm contained significantly fewer species and had lower phylogenic diversity than those >2.5 μm, we hypothesized that some species would be preferentially found on specific size fractions of particles.

We found that 14 of the 1572 OTUs (0.9%) detected differed significantly in frequency across the five particle sizes and 170 differed between sizes <2.5 μm and those >2.5 μm (Kruskal−Wallis test, Benjamini−Hochberg (FDR) $p < 0.05$). Differences between these two size ranges are dominated by OTUs that appear in the larger size fraction but not the smaller size fraction. All 14 OTUs exhibited a preference for particles above 1 μm. Of the 170 OTUs, 162 were greater in the larger size fraction than the smaller size fraction. This implies that the day-to-day differences in OTUs across all sizes are dominated by changes in the larger size particle fraction originating from mainly local sources.

Bacterial cells are typically 1−2 μm in diameter or smaller.[59] Higher abundance and alpha diversity in larger particles (>2.5 μm), as well as the individual taxon preferences for the largest three particle size fractions (Table S4; Supporting Information Additional Text), therefore implies that most microbial cells are either bound to soil particles or to each other as cell aggregates, rather than free-floating as individual cells.[60] However, abundant microbes on all particle size fractions mean that we cannot preclude the existence of some freely suspended bacteria. Some of the OTUs that were in higher abundance in the larger size fraction than the smaller size fraction have been documented to aggregate, including *Pseudomonas,*[61] *Geodermatophilus,*[62,63] and *Corynebacterium.*[64] In the atmosphere, association with particles could improve survival of airborne microbes by providing nutrients as well as protection against UV exposure and desiccation (Table S6).[65] Larger particles may provide more favorable conditions for survival or simply more crevice surface area that can harbor bacteria.

Presence of Potential Pathogens in Saharan Dust. Atmospheric transport of viruses and fungi has been associated with disease outbreaks, such as foot-and-mouth disease and sea fan disease.[66,67] To date, no studies have linked long-distance airborne transport of bacterial pathogens with outbreaks of human disease. Out of the six species and 15 individual samples tested, seven samples contained four different potentially pathogenic species: *B. cereus, E. coli, P. aeruginosa,* and *F. nucleatum* (Table S2).

The presence of potential pathogens, particularly in smaller particle sizes, is of great interest because particles <2.5 μm penetrate deeply into the lungs and can enter the bloodstream once inhaled.[68] Future studies focused on assessing the viability and strain of these potential pathogens would be highly informative for understanding the extent to which long-distance airborne transport of pathogens poses a risk to human health. For example, certain strains of *B. cereus* are the causative agent of food poisoning, eye infections, pneumonia, sepsis, and central nervous system infections.[69] This potential opportunistic pathogen was present in two of the three samples tested, both of which were the smallest size fraction (<0.5 μm). *E. coli* was present in two of the three samples tested, in the smallest two particle size fractions (<0.5 μm and 1.0−0.5 μm). Pathogenic *E. coli* strains cause diarrhea and extraintestinal infections in humans.[70] Diarrhea is a significant problem in Africa and the third leading cause of death for children under five years old.[71] Previous research showed that proximity to cattle feedlots, dry cattle pen surfaces, and airborne dust are all positively correlated with a risk for airborne transport of *E. coli*.[72] *P. aeruginosa* was present in the only sample tested, which belonged to the 10.0−2.5 μm size fraction. This potential opportunistic pathogen is most commonly associated with respiratory infections, and some pathogenic strains are particularly problematic given their potential for antibiotic resistance and ability to survive in different environments.[73] *P. aeruginosa* has been isolated from foods such as the West African Mud Creeper sold in local markets.[74]

The one prior study of airborne microbes in Mali found that up to 25% of the bacterial isolates detected were opportunistic human pathogens.[13] Many of the genera phylogenetically related to human pathogens, as well as two of the potential pathogens confirmed at the species level using PCR, exhibited abundance in the smallest particle size fractions (<2.5 μm), highlighting the potential for long-distance transport. The 72 h forward trajectories for all sampling days extended from the Northeast beyond the continent and into the Atlantic Ocean, illustrating the path of this long-distance transport (Figures S3c and S4).

Implications. Results of this work show abundant and diverse microbes in all five size fractions of dust from a desert region of Mali, West Africa. Prior studies have hypothesized that microbial abundance and diversity will be relatively low in arid desert regions[42,75] but our results show that they are similar to temperate regions. Because bacterial cells are typically 1−2 μm in diameter or smaller,[59] the presence of more detectable bacteria on larger particle size fractions suggests that most are aggregated on particles or other cells. The microbial community detected in Mali contained similarities at the phylum, class, and family levels to prior studies conducted in destination regions from the Sahara, suggesting that some of the microbial community structure is retained following long-distance transport.

We observed substantial daily variability in microbial abundance, with similar patterns across most particle size fractions. One exception was for <0.5 μm diameter particles whose abundance did not track with other size fractions. These ultrafine particles are generated primarily in the atmosphere and are difficult to suspend from local sources, suggesting a different origin than other particle size fractions.[57] Daily variability in abundance was not correlated with diversity for most of the particle size fractions, with the exception of >10 μm diameter particles. Such large particles are difficult to suspend by wind only and have a short atmospheric lifetime and therefore likely reflect local human activity.[57]

Larger particles, with more localized origins, contained the greatest proportion of species diversity. Of the 1572 OTUs detected in this study, 162 were preferentially present on particles >2.5 μm in diameter. This implies that some bacteria are preferentially aggregated on large particles. Greater abundance on larger particles may reflect more favorable conditions or a greater crevice area allowing better survival. Overall, our results suggest that the composition and sources of airborne microbes can be better discriminated by sampling programs that collect size-fractionated samples. A limitation of this study was the lack of access to a particle counter in this remote field setting, and particle concentrations were instead normalized to volumes of air sampled. Future studies of microbial composition would benefit from size-fractionated microbial samples collected simultaneously with aerosol concentrations.

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Potential human pathogens were found using PCR in all size fractions of dust tested, including the smaller particles that are more likely to undergo long-distance transport. A limitation of our work was that we were unable to test the strain and viability of these pathogens using typical culture-based assays because international transport restrictions required dust samples to be sterilized. Such bacteria are therefore excellent candidates for viability and strain testing in future studies of dust from Saharan source regions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06332.

Processing of sequences using QIIME v1.9 with the individual steps including extracting barcodes, assembling and aligning the reads, locating using the mapping file, demultiplexing and quality-filtering the sequence data, checking for chimeras, filtering out chimeric sequences, using clean, assembled reads, removing OTUs, calculating the minimum number of sequences, and performing core diversity analyses and the Kruskal–Wallis test (PDF)

Meteorological conditions, samples for PCR of potential pathogens, primer sets for PCR of potential pathogens, rarefaction curves, observed species table, OTUs that differed across particle sizes, additional text on bacterial taxa across particle sizes, PCOA by sampling day, taxa detected with features for survival under harsh conditions, regression model details, details of genera that contain pathogenic species, wind direction and forward trajectories, wind rose plots, and most abundant genera with pathogenic species(PDF)

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Notes

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Due to a production error, this article published January 21, 2021 with errors in Figure 2. The corrected Figure 2 published February 2, 2021.

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