Microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications

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The composition and prevalence of microorganisms in the middle-to-upper troposphere (8–15 km altitude) and their role in aerosol-cloud-precipitation interactions represent important, unresolved questions for biological and atmospheric science. In particular, airborne microorganisms above the oceans remain essentially uncharacterized, as most work to date is restricted to samples taken near the Earth's surface. Here we report on the microbiome of low- and high-altitude air masses sampled onboard the National Aeronautics and Space Administration DC-8 platform during the 2010 Genesis and Rapid Intensification Processes campaign in the Caribbean Sea. The samples were collected in cloudy and cloud-free air masses before, during, and after two major tropical hurricanes, Earl and Karl. Quantitative PCR and microscopy revealed that viable bacterial cells represented on average around 20% of the total particles in the 0.25- to 1-μm diameter range and were at least an order of magnitude more abundant than fungal cells, suggesting that bacteria represent an important and underestimated fraction of micrometer-sized atmospheric aerosols. The samples from the two hurricanes were characterized by significantly different bacterial communities, revealing that hurricanes aerosolize a large amount of new cells. Nonetheless, 17 bacterial taxa, including taxa that are known to use C1–C4 carbon compounds present in the atmosphere, were found in all samples, indicating that these organisms possess traits that allow survival in the troposphere. The findings presented here suggest that the microbiome is a dynamic and underappreciated aspect of the upper troposphere with potentially important impacts on the hydrological cycle, clouds, and climate.

ice nucleation | cloud condensation nuclei | microbial community | pyrosequencing | biogeography

Airborne microorganisms likely play an important role in cloud formation and precipitation for a number of reasons. First, the concentration of microbial cells (typically, 0.1–3 μm in diameter) in the lower troposphere (<5 km altitude) is thought to be comparable to nonbiological supermicron ice nuclei (IN) (1). Second, several bacterial plant pathogens are known to promote heterogeneous freezing of ice through the action of an outer membrane protein (the inaZ protein), which serves as a nucleation center (2, 3). The prime biological function of the protein is to damage the leaves of plants and facilitate plant infection by the pathogens. When suspended in the atmosphere, these organisms can act as efficient IN at temperatures as high as −2 °C (3), much higher than any nonbiological IN. Third, several bacterial species are known to act as efficient cloud condensation nuclei (CCN) (4); because of their large size and concentration, they can contribute to the population of "giant CCN" (2, 5). Giant CCN can promote the formation of precipitation by acting as collector drops that form drizzle.

However, a comprehensive understanding of the CCN and IN efficiency of different microbial types (e.g., bacteria vs. fungi) and species, as well as their spatial and temporal distribution in the troposphere, particularly at high altitudes, is essentially lacking. This severely limits our understanding of the importance of airborne microbial cells for cloud formation and the hydrological cycle (1, 6). Furthermore, most studies to date on the atmospheric microbiome are restricted to samples collected near the Earth's surface (e.g., top of mountains). The tropospheric microbial communities at high altitudes and in air masses over marine/oceanic regions remain poorly characterized, mostly due to difficulties associated with obtaining representative samples of sufficient biomass (which imposes additional challenges to recover enough DNA and protein material for analysis). Little is known about the composition and spatial and temporal variability of these microbial communities, about how they adapt to their environment, and if they are viable and can metabolize organic constituents present in the atmosphere. It is also important to understand how atmospheric processes that can aerosolize and/or precipitate microbial cells, such as major storm systems (tropical cyclones and hurricanes), affect microbial community composition and function. Advancing our understanding of these issues will also contribute to improved models of microbial disease dispersal and microbial biogeography (6–8).

A few culture-independent molecular studies, which analyzed atmospheric air or snow water collected on the ground, have appeared recently. These studies have revealed considerably more diverse bacterial and fungal communities to be present in the atmosphere of several ecosystems such as the Amazon River and urban cities compared with what was previously anticipated on the basis of the results of culture-based efforts (9–14). Furthermore, microorganisms commonly found in soil (15) and vegetation (16), including plant pathogens with IN activity such as *Pseudomonas syringae*, were frequently observed in the previous culture-independent surveys. Although the relevance of the findings of these studies for the microbial communities of the middle-to-upper troposphere remains unknown, the identification of complex microbial communities warrants further investigations of the role of the diverse microbes found in the atmosphere and their potential to serve as CCN or IN.

Toward closing these knowledge gaps, we obtained several high-altitude samples at ∼10 km above sea level during the National Aeronautics and Space Administration Genesis and Rapid Intensification Processes (GRIP) campaign, which was focused on tropical hurricanes that developed in the Caribbean

ENVIRONMENTAL SCIENCES

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Sea and the midwestern Atlantic Ocean in 2010. Here, we report on our initial efforts to characterize the microorganisms in these samples, including the analysis of sequenced amplicons of the small subunit ribosomal RNA gene (SSU rRNA), the gene that serves as the best phylogenetic marker to identify the species present in the samples and estimate their in situ abundance (17). Our results provide important insights into the species composition, concentration, and dynamics of the microbial communities of the upper troposphere above the oceans and the potential impact of airborne microbial cells on cloud formation.

Results

Samples Collected and Cell Concentrations. The GRIP campaign operated one flight off the coast of California, one intercontinental flight across the United States (transit flight from California to Florida), and seven flights in the areas of the Gulf of Mexico, the Caribbean Sea, and the midwestern Atlantic Ocean (*SI Appendix*, Fig. S1) during which samples were taken. The flights spanned a total period of about 6 wk (August 10, 2010–September 20, 2010) and included flights during Hurricanes Earl and Karl. For each hurricane, samples were collected at the hurricane's intensification (category 1) phase and later (category 2 and 3) phases. Flights also included sampling the cloudy environment before Hurricane Earl (August 17, 2010), low altitudes (September 17, 2010, during the time of Hurricane Karl but sampling air masses undisturbed by the hurricane; 1–4 km vs. ∼10 km in all other flights), and a cloud-free air mass (September 20, 2010) after the passing of Hurricane Karl. During each flight, biomass from an average of 6 m^3 of ambient air was collected per sample, using two separate sampling lines (duplicate samples; *SI Appendix*, Table S1). Each sample was collected over a period of 3 h on average (ranging from 1 to 5 h); thus, the samples were presumably representative of the air masses sampled. In addition, several filters, used as handling blanks, were connected to a sampling line with no airflow.

Both molecular and microscopic approaches were used to quantify the concentrations of bacterial and fungal cells in the samples. Quantitative PCR (qPCR) of the gene SSU rRNA revealed an average bacterial SSU rRNA gene copy number of 2.0×10^4 copies m^{-3} (ranging from 3.0×10^3 to 9.0×10^4 copies m^{-3}). Assuming that the average bacterial genome has about four rRNA copies based on 1,144 complete genomes available in the *rrnDB* database at the beginning of 2012 (18), our results revealed an average bacterial concentration of 5.1×10^3 cells m^{-3}. Field blanks showed at least an order of magnitude lower SSU rRNA gene copy abundance compared with the SSU rRNA genes in the corresponding field samples or, in about half of the cases, no detectable SSU rRNA genes (Fig. 1 *A* and *B*), suggesting that the field samples have microbial cells that truly represent the tropospheric microbial communities. [Note that the weak positive qPCR signal observed in a few of the blanks is most likely due to our approach to sterilizing the filters and filter holders, which involved autoclaving at 120 °C for 30 min. Thus, it is possible that DNA from dead (autoclaved) cells remained on the filters and provided a signal during qPCR. Consistent with these interpretations, we failed to obtain enough DNA for SSU rRNA amplicon pyrosequencing from any of the blanks (see below). Also note that more starting DNA material is required for amplicon sequencing compared with qPCR, which presumably accounts, in part, for the results obtained].

Fungal cells were also present in the samples based on qPCR analysis at about an order of magnitude lower concentration than bacterial cells, with an average concentration of 6.8×10^2 copies m^{-3} (ranging from 1.9×10^2 to 1.3×10^3 copies m^{-3}). Given also that fungal genomes typically encode a higher number of rRNA copies than bacteria (ranging form 30–100 copies per genome, depending on the species considered), these results suggest that bacteria are at least two orders of magnitude more abundant relative to the fungi at high altitudes. Particles of similar size to that of bacterial cells (0.1–3 μm) tend to have greater residence times in the atmosphere compared with larger particles (such as fungal cells and spores that are typically >3 μm in diameter), which probably accounts, at least in part, for the differences in cell concentrations observed. Bacterial and fungal cells had similar concentration patterns across all samples analyzed with the exception of the samples taken during Hurricane Earl, where bacterial cells increased and fungi cells decreased in samples taken over 2 consecutive days (Fig. 1 *A* and *B*).

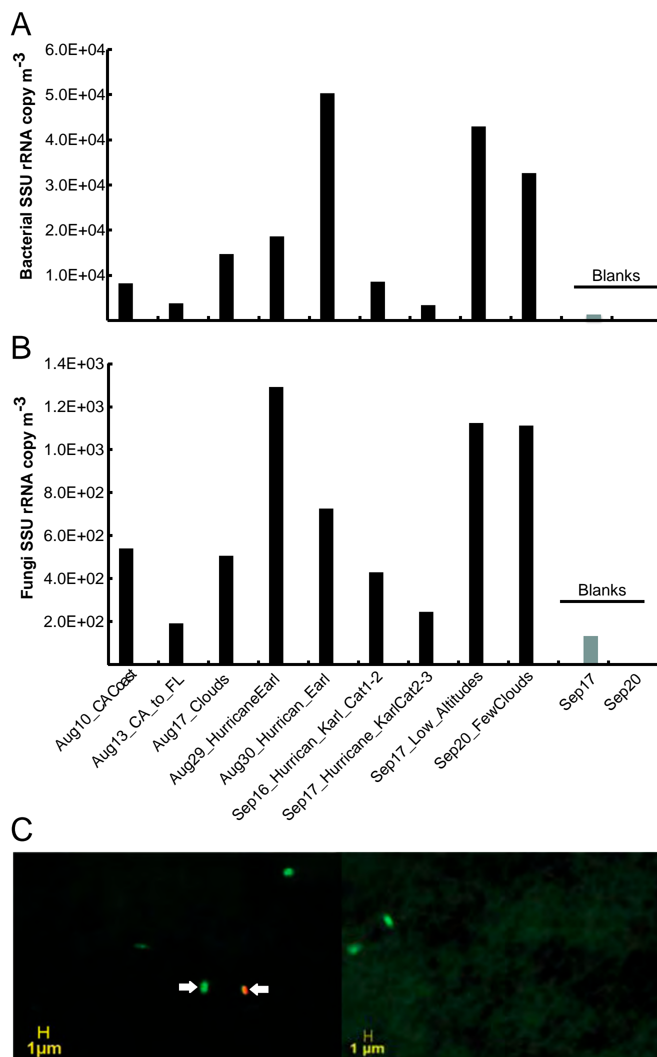

Fig. 1. Quantification of bacterial and fungal cells in samples from high altitudes in the atmosphere. Concentration of bacterial (*A*) and fungal (*B*) cells based on qPCR analysis of SSU rRNA gene copies in the samples. Note that samples are ordered by the collection time on the *x* axis except for blank samples, which are shown at the rightmost part of the graphs in light gray. (*C*) Live/dead microscopy image of two samples from the California coast and transit flights. Green-stained cells represent cells with viable/intact membrane (e.g., cell indicated by left arrow), and red/yellow-stained cells represent cells with a damaged membrane (e.g., cell indicated by right arrow).

Microscopy-based cell counting for the same samples revealed an average concentration of 1.5×10^5 cells m^{-3} (*SI Appendix*, Table S1). The higher cell concentration based on microscopy relative to qPCR by about one order of magnitude is not unexpected and is likely attributable to a smaller average SSU rRNA copy number of the organisms in the samples relative to the copy number used in the calculations and/or qPCR underestimating cell abundance due to technical limitations. The technical limitations include primer mismatches, not all cells in the sample being lysed for DNA extraction (e.g., spores can be resistant to lysis), and DNA loss during extraction. Microscopy-based estimates might also represent overestimates due, for example, to abiotic particles being stained and counted (erroneously) as cells. However, the limitations of microscopy should be comparatively less important for the results obtained. The microscopy observations also revealed that the fraction of viable

cells in the samples ranged from 60 to almost 100% of the total cells based on the live/dead cell viability test (*SI Appendix*, Table S1; Fig. 1*C*), suggesting that these organisms can survive the adverse conditions present in the atmosphere. Samples taken during hurricanes had typically higher numbers of total and viable cells compared with samples taken off the coast of California and during the transit flight (*SI Appendix*, Table S1; Fig. 1*A*), revealing that hurricanes aerosolize a large amount of new cells and that these cells remain viable for at least a few days (because our samples taken around a hurricane event spanned a period of about 1 wk).

The microscopy analysis also revealed that most bacterial cells in a sample (typically, >80% of the total) were 0.25–1 μm in diameter (*SI Appendix*, Table S2; Fig. 1*C*). Total particle concentration in the 0.25- to 1-μm diameter range, including biological and abiotic particles, was measured during each flight (in real time) using an Ultra-High Sensitivity Aerosol Spectrometer (Droplet Measurement Technologies). As expected, particle concentration decreased with altitude from an average of 5.9×10^6 particles m^{-3} at 0–1 km to 2.6×10^5 particles m^{-3} at 7–8 km (*SI Appendix*, Table S1). These results were also consistent with independent measurements obtained using an aerodynamic particle sizer (TSI Inc., model 3321; *SI Appendix*, Fig. S2). Based on the microscopy cell counts, bacterial cells constituted a substantial fraction of the total coarse-mode particles at high altitudes (8–10 km), ranging from 3 to almost 100% in some of the hurricane samples [about 20% on average (*SI Appendix*, Table S1)]. Bacterial contribution was significant even based on the qPCR results, representing about 2% of the total particles on average. These findings reveal that viable cells represent an important component of the micron-sized aerosols at high altitudes, contrasting with near-surface aerosols that are typically characterized by a much lower cell fraction on the order of 1 cell in 1 million of abiotic CCN particles (19).

Microbial Community Composition. Bacterial SSU rRNA gene amplicon sequences were obtained using pyrosequencing technology (Roche 454) and were processed, trimmed, and denoised using the QIIME pipeline (20), resulting in over 45,000 sequences across all samples (about 5,000 per sample on average). A total of 314 operational taxonomic units (OTUs), defined at the 97% nucleotide-sequence identity level, were identified among all sequences, ranging from 99 to 299 OTUs per sample. Notably, samples collected over the continental United States had lower species richness compared with the hurricane-related samples based on the Chao1 richness estimate, i.e., 129 and 113 for the California coast and transit flights, respectively, vs. an average of 241 for the hurricane samples (147 and 118 vs. 251, respectively, when we normalized for the number of sequences in each sample by randomly subsampling 3,000 sequences per sample). No other significant difference in species richness was observed. Rarefaction analysis revealed that the number of unique SSU rRNA genes in most of the samples was close to being saturated by sequencing (*SI Appendix*, Fig. S3), revealing that the tropospheric communities are less complex compared with many habitats on Earth such as soils (e.g., ref. 21). The vast majority of the OTUs were classified *Alpha-* and *Betaproteobacteria*. Based on the taxonomical classification of the SSU rRNA gene sequences recovered, *Afipia* sp. (*Alphaproteobacteria*) comprised over 50% of the total communities sampled off the California coast and during the transit flights. This group was reduced to less than 20% of the community and apparently replaced by members of the *Burkholderiales* order (*Betaproteobacteria*) as an effect of Hurricane Karl (the possibility that the high relative abundance of these groups is due to amplification biases is discussed below). The abundances of all OTUs in each sample are provided in *SI Appendix*, Table S3.

Samples related in time or space (same geographic region) tended to show more similar community composition patterns compared with unrelated samples (PerMANOVA, $P < 0.05$). For example, the samples taken off the coast of California and during the transit flight, as well as the four samples taken during and 3 d after Hurricane Karl, showed almost indistinguishable community compositions (Fig. 2*A*). However, the communities at the aftermath of the two hurricanes were dramatically different compared with those before the beginning of the hurricanes. These results suggest that hurricanes have a major impact on the composition of the tropospheric communities based apparently on the large number of new microbial cells that they aerosolize and the precipitation scavenging of preexisting cells. Note, for example, the absence of *Ralstonia* during Hurricane Earl compared with the high abundance of this group during Hurricane Karl in Fig. 2. Consistent with these interpretations, principal coordinate analysis of SSU rRNA gene sequences revealed three major clusters among the GRIP samples: two clusters representing the samples taken during each of the hurricanes and one cluster representing the samples taken off the California coast (*SI Appendix*, Fig. S4; PerMANOVA, $P < 0.05$). Nonetheless, additional samples need to be analyzed before more quantitative conclusions about the effect of tropical hurricanes on tropospheric microbial communities can emerge.

A set of 17 OTUs was present across all samples, albeit in varied abundances, indicating that these organisms represent core members of the microbiome of the middle and upper troposphere. Given that the samples analyzed represent geographically distant locations (e.g., California vs. Caribbean) and cloud-free, cloudy, and tropical storm environments, these OTUs represent organisms that apparently possess traits to survive long periods of time at high altitudes in the atmosphere. Two of the core families were the *Methylobacteriaceae* and *Oxalobacteraceae*, members of which metabolize oxalic acid, a main product of cloud-mediated chemistry and one of the most abundant dicarboxylic acids in the atmosphere (22, 23). Hence, it is conceivable that these groups could remain metabolically active in clouds, although their ability to use oxalic acid while present in the atmosphere awaits experimental verification. The relative abundance of the core OTUs varied with time and geographic location. For example, the core OTUs substantially decreased in abundance at the aftermath of Hurricane Earl but remain present, even at relatively low numbers, in the sample taken 3 d after the impact of Hurricane Karl. Microbial cells of 0.25–1 μm in diameter (Fig. 1*C*) take several days (to a few weeks) to gravitationally settle from high altitudes, and the samples analyzed here spanned a period of more than a month. Therefore, the core members are apparently able to stay aloft and remain viable (microscopic observations) for at least several days.

Habitat of Origin on Earth. Airborne bacteria are thought to originate from different habitats on the Earth's surface (ocean, soil, freshwater, etc.). To gain quantitative insights into the habitat of origin of the microbes present in the GRIP campaign samples, we identified the best match of each sequence in the GreenGenes database (24) and analyzed the habitat of origin of the best matches when available. We also performed back trajectory analysis, which showed the route of the air masses for 5 d before sampling [e.g., continental vs. oceanic mass transport (*SI Appendix*, Fig. S5)], and contrasted this information to the habitat of origin of the SSU rRNA sequences. The results revealed that the organisms sampled originated from almost all habitats on Earth and that the hurricane samples had a higher abundance of marine bacteria (Fig. 3), which was consistent with back trajectory analysis and the geographic areas where the hurricanes developed. Furthermore, only in the hurricane samples did we observe a substantial signal of bacteria known to be associated with human and animal feces such as members of the *Escherichia* (38% in the first Hurricane Earl sample) or *Streptococcus* (26% in the second Hurricane Earl sample) genera, consistent with the passing of hurricanes over populated areas. However, at the resolution provided by our approaches, i.e., resolving phyla and genera well but species poorly, we could not confirm whether or not any of these bacteria represent pathogens. These results were reproducible when the differential representation of habitats in the GreenGenes Database was normalized (*SI Appendix*, Fig. S6) or when the analysis was performed by using only one representative sequence for each OTU (as opposed to all sequences above) to avoid potential amplification biases during sequencing that affected OTU relative abundance (*Discussion*). Our results were not always consistent with back trajectory analysis, and these cases were typically attributed to

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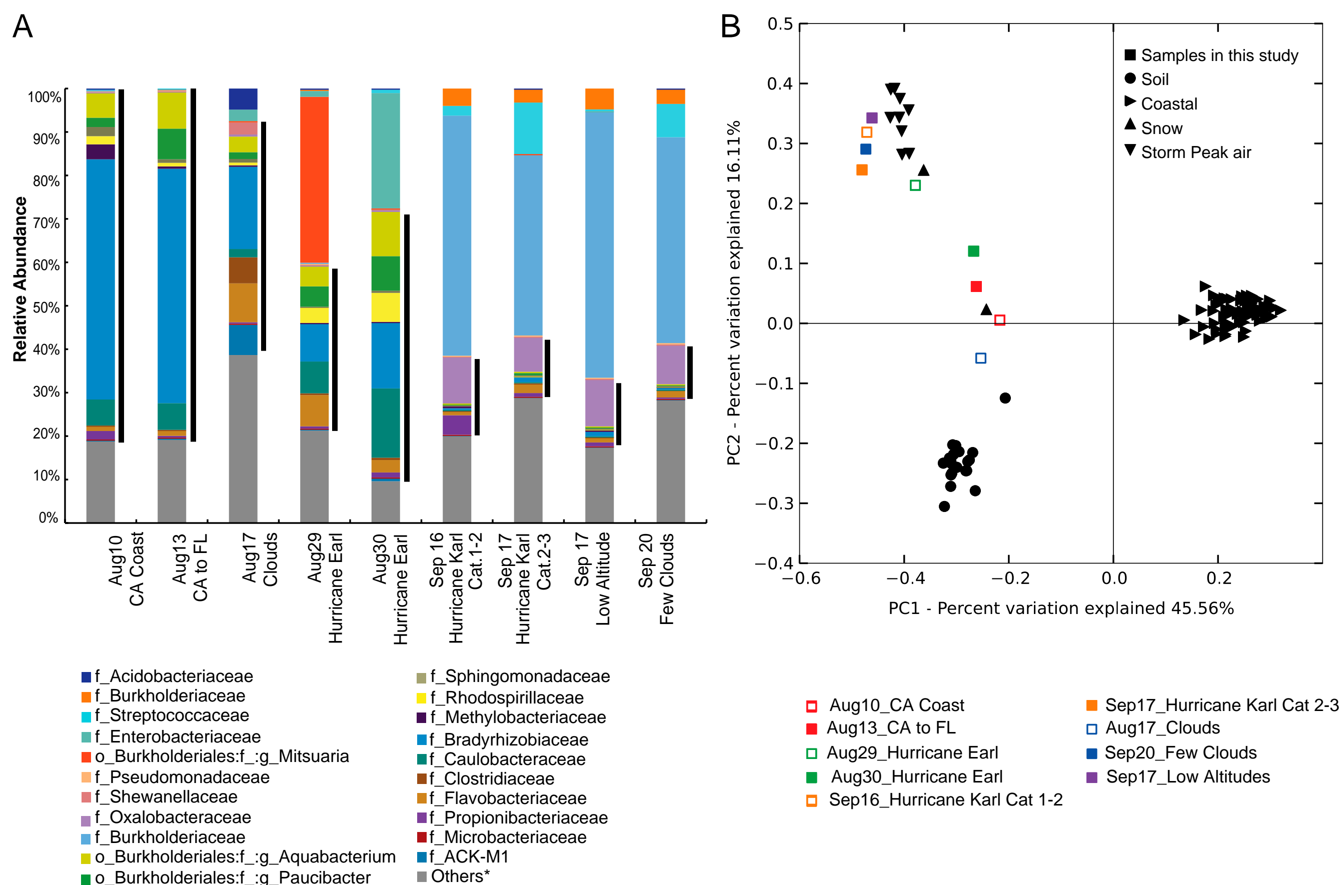

Fig. 2. Composition of tropospheric bacterial communities. (*A*) Relative abundance (*y* axis) of taxa (see key) represented by the partial SSU rRNA gene sequences recovered in each sample (*x* axis). Black vertical lines next to the bars underline the core OTUs that are present in all samples. "*Others" refers to low-abundance OTUs; for a complete list of the OTUs grouped under "*Others," refer to *SI Appendix*, Table S3. (*B*) Principal coordinates analysis based on the β-diversity values calculated by the weighted UniFrac distance metric of samples collected during the GRIP campaign and samples from previous studies (see key). Samples from this study are represented with open and closed squares and color-coded as follows: red, California/transit; blue, clouds; purple, low altitude; green, Hurricane Earl; orange, Hurricane Karl.

predictable processes or phenomena. For example, the California coast and Hurricane Earl samples were primarily characterized by oceanic transport, but we detected a relatively high abundance of freshwater and soil bacteria, respectively (Fig. 3). The former results are likely attributable to the long residence time of cells in the troposphere, longer than the 5-d period assessed by the back trajectory analysis, which is also consistent with the presence of several core OTUs in all GRIP samples. The presence of soil bacteria in Hurricane Earl samples is likely attributable to the strong winds from Africa, which preceded the period assessed by the back trajectory analysis and the development of the hurricane. These findings revealed that a "background" community was typically present in our samples and that back trajectory analysis can provide additional insight into the source region and long-range atmospheric transport of recently emitted bacteria, likely including disease-causing organisms.

Discussion

Our work shows that microbial cells, a majority of which appear to be bacterial, constitute an important component of the total super μm-sized particles in the mid/upper tropospheric air masses sampled (Fig. 1*A*; *SI Appendix*, Table S1). Based on the variety of samples analyzed, the substantial presence of bacteria cells represents a robust feature of the troposphere and not a transient effect of large storm systems. Most of the airborne cells are viable (Fig. 1*C*) and are large enough (0.25–1 μm, *SI Appendix*, Table S2) to support IN activity (25). Given also that many species (especially *Proteobacteria*) are known to be efficient nuclei for the formation of water droplets and ice crystals, and that middle-to-upper troposphere (e.g., low temperature) clouds can be affected by low-activity IN (26), airborne bacterial cells could influence cloud formation and precipitation more than previously thought. Furthermore, about 85% of the total SSU rRNA gene sequences that were recovered from the GRIP samples and were assignable to a habitat on Earth represented aquatic species (Fig. 3). This is consistent with previous studies showing that marine bacteria are more efficient CCN compared with bacteria from other ecosystems (e.g., soils) (27, 28). Assessing the CCN and IN potential of the cells in situ will provide for a more complete picture and allow parameterization of bacterial cell contribution to giant CCN and IN for use in cloud-resolving, regional, and global models. Our findings also reveal that tropical hurricanes aerosolize a large number of new cells and taxa, dramatically affecting the composition of the tropospheric communities for days after their passing (Fig. 2). Back trajectory analysis supported that air masses (and microbial cells transported with them) originated from lower altitudes and were brought aloft during the hurricanes. These findings indicate that long-range transcontinental transport of viable bacteria occurs, with potentially important implications for the biogeography of bacteria.

Our calculations also indicate that, at high altitudes, cells represent a higher fraction of the total particles than observed near the surface of the Earth. This enrichment of cells at high altitudes cannot be solely attributed to the limitations of our methods or the difficulties associated with sampling air masses high in the

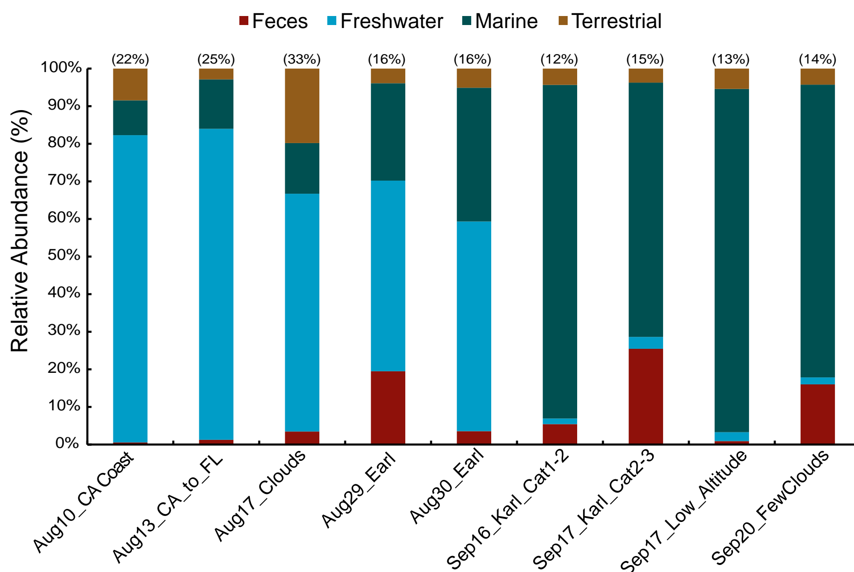

Fig. 3. Habitat of origin of the SSU rRNA gene sequences recovered in the GRIP samples. Sequences were assigned to a habitat (see key) based on the source of isolation of their best match in the GreenGenes database. The graph represents the relative abundance of each habitat (vertical axis) for each sample (*x* axis). Numbers on the top denote the fraction of sequences that were assignable to a habitat for each sample. For a similar analysis that normalized for the differential representation of habitats among the reference GreenGenes sequences, see *SI Appendix*, Fig. S6.

troposphere (discussed below). Other factors such as faster growth of abiotic particles as CCN and/or IN compared with cells and subsequent precipitation scavenging might be responsible for the patterns observed. It is also reasonable to hypothesize that different bacterial species show different CCN/IN efficiencies and, thus, at high altitudes, we have preferentially sampled species that are not as efficient CCN/IN; i.e., they are less affected by wet scavenging at lower altitudes. Consistent with this hypothesis, we found that *Gammaproteobacteria*, the most efficient IN bacteria known, did not make up a proportionally large part of the tropospheric communities (*SI Appendix*, Table S3) compared with the near-surface communities studied previously (9, 11, 12). Furthermore, model simulations have shown that CCN-active microorganisms have a shorter residence time in the atmosphere than those that are CCN-inactive (29). This hypothesis is still consistent with the idea that airborne microbial cells significantly influence cloud formation and precipitation at medium-to-high altitudes because the prevailing in situ conditions favor the nucleation of ice by even relatively inefficient IN particles. However, more observations and modeling studies are clearly required to support the above. Our findings do highlight that the life cycle of bioaerosols may differ from that of abiotic particles and underscore the need to better understand emissions, transport, and removal of bioaerosols.

The presence of 17 OTUs in all samples of our study (core microbiome), even those taken after the impact of the two hurricanes, implies that these OTUs represent organisms that have developed mechanisms—such as mechanisms to cope with UV, desiccation, and large concentrations of oxidants (OH, O_3, H_2O_2)—that have allowed them to survive long periods in the atmosphere. Previous literature indicates that this hypothesis may be true. For example, *Afipia* sp. was the most abundant core group in our samples, especially before the pass of the hurricanes. This group is commonly found in aquatic environments and is known to use dimethyl sulfone ($DMSO_2$) as a sole carbon source (30, 31). $DMSO_2$ represents an intermediate of the oxidation of dimethyl sulfide (DMS), which is commonly found in the marine atmosphere (32). Thus, *Afipia* sp. could potentially survive in clouds by using the available carbon compounds present in the atmosphere. Previous studies have also found members of the *Bradyrhizobiaceae* family (to which *Afipia* sp. is assigned) to compose around 10% of the total community in samples collected from cloud water (9), which is consistent with our findings that this group is ubiquitously present in the atmosphere. Similar to *Afipia* sp., *Oxalobacteraceae* and *Methylobacterium*, two other core groups, can also use C1–C4 compounds that are ubiquitously present in the atmosphere and concentrated (in the millimeter range) in cloud water.

The results presented here are largely consistent with existing knowledge but with a few notable exceptions. For example, Harrison et al. estimated the average bacterial cell concentration in near-surface air over coastal ecosystems to be 7.6×10^4 cells m^{-3} (33). Bacterial cell concentration in the cloud-free, high-altitude sample collected during the GRIP campaign based on our microscopy-based counts is about twofold lower (it is also likely that the real difference is even greater because the methods used in the previous study are more comparable to our qPCR than the microscopy methods). However, the previous study was conducted at sea level whereas GRIP samples were collected at 8 km or higher in the troposphere, and aerodynamic particle sizer analysis showed that coarse-mode particle concentration at these altitudes is at least an order of magnitude lower compared with at the Earth's surface (*SI Appendix*, Fig. S2). Principle Coordinates Analysis (PCoA) also revealed that the microbial communities in the GRIP samples were much more similar to those associated with air and snow collected at the top of high mountains compared with oceanic or soil communities (Fig. 2*B*). These results show that sampling air at the top of mountains or snow on the ground may reflect the medium-to-upper troposphere. Despite the similarities between ground and snow samples and the ones collected in this study, however, there are several striking and profound differences that suggest a very dynamic and diverse tropospheric microbiome that merits considerable more attention. For example, substantially fewer OTUs were found in this study compared with samples collected at 3.2 km at the top of Colorado mountains (9), indicating that not all microbial cells can apparently reach or survive high-altitude conditions. Furthermore, contrary to previous surveys of near-surface atmospheric samples (9, 11, 12), *Gammaproteobacteria* did not make up a proportionally large part of the tropospheric communities sampled. These findings might reflect the influence of atmospheric processes and transport of the bacterial cells because several members of *Gammaproteobacteria*, for example, are known to be CCN- and IN-active.

The high abundance (>40% of the total community) of a few OTUs—i.e., the *Afipia* sp. in the California coast and the transit flight samples and the *Burkholderiales* order in Hurricane Karl samples—was somewhat surprising because the conditions prevailing in the atmosphere are not expected to favor the high abundance or fast growth of a few individual species. Furthermore, back trajectory analysis was not always consistent with the high abundance of these OTUs. For example, the majority of sequences that made up the *Afipia* sp. OTU in the California coast sample best matched sequences of freshwater organisms in the GreenGenes database; yet, the air masses sampled in this case were influenced primarily by oceanic, not continental, transport.

ENVIRONMENTAL SCIENCES

Therefore, the high abundance of a few OTUs might be due, in part, to experimental artifacts and noise. For example, we (34) and others (35) have noted that amplification biases can affect the results of amplicon pyrosequencing. Due to these limitations, we also performed a large part of our analyses using OTU presence/absence as opposed to relative abundance (Fig. 3), which did not differentiate our conclusions substantially. Furthermore, the *Afipia* sp. and *Burkholderiales* OTUs were present in all samples (core OTUs), and previous studies have also frequently recovered these groups from air samples (9, 10). Thus, the qualitative trends in OTU distribution and relative abundance in different samples reported here should be robust.

It is technically challenging to achieve high sampling flow rates and filter large quantities of air at high altitudes. Our experimental design represented a compromise between obtaining high volumes of air and having a controlled, enclosed system to avoid contamination. The low biomass collected, and as a result the low DNA yields, imposed additional challenges for cell counting and molecular work (qPCR and SSU rRNA gene amplicon sequencing). Accordingly, at least some variation in the results obtained is presumably attributable to experimental noise. However, this noise was not high enough to confound our results as evidenced by several metrics, such as the at least one order of magnitude higher cell counts in field samples vs. blanks; the consistency between qPCR, SSU rRNA gene sequencing, and microscopy results; the high similarity in community composition among related samples [e.g., same hurricane (Fig. 2*A*)] but not among unrelated ones (different hurricanes); and several results that were consistent with expectations and/or previous literature (e.g., higher cell counts in low altitude and cloud samples compared with samples from high altitudes and no clouds, respectively).

The results presented here represent a culture-independent analysis of the microbial communities of the middle-to-upper troposphere above the oceans and advance our understanding of the composition of these communities and their shifts over time, space, and environmental perturbations caused by tropical storms or hurricanes. This information is important for modeling the dispersal of microbial diseases and for determining which microbes show limits (or no limits) in terms of dispersion through the atmosphere. Our results also indicate that airborne microbial cells may quantitatively be more important for cloud formation and precipitation than previously anticipated. Clearly, more attention should be given to these microbes and their role in the atmosphere compared with what has been accomplished to date. Microbes are known to actively contribute to, if not drive, the geochemistry in all habitats on Earth. The results reported here indicate that airborne microbes may have a similarly important role in the (bio)chemistry of the atmosphere and the hydrological cycle.

Materials and Methods

Details of all methods used in this study are described in *SI Appendix, SI Materials and Methods*. *SI Materials and Methods* include details on (*i*) sample collection; (*ii*) microscopy and molecular techniques including DNA extraction, qPCR protocols, and sequencing of SSU rRNA gene amplicons; and (*iii*) computational analyses and methodologies. Additional references provide information about procedures and analytical techniques.

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