Continental-scale distributions of dust-associated bacteria and fungi

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It has been known for centuries that microorganisms are ubiquitous in the atmosphere, where they are capable of long-distance dispersal. Likewise, it is well-established that these airborne bacteria and fungi can have myriad effects on human health, as well as the health of plants and livestock. However, we have a limited understanding of how these airborne communities vary across different geographic regions or the factors that structure the geographic patterns of near-surface microbes across large spatial scales. We collected dust samples from the external surfaces of ∼1,200 households located across the United States to understand the continental-scale distributions of bacteria and fungi in the near-surface atmosphere. The microbial communities were highly variable in composition across the United States, but the geographic patterns could be explained by climatic and soil variables, with coastal regions of the United States sharing similar airborne microbial communities. Although people living in more urbanized areas were not found to be exposed to distinct outdoor air microbial communities compared with those living in more rural areas, our results do suggest that urbanization leads to homogenization of the airborne microbiota, with more urban communities exhibiting less continental-scale geographic variability than more rural areas. These results provide our first insight into the continental-scale distributions of airborne microbes, which is information that could be used to identify likely associations between microbial exposures in outdoor air and incidences of disease in crops, livestock, and humans.

aerobiology | microbial ecology | microbial dispersal | urbanization | allergens

For nearly 2 centuries, we have known that microbes are ubiquitous in dust and outdoor air (1–3). In the near-surface atmosphere, microbial cells likely account for a significant fraction of aerosolized organic carbon (4), with microbial cell numbers typically ranging from 10^4 to 10^6 cells·m^{-3} over land (5). This means we inhale thousands of microbial cells every hour spent outdoors, and the potential effects of these airborne microbes on human health and the health of plants and animals are well recognized. As just one example, there are currently ∼16 million people living in the United States suffering from allergic asthma (6), and there has been considerable attention focused on understanding the airborne and dust-associated microbes influencing asthma and how those microbial triggers vary across space and time (6–9). In addition, the number of virulent fungal infections affecting human populations, wildlife, and plant crops is increasing (10, 11). The effects of those microbes capable of atmospheric transport can even extend to entire ecosystems: Microbes in Saharan dust clouds, for example, have been shown to affect the ecology of alpine lakes in Spain (12) and coral reefs in the Caribbean Sea (13).

Despite the well-recognized importance of airborne microbes and the long history of research on microbial transport through the atmosphere (3), we have only recently been able to describe the full extent of microbial diversity found in the atmosphere by using molecular approaches to characterize the microbial taxa that are difficult to identify via cultivation-dependent or microscopy-based surveys. Such molecular approaches not only have yielded new insight into the enormous diversity of airborne microbes (14, 15) but also have been instrumental in helping us understand how the composition of the airborne microbial communities varies across time and space (16, 17). However, nearly all of this work has focused on local-scale variability, examining the bacterial or fungal communities found in outdoor air at selected sites. What is missing is a continental-scale understanding of microbial diversity in the near-surface atmosphere and its deposition patterns over land. We do not know whether there are distinct microbial taxa found in the outdoor air from different geographic regions, nor do we know what biotic and abiotic factors may be driving the geographic patterns of dust-associated microbial communities across larger spatial scales. There is some evidence to suggest there are regional differences in exposures to specific bacterial and fungal taxa (18) that may be associated with geographic patterns in allergenic disease (19), but the continental-scale biogeographic patterns exhibited by the broad range of microbes that can be found in outdoor air remain undetermined.

Here we used dust samples collected from the external surfaces of ∼1,200 homes located across the continental United States to gain our first insight into the continental-scale patterns of bacterial and fungal diversity in the near-surface atmosphere

Significance

We inhale thousands of microbial cells when we breathe in outdoor air, and some of these airborne microbes can serve as pathogens or triggers of allergic disorders. Using settled dust samples from ∼1,200 locations, we generated the first atlas, to our knowledge, of airborne bacterial and fungal distributions across the continental United States. We found that airborne microbial communities, such as terrestrial plants and animals, exhibit nonrandom geographic patterns, and we identified the factors that shape the continental-scale distributions of microbial taxa. Furthermore, we found that the airborne microbes found in urban and more rural areas are not distinct in composition, but the dust-associated communities found in more urbanized areas are more homogeneous across the United States.

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and the factors driving the distributions of these airborne microbial taxa. Specifically, we investigated two questions: First, to what extent are the diversity and types of bacteria and fungi found in settled dust collected from outside homes a function of geographic location, climatic variables, soil characteristics, crop production, and land use type? Second, we sought to determine whether urbanization has a significant effect on bacterial and fungal exposures, given that it has long been assumed that airborne microbial exposures differ across urban and rural areas (3), with recent work suggesting that these differential microbial exposures may be one explanation for the higher rates of allergic disorders often observed in more urbanized areas (7, 8).

Results and Discussion

Microbial Diversity of Dust Samples. Our results stand in contrast to culture-based surveys, which typically show that only a handful of bacterial or fungal taxa dominate most air and settled dust samples (e.g., refs. 20, 21). The average dust sample harbored ∼4,700 and ∼1,400 bacterial and fungal phylotypes, respectively, of the total of ∼112,000 and ∼57,000 bacterial and fungal phylotypes observed across all samples (*SI Appendix*, Fig. S3). The number of different bacterial or fungal phylotypes (richness) found in each sample (both correlated; Pearson's $r = 0.69$; $P < 0.001$) did not vary in any consistent manner with geographic distance [$r_M = 0.03$ ($P = 0.03$); $r_M = 0.02$ ($P = 0.03$); Mantel tests for bacterial and fungal richness differences, respectively]. Although previous culture-independent studies have shown high levels of bacterial diversity in dust samples comparable to what was observed here (12, 14, 22), we also found an equally impressive amount of fungal diversity in the collected dust samples, with 74% of these fungal taxa being undescribed (i.e., not represented in the reference sequence database).

Dust samples were dominated by Proteobacteria of the class Alphaproteobacteria (*SI Appendix*, Fig. S4) and Ascomycota from the orders Capnodiales and Pleosporales (*SI Appendix*, Fig. S5). Most phylotypes were restricted to a small subset of samples; 88% and 94% of the bacterial and fungal phylotypes, respectively, were detected in only 10% of the collected samples. Only 0.03% and 0.02% of the bacterial and fungal phylotypes were found in more than 90% of the samples, and these ubiquitous and abundant phylotypes belonged to the genera *Sphingomonas* and *Hymenobacter* for bacteria and to *Cladosporium*, *Toxicocladosporium*, and *Alternaria* for fungi (*SI Appendix*, Fig. S6); these are taxa that are commonly found in the atmosphere (12, 14, 15, 21). In other words, although most people are likely exposed to these common taxa when breathing outdoor air, the aerosolized bacteria and fungi are highly variable in composition across the continental United States (see variability in *SI Appendix*, Figs. S4 and S5). Given that many bacteria and fungi are capable of long-distance dispersal through the atmosphere (5, 10, 13), one might expect greater homogeneity in the composition of the bacterial and fungal communities found in outdoor air. This is clearly not the case.

Factors Structuring Community Similarity Patterns. The bacterial and fungal communities were highly variable in composition across the collected samples (*SI Appendix*, Figs. S2 and S3), and this variability was predictable. Community similarity patterns between bacteria and fungi were correlated ($r_M = 0.52$; $P < 0.01$; Mantel test), suggesting similar factors structure these communities in the dust settled on the outside surface of homes across the United States. For both groups of microorganisms, community similarity was related to the geographic location of the sample (in contrast to the pattern for richness), although this relationship was weaker for bacteria. Sites that were located closer in proximity tended to share more similar microbial communities than sites located further apart [$r_M = 0.13$ ($P < 0.01$); $r_M = 0.29$ ($P < 0.01$); Mantel test for bacterial and fungal communities, respectively]. The effects of geographic distance were most obvious at local to regional scales. At sites >750 km apart, there was no significant geographic structure in community similarity (*SI Appendix*, Fig. S7 and Fig. 1), as evidenced by the samples collected from coastal regions in the eastern and western United States sharing similar communities (Fig. 1). Although marine bacteria are capable of being aerosolized (23), this bicoastal pattern is not a result of the presence of marine-associated microbes, as their geographic distribution was patchy, with such

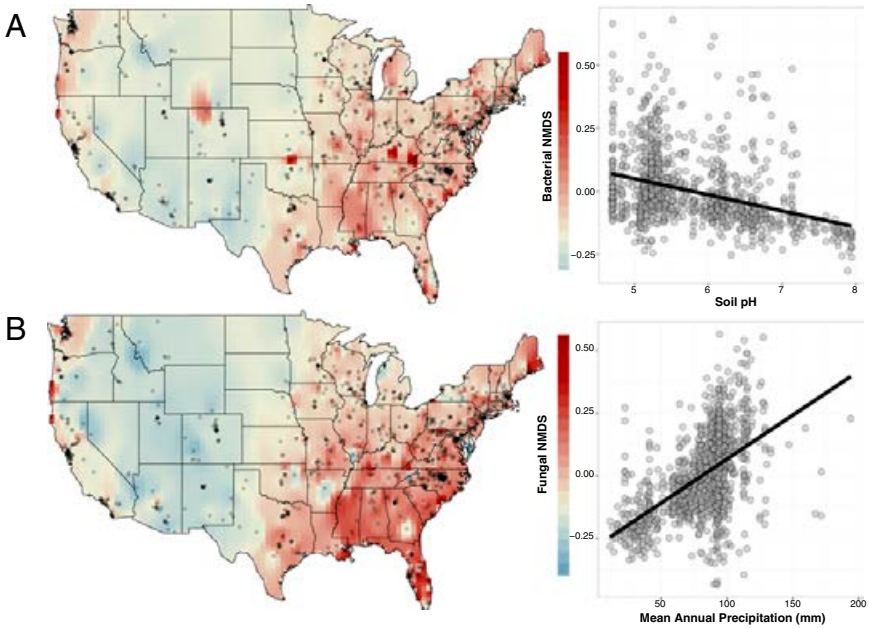

Fig. 1. Maps of overall community similarity (nonmetric multidimensional scaling axis scores) and the relationships between community similarity and environmental parameters for (*A*) bacterial communities ($r = -0.40$; $P < 0.001$; soil pH) and (*B*) fungal communities ($r = 0.56$; $P < 0.001$; mean annual precipitation). Similar colors indicate bacterial or fungal communities that are more similar in overall community composition. Points represent sampling locations.

microbes being rare and only present in a handful of samples (*SI Appendix*, Fig. S8 and Table S2). As some of these marine bacterial taxa are also common in many freshwater environments (e.g., *Prochlorococcus*, *Synechococcus*), their presence in locations far from oceans also may be a result of aerosolization of bacteria from lakes or other nearby water bodies.

We then determined what factors are correlated with the observed similarity patterns in community composition (Fig. 1). Previous regional-scale studies have identified vegetation, land-use characteristics (17, 24), and climatic conditions (14) as factors influencing airborne microbial communities. Others factors such as human population and livestock density or house characteristics were included as additional candidate explanatory variables in this study. A list of these variables is provided in *SI Appendix*, Table S1. Our results, using multiple regression on distance matrices (MRM), indicated that the composition of the dust-associated microbial communities was significantly correlated with a combination of soil and climatic variables. The best predictors of bacterial community composition, in order of importance, were soil pH, mean annual precipitation, net primary productivity, and mean annual temperature [overall correlation with community similarity: $r_M = 0.25$ ($P < 0.01$), Mantel test; MRM: $R^2 = 0.06$ ($P < 0.001$); see Fig. 1*A* for the relationship between the main bacterial ordination axis and soil pH]. Similarly, the best predictors of fungal community composition were, in order of importance, mean annual precipitation, soil pH, net primary productivity, the diversity of vascular plants, and mean annual temperature [overall correlation with community similarity: $r_M = 0.38$ ($P < 0.01$), Mantel test; MRM: $R^2 = 0.15$ ($P < 0.001$); see Fig. 1*B* for the relationship between the main fungal ordination axis and mean annual precipitation]. As climate, soil characteristics, and plant productivity are tightly linked, we cannot assign unequivocal cause and effects relationships. That is, we do not know whether the observed patterns in fungal community structure are driven directly by climate or by differences in plant productivity (which will be approximately collinear with climate at the continental-scale of inquiry). The same caveat applies to the apparent effects of soil characteristics and climate on bacterial community structure.

The environmental associations were also evident when we looked at the geographic distributions of individual taxa. For example, the strong influence of soil characteristics in shaping dust bacterial community composition is consistent with the distributions of *Cellulomonas*, one of the more abundant actinobacterial genera (Fig. 2*A*; Pearson's correlation with soil pH: $r = 0.47$; $P < 0.001$), and *Terriglobus*, the most abundant genus of Acidobacteria (Fig. 2*B*; Pearson's correlation with soil pH: $r = -0.31$; $P < 0.001$). These patterns agree with previous work showing that soil bacterial communities are strongly structured by pH (25), with Acidobacteria dominating in low-pH soils and actinobacterial taxa being relatively more abundant in high-pH soils such as those found in deserts (26, 27). Many fungal taxa also exhibited distinct geographic patterns. Most notably, the genus *Alternaria*, which contains species that are well-known triggers of allergenic disease (28) and is commonly found in both indoor and outdoor dust samples (15), was highest in abundance in samples collected from the Great Plains region of the United States, with relative abundances that often exceeded 50% of the fungal sequences from individual samples (Fig. 2*C*). Likewise, we found that the genus *Cladosporium*, the most abundant genus in our samples (often representing >75% of all fungal sequences from individual samples) and one of the more common fungal groups identified in other studies of airborne and dust-associated fungi (e.g., refs. 21, 29), was dominant in humid areas with low-pH soils (Fig. 2*D*). This biogeographic pattern is noteworthy, given that *Cladosporium* is a common allergen and members of

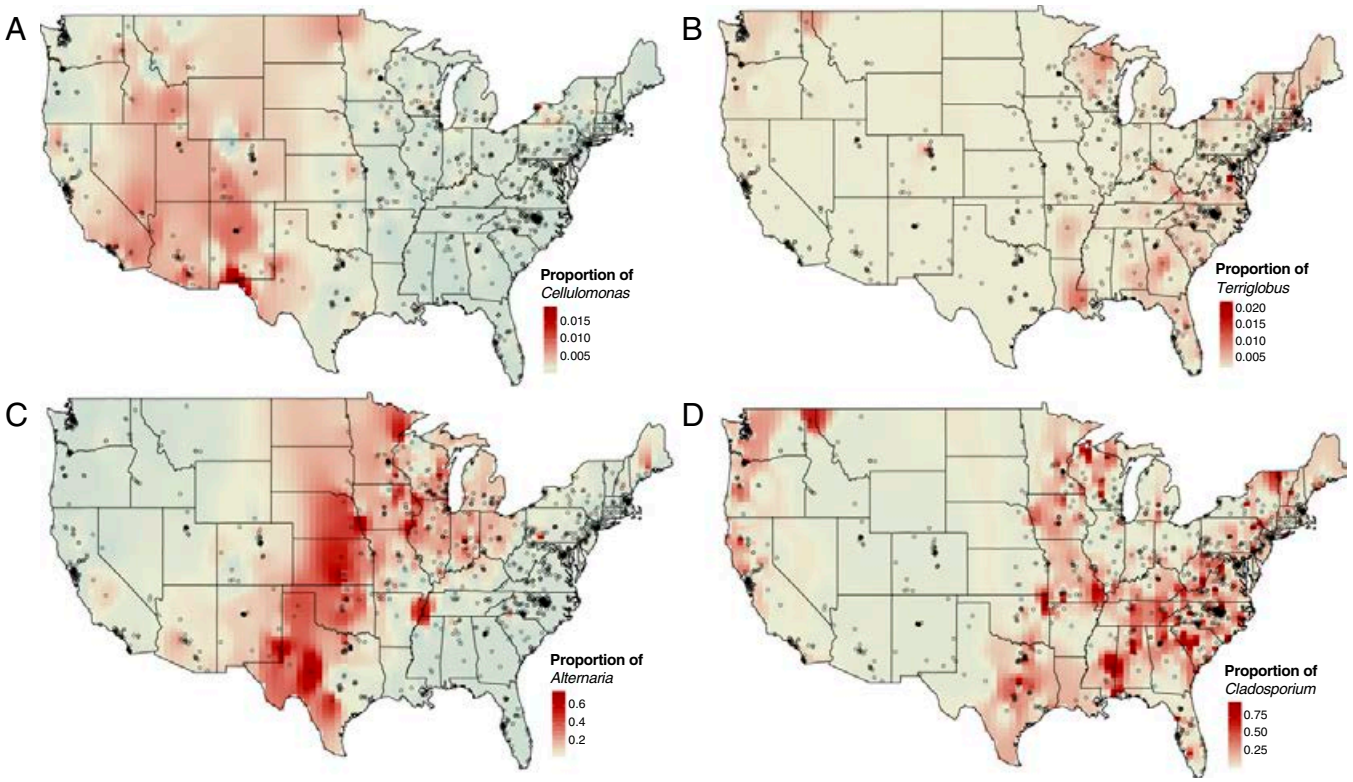

Fig. 2. Maps showing the relative abundance of specific bacterial and fungal genera across the continental United States: (*A*) *Cellulomonas*, (*B*) *Terriglobus*, (*C*) *Alternaria*, and (*D*) *Cladosporium*. Warmer colors indicate higher relative abundances. Points represent sampling locations.

this genus include major plant pathogens. Together, these results highlight that many microbial taxa exhibit local- and regional-scale geographic distribution patterns and that we can build range maps of bacterial and fungal taxa comparable to the maps long built by plant and animal ecologists to quantify biogeographic patterns and predict responses to climate change (30).

Our results indicate that the microbial communities found in outdoor air exhibit local and regional-scale geographic patterns, even though airborne microbes are known to disperse many thousands of kilometers (10, 12, 13, 31) and we might expect the complexities of wind patterns in the near-surface atmosphere to obscure the biogeographic distributions of microbes in settled dust. Some of the geographic patterns in airborne microbial communities could be driven by differences in dispersal capabilities. Not all taxa are equally adept at dispersal and survival on the sampled house surfaces, and this variation in the abilities of microbes to disperse to a given location and persist at that location could be an important mechanism generating the biogeographic patterns observed here (32). Likewise, it is important to mention that many local-scale factors that were not effectively captured with our data or statistical models could be responsible for generating some of the observed biogeographic patterns. For example, the types of vegetation found neighboring the homes or other local-scale sources of microbes to the atmosphere (e.g., construction sites, dirt roads) may be influencing the structure of these dust-associated communities at smaller spatial scales.

Effect of Urbanization. Urbanization tends to homogenize plant and animal communities, with more urbanized areas tending to share a large number of plant and animal taxa in common (33). At the same time, urbanization promotes the establishment of nonnative plant and animal species, and thus, cities can often have higher species richness than surrounding rural areas (e.g., refs. 34, 35). We do not know how urbanization affects airborne microbial communities, with some studies suggesting microbial diversity should be lower in more urbanized areas (8) and other studies showing little to no effect of urbanization on microbial diversity (17). This question is important, given that rates of allergenic asthma are higher for the children living in more urbanized areas (reviewed in ref. 9). Although many factors contribute to allergenic disease (19), it has been hypothesized that these geographic differences in allergy rates can be attributed to people living in more urbanized areas being exposed to lower levels of microbial diversity (7, 8). However, at least outdoors, we found no significant effect of urbanization on bacterial or fungal diversity levels ($P > 0.05$, Mann-Whitney test; *SI Appendix*, Fig. S9). Likewise, we found that more urbanized sites did not have microbial communities compositionally distinct from those found in less-urbanized or rural locations ($R^2 < 0.005$ for both bacterial and fungal communities, permutational analysis of variance (PERMANOVA); *SI Appendix*, Fig. S9). We also did not observe any effect of urbanization on bacterial or fungal community composition when we treated urbanization as a quantitative variable (i.e., population density, $R^2 < 0.005$, PERMANOVA), rather than a qualitative one (i.e., urban and rural classification).

Although we observed no significant effects of urbanization on the overall diversity and composition of dust-associated microbial communities, we expected the specific sources of airborne microbes to vary across urban to rural gradients (24). To test this hypothesis, we identified specific bacterial taxa indicative of specific source environments from which dust-associated microbes are most likely to originate (*SI Appendix*, Table S2), including soil, marine waters, feces and skin of humans or other animals, or plants. On the basis of these source assignments, we could then test how urbanization affects the relative importance of specific sources of bacteria to the atmosphere. For example, we would expect fecal bacteria to be more abundant in rural areas close to high densities of livestock (24, 36), and skin-associated microbes to be more common in cities and populated areas. However, we did not detect any strong or significant effect of urbanization on the proportion of different bacterial indicator taxa (no difference greater than 1% or $P < 0.05$, Mann-Whitney test; Fig. 3). Overall, bacterial communities in the dust samples were primarily derived from bacteria likely to be found on plants and soil, regardless of the degree of urbanization (Fig. 3), a finding in line with other work showing that these sources are the dominant contributors of bacteria to the near-surface atmosphere in most terrestrial environments (13, 15). The imprint of human-associated bacteria was low and primarily a result of skin, rather than stool (with the exception of a single rural sample that was dominated by stool indicator taxa, mainly *Bacteroides* and *Parabacteroides*; Fig. 3). Neither marine waters (and potentially other water bodies including lakes or rivers) nor insects appeared to be important sources of bacteria of the atmosphere (Fig. 3). In the case of the few samples (particularly from urban areas) in which we observed high abundances of bacteria indicative of insects (notably *Rickettsiella*, Entomoplasmatales, *Buchnera*, and *Wolbachia*), these extreme values might be a result of the presence of dead insects (or fragments/depositions of insects) on the outdoor frame of those homes. Although we can demonstrate the importance of plants and soils as sources of microbes in settled dust, our data suggest that people living in more rural areas are not exposed outdoors to bacteria derived from different source environments than those living in more urbanized areas.

However, in line with the patterns commonly observed for plant and animal communities (33), the bacterial and fungal communities found in the dust from more urbanized locations tended to be more homogeneous. In other words, the microbial communities found in cities were not as spatially variable across the continental United States as in those communities found in more rural areas (Fig. 4). People living in more urbanized areas are not likely exposed to different airborne bacteria and fungi outdoors than those living in more rural areas, but urban areas tend to harbor microbial communities that are less spatially

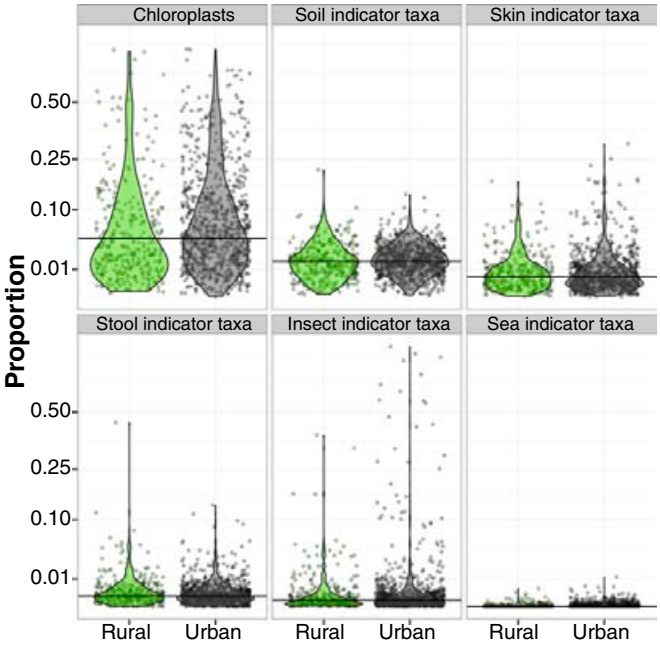

Fig. 3. Proportion of bacterial sequences identified as indicator taxa of potential source environments in urban and rural samples. Chloroplasts were used as indicators of plant influence. Source environments are ordered from left to right and top to bottom according to their median value (medians are shown as horizontal lines). Note that because of the highly skewed distribution, the *y* axis is squared.

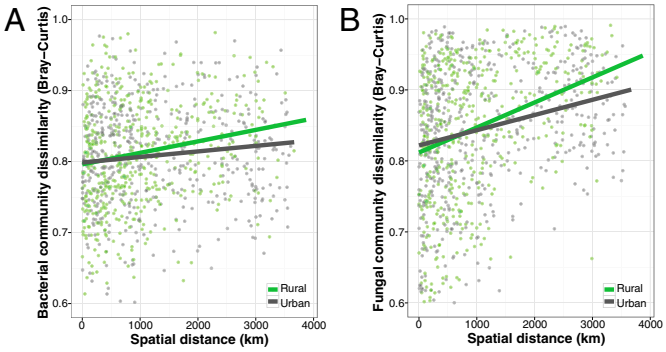

Fig. 4. Relationship between community dissimilarity and spatial distance for (*A*) bacterial and (*B*) fungal urban and rural samples. The slopes were significantly different in both cases (bacteria urban = $0.9{\cdot}10^{-5} \pm 1.1{\cdot}10^{-7}$; bacteria rural = $1.7{\cdot}10^{-5} \pm 3.9{\cdot}10^{-7}$; fungi urban = $2.6{\cdot}10^{-5} \pm 1.3{\cdot}10^{-7}$; fungi rural = $4.1{\cdot}10^{-5} \pm 4.4{\cdot}10^{-7}$).

variable than those found in more rural areas. This observed homogenization of dust-associated microbial communities might be a result of a homogenization of environmental conditions and land-use types in urban areas (33) or the homogenization in plant communities or other potential biotic sources of bacteria and fungi to the atmosphere.

Methods

Sample Collection. Outdoor dust samples were collected by volunteers participating in the Wild Life of Our Homes project (homes.yourwildlife.org), a continental-scale citizen science project. We recruited participants from all 50 states and the District of Columbia through our website, social media, and email campaigns over the period from January 2012 to March 2013. Enrolled participants (n = 1,430) were provided a written informed consent form approved by the North Carolina State University's Human Research Committee (approval no. 2177), as well as instructions for sampling and a microbe sampling kit containing dual-tipped sterile BBL CultureSwabs. Here we focus on a subset of samples that participants collected from the upper door trim on the outside surface of an exterior door, a sampling location that is found in every home, is unlikely to be cleaned frequently, and serves as a passive collector of outdoor aerosols and dust with little to no direct contact from the home occupants. The amount of time dust has accumulated on these surfaces is unknown and likely variable across homes, so we cannot use this sample set to assess temporal variation in the microbial communities. Participants returned swabs by first-class mail over the period from March 2012 to May 2013, and these swabs were stored in a −20 °C freezer until processed. A map of sampling locations and information on the number of samples collected per US state are provided in *SI Appendix*, Fig. S1.

Molecular Analyses. Swabs were prepared for sequencing using the direct PCR approach described previously (37). Swab tips were placed directly into 2-mL 96-well plates (Axygen Inc.), along with the appropriate negative control samples. Plates were processed using the Extract-*N*-Amp PCR kit (Sigma-Aldrich, Inc.), following a modified version of the manufacturers' instructions. After each well received 250 μL of the Extract-*N*-Amp Extraction solution, the plate was sealed securely with a 96-round well Impermamat Silicon Sealing Mat (Axygen, Inc.) and heated at 90 °C for 10 min in a dry bath. Extract-*N*-Amp Dilution solution was then added to the wells at a 1:1 ratio to the extraction solution and mixed gently by pipetting. The plate was resealed with the mat and stored at 4 °C. PCR was conducted in 20-μL triplicate reactions per sample, using 10 μL Extract-*N*-Amp Ready Mix, 1 μL of the forward and reverse primers, 5 μL PCR-grade water, and 4 μL of the Extract-*N*-Amp sample solutions from the 96-well plate.

Microbial diversity was assessed using high-throughput sequencing methods to characterize the variation in marker gene sequences. For bacterial and archaeal analyses, we sequenced the V4 hypervariable region of the 16S rRNA gene using the 515-F (GTGCCAGCMGCCGCGGTAA) and 806-R (GGACTACHVGGGTWTCTAAT) primer pair (26). For the fungal analyses, we sequenced the first internal transcribed spacer (ITS1) region of the rRNA operon, using the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) primer pair (38). The primers included the appropriate Illumina adapters, with the reverse primers also having an error-correcting 12-bp barcode unique to each sample to permit multiplexing of samples. Archaeal sequences represented a very small proportion of the recovered sequences (0.13% of the 16S rRNA gene sequences). PCR products from all samples were quantified using the PicoGreen dsDNA assay and pooled together in equimolar concentrations for sequencing on either an Illumina HiSeq or MiSeq instrument. All sequencing runs were completed at the University of Colorado Next Generation Sequencing Facility.

Sequence Processing. The 100-bp sequences were demultiplexed using a custom Python script (https://github.com/leffj/helper-code-for-uparse), with quality filtering and phylotype clustering conducted using the UPARSE pipeline (39). For quality filtering, we used a maximum e-value of 0.5 (indicating that on average, a maximum of 0.5 nucleotides were incorrectly assigned in every sequence). Sequences were also dereplicated, and singleton sequences were removed before phylotype determinations. Representative sequences from the phylotypes that were not ≥75% similar to sequences contained in either the Greengenes 13_8 database (40) or the UNITE May 2014 database (41) for 16S and ITS rRNA sequences, respectively, were discarded. Raw sequences were then mapped to phylotypes at the 97% similarity threshold. Phylotype taxonomy was determined using the Ribosomal Database Project classifier with a confidence threshold of 0.5 (42), trained on the respective databases for 16S and ITS rRNA sequence. 16S rRNA sequences resulting from direct PCR reagent contaminants (37) were filtered from all samples by removing those classified as *Mycoplasma*, *Pseudomonas*, and *Serratia*, which represented a median 59%, 9%, and 4% of sequences across the controls, respectively. In addition, sequences representing any phylotypes present in ≥25% of control samples and those classified as mitochondria or chloroplast were removed (except during analysis of plant presence described here).

To remove potential amplicon sequencing biases, we first removed samples with fewer than 10,000 sequences, and then we normalized the sequence counts using a cumulative-sum scaling, which scales counts by dividing up to a percentile empirically determined (43). Richness and community similarity values after normalization were highly correlated, with values obtained after rarefying to 10,000 sequences per sample (*SI Appendix*, Fig. S2). The total number of samples included in downstream analyses was 1,187 for bacteria and 1,289 for fungi.

Sample Characterization. Latitude and longitude coordinates were derived from location information (address), and the coordinates were used to obtain georeferenced variables for each household. We compiled a set of 31 descriptors: elevation, distance to the coast, climatic variables, plant productivity, soil factors, other organisms' (including humans and livestock) variables, and house characteristics (*SI Appendix*, Table S1). Urban and rural classifications for all sampled locations were obtained from the 2010 US Census Bureau data (www.census.gov). Urban areas are classified as densely developed (>50,000 people) residential and commercial territories; 28% of the sampled locations were classified as rural.

Identification of Bacterial Source Indicators. To identify potential taxa indicative of specific source environments (i.e., soil, oceans, human skin, and human/livestock feces), we used Indicator Value analyses (44), as described in ref. 22. We used the proportion of chloroplast sequences in each sample as a proxy for plant presence. To identify those bacteria likely derived from insects, we compiled a list of exclusive symbiont and/or insect pathogens from the literature (45, 46). For the complete list of bacterial indicator taxa, see *SI Appendix*, Table S2.

Statistical Analyses. Microbial community similarity was represented by nonmetric multidimensional scaling, using the Bray-Curtis dissimilarity distance metric after Hellinger standardization (47). Bray-Curtis abundance-based dissimilarity matrices were highly correlated with Jaccard incidence-based matrices [$r_M = 0.86$ ($P < 0.001$); $r_M = 0.92$ ($P < 0.001$); Mantel test for bacteria and fungi, respectively]. As some of the descriptors were correlated, we reduced collinearity before our analyses by identifying highly correlated variables by principal component analyses and by examining the variance inflation factor (48). The final set of factors consisted of 18 variables (*SI Appendix*, Table S1). To find the best subset of explanatory variables, we first ran bioenv (49) to maximize the correlation with community dissimilarity. We then performed MRM to estimate the overall explanatory power of the model (50). To test whether community composition was different between urban and rural areas, we used PERMANOVA based on 1,000 permutations (51).

All multivariate statistical analyses were carried out in the R environment (www.r-project.org), using the vegan (vegan.r-forge.r-project.org/), labdsv (ecology.msu.montana.edu/labdsv/R/), and ecodist (cran.r-project.org/web/

packages/ecodist/) packages. Nonmetric multidimensional scaling axis scores and the proportional abundances of taxa were mapped by inverse distance weighting interpolation on 100 × 100 grid cells, using the gstat package (https://r-forge.r-project.org/projects/gstat/).

Conclusion

We show that the continental-scale distributions of bacteria and fungi in settled dust are correlated with regional environmental factors. These geographic patterns are surprisingly robust, considering the potential for long-distance dispersal of microbes in aerosolized dust particles, the complexity of wind patterns in the near-surface atmosphere, and the difficulties associated with quantifying specific local-scale factors that may influence the types of bacteria and fungi that can be introduced into the atmosphere.

The health effects of this work remain unknown. We do know that bacterial and fungal exposures are distinct across the United States, and some of this variability can be explained by environmental conditions; namely, climatic and soil characteristics. We also know that allergen sensitivities and incidences of asthma, including allergen sensitivities to particular microbial triggers, can vary across the United States (28). Our study was not designed to collect specific allergen sensitivity data for the individuals living in each home, but clearly there are opportunities for future work investigating how the diversity, distribution, and composition of these dust-associated bacteria and fungi may shape incidences of disease across the United States.

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