Microorganisms are abundant in the upper atmosphere, particularly downwind of arid regions, where winds can mobilize large amounts of topsoil and dust. However, the challenge of collecting samples from the upper atmosphere and reliance upon culture-based characterization methods have prevented a comprehensive understanding of globally dispersed airborne microbes. In spring 2011 at the Mt. Bachelor Observatory in North America (2.8 km above sea level), we captured enough microbial biomass in two transpacific air plumes to permit a microarray analysis using 16S rRNA genes. Thousands of distinct bacterial taxa spanning a wide range of phyla and surface environments were detected before, during, and after each Asian long-range transport event. Interestingly, the transpacific plumes delivered higher concentrations of taxa already in the background air (particularly Proteobacteria, Actinobacteria, and Firmicutes). While some bacterial families and a few marine archaea appeared for the first and only time during the plumes, the microbial community compositions were similar, despite the unique transport histories of the air masses. It seems plausible, when coupled with atmospheric modeling and chemical analysis, that microbial biogeography can be used to pinpoint the source of intercontinental dust plumes. Given the degree of richness measured in our study, the overall contribution of Asian aerosols to microbial species in North American air warrants additional investigation.

MATERIALS AND METHODS

Sample collection. Microbes were collected on sterile polysulfone filters (pore size, 0.8 μm) connected to a previously described air-sampling device at MBO (13). Briefly, a high-volume pump pulled ~0.5 m3 min⁻¹ of air through individual filters over 12-h intervals, and then samples were removed from the device and stored at ~80°C. During sampling periods, meteorological and atmospheric chemistry data were collected for aerosol elemental composition (e.g., ammonium sulfate [NH₄SO₄], soil, and trace metals), carbon monoxide (CO), ozone (O₃), water (H₂O) vapor, total gaseous mercury (THg), temperature, atmospheric pressure, wind speed, and direction. Details of MBO instruments, calibrations, and element concentration calculations have been published elsewhere (4, 10, 13).

Atmospheric modeling. We calculated 240-h backward trajectories initialized from MBO during peak aerosol periods to establish the long-range transport history of arriving air masses. Trajectories were calculated with the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYPLIT) model (14), version 4, which uses global meteorological data from the Global Data Assimilation System archive. The data set has a time resolution of 3 h, a spatial resolution of 1° latitude by 1° longitude, and a vertical resolution of 23 pressure surfaces between 1,000 and 20 hPa. Trajectories were used to reexamine the air samples with a more comprehensive molecular tool testing the hypothesis that transpacific plumes deliver rich microbial populations to North America.
Transoceanic aerosol plumes were also modeled with the Navy Research Laboratory Aerosol Analysis and Prediction System (NAAPS; http://www.nrlmry.navy.mil/aerosol/) to compare them with HYSPLIT, version 4, long-range transport patterns. The NAAPS model produces a total aerosol forecast at an optical depth of 350 nm that includes sulfate, smoke, dust, and sea salt mass concentrations. We examined air samples from the periods from 15 to 25 April 2011 and from 7 to 17 May 2011.

DNA extraction and PCR amplification. To extract DNA, samples were processed with MO BIO PowerWater (PW) kits (product 14900-0.5% divergence). In total, there were 59,959 potential clusters spanning 2 domains (archaea and bacteria), 147 phyla, 1,123 classes, and 1,219 orders. Each OTU was assigned to 1 of 1,464 families (19). Experimentally, hybridization took place for 16 h in an oven at 48°C and 60 rpm before the PhyloChip array was washed, stained, and scanned using a GeneChip 3000 7G scanner (Affymetrix, Santa Clara, CA). Fluorescence intensity was captured using Affymetrix software (GeneChip Microarray Analysis Suite) and calculated using preestablished formulas (17).

Table 1. April plume sample manifest

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date (mo/day/yr) and time (UTC)</th>
<th>Category</th>
<th>Avg aerosol level (µg m⁻³)</th>
<th>DNA concn (ng µl⁻¹)</th>
<th>PCR yield (ng)</th>
<th>No. of OTUs detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abk142</td>
<td>4/21/2011 20:00 to 4/22/2011 08:00</td>
<td>Background</td>
<td>1.56</td>
<td>2.2</td>
<td>1,394</td>
<td>2,325</td>
</tr>
<tr>
<td>Adu143</td>
<td>4/22/2011 08:00 to 4/22/2011 19:00</td>
<td>Plume</td>
<td>23.3</td>
<td>15.3</td>
<td>1,442</td>
<td>2,197</td>
</tr>
<tr>
<td>Adu144</td>
<td>4/22/2011 19:00 to 4/23/2011 07:00</td>
<td>Plume</td>
<td>10.3</td>
<td>1.5</td>
<td>1,328</td>
<td>2,808</td>
</tr>
<tr>
<td>Adu145</td>
<td>4/23/2011 07:00 to 4/23/2011 19:00</td>
<td>Plume</td>
<td>10.7</td>
<td>1.7</td>
<td>1,347</td>
<td>2,620</td>
</tr>
<tr>
<td>Adu146</td>
<td>4/23/2011 19:00 to 4/24/2011 07:00</td>
<td>Plume</td>
<td>10.2</td>
<td>1.8</td>
<td>1,330</td>
<td>2,742</td>
</tr>
<tr>
<td>Abk147</td>
<td>4/24/2011 07:00 to 4/24/2011 19:00</td>
<td>Background</td>
<td>0</td>
<td>2.0</td>
<td>991</td>
<td>2,114</td>
</tr>
</tbody>
</table>
Microarray data accession number. The entire microarray data set with OTU annotations can be accessed at the Greengenes database (http://greengenes.secondgenome.com/downloads/phylochip_datasets).

RESULTS AND DISCUSSION

First, it was essential to establish possible source regions and transport histories for the events of interest using meteorological and chemical data. Kinematic back trajectories were modeled for each episode with HYSPLIT, revealing key differences in origin, mixing, and vertical transport. Ten-day back trajectories for the April event began near China, the Korean Peninsula, or Japan and showed the air mass rapidly lifting to an altitude of ~8 km (Fig. 1a). Low humidity and high O₃ indicate that the pollution plume may have mixed with air from the upper troposphere/lower stratosphere during transport (13). Airborne THg and CO can be used as tracers for ALRT (4), and enhancement ratios between the species (see Fig. S1 in the supplemental material) in the April plume were consistent with previously identified Asian pollution events at the collection site (10). Soil and NH₄SO₄ concentrations were also correlated (see Fig. S2 in the supplemental material), indicating a similar origin and an airborne time sufficient for homogenization (22). The NAAPS model provided another check for ALRT by depicting the transpacific migration of airborne sulfate, dust, smoke, and sea salt at a total aerosol optical depth of 0.1 to 0.2 (see Movie S1 in the supplemental material). For the May episode, 10-day HYSPLIT back trajectories show an air mass originating over the Pacific Ocean and mixing into the marine boundary layer (Fig. 1b). According to the model, the air was swept through a storm loop off the coast of Alaska before ascending into the free troposphere and MBO. Depleted levels of THg/CO (see Fig. S1 in the supplemental material) and NH₄SO₄/soil (see Fig. S3 in the supplemental material) align with the possibility of a boundary layer excursion. Compared to what was observed in the April episode, the May NAAPS data (see Movie S2 in the supplemental material) show denser emissions in Asia and a larger transpacific plume.

Air samples (spanning 12-h intervals) from before, during, and after ALRT events were analyzed for microbial richness and abundance using a PhyloChip 16S rRNA microarray (3, 15–18). Tables 1 and 2 provide the aerosol level, microbial concentration (ng DNA), and community richness (number of OTUs) for each sampling interval and category (plume or background). Overall, 2,808 bacterial OTUs were detected at the peak of the April episode (694 above background levels); 2,864 bacterial OTUs were detected at the peak of the May episode (629 above background levels). On the basis of incidence data alone, bacterial richness was highest during plumes and lowest before and after plumes (i.e., from background air). Bacterial taxa spanned a broad range of phyla, including Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes. Six species of archaea were also measured in 8 samples (see Table S1 in the supplemental material).

 Principle coordinate analysis (PCoA) was used to measure dissimilarity in ALRT plumes. Each point on the ordination plots represents an entire microbial community sample. Figure 2 assembled incidence data from 38,546 possible taxa and partitioned the samples into two distinct categories: plume and background. Significant clusters (P = 0.032) emerged with (i) April and May background samples and (ii) April and May plume samples, supporting the idea that ALRT events were more similar than different. Comparable clustering was observed (P = 0.044) in a follow-up analysis using abundance data from 2,514 possible taxa (see Fig. S4 in the supplemental material). Incidence data for only 86 selected taxa at peak plume sampling intervals (Adu143 to 0.2 (see Movie S1 in the supplemental material). For the May NAAPS data (see Table S1 in the supplemental material).

**FIG 2** Principle coordinate analysis of background samples (green) and plume samples (blue). Analysis was based on the unweighted UniFrac distance between samples from 38,546 possible taxa with incidence differences. For axis 1, 29% of the variation was explained; for axis 2, 9% of the variation was explained. The partitioning shows the similarity in community composition between plume samples (abbreviated du) and the similarity in community composition background samples (abbreviated bk), regardless of plume timing (April or May). Note that Adu143 and Tdu176 were transitional samples at the onset of a plume (i.e., a mixture of background and plume).
Adu145, Tdu147 to Tdu179) revealed a microbiome contrast between April and May plumes (see Fig. S5 in the supplemental material).

Figure 3 supports the idea that ALRT plumes delivered similar microbiota, despite unique transport histories, in April and May: although species richness levels varied between the events (Tables 1 and 2), the proportion of 15 common bacterial families was essentially parallel. An important point, however, is that while the relative abundance remained steady across ALRT plumes, the absolute abundance of 312 OTUs changed significantly ($P < 0.001$).

To illustrate this observation, a circular tree (see Fig. S6 in the supplemental material) was constructed to display differentially abundant OTUs and their taxonomic relationship on the basis of 16S rRNA gene alignment. Welch test $P$ values were used to reduce the number of significantly different families to 83, and the one OTU with the greatest abundance difference from each family was included in the tree. Heat-map values revealed increases within the class Clostridia and families Sphingomonadaceae, Rhodobacteraceae, and Isosphaeraceae relative to the combined means of baseline samples. Altogether, Proteobacteria ($n = 29$), Actinobacteria ($n = 19$), and Firmicutes ($n = 19$) totaled 80% of the phyla whose abundance increased during ALRT plumes. A higher level of Actinobacteria and Firmicutes is noteworthy because the families include many spore-forming and Gram-positive species capable of surviving extreme conditions associated with long-range upper atmospheric transport. Curiously, 4 out of 5 of the families that decreased in abundance during plumes were Proteobacteria from marine environments (including Alteromonadaceae, Vibrionaceae, and the OM60 family within the Oceanospirillales).

After establishing that ALRT plumes delivered higher concentrations of microbes already present in the North American background air, we focused on variations within specific taxa using prediction analysis for microarrays (PAM) (23). Figure 4 highlights taxa with possible Asian or oceanic origins, including isolates from a Chinese forest (OTU 51259), Dongping Lake sediments (OTU 51013), and marine microbial mats (OTU 75349). The alignment between taxon source regions and probable emission sites identified by atmospheric data was striking; however, annotations from the 16S rRNA sequence database can be inaccurate. Source verification of probe-detected taxa would require sample sequencing (which was outside the scope of our current study). Another possible agreement between atmospheric trans-
port models and microbial biogeography was the detection of *Aeropyrum* spp. in the May episode. Finding marine archaea (24) in the free troposphere above central Oregon also supports microbial ALRT, but biogeography alone cannot be the only means of inferring distant provenance. Only after considering the location of our field site, prevailing wind direction, and long-range transport validation through a number of independent atmospheric data sets [including (i) HYSPLIT, version 4, kinematic back trajectories, (ii) aged aerosol data (i.e., NH₄SO₄ and soil), (iii) plume chemical composition (i.e., CO and THg), and (iv) the NAAPS model] could we be confident about the transoceanic origin of air samples.

Soon, it may be useful to think about microorganisms as air pollution (e.g., how aerosols were depicted in Movies S1 and S2 in the supplemental material, moving in plumes through a global background layer). Our main finding—that transpacific dust plumes deliver elevated levels of species already in the background air—suggests that microbes pool like other types of pollution over the Pacific Ocean. However, a transpacific monitoring network with sampling sites in eastern Asia and western North America is needed to establish an aerobiology data set comparable to that for the NAAPS model. Such an undertaking would require seasonal measurements from a variety of natural (desert dust, marine sea spray, etc.) and artificial (livestock feedlots, wastewater-treatment facilities, etc.) upwind emission sources (25), monitored, ideally, through a combination of ground- and aircraft-based platforms.

**FIG 4** Significant abundance variations in specific taxa between background (green) and plume (blue) periods. Combined data for April and May events are shown. HybScores are on the y axis, and the sample order (from left to right) follows the order used in Tables 1 and 2 (e.g., Abk142 is the leftmost data point). Numbers in parentheses are OTU identification numbers.
Global sampling efforts using rRNA microarrays might consider employing the same commercial products to reduce false hybridizations and other sources of variation [17]. Standardizing air collection techniques, DNA extraction, PCR amplification, and microarray protocols would be useful for comparisons between field sites. Even though microarrays offer improved sensitivity to microbial taxa (our first investigation of the same ALRT samples detected only 18 species of bacteria [13]), culture-based aerobiology data still have value: understanding what species remain viable after intercontinental atmospheric transport informs questions related to disease propagation.

Airborne microorganisms originate from the surface and must eventually return to it. Consequently, the atmosphere has generally been considered a conduit for life rather than a true ecosystem. However, our study revealed a microbial richness that rivals that of surface ecosystems and the presence of many phyla with adaptations for extended viability during atmospheric transport (e.g., spore-forming and Gram-positive bacteria). In addition, the potential for dynamic microbial interactions with the environment, such as in situ metabolism [26], the stimulation of cloud formation and precipitation [5], and selection pressures from UV radiation [27] all support the idea that the atmosphere might be considered an ecosystem in its own right. No matter how it is classified, as desertification injects more dust into the atmosphere (6, 12) and humans grow increasingly vulnerable to changing patterns of weather and disease, it will be important to monitor microbial populations on intercontinental winds.

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REFERENCES