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Supplementary Information for

Just passing through: neither time nor environment affect fungal aerobiota over a 13-year time series at the Mauna Loa Observatory

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Supplementary Information Text

Methods.

Sample Collection

The atmospheric particles were passed alternately through 10 μm and 1μm impactors, with the switching occurring every six minutes. The 0.78 cm^2 filters were made of borosilicate glass fibers (Pall Corporation, filter type E70-2075W), and with the 10 μm impactor in place, captured particles with aerodynamic diameters between 50 nm and 10 μm. Larger particles that are porous or low-density could have also been captured. The filters sampled approximately 1.44 m^3 of air per day, and were collected with forceps and placed into individual plastic bags when the filter transmittance dropped to below 70% of that for a fresh filter.

Weather and Atmospheric variables from Mauna Loa Observatory

These data were downloaded in February 2016 from the Global Monitoring Division of the Earth System Research Laboratory ftp server located at https://www.esrl.noaa.gov/gmd/dv/data/index.php?site=mlo and included wind speed, wind direction, wind steadiness factor, barometric pressure, air temperature two meters and ten meters above the surface, air temperature at the tower, humidity, accumulated precipitation, carbon dioxide in the air, aerosol scattering coefficients, and aerosol particle concentration.

DNA extraction and sequencing

DNA was extracted from filters and five negative extraction controls using MoBio Powersoil-htp kits (QIAGEN, Venlo, The Netherlands) following the manufacturer's protocol. DNA templates, including a PCR blank, were amplified using Q5 Taq-Polymerase (NEB, Ipswitch, MA, USA) and the primer set ITS1F and ITS2 (1), modified with the addition of Illumina adaptors and bi-directional barcode indices (see 2, 3). The resulting barcoded libraries were cleaned and normalized with Charm Just-a-Plate(TM) (Charm Biotech, San Diego, CA, USA), and sequenced as 84% of a single run

on the Illumina MiSeq platform with V3 chemistry and a 2 x 300 base pair protocol at the Hawaii Institute for Marine Biology Genetics Core Facility.

The sequencing run resulted in approximately 3.2 million reads. The paired end reads were merged using PEAR (4). The reads were filtered so that reads containing ambiguous bases or at least one phred score lower than 25 were removed. Chimeric reads were removed using reference based Vsearch (5). The ITS1 region was extracted using ITSx to remove the highly conserved flanking regions (18S and 5.8S) (6), resulting in 2,966,323 high quality reads across all samples, five extraction controls, and seven negative filter controls. Samples with fewer than 3000 reads were excluded from further analysis. There were 172 filters that were successfully sequenced with an average of 16,764 (SD: 7,996) reads. These reads were assigned to operational taxonomic units (OTUs) in QIIME version 1.9 (7) using the uclust algorithm (8). The UNITE database version 7.0, dated 1/31/2016, was used as the reference for open reference clustering (9).

Taxonomic Assignments

If the UNITE and NCBI taxonomies matched, this taxonomic assignment was used. If one assignment was deeper (closer to the species level, i.e. genus) than the other but otherwise matched, the deeper taxonomic assignment was used. If the species level assignment matched but the middle taxonomic levels were missing, labeled as *Incertae sedis*, or in disagreement, the NCBI taxonomy was used. If a final taxonomy could not be assigned in this way, as was the case for 13 (8.125%) OTUs, the representative sequence was matched to the NCBI GenBank database and assigned to the genus if the query coverage was 100% with greater than 97% identity; assigned to the family if the query coverage was greater than 93% with 100% identity; assigned to the order if the query coverage was 100% with greater than 82% identity. If the top two hits had identical scores, preference was given to the vouchered or cultured hit. If a vouchered or cultured hit was not available, the taxonomy was called at the deepest matching taxonomic level.

OTUs assigned to non-fungal taxonomies (N=22) and those present in less than 1% of samples (N=18) were removed. The 18 fungal OTUs that were removed represented less than 2.8% of reads in any given sample and resembled the retained

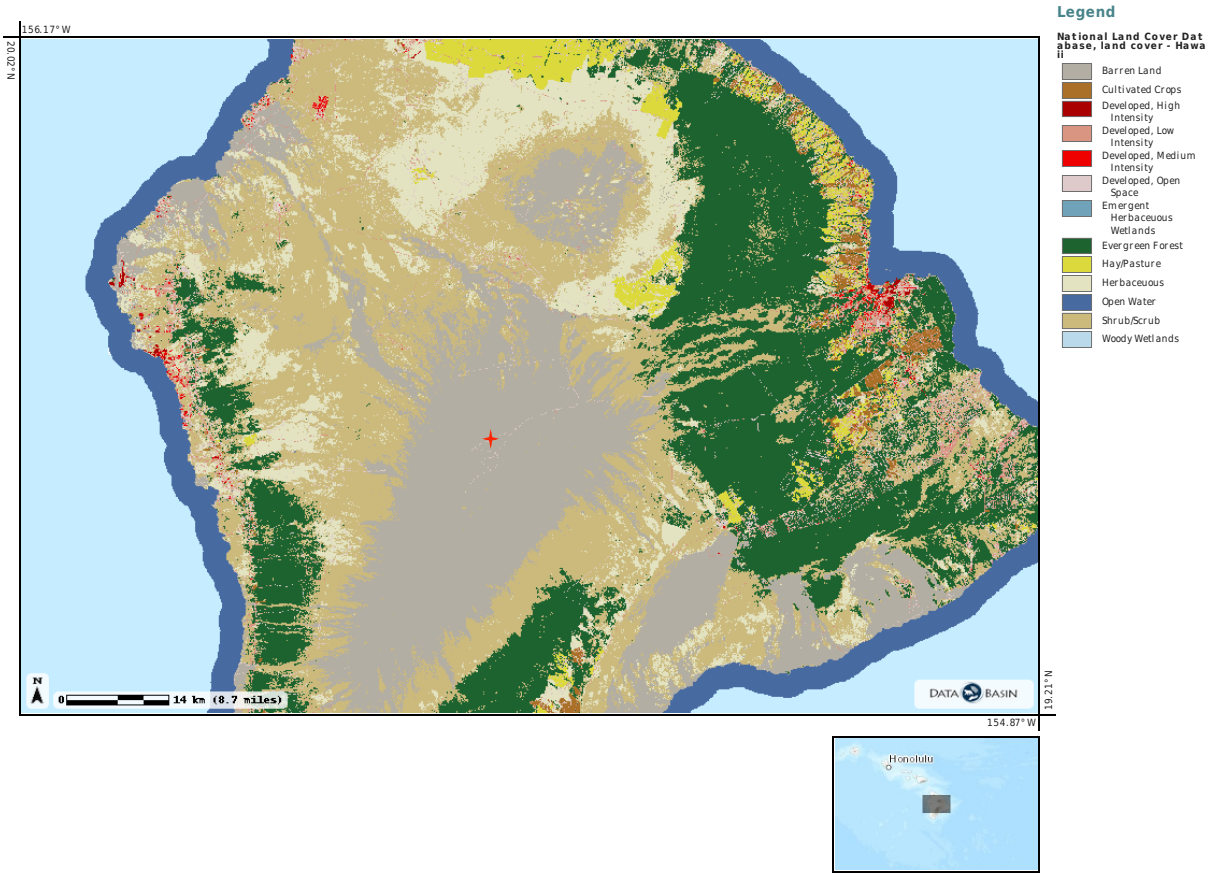
OTUs taxonomically, therefore removing them had minimal impact on results for all downstream statistical analyses.

Hill Numbers as estimators of alpha diversity

The first Hill number (q=0) is simply an estimate of richness without regard for relative abundance. The second two Hill numbers (q=1 and q=2) are the exponential of Shannon entropy and the inverse Simpson concentration, respectively, which both incorporate species (or OTU) abundance into their estimates. Shannon is the effective number of common OTUs whereas Simpson is the effective number of dominant OTUs in the assemblage (10).

Niche and neutral modeling

The *sads* R package (11) was used to generate 1000 permutations of species distributions for each assembly model based on the maximum likelihood parameters using a random number seed of 96822. The Volkov method (12) was used to generate permutations for a neutral model of assembly, while a broken stick model (13) was used for niche-based assembly.



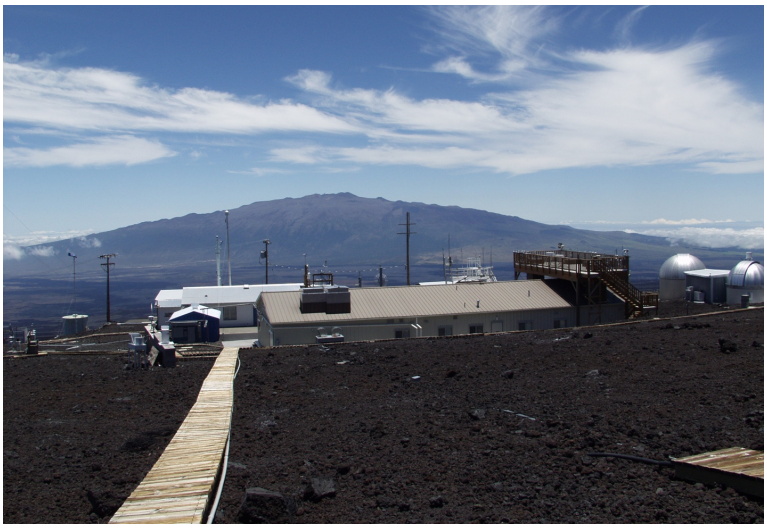


Fig. S1. **Top: Location of MLO and surrounding land cover type.** Red cross indicates location of MLO. Legend describes land cover types by color. MLO is surrounded by ~650 km2 of lava, with the closest viable fungal habitat located in a pasture ~6.5 km to the north. Bottom: Photograph of MLO and the surrounding volcanic desert The high elevation (3,397 masl) location and relatively recent volcanic activity has created a volcanic desert where few to no hosts supporting microorganisms such as fungi can survive. Photo curtesy of NOAA.

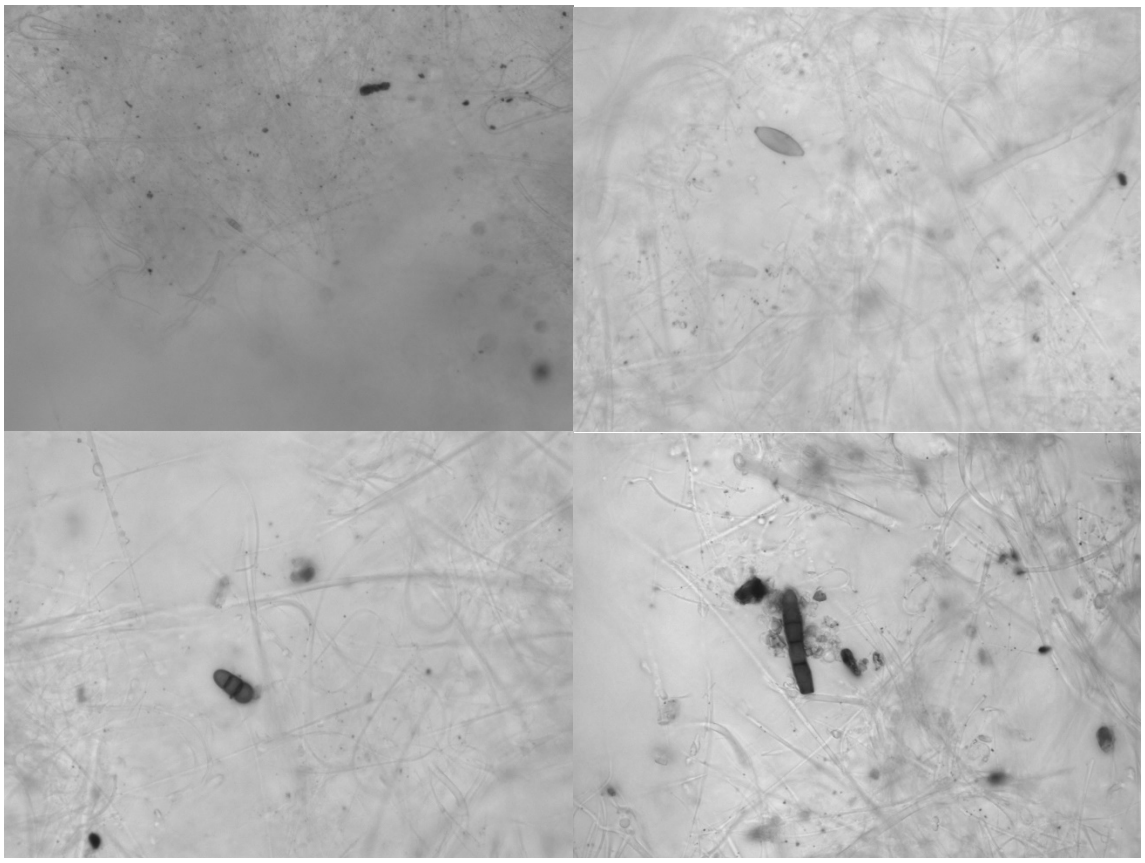


Fig. S2. **Spores trapped in particle filters**. All images were taken with a compound light microscope at 400x magnification. These images show the range of spore morphology trapped in the borosilicate glass filters used to measure atmospheric particle density.

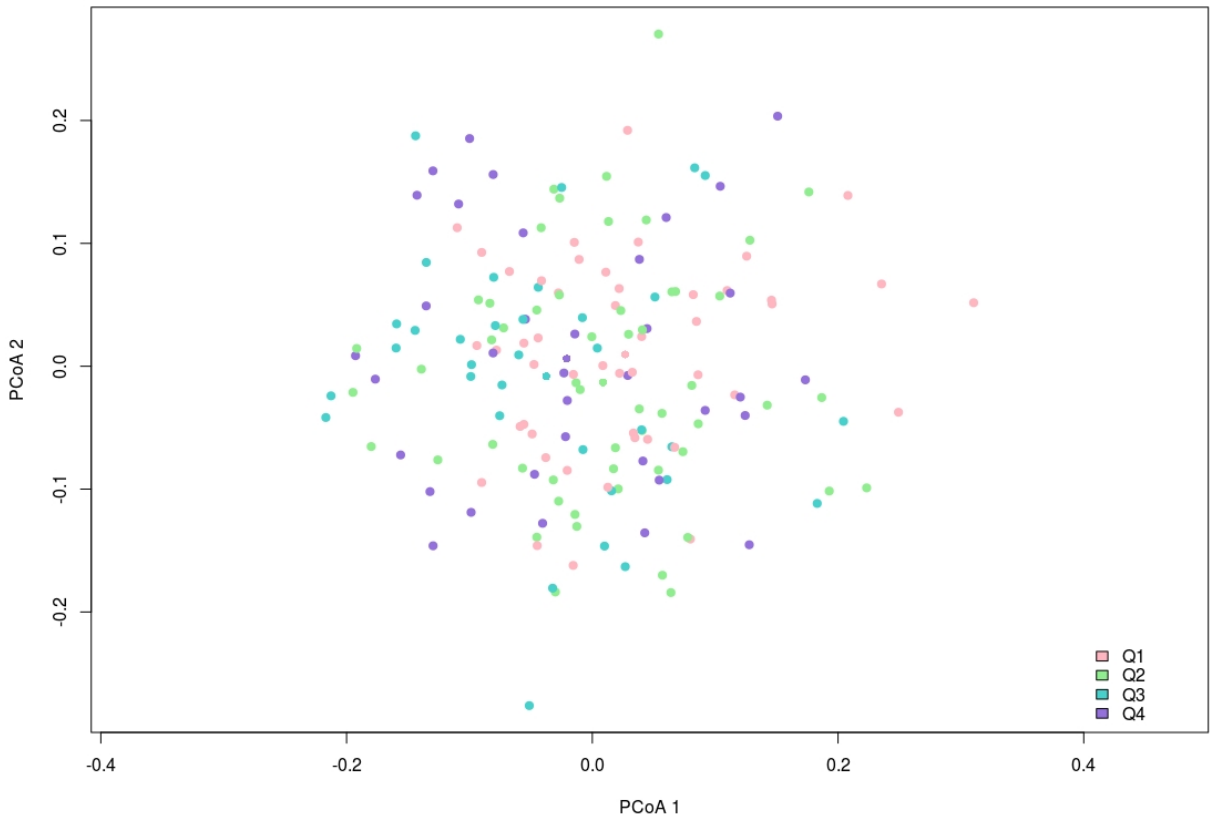


Fig. S3. **Fungal Aerobiota at MLO By Season** Principle coordinate analysis (PCoA) plot of the fungal aerobiota based on the Jaccard distance between samples. No separation or pattern among quarters was observed.

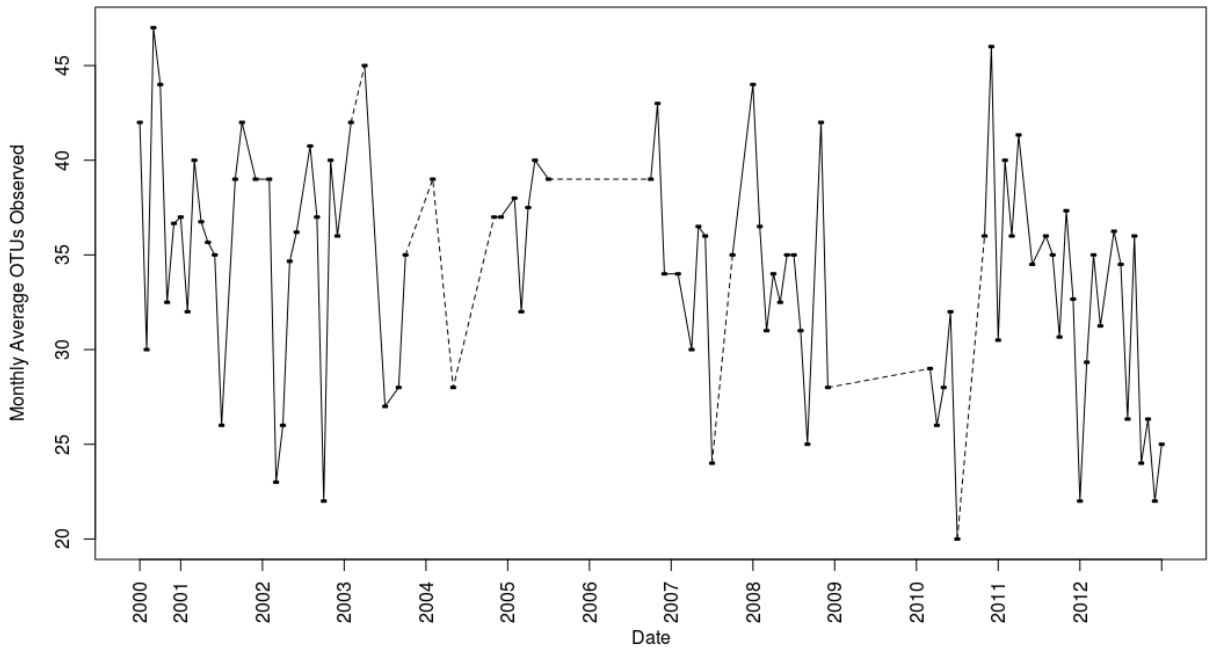


Fig S4. **Fungal Richness at MLO Over Time** Plot of the average number of observed OTUs over the study course from June 2000 to December 2012. Samples were averaged by month to smooth the curve and a dashed line indicates that more than one month passed between samples. The number of observed OTUs showed no discernable trends over season or time.

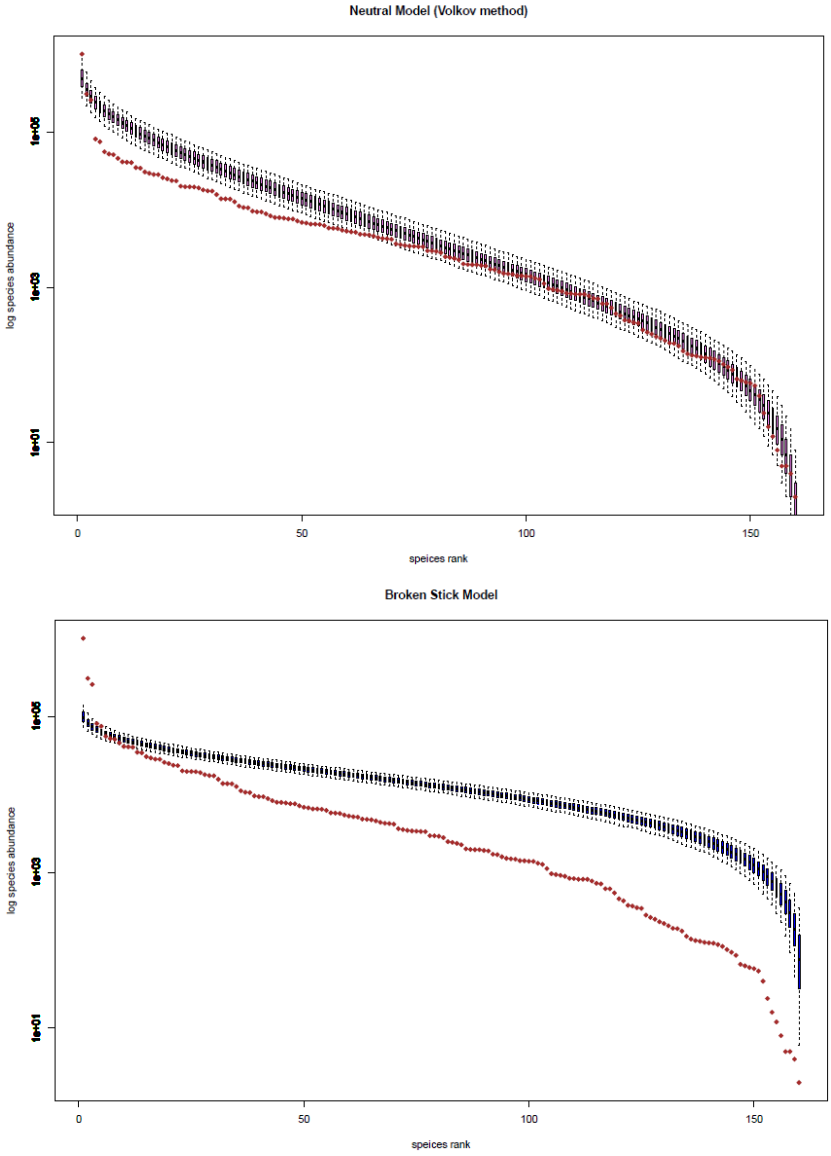


Fig. S5. **Assembly Models for the Fungal Aerobiota at MLO** Under the neutral model (top graph), the log species abundance (y-axis) of 100 of the 160 OTUs (show by red diamonds) fell within the 95% confidence interval of the permuted mean log species abundance (shown by box and whiskers). Mean log species abundances were permuted 1000 times based on parameters estimated from the observed fungal aerobiota at MLO, $\theta = 14.18$ and $m = 0.0014$. Under the broken stick model (bottom graph), the log species abundance of 5 of the 160 OTUs (shown by red diamonds) fell within the 95% confidence interval of the permuted mean log species abundance (shown by box and whiskers). Mean log species abundances were permuted 1000 times based on the size (read count) and number of OTUs.

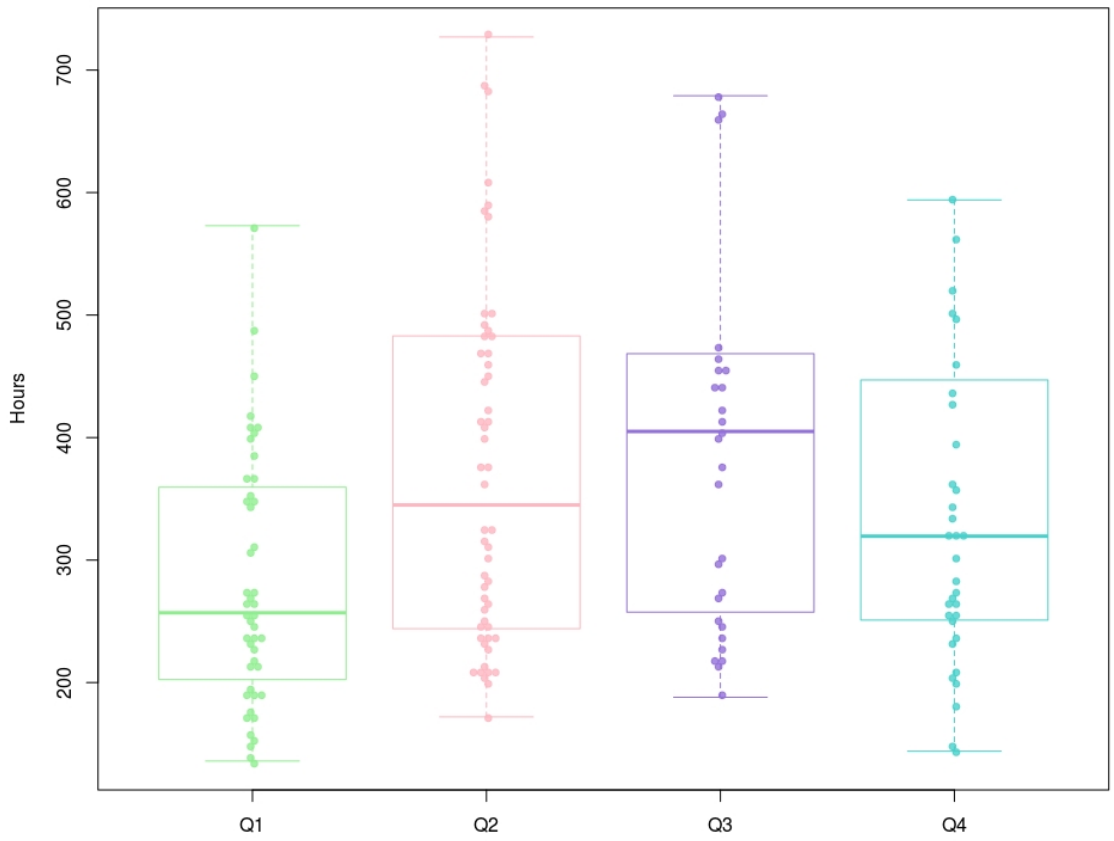


Fig S6. **Hours since airmasses were last over land by quarter**. Box-and-whisker style plots of the number of hours prior to arriving at MLO that the airmasses were last over a landmass broken down by quarter. The "boxes" cover the inter-quartile range and "whiskers" cover 1.5 times the inter-quartile range. The mid-line indicates the median number of hours for that quarter. While the boxes overlap overall, there is enough difference between Q1 and Q3 to drive a significant difference (ANOVA p-value = 0.002).

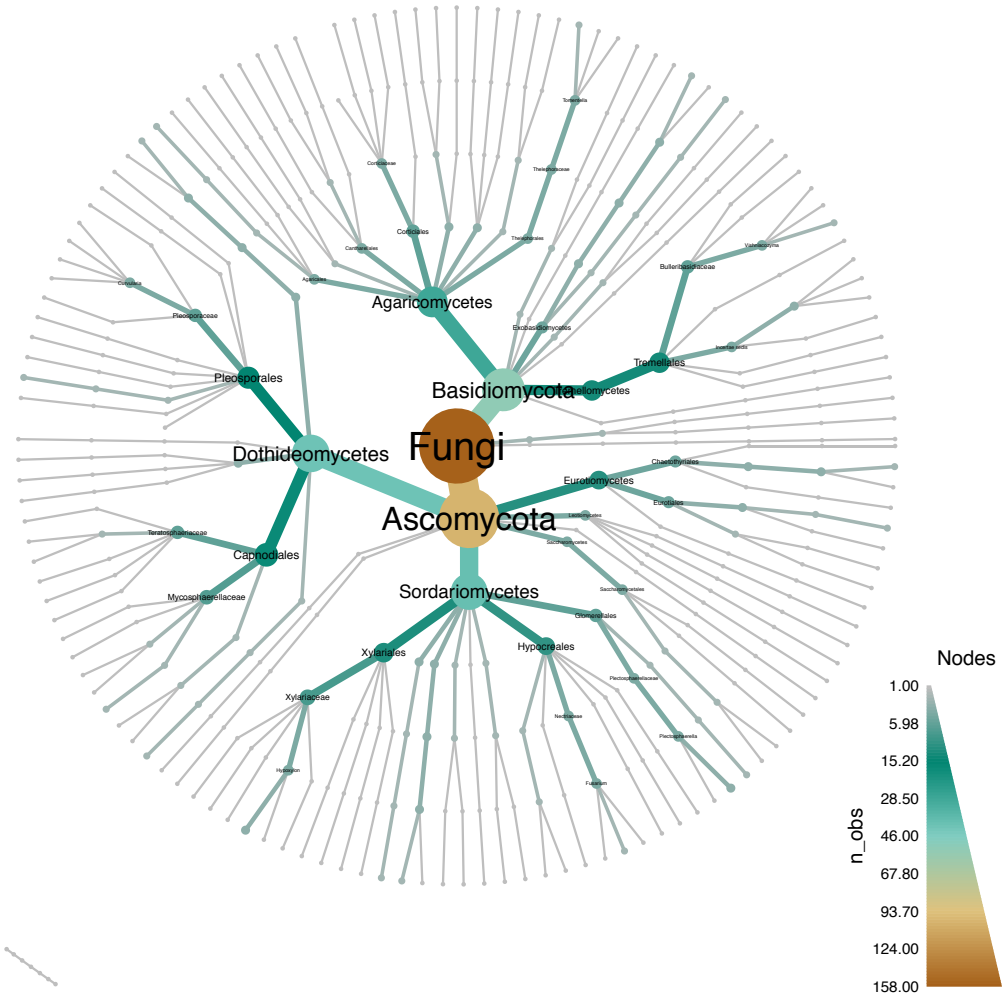


Fig. S7. **Phylogeny of the fungal aerobiota at MLO** Each terminal branch represents an OTU and the size of the internal nodes is proportional to the percentage of OTUs contained in that node.

Table S1. **Average assemblage dispersion and beta diversity by quarter**. Distances are presented at mean (SD). The Mantel permuted p-value is the result of testing for increases in distance as time increased.

Quarter	Jaccard Distance Between Samples	Dispersion (β_{SIM})	Distance to Median	Mantel permuted p-value
Q1	0.686 (0.061)	0.953	0.480 (0.043)	0.328
Q2	0.681 (0.059)	0.958	0.477 (0.041)	0.572
Q3	0.679 (0.057)	0.987	0.473 (0.037)	0.338
Q4	0.678 (0.064)	0.941	0.472 (0.048	0.245

Dataset S1. **Samples from MLO** Each sample that was successfully sequenced is represented by a row in the table. In addition to the date of sampling, all locally collected weather variables for that date are included in the table.

See file DatasetS1_Samples.xlsx

Dataset S2. **Operational Taxonomic Units and their Taxonomic Identifications** Spore measurements, trophic mode, guild, and associated references for each OTU, based on its taxonomic assignment, are included in the table.

See file DatasetS2_TaxonomyGuildsandSpores.xlsx

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