

Characterization of active and total fungal communities

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Characterization of active and total fungal communities in the atmosphere over the Amazon rainforest

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Abstract

Fungi are ubiquitous in the atmosphere and may play an important role in atmospheric processes. We investigated the composition and diversity of fungal communities over the Amazon rainforest canopy and compared these communities to fungal communities found in terrestrial environments. We characterized the total fungal community and the metabolically active portion of the community using high-throughput DNA and RNA sequencing and compared these data to predictions generated by a mass-balance model. We found that the total community was primarily comprised of fungi from the phylum Basidiomycota. In contrast, the active community was primarily composed of members of the phylum Ascomycota and included a high relative abundance of lichen fungi, which were not detected in the total community. The relative abundance of Basidiomycota and Ascomycota in the total and active communities was consistent with our model predictions, suggesting that this result was driven by the relative size and number of spores produced by these groups. When compared to other environments, fungal communities in the atmosphere were most similar to communities found in tropical soils and leaf surfaces, suggesting that inputs of fungi to the atmosphere are from local, rather than distant, sources. Our results demonstrate that there are significant differences in the composition of the total and active fungal communities in the atmosphere, and that lichen fungi, which have been shown to be efficient ice nucleators, may be abundant members of active atmospheric fungal communities over the forest canopy.

1 Introduction

Fungi are critical to the functioning of terrestrial ecosystems and may also play an important role in the functioning of the atmosphere. Fungi are abundant and ubiquitous in the atmosphere, with an estimated global land surface emission rate of 50 Tg year^{-1} for fungal spores alone. Fungal bioaerosols are not only abundant but also affect phys-

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ical and chemical processes in the atmosphere. Fungal spores and cellular fragments affect precipitation by acting as ice and cloud condensation nuclei (Després et al., 2012; Morris et al., 2013; Pouleur et al., 1992; Richard et al., 1996), and metabolically active fungi sampled from the atmosphere are capable of transforming compounds known to play a major role in atmospheric chemistry, including carboxylic acids (Ariya, 2002; Côté et al., 2008; Vaïtilingom et al., 2013), formaldehyde, and hydrogen peroxide (Vaïtilingom et al., 2013).

The in situ function of airborne fungi will depend on the physiological state of fungal cells. Metabolically active vegetative cells have the potential to transform atmospheric compounds and ultimately alter atmospheric chemistry, whereas dormant spores do not. The ice nucleation efficiency of fungal cells also likely depends on their physiological state; vegetative cells derived from potentially active fungi are more efficient ice nucleators than spores. Vegetative forms of *Fusarium* (a filamentous fungi) as well as several lichen fungi have been shown to nucleate ice at temperatures as warm as -1°C (Després et al., 2012) (Fig. S1 in the Supplement), and ice nucleation by hyphae has been observed at -2.5°C (Pouleur et al., 1992). In contrast, dormant spores – particularly those with surface hydrophobins – are generally poor ice nucleators. For example, ice nucleation of rust (*Puccinia*) spores requires temperatures lower than -10°C (Morris et al., 2013), and *Penicillium* spores nucleate ice at temperatures of approximately -22°C (Iannone et al., 2011).

Despite its importance, we know relatively little about the physiological state of fungal cells in the atmosphere. Specifically we know little about the taxonomic composition of metabolically active airborne fungi and how this compares to the composition of the total fungal community. One way to survey the total and active communities is to measure community composition from rDNA (i.e. rRNA genes) and rRNA in ribosomes. Sequencing rDNA provides information about the total community, which includes both active and dormant individuals, whereas rRNA sequences provides information about the potentially active community, because ribosomes are more abundant in active cells than dormant cells (Prosser, 2002). This approach has been applied to study active fun-

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gal communities in soils and on decaying plant material (Baldrian et al., 2012; Barnard et al., 2013, 2014; Rajala et al., 2011) but has not been applied to fungal communities in the atmosphere.

Information about the taxonomic composition of airborne fungi that are present in different physiological states could be used to advance atmospheric science in multiple ways. For example, such data could be used to improve estimates of the ice nucleating capacity of fungal bioaerosols. Recent estimates of the ice nucleating capacity of fungal bioaerosols based on culture-based approaches – the abundance of colony forming units (CFUs) – have led some scientists to conclude that atmospheric fungi have a low ice nucleation efficiency (Iannone et al., 2011). However, these culture-based data may be misleading, as the vast majority of fungi require identification using culture-independent approaches (Borneman and Hartin, 2000). Culture-independent identification of active fungal taxa sampled from the atmosphere could be used to direct selective culturing of potentially important fungi to test their ice nucleation efficiencies and their metabolic capabilities in the laboratory.

In this study, we used culture-independent approaches to measure the composition of total and active atmospheric fungal communities in situ and a mass-balance model to aid in the interpretation of our results. Our study system is the atmosphere above the Amazon rainforest canopy. We chose this system because fungal bioaerosols make up a substantial proportion of aerosol particulate matter over the Amazon (Elbert et al., 2007; Heald and Spracklen, 2009) and are estimated to be a dominant force responsible for cloud formation over the Amazon (Pöschl et al., 2010). We used a combined approach of DNA and RNA sequencing to address the following questions: (1) What is the composition of total airborne fungal communities? (2) What is the composition of active airborne fungal communities? (3) What likely drives differences in the composition of the total and active airborne fungal communities? (4) Is the diversity and structure of fungal communities in the atmosphere similar to that found in terrestrial environments?

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2 Methods

2.1 Sample collection

Sampling was conducted on the ZF2 K34 flux tower (−2.60907° S, −60.20917° W, 67 m a.s.l.) in the Reserva Biologica do Cueiras in central Amazonia, about 60 km NNW of Manaus, Brazil. The site is operated by the Instituto Nacional de Pesquisas da Amazonia (INPA) under the Large Scale Biosphere–Atmosphere Experiment in Amazonia (LBA) program (Martin et al., 2010). Tower height is approximately 54 m. Surrounding vegetation is undisturbed, mature, terra firme rainforest, with a leaf area index of 5–6 and an average canopy height of 30 m. Samples were collected at the end of dry season over four days, 8–11 December 2010, from a height of 48 m above the forest floor. Aerosol samples were collected using SKC Biosamplers (BioSampler SKC Inc.). Samplers were filled with 20 mL of a water-based preservation solution (LifeGuard Soil Preservation Solution, MO BIO Laboratories, Inc) to prevent DNase and RNase activity and maintain cells in stasis to allow accurate community profiling of the total and active fungal community. Twelve impingers were operated at 12.5 L min^{−1} from approximately 09:00–04:00 a.m. each day. At the end of each day, the sampling liquid from all impingers was pooled and stored at −20 °C.

2.2 Nucleic acid isolation and cDNA synthesis

Samples were transported on ice to the University of Oregon where the liquid sample from each day was separated into two aliquots, one to be used for DNA extraction and the other for RNA extraction. The divided samples were filtered through 0.22 µm cellulose nitrate filters. DNA was extracted from filters using the MO BIO PowerWater DNA Isolation Kit according to the manufacturer's instructions with a 100 µL elution volume. RNA was extracted from filters using the MO BIO PowerWater RNA Isolation Kit with the following modifications. The DNase steps included in the kit were omitted. RNA was eluted in 50 µL. The extracted RNA was treated with DNase I (RNase-free) (Fermen-

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tas International, Inc) according to the manufacturer's instructions. DNase reactions were cleaned (Zymo Research Clean and Concentrate-5) and eluted into 50 μ L. cDNA was synthesized from the total RNA extract using the SuperScript II First-Strand Synthesis System (Invitrogen, Life Technologies Corporation) with random hexamers. All RNA was converted into cDNA in six synthesis reactions and one reverse transcriptase negative control reaction.

2.3 Library preparation and sequencing

To increase the concentration of cDNA to levels required for sequencing, we used multiple displacement amplification (GenomiPhi V2, GE Healthcare) according to the protocol described in Gilbert et al. (2010) including second-stand synthesis, amplification, and de-branching of amplification products. The fully de-branched products were sheared by sonication (24 cycles, 30 s each) using the Bioruptor sonication system (Diagenode). cDNA fragments were end-repaired (End-It DNA End-Repair Kit, Epicentre Biotechnologies), cleaned and concentrated (Zymo Research Clean and Concentrate-5) and eluted in 40 μ L. A-overhangs were added to the end-repaired fragments using Klenow exo(-) (Epicentre Biotechnologies) in a 50 μ L reaction. Reaction products were cleaned and concentrated (Zymo Research Clean and Concentrate-5). Standard paired-end, barcoded Illumina adaptors (Table S1 in the Supplement) were ligated to the fragments using T4 ligase (Fermantas). Reaction products were cleaned and concentrated (Zymo Research Clean and Concentrate-5) and eluted in 12 μ L. To enrich fragments with ligated adaptors, PCR amplification was performed using primers containing the flowcell adaptor and complementary to the Illumina sequencing primer (Table S1). PCR reactions were performed using Phusion DNA polymerase (New England Biolabs) with 12 μ L template, 10 μ L 5 \times HF buffer, 1 μ L 10 mM dNTPs, 2 μ L 10 mM primer mix, 0.5 μ L enzyme and 25.5 μ L water for a final reaction volume of 50 μ L. PCR cycling conditions were as follows: 30 s denaturation at 98 $^{\circ}$ C followed by 18 cycles of 98 $^{\circ}$ C for 30 s, 65 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 30 s following by a final extensions at 72 $^{\circ}$ C for 5 min. PCR products were size fractionated by gel electrophoresis (2.5 %, low-melt

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agarose). Products in the range of 150–500 bp were excised, and DNA from the excised gel pieces was extracted (QiagenMinElute Gel Extraction) and eluted into 20 μ L. DNA was quantitated using a Qubit 2.0 Fluorometer (Invitrogen, Life Technologies Corporation) and combined in equal molar concentrations. Shotgun metatranscriptome libraries were sequenced (150 base pairs, paired-end) on the Illumina HiSeq 2000 (Illumina, Inc.) platform at the University of Oregon Genomics Core Facility. LSU rDNA amplicons were sequenced (250 base pairs, paired-end) on the Illumina MiSeq platform at the Dana–Farber Cancer Institute Molecular Biology Core.

The D1-D2 region of the large subunit (LSU) rRNA gene was targeted using PCR with the primers LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR3 (5'-CCGTGTTTCAAGACGGG-3') (<http://sites.biology.duke.edu/fungi/mycolab/primers.htm>). LSU amplicon libraries were prepared using a two-stage PCR procedure as described in (Kembel and Mueller, 2014) using unique combinatorial barcodes (Gloor et al., 2010) to identify samples (Table S2).

2.4 Sequence pre-processing

2.4.1 Metatranscriptome

Overlapping paired end reads were aligned and joined using fastq-join (<https://code.google.com/p/ea-utils/wiki/FastqJoin>). Joined reads and non-overlapping single-end reads were trimmed and filtered using PrinSeq (Schmieder and Edwards, 2011). Sequences < 75 bp, > 2% Ns, and/or mean quality score < 20 were removed. Sequence artifacts defined as exact duplicates with > 5000 sequences were removed. Sequences in the 10 December sample were primarily artifacts, so this metatranscriptome sample was excluded from further analysis. Putative rRNAs in the remaining sequences were identified using SortMeRNA (Kopylova et al., 2012) with the non-redundant version of the following databases: rfam 5.8S (version 11.0) (Burge et al., 2013); Unite (November 2011 version) (Köljalg et al., 2013), and Silva 18S and Silva 28S (Release

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Following sequence processing and quality filtering, a total of 55 414 amplicon and 1 915 994 metatranscriptome LSU sequences generated for this study and 1 577 458 LSU sequences from soil and phyllosphere studies were retained (Table S3). For analyses using only samples from this study, the data were rarefied to 5300 sequences per sample. For analyses that compare samples in this study to samples from other studies, the data were rarefied to 500 sequences per sample.

2.6 Statistical analyses and data availability

All statistical analyses were conducted in R (R Core Team, 2014) primarily using the *vegan* (Oksanen et al., 2013) package for ecological statistics and the *ggplot2* (Wickham, 2009) package for visualizations.

Sequence files and metadata have been deposited in Figshare (doi:10.6084/m9.figshare.1335851). Data from other studies used for cross environment analyses are available using the databases and identifiers referenced in the respective manuscripts.

2.7 Mass-balance model

We use a global, well-mixed, one-box material-balance model to predict the relative abundances of fungal cells measured as gene copies sampled in the active and total portions of atmospheric bioaerosols. Model description and details are available in the Appendix.

3 Results and discussion

3.1 Basidiomycota dominate total airborne fungal communities

Measurements of airborne fungi using culture-based methods such as quantifying spore and colony-forming unit counts have been conducted for centuries (Després

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et al., 2012). In comparison, there have been few culture-independent studies of the fungal composition of atmospheric samples (e.g. Boreson et al., 2004; Bowers et al., 2013; Fierer et al., 2008; Fröhlich-Nowoisky et al., 2009, 2012; Pashley et al., 2012; Yamamoto et al., 2012). Using a culture-independent approach, we found the composition of total airborne fungal communities primarily included taxa belonging to the phyla Ascomycota and Basidiomycota (Fig. 1). This result is similar to what is observed in environments on the Earth's surface (James et al., 2006) and what has been reported in other studies of fungi in the atmosphere (Bowers et al., 2013; Fröhlich-Nowoisky et al., 2009, 2012; Yamamoto et al., 2012).

Basidiomycota dominated the total airborne community in our air samples (mean relative abundance = $90.2 \pm 6.9\%$) (Fig. 1). Within the phylum Basidiomycota, Agaricomycetes were the most abundant class in our samples. Agaricomycetes have been previously detected in air samples (Fröhlich-Nowoisky et al., 2012; Woo et al., 2013; Yamamoto et al., 2012) and are common in tropical soils (Tedersoo et al., 2014) and leaf surfaces (Kembel and Mueller, 2014). Within the Agaricomycetes, the most abundant order was the Polyporales (mean = $55.7 \pm 2.3\%$). Polyporales have been detected in culture-independent studies of urban aerosols (Yamamoto et al., 2012) and culturable representatives have been isolated from cloud water (Amato et al., 2007). Given that these are largely saprotrophic (i.e. wood-decay) fungi (Binder et al., 2013; Larson et al., 2007), it is parsimonious to assume there is a significant local source of Polyporales on the forest floor.

The presence of Agaricomycetes may have implications for atmospheric processes. Ice nucleation efficiency within the Agaricomycetes is variable, with some taxa capable of nucleating ice at temperatures as warm as -17°C (Haga et al., 2014) (Fig. S1). These temperatures are warmer than what has been measured for *Penicillium* spores (Iannone et al., 2011) although not as warm as what has been measured for other spore types (Morris et al., 2013), hyphal fragments (Pouleur et al., 1992), and lichen fungi (Després et al., 2012). Despite the low ice nucleation efficiency of some taxa in this group, given the high abundance of Agaricomycetes over the forest canopy, this

group could still have a significant impact on cloud formation and precipitation in the tropics.

3.2 Ascomycota dominate active airborne fungal communities

The composition of total and active fungal communities over the Amazon rainforest canopy significantly differed (ADONIS, $R^2 = 0.342$, $p = 0.029$). The active community in the atmosphere over the forest canopy was dominated by Ascomycota (mean relative abundance = $80.4 \pm 20\%$) (Fig. 1). Basidiomycota comprised a smaller fraction of the sampled genes (mean = $7.3 \pm 6.8\%$) with the remainder of identified sequences belonging to the phyla Chytridiomycota and Glomeromycota. This result makes sense in light of the natural histories of many of the Ascomycota, which have single-celled or filamentous vegetative growth forms that are small enough to become aerosolized, while many of the Basidiomycota are too large to be easily aerosolized, other than in the form of metabolically inactive spores.

The most abundant classes of Ascomycota detected were Sordariomycetes (mean = $27.1 \pm 6.6\%$), and Lecanoromycetes (mean = $17.5 \pm 7.6\%$). Sordariomycetes have been detected in air samples (Fröhlich-Nowoisky et al., 2009, 2012; Yamamoto et al., 2012) and have been shown to be abundant on tropical tree leaves (Kembel and Mueller, 2014) and tropical soils (Peay et al., 2013). In most ecosystems, Sordariomycetes are endophytes, pathogens, and saprotrophs (Zhang et al., 2007). Xylariales, which includes both endophytes and plant pathogens (Zhang et al., 2007), was the most abundant order within the Sordariomycetes in our samples.

Lecanoromycetes were the second most abundant class of Ascomycota detected over the rainforest canopy. This group has been detected in other culture-independent studies of fungi in the atmosphere (Fröhlich-Nowoisky et al., 2012; Yamamoto et al., 2012). The Lecanoromycetes contain 90% of the lichen-associated fungi (Miadlikowska et al., 2007). Lichens are a symbiosis between a fungus and a photosynthetic partner such as eukaryotic algae or cyanobacteria. Lichens are known to be hardy and may be particularly well-adapted for long distance transport and metabolic activity in

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the atmosphere. Lichens are often the dominant life forms in environments that have conditions similar to those found in the atmosphere, including low water (Kranner et al., 2008) and nutrient availability, wide temperature variations, and high UV irradiance (e.g. Solhaug et al., 2003; Onofri et al., 2004).

Another notable trait of lichens is their efficient ice nucleation capacity. Although there have been no investigations specifically on the most abundant lichen species detected in this study, *Physcia stellaris* (mean = $8.3 \pm 3.8\%$) and *Rinodina milvina* (mean = $4.8 \pm 3.4\%$), there have been multiple studies of the ice nucleation efficiency of many other lichen fungi species. Ice nucleation activity of lichens has been measured at temperatures warmer than -8°C , including 13 of 15 taxa tested by Henderson-Begg and colleagues (Henderson-Begg et al., 2009) and 9 of 15 taxa tested by Kieft (Kieft, 1988). These studies have demonstrated that lichens are among the most efficient biological ice nucleators. Therefore their presence in the atmosphere may have a significant impact on cloud formation and precipitation. This ice nucleation capacity may also enable lichens to control the extent of their dispersal through the atmosphere. It is possible that lichens achieve this by nucleating ice formation, which leads to precipitation and ultimately deposition. This phenomenon has been shown to occur in some phytopathogenic bacteria (Morris et al., 2008, 2010) and potentially fungi as well (Morris et al., 2013).

3.3 Dominance of Basidiomycota in total communities and Ascomycota in active communities is consistent with mass-balance predictions

Our mass balance model (Appendix) predicted Basidiomycota would dominate the total community because they produce orders of magnitude more spores and have smaller aerodynamic diameters compared to Ascomycota. Consistent with this prediction, the total airborne community was dominated by Basidiomycota in our air samples (mean relative abundance = $90.2 \pm 6.9\%$) (Fig. 1). There have been some empirical studies reporting the opposite pattern, with a higher relative abundance of Ascomycota compared to Basidiomycota (Bowers et al., 2013; Fierer et al., 2008; Pashley et al., 2012).

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There has been one study focused on airborne fungal communities in the Amazon Basin (Fröhlich-Nowoisky et al., 2012). Although the site of this study was the atmosphere above a rural pasture (vs. a tropical rainforest, as in our study) these investigators also found that Basidiomycota dominate airborne fungal communities

5 Our mass-balance model explains the differences in composition between the total and active air communities. However some of the differences we observed may be partially attributable to the use of different approaches in characterizing the total and active communities. In this study, the total community was characterized by PCR-based amplification and sequencing of LSU genes, whereas the active community was characterized through random sequencing of all the RNA present in the samples. Shotgun metatranscriptome sequencing and PCR-based community characterization approaches each have their own biases (Hong et al., 2009; Morgan et al., 2010). Our data suggest that the selection of LSU primers led to biased results. For example, the high relative abundance of lichen fungi in the active community was unexpected because this group was not present in the total community and has only been detected in low abundance in other PCR-based studies of fungi in the atmosphere (Fröhlich-Nowoisky et al., 2012). We tested the LR0R-LR3 primer pair using the SILVA Test-Prime tool (Klindworth et al., 2013) and found coverage of the Lecanoromycetes with this primer pair was 71.4%. Importantly, the order Teloschistales, which contains the most abundant species in the active community, would not be detected with this primer pair. However the general pattern that Ascomycota were much less abundant than Basidiomycota in the total community is not likely due to primer bias as overage of the phylum Ascomycota by the LR0R-LR3 primer pair is 85.5% according to TestPrime. Our findings underscore the value of using a combination of PCR-based and shotgun-based sequencing approaches, particularly in environments that are understudied and where little is known about microbiome structure and function.

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3.4 Fungal air communities above the forest canopy are most similar in composition to tropical phyllosphere and soil communities

We compared total and active fungal air communities to communities from tropical, temperate, and tundra soils and from the surfaces of tropical tree leaves. Community composition significantly differed across environment types (ADONIS, $R^2 = 0.167$, $p = 0.001$), and fungal communities in the atmosphere were compositionally distinct from communities in other environments (Fig. 2). Ascomycota was the most abundant phylum across all soil and phyllosphere samples (soil mean relative abundance = $78.4 \pm 14.9\%$, phyllosphere = $90.9 \pm 4.9\%$) followed by Basidiomycota (soil mean relative abundance = $19.0 \pm 14.9\%$, phyllosphere = $7.4 \pm 4.5\%$) (Fig. 3). We expected communities to be distinct across habitat types because environmental conditions may differ across the habitat types and select for different communities. However, in the atmosphere, dispersal and mixing of fungi from multiple habitat types may be driving the observed community composition differences instead of environmental selection.

The diversity of fungal communities in the atmosphere is within the range of diversities reported for terrestrial environments, including those of tropical leaf surfaces, tropical soils, temperate grassland soils, and tundra soils. Overall taxonomic richness, defined as the number of OTUs, significantly varied among environment types (ANOVA, $F(5237) = 66.89$, $p < 0.001$) (Fig. S2). Tukey's HSD post-hoc comparisons indicated that the richness of air communities, both total and active, was greater than tundra soil communities and did not significantly differ from temperate grassland soil communities. In general, air communities were less diverse than tropical forest phyllosphere and soil communities with the exception of tropical forest soils and active air communities, which did not significantly differ. Similar patterns have been observed in soil communities where taxonomic richness in arctic soils was significantly lower than soils from temperate and tropical ecosystems (Fierer et al., 2012).

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Total air communities were most similar to tropical phyllosphere communities (mean Sørensen similarity = 0.015 ± 0.009 ; statistic) and active air communities were most similar to tropical soil communities (mean Sørensen similarity = 0.010 ± 0.007) (Fig. S3). These results suggest that inputs of fungi into the atmosphere over the canopy are derived from local, as opposed to long-distance, sources. This suggestion makes sense since fungal spores and hyphae are relatively large aerosol particles with short residence times in the atmosphere, limiting opportunities for long-distance dispersal. While these results are suggestive, detailed information is lacking regarding the potential influence of terrestrial source environments and their role in structuring airborne fungal communities.

4 Conclusion

Fungi in the atmosphere play an important role in atmospheric processes including precipitation development through ice nucleation. This is of particular significance in the atmosphere over the Amazon rainforest canopy where fungi constitute a large fraction of the total aerosol content (Elbert et al., 2007; Heald and Spracklen, 2009) and precipitation is aerosol-limited (Pöschl et al., 2010). Our study represents the first culture-independent survey of fungal communities over the Amazon rainforest canopy. It is also the first to measure metabolically active microbial communities in the atmosphere using an RNA-based approach. Using this RNA-based approach, we found evidence for the presence of potentially active fungi in the atmosphere, including lichen fungi. While an understanding of the structure of fungal communities in the atmosphere is beginning to emerge, studies on the function of these communities have lagged behind. We suggest that future research focus on understanding the functional capacity of airborne microbes with traits particularly well-suited for survival and metabolic activity in extreme environments. As with any environment, understanding both the structure and function of microbial communities in the atmosphere is needed to assess their potential impact on ecosystem processes such as water and carbon cycling. This study opens

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the door for future investigations of the diversity and function of fungal communities in the atmosphere.

Appendix: Mass-balance model

We use a global, well-mixed, one-box material-balance model to explain the relative abundances of fungal cells measured as gene copies sampled in the active and total portions of atmospheric bioaerosols. By material-balance, for any taxon i within a biological community, the change in time in the abundance of fungal gene copies, N_i , must be equal to the difference in source and sinks:

$$\frac{dN_i}{dt} = \sum \text{sources} - \sum \text{sinks} \quad (\text{A1})$$

Here we assume sources are equal to the emission of fungal gene copies from the Earth's surface into the atmosphere, E_i (gene copies hour⁻¹). We assume sinks are equal to deposition of fungal gene copies out of the atmosphere back to the Earth's surface, $D_i = N_i k_i$, (gene copies hour⁻¹), where k_i (1 hour⁻¹) represents a first order deposition coefficient. We can rewrite Eq. (A1) as:

$$\frac{dN_i}{dt} = E_i - N_i k_i \quad (\text{A2})$$

We expect the terms E_i and k_i to vary as a function of life history traits including the method of cell release into the atmosphere, the physiological state of sampled cells, and the aerodynamic diameter of fungal taxa. In this case, Eq. (A2) does not directly represent the entire airborne fungal gene copy abundance. We assume that a first order approximation of fungal bioaerosol behavior may be obtained by subdividing the particle distribution into two modes: vegetative cells, $N_{i,\text{veg}}$, and spores, $N_{i,\text{spores}}$. We thus model fungal gene copy abundance as:

$$N_i = N_{i,\text{veg}} + N_{i,\text{spores}}$$

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Based on these assumptions, it follows that the expected number of Ascomycota spores in the atmosphere will be less than the number of Basidiomycota spores:

$$\frac{E_{A, \text{ spore}}}{k_{A, \text{ spore}}} \ll \frac{E_{B, \text{ spore}}}{k_{B, \text{ spore}}}$$

or

$$N_{A, \text{ spores}} \ll N_{B, \text{ spores}}$$

We next consider fungal vegetative cells. Vegetative forms of Ascomycota are generally smaller than vegetative forms of Basidiomycota (Moore et al., 2011). Many Ascomycota grow as filaments or single cells which are small enough to be aerosolized (Després et al., 2012). In contrast, many Basidiomycota grow as mushrooms, which are too large to be aerosolized (although debris from mushrooms and their mycelia can be aerosolized). Due to this difference in the vegetative forms of each group, we expect emission rate of vegetative Ascomycota to be greater than Basidiomycota:

$$E_{A, \text{ veg}} > E_{B, \text{ veg}}$$

No comparative data currently exists on the relative deposition rate of vegetative cells across fungal taxa. Research has shown that at the phylum level, the aerodynamic diameter of Ascomycota is greater than that of Basidiomycota, resulting in a greater deposition rate overall for Ascomycota (Yamamoto et al., 2014). However this work did not differentiate between vegetative cells and spores, and there is no a priori reason to assume that the deposition rate of Ascomycota vegetative cells are less than or greater to that of Basidiomycota cells. For this reason, we make the null assumption that the deposition rate of each group is equal:

$$k_{d, A, \text{ veg}} = k_{d, B, \text{ veg}}$$

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Based on these assumptions, we expect the number of vegetative Ascomycota genes to be greater than the number of vegetative Basidiomycota genes:

$$\frac{E_{A, \text{veg}}}{k_{A, \text{veg}}} > \frac{E_{B, \text{veg}}}{k_{B, \text{veg}}}$$

or

$$5 \quad N_{A, \text{veg}} > N_{B, \text{veg}} \quad (\text{A3})$$

Equation (A3) predicts that Ascomycota will dominate the active fungal community in the atmosphere.

Finally, we relate the abundance of Ascomycota and Basidiomycota gene copies in their totality to ask if $N_A < N_B$ or $N_A \geq N_B$. $N_A < N_B$ if and only if:

$$10 \quad N_{A, \text{veg}} + N_{A, \text{spores}} < N_{B, \text{veg}} + N_{B, \text{spores}}$$

Rearranging terms and dividing both sides of the equation by $N_{B, \text{spores}}$ yields the inequality:

$$\frac{N_{A, \text{veg}} - N_{B, \text{veg}}}{N_{B, \text{spores}}} + \frac{N_{A, \text{spores}}}{N_{B, \text{spores}}} < 1$$

or

$$15 \quad \frac{N_{A, \text{veg}} - N_{B, \text{veg}}}{N_{B, \text{spores}}} < 1 - \varepsilon$$

where $\varepsilon = \frac{N_{A, \text{spores}}}{N_{B, \text{spores}}}$. Empirical data on the discharge of Ascomycota and Basidiomycota spores from fruiting bodies suggests that $\varepsilon \leq 0.01$ (Elbert et al., 2007). In this case $N_A < N_B$ if and only if:

$$\frac{N_{A, \text{veg}} - N_{B, \text{veg}}}{N_{B, \text{spores}}} < 0.99 \quad (\text{A4})$$

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taxo conceived and designed the experiments. F. Y. Ishida collected the samples and reviewed drafts of the paper. R. C. Mueller conceived and designed the experiments, reviewed drafts of the paper and contributed reagents/materials/analysis tools. S. R. Saleska conceived and designed the experiments. K. T. Wiedemann collected the samples. B. J. M. Bohannan conceived and designed the experiments, collected the samples, and reviewed drafts of the paper. J. L. Green conceived and designed the experiments, wrote the paper, reviewed drafts of the paper, and contributed reagents/materials/analysis tools.

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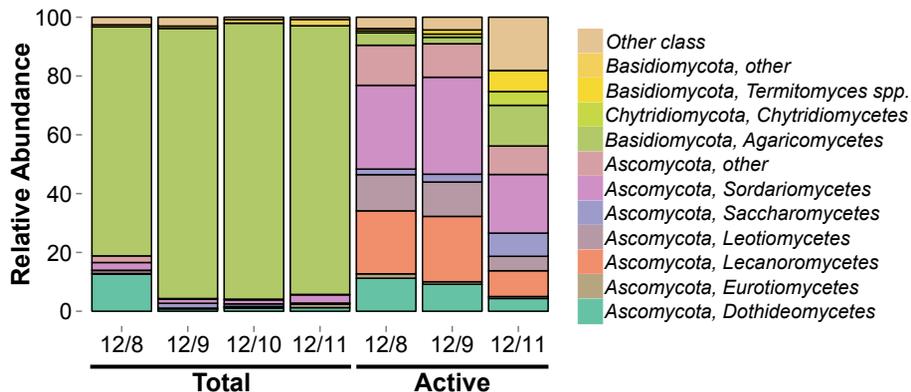


Figure 1. Basidiomycota dominate the total fungal community.

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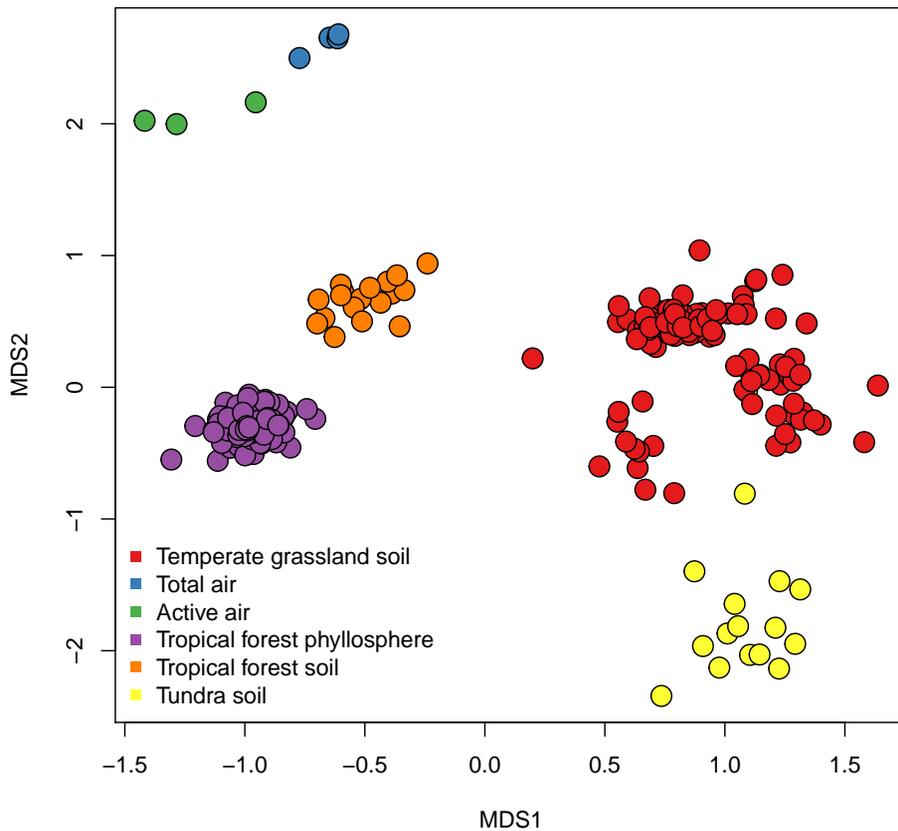


Figure 2. The compositions of the total and active fungal communities are distinct. Sørensen similarities are depicted, ordinated via NMDS.

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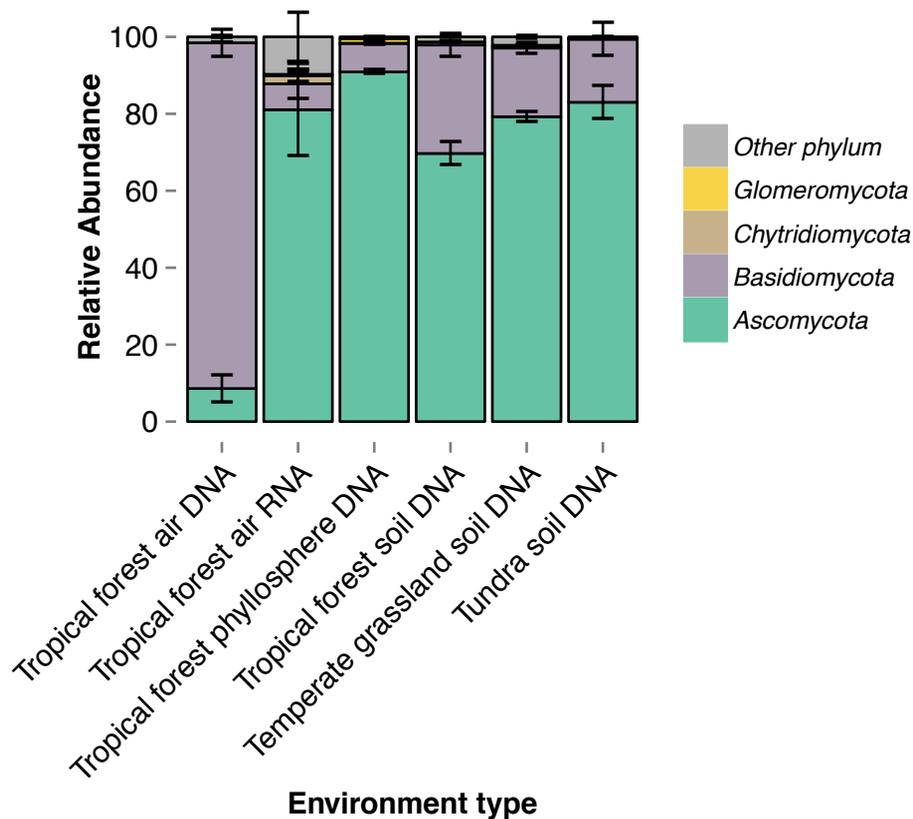


Figure 3. The active atmospheric fungal community over the Amazon rainforest is most similar to fungal communities found in tropical soils and on plant leaves.

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