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

A. M. Womack[1], P. E. Artaxo[2], F. Y. Ishida[3,4], R. C. Mueller[1,5], S. R. Saleska[6], K. T. Wiedemann[2,7], B. J. M. Bohannan[1], and J. L. Green[1,8]

[1]{Institute of Ecology and Evolution, University of Oregon, Eugene, OR USA}

[2]{Insititute of Physics, University of São Paulo, São Paulo, Brazil}

[3]{Instituto Nacional de Pesquisas da Amazonia, Manaus, Brazil}

[4]{School of Marine and Tropical Biology, James Cook University, Cairns, Qld, Australia}

[5]{Los Alamos National Laboratory, Los Alamos, NM, USA}

[6]{Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA}

[7]{School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA}

[8]{Santa Fe Institute, Santa Fe, NM, USA}

Correspondence to: Ann M. Womack (womack.ann@gmail.com)

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-throughout 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. 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 for fungal spores alone (Elbert et al, 2007). Fungal bioaerosols are not only abundant but also affect physical and chemical processes in the atmosphere. Fungal spores, cellular fragments, and cell-free biological particles have the potential to 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 for dormant spores this metabolic capability is greatly reduced (Sussman and Douthit, 1973). 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) (Supplement figure 1), and ice nucleation by hyphae has been observed at -

1   2.5°C. In contrast, dormant spores – particularly those with surface hydrophobins – are generally

2   poor ice nucleators. For example, ice nucleation of rust (*Puccinia*) spores requires temperatures

3   lower than -10°C (Morris et al., 2013), and *Cladsporium* spores nucleate ice at temperatures of

4   approximately -28.4°C (Iannone et al., 2011).

5   Despite its importance, we know relatively little about the physiological state of fungal cells in

6   the atmosphere. Specifically, we know little about the taxonomic composition of metabolically

7   active airborne fungi and how this compares to the composition of the total fungal community.

8   One way to survey the total and active communities is to measure community composition from

9   rDNA (i.e. rRNA genes) and rRNA in ribosomes. Sequencing rDNA provides information about

10 the total community, which includes both active and dormant individuals, whereas rRNA

11 sequences provide information about the potentially active community, because ribosomes are

12 more abundant in active cells than dormant cells (Prosser, 2002). This approach has been applied

13 to study active fungal communities in soils and on decaying plant material (Baldrian et al., 2012;

14 Barnard et al., 2013, 2014; Rajala et al., 2011) but has not been applied to fungal communities in

15 the atmosphere.

16 Information about the taxonomic composition of airborne fungi that are present in different

17 physiological states can be used to advance atmospheric science. For example, such data can be

18 used to improve estimates of the ice nucleating capacity of fungal bioaerosols. Historically, the

19 composition of fungal communities in the atmosphere has been measured using culture-based

20 approaches such as the abundance of colony forming units of specific taxa. This has led some

21 scientists to conclude that fungal communities in the atmosphere have a low capacity for ice

22 nucleation because taxa that appear abundant using plate counts have a low ice nucleation

23 efficiency (Iannone et al., 2011, but see Pummer et al., 2013). This data may be misleading, as

24 the vast majority of fungi require identification using culture-independent approaches (Borneman

25 and Hartin, 2000). Today, culture-independent identification of active fungal taxa sampled from

26 the atmosphere can be used to direct selective culturing of potentially important fungi in the

27 laboratory, where their ice nucleation efficiencies and their metabolic capabilities can be further

28 tested.

29 In this study, we used culture-independent approaches to measure the composition of total and

30 active atmospheric fungal communities *in situ* and a mass-balance model to aid in the

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1 interpretation of our results. Our study system is the atmosphere above the Amazon rainforest

2 canopy. We chose this system because fungal bioaerosols make up a substantial proportion of

3 aerosol particulate matter over the Amazon (Elbert et al., 2007; Heald and Spracklen, 2009) and

4 are estimated to be a dominant force responsible for cloud formation over the Amazon (Pöschl et

5 al., 2010). We used a combined approach of DNA and RNA sequencing to address the following

6 questions: 1) What is the composition of total airborne fungal communities? 2) What is the

7 composition of active airborne fungal communities? 3) What likely drives differences in the

8 composition of the total and active airborne fungal communities? 4) Is the diversity and structure

9 of fungal communities in the atmosphere similar to that found in terrestrial environments?

10

11 **2   Methods**

12 **2.1   Sample collection**

13 Sampling was conducted on the ZF2 K34 flux tower (S -2.60907, W -60.20917, 67 m a.s.l.) in the

14 Reserva Biologica do Cueiras in central Amazonia, about 60 km NNW of Manaus, Brazil. The

15 site is operated by the Instituto Nacional de Pesquisas da Amazonia (INPA) under the Large

16 Scale Biosphere-Atmosphere Experiment in Amazonia (LBA) program (Martin et al., 2010).

17 Tower height is approximately 54 m. Surrounding vegetation is undisturbed, mature, terra firme

18 rainforest, with a leaf area index of 5–6 and an average canopy height of 30 m. Samples were

19 collected at the end of dry season over four days, December 8-11, 2010, from a height of 48m

20 above the forest floor. Environmental conditions during the four-day sampling period were

21 typical for the location in early December with partial clouds and temperatures ranging from

22 approximately 28.5°C to 32.1°C. Heavy rain and thunderstorms occurred on 12/8 and 12/11.

23 Aerosol samples were collected using SKC Biosamplers (BioSampler SKC Inc.). Samplers were

24 filled with 20 mL of a water-based preservation solution (LifeGuard Soil Preservation Solution,

25 MO BIO Laboratories, Inc) to prevent DNase and RNase activity and maintain cells in stasis to

26 allow accurate community profiling of the total and active fungal community. Twelve impingers

27 were operated at 12.5 L/min from approximately 9:00 am – 4:00 pm each day. At the end of each

28 day, the sampling liquid from all impingers was pooled and stored at -20°C. Impingers were

cleaned each day by rinsing in 70% ethanol followed by sterilization using a portable pressure cooker.

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 sterile, individually wrapped, 0.22 μm cellulose nitrate filters (Nalgene Analytical Test Filter Funnels, Thermo Fisher Scientific). 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) (Fermentas International, Inc) according to the manufacturer's instructions. DNase reactions were cleaned (Zymo Research Clean and Concentrate-5) and eluted into 50 μl. 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. Three field blanks were generated by filtering unused LifeGuard Solution through new, sterile filters. Blanks were processed in parallel to the RNA and DNA samples including extraction, PCR amplification, and library preparation. Following library preparation, blank samples were visualized on an agarose gel and no visible bands were observed.

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 seconds 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 μl. A-overhangs were added to the

1   end-repaired fragments using Klenow exo(-) (Epicentre Biotechnologies) in a 50 µl reaction.

2   Reaction products were cleaned and concentrated (Zymo Research Clean and Concentrate-5).

3   Standard paired-end, barcoded Illumina adaptors (Supplement table 1) were ligated to the

4   fragments using T4 ligase (Fermantas). Reaction products were cleaned and concentrated (Zymo

5   Research Clean and Concentrate-5) and eluted in 12 µl. To enrich fragments with ligated

6   adaptors, PCR amplification was performed using primers containing the flowcell adaptor and

7   complementary to the Illumina sequencing primer (Supplement Table 1). PCR reactions were

8   performed using Phusion DNA polymerase (New England Biolabs) with 12 µl template, 10 µl 5x

9   HF buffer, 1 µl 10 mM dNTPs, 2 µl 10 mM primer mix, 0,5 µl enzyme and 25.5 µl water for a

10 final reaction volume of 50 µl. PCR cycling conditions were as follows: 30 seconds denaturation

11 at 98°C followed by 18 cycles of 98°C for 30 seconds, 65°C for 30 seconds and 72°C for 30

12 seconds following by a final extension at 72°C for 5 minutes. PCR products were size

13 fractionated by gel electrophoresis (2.5%, low-melt agarose). Products in the range of 150-500 bp

14 were excised, and DNA from the excised gel pieces was extracted (QiagenMinElute Gel

15 Extraction) and eluted into 20 µl. DNA was quantitated using a Qubit 2.0 Fluorometer

16 (Invitrogen, Life Technologies Corporation) and combined in equal molar concentrations.

17 Shotgun metatranscriptome libraries were sequenced (150 base pairs, paired-end) on the Illumina

18 HiSeq 2000 (Illumina, Inc.) platform at the University of Oregon Genomics Core Facility. LSU

19 rDNA amplicons were sequenced (250 base pairs, paired-end) on the Illumina MiSeq platform at

20 the Dana-Farber Cancer Institute Molecular Biology Core.

21 The D1-D2 region of the large subunit (LSU) rRNA gene was targeted using PCR with the

22 primers LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR3 (5'-CCGTGTTTCAAGACGGG-3')

23 (http://sites.biology.duke.edu/fungi/mycolab/primers.htm). LSU amplicon libraries were prepared

24 using a two-stage PCR procedure as described in (Kembel and Mueller, 2014) using unique

25 combinatorial barcodes (Gloor et al., 2010) to identify samples (Supplement table 2).

26 **2.4   Sequence pre-processing**

27 **2.4.1 Metatranscriptome**

1   Overlapping paired end reads were aligned and joined using fastq-join (https://code.google.com/

2   p/ea-utils/wiki/FastqJoin). Joined reads and non-overlapping single-end reads were trimmed and

3   filtered using PrinSeq (Schmieder and Edwards, 2011). Sequences <75 bp, > 2% Ns, and/or mean

4   quality score <20 were removed. Sequence artifacts defined as exact duplicates with >5,000

5   sequences were removed. Sequences in the Dec. 10 sample were primarily artifacts, so this

6   metatranscriptome sample was excluded from further analysis. Putative rRNAs in the remaining

7   sequences were identified using SortMeRNA (Kopylova et al., 2012) with the non-redundant

8   version of the following databases: rfam 5.8S (version 11.0) (Burge et al., 2013); Unite

9   (November 2011 version) (Kõljalg et al., 2013), and Silva 18S and Silva 28S (Release 115)

10 (Quast et al., 2013). Of 5,165,185 quality-filtered reads, 1,915,994 with an average length of

11 137.5 bp were identified as putative rRNAs (Supplement table 3).

12 **2.4.2 LSU amplicons**

13 Forward and reverse barcodes were combined to make a 12 bp barcode on the forward read. Only

14 forward reads derived from the LR3 region were used for analysis. This region has been shown to

15 have high species-level resolution even with short read lengths (Liu et al., 2012).

16 **2.4.3 Multi-environment sequences**

17 LSU sequences from four soil studies (Barnard et al., 2013; Kerekes et al., 2013; Penton et al.,

18 2013, 2014) and one phyllosphere study (Kembel and Mueller, 2014) were compared to air

19 samples collected for this study (Supplement table 4). Raw sequence data and associated

20 metadata were downloaded from publically available databases. 12 bp barcodes were added to all

21 sequences to identify each sample in downstream analysis.

22 **2.5   LSU amplicon and metatranscriptome sequence processing**

23 All sequences were processed in QIIME version 1.7 (Caporaso et al., 2010). Briefly, libraries

24 were individually demultiplexed and filtered for quality. Sequences with an average quality score

25 less than 20, shorter than 150 bp and with greater than 2 primer mismatches were discarded. The

26 same parameters were used across all samples except the metatranscriptome rRNAs were a size

27 cut off of greater than 75 bp was used. In order to decrease computation time, sequences from

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Kembel and Mueller (2014) and Penton *et al*. (2014) were randomly subsampled to 25% and 60% of the total number of sequences, respectively. Sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using closed reference BLAST (Altschul et al., 1990) against the Ribosomal Database Project Fungal LSU training set 1 (Cole et al., 2014). Taxonomy was assigned to each OTU was that of the most similar representative in the RDP database.

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 (Supplement table 3). For analyses using only samples from this study, the data were rarefied to 5,300 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 (http://dx.doi.org/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 Appendix A.

3   Results & 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 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 (Figure 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±6.9%) (Figure 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±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). At the genus level, there were several taxa detect in the total community with ice nucleation activity including Acremonium, Cladosporium, Fusarium, and Rhizopus (Table S4).

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) (Supplement figure 1). 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 biological particles including other spore types (Morris et al., 2013), suspensions of *Fusarium* cultures (containing spores and hyphae) (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.

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The patterns we report reflect a snapshot in space and time. As in other environmental systems, the composition of total fungal communities in the atmosphere will vary across different spatial and temporal scales. Research has shown, for example, that concentrations of fungal spores in the atmosphere vary diurnally and seasonally. This variation is driven by complex interactions between fungal dispersal mechanisms and environmental conditions, particularly moisture and wind speed. (Lacey, 1996). Our samples were collected during the day, and spores released by mechanical disturbances often peak in abundance in the air during midday when wind speeds are highest (Lacey, 1996). Taxa that require dry conditions for dispersal also tend to release spores during the day, and taxa that require high relative humidity, including many Basidiomycota, tend to release spores at night when humidity is highest (Elbert et al., 2007; Lacey, 1996). In addition to humidity, precipitation events can also affect the dispersal of fungi. Overall concentrations of spores have been shown to increase in the atmosphere due to convective instability preceding thunderstorms (Burch and Levetin, 2002), and Ascomycota concentrations increase during and immediately after rainstorms (Elbert et al., 2007). Our samples were collected at the end of the dry season. If we had sampled during the wet season, it is possible we would have observed a higher relative abundance of Ascomycota in the total community since the dispersal of Ascospores has been shown to increase before and after rain storms.

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±20%) (Figure 1). Basidiomycota comprised a smaller fraction of the sampled genes (mean = 7.3±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, whereas many of the Basidiomycota are too large to be easily aerosolized, other than in the form of metabolically inactive spores. It is possible that if we had sampled at night rather than during the day, we would have observed a higher relative abundance of Basidiomycota in the active community. The abundance of vegetative Ascomycota fragments

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may peak during the day when wind speeds are high, assuming they are passively dispersed by wind and convection (as opposed to active mechanisms many fungi use to disperse spores). And, Basidiospores have been shown to be particularly abundant in the Amazon atmosphere at night (Elbert et al., 2007). As with the total community, we expect that the composition of active fungal communities in the atmosphere will vary across different spatial and temporal scales.

The most abundant classes of Ascomycota detected were Sordariomycetes (mean = 27.1±6.6%), and Lecanoromycetes (mean = 17.5±7.6%). Sordariomycetes have been previously detected in culture-independent 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 saprotophs (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. Several genera with known ice nucleation capability were detected in the active community including Agaricus, Amanita, Aspergillus, Boletus, Lepsita, Mortierella Puccinia, Rhizopus, and the lichen fungus Cladonia (Table S4). Below we focus our discussion on the class Lecanoromycetes, an understudied but potentially important group of fungi in the atmosphere.

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 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, Gauslaa, Nybakken, & Bilger, 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±3.8%) and *Rinodina milvina* (mean = 4.8±3.4%), there have been multiple studies of the ice nucleation efficiency of many other lichen fungi species. Ice nucleation

1   activity of lichens has been measured at temperatures warmer than -8°C, including 13 of 15 taxa

2   tested by Henderson-Begg and colleagues (Henderson-Begg et al., 2009) and 9 of 15 taxa tested

3   by Kieft (Kieft, 1988). These studies have demonstrated that lichens are among the most efficient

4   biological ice nucleators. Therefore, their presence in the atmosphere may have a significant

5   impact on cloud formation and precipitation. This ice nucleation capacity may also enable lichens

6   to control the extent of their dispersal through the atmosphere. It is possible that lichens achieve

7   this by nucleating ice formation, which leads to precipitation and ultimately deposition. This

8   phenomenon has been shown to occur in some phytopathogenic bacteria (Morris et al., 2008,

9   2010) and may occur in fungi as well (Morris et al., 2013).

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10 **3.3   Dominance of Basidiomycota in total communities and Ascomycota in**

11   **active communities is consistent with mass-balance predictions**

12 Our mass balance model (Appendix A) predicted Basidiomycota would dominate the total

13 community because they produce orders of magnitude more spores and have smaller

14 aerodynamic diameters compared to Ascomycota. Consistent with this prediction, the total

15 airborne community was dominated by Basidiomycota in our air samples (mean relative

16 abundance = 90.2±6.9%) (Figure 1). There have been some empirical studies reporting the

17 opposite pattern, with a higher relative abundance of Ascomycota compared to Basidiomycota

18 (Bowers et al., 2013; Fierer et al., 2008; Pashley et al., 2012). There has been one study focused

19 on airborne fungal communities in the Amazon Basin (Fröhlich-Nowoisky et al., 2012). Although

20 the site of this study was the atmosphere above a rural pasture (versus a tropical rainforest, as in

21 our study) these investigators also found that Basidiomycota dominate airborne fungal

22 communities

23 Our mass-balance model explains the differences in composition between the total and active air

24 communities. However, some of the differences we observed may be partially attributable to the

25 use of different approaches in characterizing the total and active communities. In this study, the

26 total community was characterized by PCR-based amplification and sequencing of LSU genes,

27 whereas the active community was characterized through random sequencing of all the RNA

28 present in the samples. Shotgun metatranscriptome sequencing and PCR-based community

29 characterization approaches each have their own biases (Hong et al., 2009; Morgan et al., 2010).

1   Our data suggest that the selection of LSU primers led to biased results. For example, the high

2   relative abundance of lichen fungi (class Lecanoromycetes) in the active community was

3   unexpected because this group was not detected in the total community and has only been

4   detected in low abundance in other PCR-based studies of fungi in the atmosphere (Fröhlich-

5   Nowoisky et al., 2012). We tested the primer pair used in this study (LR0R-LR3) using the

6   SILVA TestPrime tool (Klindworth et al., 2013) and found coverage of the Lecanoromycetes

7   with this primer pair was 71.4%. Within the class Lecanoromycetes, the order Teloschistales,

8   which contains the most abundant species detected in the active community, would not have been

9   detected with this primer pair. However, the general pattern that Ascomycota were much less

10 abundant than Basidiomycota in the total community is not likely due to primer bias as overage

11 of the phylum Ascomycota by the LR0R-LR3 primer pair is 85.5% according to TestPrime. Our

12 findings underscore the value of using a combination of PCR-based and shotgun-based

13 sequencing approaches, particularly in environments that are understudied and where little is

14 known about microbiome structure and function.

15 ### 3.4   Fungal air communities above the forest canopy are most similar in
16 composition to tropical phyllosphere and soil communities

17 We compared total and active fungal air communities to communities from tropical, temperate,

18 and tundra soils and from the surfaces of tropical tree leaves. Community composition

19 significantly differed across environment types (ADONIS, R^2 = 0.167, p = 0.001), and fungal

20 communities in the atmosphere were compositionally distinct from communities in other

21 environments (Figure 2). Ascomycota was the most abundant phylum across all soil and

22 phyllosphere samples (soil mean relative abundance = 78.4±14.9%, phyllosphere = 90.9±4.9%)

23 followed by Basidiomycota (soil mean relative abundance = 19.0±14.9%, phyllosphere =

24 7.4±4.5%) (Figure 3). We expected communities to be distinct across habitat types because

25 environmental conditions may differ across the habitat types and select for different communities.

26 However, in the atmosphere, dispersal and mixing of fungi from multiple habitat types may be

27 driving the observed community composition differences instead of environmental selection.

28 The diversity of fungal communities in the atmosphere is within the range of diversities reported

29 for terrestrial environments, including those of tropical leaf surfaces, tropical soils, temperate

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grassland soils, and tundra soils. Overall taxonomic richness, defined as the number of OTUs, significantly varied among environment types (ANOVA, $F_{(5,237)}$ = 66.89, $p < 0.001$) (Supplement figure 2). 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).

Total air communities were most similar to tropical phyllosphere communities (mean Sørensen similarity = 0.015±0.009; Tukey's HSD, $p < 0.001$) and active air communities were most similar to tropical soil communities (mean Sørensen similarity = 0.010±0.007, Tukey's HSD, $p < 0.001$) (Supplement figure 3). 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 (class Lecanonomycetes) and the following genera: Agaricus; Amanita; Aspergillus; Boletus; Cladonia; Lepsita; Mortierella; Puccinia; and Rhizopus. 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 the door for future investigations of the diversity and function of fungal communities in the atmosphere.

Author contributions

A. M. Womack conceived and designed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, and reviewed drafts of the paper. P. E. Artaxo 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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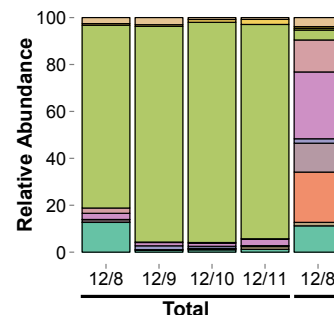17 98(6), 1076–1087, doi:10.3852/mycologia.98.6.1076, 2007.
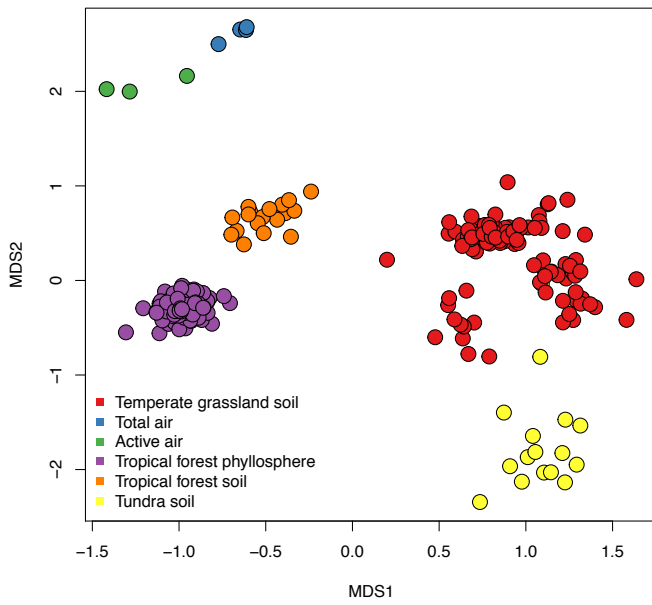
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19 **Figures**
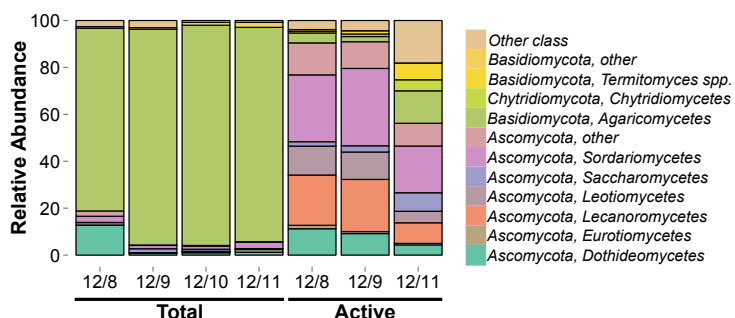
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21 Figure 1. Basidiomycota dominate the total fungal community (mean relative abundance =
22 90.2±6.9%). Bars are colored according to class-level taxonomic assignments. Taxonomy was
23 assigned to representative sequences from each OTU.
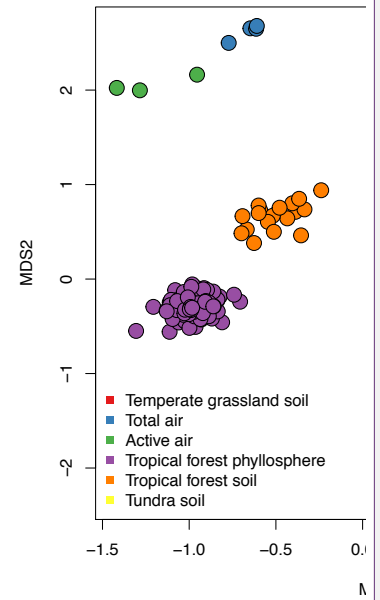
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2   Figure 2. OTU-based community composition significantly differed across environment types

3   (ADONIS, R^2 = 0.167, p = 0.001). Total and active communities in air samples (upper left)

4   clustered together and separate from other environments indicating these communities are distinct

5   from communities found in soils and on leaf surfaces. Sørensen similarities are depicted,
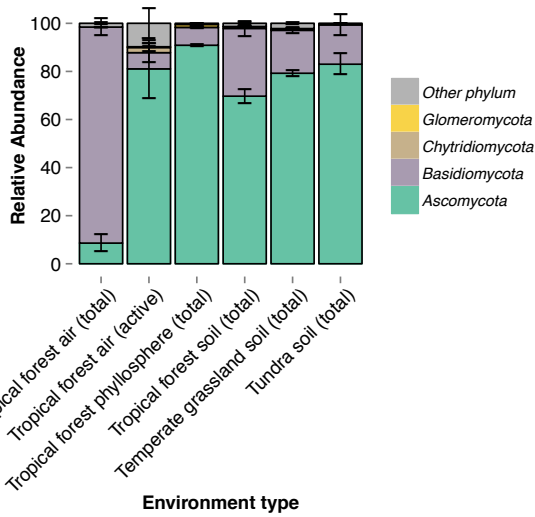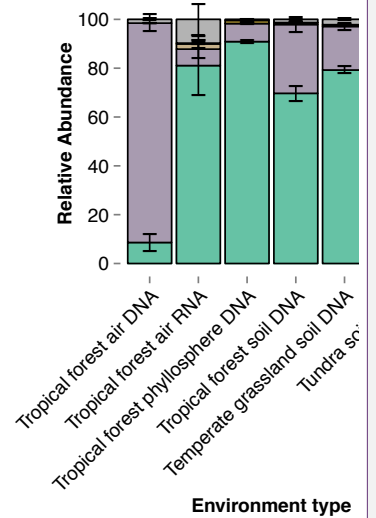
6   ordinated via NMDS.

1

2 Figure 3. Relative abundances of fungal phyla across environment types. The active atmospheric
3 fungal community over the Amazon rainforest was more similar in phylum-level composition to
4 fungal communities found in tropical soils and on plant leaves than was the total community.
5 Error bar represent standard deviations.

6

7 **Appendix A**

8 **Mass-balance model**

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

14
$$\frac{dN_i}{dt} = \sum sources - \sum sinks \qquad (A1)$$

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). 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), where k_i (1/hour) represents a first order deposition coefficient. We can rewrite Equation (A1) as:

$$\frac{dN_i}{dt} = E_i - N_i k_i$$

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, Equation (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, $N_{i,veg}$, and spores, $N_{i,spores}$. We thus model fungal gene copy abundance as:

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

We can then write and solve parallel versions of Equation (A2) for each mode. At steady state, the expected gene copy abundance taxa i in each mode is:

$$N_{i,veg} = \frac{E_{i,veg}}{k_{i,veg}}$$

$$N_{i,spore} = \frac{E_{i,spore}}{k_{i,spore}}$$

Our interest lies in the two most common fungal phyla sampled in the atmosphere: Ascomycota, N_A, and Basidiomycota, N_B. To make predictions about the expected relative abundance of gene copies in these two groups, we make informed assumptions about the relative magnitude of their respective emission and deposition rates. We begin by considering fungal spores. Although a few empirical studies have suggested that Ascomycota are more abundant than Basidiomycota in likely source environments including tropical soils (Kerekes et al., 2013) and leaf surfaces (Kembel and Mueller, 2014), Basidiomycota (e.g. Agaricomycetes, the most abundant class of Basidiomycota in our samples) produce orders of magnitude more spores per individual than Ascomycota (Elbert et al., 2007; Pringle, 2013). For this reason, we assume the emission rate of Basidiomycota spores is much greater than that of Ascomycota spores:

26

1 $$E_{A,spores} \ll E_{B,spores}$$

2 Culture-based microscopy data suggests that spores of Ascomycota are typically larger than

3 spores of Basidiomycota (Elbert et al., 2007; Ingold, 2001; Yamamoto et al., 2014). Owing to the

4 difference in spore size, we expect deposition rate of Ascomycota spores to be greater than that of

5 Basidiomycota spores:

6 $$k_{d,A,spores} > k_{d,B,spores}$$

7 Based on these assumptions, it follows that the expected number of Ascomycota spores in the

8 atmosphere will be less than the number of Basidiomycota spores:

9 $$\frac{E_{A,spore}}{k_{A,spore}} \ll \frac{E_{B,spore}}{k_{B,spore}}$$

10 or

11 $$N_{A,spores} \ll N_{B,spores}$$

12 We next consider fungal vegetative cells. Vegetative forms of Ascomycota are generally smaller

13 than vegetative forms of Basidiomycota (Moore et al., 2011). Many Ascomycota grow as

14 filaments or single cells which are small enough to be aerosolized (Després et al., 2012). In

15 contrast, many Basidiomycota grow as mushrooms, which are too large to be aerosolized

16 (although debris from mushrooms and their mycelia can be aerosolized). Due to this difference in

17 the vegetative forms of each group, we expect emission rate of vegetative Ascomycota to be

18 greater than Basidiomycota:

19 $$E_{A,veg} > E_{B,veg}$$

20 No comparative data currently exists on the relative deposition rate of vegetative cells across

21 fungal taxa. Research has shown that at the phylum level, the aerodynamic diameter of

22 Ascomycota is greater than that of Basidiomycota, resulting in a greater deposition rate overall

23 for Ascomycota (Yamamoto et al. 2014). However, this work did not differentiate between

24 vegetative cells and spores, and there is no *a priori* reason to assume that the deposition rate of

25 Ascomycota vegetative cells are less than or greater to that of Basidiomycota cells. For this

26 reason, we make the null assumption that the deposition rate of each group is equal:

27

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

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

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

or

$$N_{A,veg} > N_{B,veg} \tag{A3}$$

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

Finally, we relate the abundance of Ascomycota and Basiodiomycota 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:

$$N_{A,veg} + N_{A,spores} < N_{B,veg} + N_{B,spores}$$

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

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

or

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

where $\varepsilon = \frac{N_{A,spores}}{N_{B,spores}}$. 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:

$$\frac{N_{A,veg} - N_{B,veg}}{N_{B,spores}} < 0.99 \tag{A4}$$

We expect Equation A4 to hold due to the likelihood that spores greatly out number vegetative cells in the atmosphere in both phyla. Spores can be actively discharged into the air, whereas vegetative cells are not actively propelled into the atmosphere and require aerosolization by

28

mechanical forces like wind. Furthermore, empirical data suggests that vegetative cell fragments constitute a small fraction (0.2-16% (Green et al., 2011)) of the total fungal biomass in the atmosphere. For these reasons, we predict that

$$N_A < N_B$$

Based on the conclusions of this model, we expect Basidiomycota will dominate the total community, and Ascomycota will dominate the active community in the atmosphere. We note there are many limitations to our model. First, we model fungal gene copy abundances assuming a well-mixed atmosphere at steady state. Yet the atmosphere is a highly heterogeneous and dynamic environment; the sampled air volume was likely neither well mixed nor at steady state over the time intervals we measured. Second, we use a global model with emission and deposition as the sole input and output, whereas a local model that incorporated site-specific environmental fate and transport terms would likely provide more accurate expectations. Third, due to a paucity of data, our estimates of fungal gene abundance levels rely on assumptions about the emission and deposition rates of vegetative cells and spores across fungal taxonomic groups. Empirically derived estimates of these model parameters would significantly improve our approach. Fourth, we do not know to what extent vegetative cells and spores are associated with other particulate matter and how this affects their deposition and emission rates. Adopting an approach to empirically estimate the aerodynamic diameter of these fungal cell types across taxonomic groups would allow for improved estimates of deposition rates (Yamamoto et al., 2014).

29