„Characterization of primary biogenic aerosol particles by DNA analysis: Diversity of airborne *Ascomycota* and *Basidiomycota*"

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Janine Fröhlich

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Abstract

Primary biogenic aerosol (PBA) particles account for large proportions of air particulate matter, and they can influence the hydrological cycle and climate as nuclei for water droplets and ice crystals in clouds, fog, and precipitation. Moreover, they can cause or enhance human, animal, and plant diseases. The actual abundance and properties of PBA particles and components in the atmosphere are, however, still poorly understood and quantified.

In this study, the identity, diversity, and frequency of occurrence of PBA particles were investigated by DNA analysis. Methods for the extraction, amplification, and analysis of DNA from aerosol filter samples were developed and optimized for different types of organisms, including fungi, bacteria, and plants. The investigations were focused on fungal DNA, and over 2500 sequences were obtained from air samples collected at different locations and climatic zones around the world (tropical, mid-latitude, sub-polar; continental, marine).

Nearly all fungal DNA sequences could be attributed to the phyla of *Ascomycota* and *Basidiomycota*. With regard to species richness, the ratio of *Basidiomycota* to *Ascomycota* was much higher in continental air samples (~60:40) than in marine air samples (~30:70). Pronounced differences in the relative abundance and seasonal cycles of various groups of fungi were detected in coarse and fine particulate matter from continental air, with more plant pathogens in the coarse and more human pathogens and allergens in the respirable fine particle fraction (<3 μm). The results of this study provide new information and insights into the sources of PBA particles and the interactions of the biosphere with the atmosphere, climate, and public health.

Zusammenfassung

Primäre biogene Aerosolpartikel (PBA) haben großen Anteil an der Zusammensetzung und an den Auswirkungen atmosphärischer Aerosole. Sie können den Wasserkreislauf und das Klima als Kondensationskerne für Wassertropfen und Eiskristalle in Wolken, Nebel und Niederschlag beeinflussen, und sie können Krankheiten an Mensch, Tier und Pflanze auslösen oder verstärken. Die genauen Eigenschaften von PBA sind bislang jedoch kaum bekannt.

In dieser Arbeit wurde die Identität, Diversität und die Häufigkeit des Vorkommens von PBA mittels DNA-Analysen untersucht. Methoden für die Extraktion, Amplifizierung und Analyse von DNA aus Luftfilterproben wurden entwickelt und für verschiedene Organismen wie Pilze, Bakterien und Pflanzen optimiert. Die Untersuchungen dieser Arbeit konzentrierten sich auf den Nachweis von Pilz-DNA in Luftproben, und mehr als 2500 DNA-Sequenzen konnten aus Proben von verschiedensten Standorten und Klimazonen rund um die Welt gewonnen werden (Tropen, Mittlere Breiten, Polargebiete; kontinental, marin).

Fast alle DNA-Sequenzen konnten den Abteilungen der *Ascomycota* und *Basidiomycota* zugeordnet werden. Bezüglich der Artenvielfalt war das Verhältnis von *Basidio-* zu *Ascomycota* in kontinentalen Luftproben (~60:40) deutlich höher als in marinen Proben (~30:70). Deutliche Unterschiede zeigten sich auch in der relativen Häufigkeit und in den saisonalen Zyklen verschiedener Pilzgruppen in Grob- und Feinstaubproben aus kontinentaler Luft. Dabei war der Anteil an phytopathogenen Spezies in der Grobstaubfraktion höher, während humanpathogene und allergene Spezies eher in der lungengängigen Feinstaubfraktion gefunden wurden. Die Ergebnisse dieser Arbeit liefern neue Information und Einsicht in die Quellen von PBA und in die Wechselwirkung von Biosphäre, Atmosphäre, Klima und Gesundheit.

1 Introduction

1.1 Atmospheric aerosols and airborne fungi

The effects of aerosols on the atmosphere, climate, and health are among the central topics in current environmental research. Aerosol particles scatter and absorb solar and terrestrial radiation, provide condensation nuclei for cloud droplets, and effect atmospheric chemistry. Moreover, they play an important role in the spreading of organisms and reproductive materials, and they can cause or enhance human, animal, and plant diseases (Pöschl, 2005; Després et al., 2007).

An aerosol is generally defined as a suspension of a liquid or solid particle in a gas, with particle diameters in the range of ~0.001 μm (nucleation mode particles) up to ~100 μm (e.g., large dust particles or plant fragments; Seinfeld and Pandis, 1998). Atmospheric aerosol particles originate from a wide variety of natural and anthropogenic sources, including primary particle emission such as biomass burning, combustion processes, volcanic eruptions, and wind-driven or traffic-related suspension of road, soil, and mineral dust, sea salt and biological materials as well as secondary particle formation by gas-to-particle-conversion in the atmosphere (Pöschl, 2005).

Primary biogenic aerosol (PBA) particles are emitted directly from the biosphere to the atmosphere and represent a significant fraction of the aerosol particles in the atmosphere (up to ~50% of the mass concentration; Jaenicke, 2005; Elbert et al., 2007). Air particulate matter of biological origin includes viable cells like pollen, bacteria, and fungal spores, dead microorganisms and other non viable materials like plant, animal and fungal fragments, allergenic compounds, mycotoxins, and endotoxins (Matthias-Maser and Jaenicke, 1993; Griffiths and DeCosemo, 1994; Artaxo and Hansson, 1995; Newson et al., 2000; Mitakakis et al., 2001; Gorny et al., 2002; Douwes et al., 2003; Boreson et al., 2004; Jaenicke, 2005; Pöschl, 2005; Després et al., 2007; Georgakopoulus et al., 2008). The actual abundance and origin of PBA particles and components are, however, still poorly understood and quantified.

Especially, the knowledge about dead or fragmented biological particles and non cultivable microorganisms (bacteria, fungi) in the atmosphere is greatly limited. This is largely due to a lack of efficient measurement methods. In most earlier studies microscopy, protein staining, or cultivation of viable airborne bacteria and fungi have been applied. These methods, however, do not allow a comprehensive characterization of the origin of biological materials independent of particle size, integrity, and viability. In contrast, the application of molecular genetic tools for the analysis of DNA offers a very straightforward way to identify the origin of biological matter from both living and dead, cultivable and non cultivable, complete and fragmented organisms (Després et al., 2007; Fröhlich-Nowoisky et al., 2009).

The importance of fungi for aerobiology is based on the production of enormous numbers of spores and other fragments (e.g., broken conidia or hyphae) which constitute a major fraction of PBA. In example Womiloju et al. (2003) reported that material of fungi contribute 4-11% of the mass of fine particulate matter (PM2.5, aerodynamic diameter ≤2.5 μm) and Bauer et al. (2008b) found that fungal spores account for up to 21% of PM10 (≤10 μm) mass. On average, the number and mass concentrations of fungal spores in continental boundary layer air are on the order of ~10^3-10^4 m^{-3} and ~1 μg m^{-3}, respectively, the global emission rate is estimated to be on the order of ~50 Tg yr^{-1} (Elbert et al., 2007). The actual concentration of fungal spores in air is highly variable and depends on many factors including temperature, humidity, wind, and interactions with other trace substances, clouds, and precipitation. As outlined by Elbert et al. (2007) certain types of fungal spores are preferably emitted under humid conditions (in particular actively wet discharged asco- and basidiospores) whereas others are preferably emitted under dry conditions (dry discharged spores). The number, dispersion potential, and survival of fungal spores vary between species (Moletta et al., 2007) and may be influenced by climate change (Klironomos et al., 1997). Studies which analyzed the influence of elevated CO_2 concentrations on soil found an increase of fungal biomass and changes in fungal species composition (Klamer

et al., 2002). Thus, climate change could also have a strong impact on the concentration and composition of airborne spores, which in turn may influence the effects of fungi on human health, the biosphere, and climate and result in positive or negative feedback mechanisms (Jones and Harrison, 2004; Pöschl, 2005; Elbert at al., 2007).

1.2 Environmental and health effects of airborne fungi

Besides bacteria, fungi have been also found in cloud water, fog, and precipitation (Bauer et al., 2002). They can act as cloud condensation and ice nuclei at relatively warm temperatures and influence the formation of precipitation, the hydrological cycle, and climate (Kieft and Ahmadjian, 1989; Pouleur et al., 1992). Moreover, fungi might influence the chemical composition of cloud and rain water by metabolic transformation of organic trace substances such as dicarboxylic acids (Ariya et al., 2002; Deguillaume et al., 2008).

Fungi are found in almost every environment (Bridge and Spooner, 2001; Göttlich et al., 2002; Shearer et al., 2004; Damare et al., 2006; Elbert et al., 2007). In soil fungi constitute more of the biomass than bacteria (Thorn, 1999). They are a diverse group of organism and exist in a variety of forms like mushrooms, single cell yeasts, and molds. The number of different fungal species existing on earth is assumed to be in the range of 1–1.5 million. Some 70000 to 100000 have been described to date (Hawksworth and Rossman, 1997). Most of the fungal species found in the biosphere and atmosphere belong to the phyla *Ascomycota* (AMC, sac fungi) and *Basidiomycota* (BMC, club fungi) like described in Elbert et al. (2007) and references therein. The subkingdom of *Dikarya* which includes the AMC and BMC accounts for 98% of the known species in the biological kingdom of *Eumycota* (fungi; James et al., 2006).

As already outlined above, air is the natural medium for the dispersal of fungal spores, and these play a crucial role in the spread of diseases (Brown and Hovmøller, 2002). Many fungi like smuts, rusts, and mildews are

important plant pathogens. Fungi are also known to be associated with allergic reactions and health effects in human, animals, and insects (Bernton and Thom, 1937; Gravesen, 1979; Evans et al., 1988; Burge, 1989; Burge, 1990; Madelin, 1994; Fukatsu et al., 1997; Torricelli et al., 1997; Kurup et al., 2000; Newson et al., 2000; Berbee, 2001; Mitakakis et al., 2001; Almaraz et al., 2002; Helbling et al., 2002; Douwes et al., 2003; Adhikari et al., 2004; Casadevall, 2005; Aime et al., 2006; Segal and Walsh, 2006; Santos and Goossens, 2007; Eichmann and Hückelhoven, 2008). Fungi produce toxic metabolites like mycotoxins or allergenic substances which play a role in inflammatory responses, resulting in respiratory symptoms and cancer (Davis et al., 1988; Eduard and Heederik, 1998; Douwes et al., 2003; Nielsen, 2003; Lee et al., 2006a; Niessen and Steve, 2008). Roughly 25-30% of allergic asthma cases are fungal induced (Kurup et al., 2002).

Fungal genera associated with immunological reactions are e.g., *Alternaria spp., Cladosporium spp.*, and *Aspergillus spp*. (Bernton and Thom, 1937; Madelin, 1994; Mitakakis et al., 2001; Chew et al., 2003; Douwes et al., 2003; McDevitt et al., 2004). The mold *Cladosporium* can also interact with airborne pollen increasing allergic problems (Oliveira et al., 2005). Spores of these fungi are found worldwide in the atmosphere as well as in indoor air (Burge, 2002; Adhikari et al., 2004; Kellogg et al., 2004; Levetin, 2004; Ho et al., 2005; Lee et al., 2006b; Wu et al., 2007; O'Gorman and Fuller, 2008).

Besides viable spores, also dead fungal cells and cell debris can be allergenic and provoke health effects (Lehrer et al., 1986; Weissman et al., 1987; Davis et al., 1988; Griffiths and DeCosemo, 1994; Gorny et al., 2002; Douwes et al., 2003; Green et al., 2006). Fungal fragments like cell walls or cytoplasmatic material are easily suspended and inhaled as fine air particulate matter (Glikson et al., 1995; Cho et al., 2005; Green et al., 2006).

Beyond pathogenicity and allergenicity, fungi fulfill important functions in the biosphere and in human activities. Many species exist in symbiosis with plants (e.g., ecto- and endomycorrhizae; Read, 1997; Finley et al., 2004) and play important roles in nutrient cycling – for example in the decomposition of

plant polymers such as lignin or cellulose (Thorn and Wellington, 1997). Fungi are also widely used as biocontrol agents in agriculture (Van Driesche et al., 1996; Thorn and Wellington, 1997; Mims and Richardson, 2005; Mielnichuk and Lopez, 2007; Samils et al., 2008) and for fermentation and other processes in food and biotechnological industries (Porter and Fox, 1993).

1.3 Detection and identification of airborne fungi

Earlier studies have mostly used cultivation techniques, microscopy, and chemical tracers to identify and quantify airborne fungi (Bauer et al., 2002; Shelton et al., 2002; Chew et al., 2003; Medrela-Kuder, 2003; Elbert et al., 2007). However, only ~17% of known fungal species can be grown in culture (Bridge and Spooner, 2001), and non cultivable cells, dead cells, or cell debris are not detected by cultivation at all, but may still exert health effects as outlined above. Microscopic analyses, on the other hand, are not reliable for the detection of small nondescript spores and hyphae or fragments of fungal tissues (Pitt et al., 1997). Some fungi may remain morphologically undistinguishable or can only be identified to a class or family level. Chemical tracers like the sugar alcohols mannitol and arabitol can be used to assess the abundance of fungal spores in air particulate matter (Elbert et al., 2007; Bauer et al., 2008a), but they do not provide information about diversity. Thus, traditional cultivation, microscopy, and chemical tracer techniques are not sufficient and need to be complemented by molecular genetic techniques to fully characterize the diversity and potential effects of airborne fungi (Bridge and Spooner, 2001; Anderson and Cairney, 2004).

Only recently, first applications of the polymerase chain reaction (PCR) have been reported for the amplification and analysis of deoxyribonucleic acid (DNA) from PBA particles. These studies had been focused on the sequence analysis of DNA from specific groups of organisms, mostly from bacteria or fungi collected at one or two sampling locations (Radosevich et al., 2002; Boreson et al., 2004; Hughes et al., 2004; Maron et al., 2005; Kuske et al., 2006; Brodie et al., 2007; Fierer et al., 2008). Després et al., (2007) combined

sequence analyses of different groups of organisms (bacteria, fungi, plants, animals) with terminal-restriction fragment length polymorphism (T-RFLP) investigations and DNA quantification in urban, rural, and high-alpine aerosol samples.

A taxonomic identification of organisms is possible with use of e.g., rRNA (ribosomal ribonucleic acid) genes for amplification and sequencing. This gene cluster is a multicopy region and provides a good target region for analyzing up to the species level. The rRNA genes have been strongly conserved during evolution, due to the central role of ribosomes in protein synthesis. The rRNA genes are separated by two internal transcribed spacer (ITS) regions which are highly variable in length and sequence composition. For fungi the ITS regions provide greater taxonomic resolution than sequences generated from coding regions (White, 1990; Grades and Bruns, 1993; Green et al., 2004; Reddy et al., 2005).

1.4 Research objectives

To characterize the importance of airborne fungi and other PBA within climate and health issues, it is necessary to have a better understanding of their character, their abundance in various regions, annual cycles, fine and coarse particle fractions, and their diversity. Thus, the main objectives of this thesis were the following:

1. Develop and prove the applicability of DNA-based methods for the analysis of PBA particles in air filter samples (Appendix C1: Després et al., 2007).

2. Investigate the identity and diversity of PBA in urban, rural, and high-alpine air (Appendix C1: Després et al., 2007).

3. Characterize the seasonal variability of airborne fungi - phyla, classes, families, genera, and species - in continental boundary layer air (Appendix C2: Fröhlich-Nowoisky et al., 2009).

4. Compare the diversity and relative abundance of airborne fungi in coarse and fine particle samples (Appendix C2: Fröhlich-Nowoisky et al., 2009).

5. Compare the diversity and relative abundance of airborne fungi in aerosol samples from different locations and climatic zones around the world (Appendix C3: Fröhlich-Nowoisky et al., to be submitted, 2009).

2 Results and Conclusions

1. Methods for the characterization of PBA particles by DNA analysis have been developed and established for different types of organisms (bacteria, fungi, plants, animals; Appendix C1: Després et al., 2007; Appendix C2: Fröhlich-Nowoisky et al., 2009).

2. Investigations of blank and background samples showed that the sampling, sample handling, and analytical procedures have to be carefully controlled to avoid artifacts in molecular genetic analyses of atmospheric aerosol samples (Appendix C1: Després et al., 2007; Appendix C2: Fröhlich-Nowoisky et al., 2009; Appendix C3: Fröhlich-Nowoisky et al., to be submitted, 2009).

3. Careful selection and combination of multiple PCR primer pairs and other materials for the extraction and amplification of DNA have been identified as key elements for achieving high coverage of species richness (Appendix C2: Fröhlich-Nowoisky et al., 2009).

4. The species richness of fungi in continental air was found to be higher than indicated by earlier studies. Coarse and fine particle samples exhibited similar levels of species richness throughout the year but pronounced differences in the relative abundance of various groups of fungi and their seasonal cycles, with more plant pathogens in the coarse and more human pathogens and allergens in the respirable fine particle fraction (Appendix C2: Fröhlich-Nowoisky et al., 2009).

5. Almost all of the 2500 fungal DNA sequences obtained from air samples collected at different locations and climatic zones around the world could be attributed to the phyla of *Ascomycota* and *Basidiomycota*. With regard to species richness, the ratio of *Basidiomycota* to *Ascomycota* was much higher in

continental air samples (~60:40) than in marine air samples (~30:70; Appendix C3: Fröhlich-Nowoisky et al., to be submitted, 2009).

Information about the diversity and abundance of airborne fungi and other PBA is relevant for many areas of fundamental and applied research such as biogeosciences, global climate and ecology, human medicine, veterinary science, industrial and environmental health and hygiene, agriculture and bioengineering. Two of the many interesting and potentially important perspectives for future investigations of airborne fungi are the spread of genetically modified organisms and climate-related changes in the diversity and abundance of fungi on regional and global scales.

3 References

Adhikari, A., Sen, M. M., Gupta-Bhattacharya, S., and Chanda, S.: Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations, Sci. Tot. Environ., 326, 123-141, 2004.

Aime, M. C., Matheny, P. B., Henk, D. A., Frieders, E. M., Nilsson, R. H., Piepenbring, M., McLaughlin, D. J., Szabo, L. J., Begerow, D., Sampaio, J. P., Bauer, R., Weiss, M., Oberwinkler, F., and Hibbett, D.: An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences, Mycologia, 98, 896-905, 2006.

Almaraz, T., Roux, C., Maumont, S., and Durrieu, G.: Phylogenetic relationships among smut fungi parasitizing dicotyledons based on ITS sequence analysis, Mycol. Res., 106, 541-548, 2002.

Anderson, I. C. and Cairney, J. W. G.: Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques, Environ. Microbiol., 6, 769-779, 2004.

Ariya, P. A., Nepotchatykh, O., Ignatova, O., and Amyot, M.: Microbiological degradation of atmospheric organic compounds, Geophys. Res. Letters, 29, 2002.

Artaxo, P. and Hansson, H.-C.: Size distribution of biogenic aerosol particles from the Amazon basin, Atmos. Environ., 29, 393-402, 1995.

Bauer, H., Claeys, M., Vermeylen, R., Schueller, E., Weinke, G., Berger, A., and Puxbaum, H.: Arabitol and mannitol as tracers for the quantification of airborne fungal spores, Atmos. Environ., 42, 588-593, 2008a.

Bauer, H., Kasper-Giebl, A., Loflund, M., Giebl, H., Hitzenberger, R., Zibuschka, F., and Puxbaum, H.: The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols, Atmos. Res., 64, 109-119, 2002.

Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitzenberger, R., Marr, I. L., and Puxbaum, H.: Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol, Atmos. Environ., 42, 588, 2008b.

Berbee, M. L.: The phylogeny of plant and animal pathogens in the Ascomycota, Physiol. Mol. Plant Pathol., 59, 165-187, 2001.

Bernton, H. S. and Thom, C.: The role of Cladosporium, a common mold, in allergy, J. Allergy, 8, 363-370, 1937.

Boreson, J., Dillner, A. M., and Peccia, J.: Correlating bioaerosol load with PM2.5 and PM10cf concentrations: a comparison between natural desert and urban-fringe aerosols, Atmos. Environ., 38, 6029-6041, 2004.

Bridge, P. and Spooner, B.: Soil fungi: diversity and detection, Plant Soil, 232, 147-154, 2001.

Brodie, E. L., DeSantis, T. Z., Parker, J. P. M., Zubietta, I. X., Piceno, Y. M., and Andersen, G. L.: Urban aerosols harbor diverse and dynamic bacterial populations, P. Natl. Acad. Sci. USA, 104, 299-304, 2007.

Brown, J. K. M. and Hovmøller, M. S.: Epidemiology - Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease, Science, 297, 537-541, 2002.

Burge, H. A.: Indoor air and infectious disease, Occup. Med., 4, 713–721, 1989.

Burge, H. A.: Bioaerosols: prevalence and health effects in the indoor environment, J. Allergy Clin. Immunol., 86, 687–701, 1990.

Burge, H. A.: An update on pollen and fungal spore aerobiology, J. Allergy Clin. Immunol., 110, 544-552, 2002.

Casadevall, A.: Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection?, Fungal Genet. Biol., 42, 98-106, 2005.

Chew, G. L., Rogers, C., Burge, H. A., Muilenberg, M. L., and Gold, D. R.: Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics, Allergy, 58, 13-20, 2003.

Cho, S. H., Seo, S. C., Schmechel, D., Grinshpun, S. A., and Reponen, T.: Aerodynamic characteristics and respiratory deposition of fungal fragments, Atmos. Environ., 39, 5454-5465, 2005.

Damare, S., Raghukumar, C., and Raghukumar, S.: Fungi in deep-sea sediments of the Central Indian Basin, Deep Sea Research Part I: Oceanographic Res. Papers, 53, 14-27, 2006.

Davis, W. E., Horner, W. E., Salvaggio, J. E., and Lehrer, S. B.: Basidiospore Allergens - Analysis of Coprinus-Quadrifidus Spore, Cap, and Stalk Extracts, Clin. Allergy, 18, 261-267, 1988.

Deguillaume, L., Leriche, M., Amato, P., Ariya, P. A., Delort, A. M., Pöschl, U., Chaumerliac, N., Bauer, H., Flossmann, A. I., and Morris, C. E.: Microbiology and atmospheric processes: chemical interactions of Primary Biological Aerosols, Biogeosciences, 5, 1073-1084, 2008.

Després, V. R., Nowoisky, J. F., Klose, M., Conrad, R., Andreae, M. O., and Pöschl, U.: Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes, Biogeosciences, 4, 1127-1141, 2007.

Douwes, J., Thorne, P., Pearce, N., and Heederik, D.: Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects, Ann. Occup. Hyg., 47, 187-200, 2003.

Eduard, W. and Heederik, D.: Methods for quantitative assessment of airborne levels of non-infectious micro-organisms in highly contaminated work environments, Am. Ind. Hyg. Assoc. J., 59, 1998.

Eichmann, R. and Hückelhoven, R.: Accommodation of powdery mildew fungi in intact plant cells, J. Plant Physiol., 165, 5-18, 2008.

Elbert, W., Taylor, P. E., Andreae, M. O., and Pöschl, U.: Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions, Atmos. Chem. Phys., 7, 4569-4588, 2007.

Evans, H.C.: Coevolution of entomogenous fungi and their insect hosts, in: Coevolution of fungi in plants and animals, Pirozynski, K. A., Hawksworth, D. L. (eds.), London & New York, Academic Press, 149-170, 1988.

Fierer, N., Liu, Z. Z., Rodriguez-Hernandez, M., Knight, R., Henn, M., and Hernandez, M. T.: Short-term temporal variability in airborne bacterial and fungal populations, Appl. Environ. Microbiol., 74, 200-207, 2008.

Finley, R. D.: Mycorrhizal fungi and their multifunctional roles, Mycologist, 18, 91-96, 2004.

Fröhlich-Nowoisky, J. Després, V.R., and Pöschl, U.: High diversity of fungi in air particulate matter, 2009, submitted.

Fukatsu, T., Sato, H., and Kuriyama, H.: Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus Paecilomyces tenuipes, J. Invertebr. Patho., 70, 203–208, 1997.

Gardes, M. and Bruns, T. D.: Its Primers with Enhanced Specificity for Basidiomycetes - Application to the Identification of Mycorrhizae and Rusts, Mol. Ecol., 2, 113-118, 1993.

Georgakopoulos, D. G., Després, V., Fröhlich-Nowoisky, J., Psenner, R., Ariya, P. A., Pósfai, M., Ahern, H. E., Moffett, B. F., and Hill, T. C. J.: Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles, Biogeosciences Discuss., 5, 1469-1510, 2008.

Glikson, M., Rutherford, S., Simpson, R. W., Mitchell, C. A., and Yago, A.: Microscopic and Submicron Components of Atmospheric Particulate Matter During High Asthma Periods in Brisbane, Queensland, Australia, Atmos. Environ., 29, 549-562, 1995.

Gorny, R. L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M., and Grinshpun, S. A.: Fungal fragments as indoor air biocontaminants, Appl. Environ. Microbiol., 68, 3522-3531, 2002.

Göttlich, E., van der Lubbe, W., Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., Flemming, H.-C., and de Hoog, S.: Fungal flora in groundwater-derived public drinking water, Int. J. Hyg. Environ. Health, 205, 269-279, 2002.

Gravesen, S.: Fungi as a cause of allergic disease, Allergy, 34, 135–154, 1979.

Green, S. J., Freeman, S., Hadar, Y., and Minz, D.: Molecular tools for isolate and community studies of Pyrenomycete fungi, Mycologia, 96, 439-451, 2004.

Green, B. J., Tovey, E. R., Sercombe, J. K., Blachere, F. M., Beezhold, D. H., and Schmechel, D.: Airborne fungal fragments and allergenicity, Med. Mycol., 44, 245-255, 2006.

Griffiths, W. and DeCosemo, G.: The assessment of bioaerosols: A critical review, J. Aerosol Sci. 25, 1425-1458, 1994.

Hawksworth, D. L. and Rossman, A. Y.: Where Are All the Undescribed Fungi?, Phytopathology, 87, 888-891, 1997.

Helbling, A., Brander, K. A., Horner, W. E., and Lehrer, S. B.: Allergy to basidiomycetes, in: Fungal Allergy and Pathogenicity, Breitenbach, M., Crameri, R. and Lehrer, S. B. (eds.), Karger, Basel, 81, 28-47, 2002.

Ho, H.-M., Rao, C. Y., Hsu, H.-H., Chiu, Y.-H., Liu, C.-M., and Chao, H. J.: Characteristics and determinants of ambient fungal spores in Hualien, Taiwan, Atmos. Environ., 39, 5839-5850, 2005.

Hughes, K., McCartney, H., Lachlan-Cope, T., and Pearce, D.: A preliminary study of airborne microbial biodiversity over peninsular Antarctica, Cell. Mol. Biol., 50, 537-542, 2004.

Jaenicke, R.: Abundance of cellular material and proteins in the atmosphere, Science, 308, 73-73, 2005.

James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H. T. et al.: Reconstructing the early evolution of Fungi using a six-gene phylogeny, Nature, 443, 818-822, 2006.

Jones, A. M. and Harrison, R. M.: The effects of meteorological factors on atmospheric bioaerosol concentrations - a review, Sci. Tot. Environ., 326, 151-180, 2004.

Kellogg, C. A., Griffin, D. W., Garrison, V. H., Peak, K. K., Royall, N., Smith, R. R., and Shinn, E. A.: Characterization of aerosolized bacteria and fungi from desert dust events in Mali, West Africa, Aerobiologia, 20, 99-110, 2004.

Kieft, T. L. and Ahmadjian, V.: Biological Ice Nucleation Activity in Lichen Mycobionts and Photobionts, Lichenologist, 21, 355-362, 1989.

Klamer, M., Roberts, M. S., Levine, L. H., Drake, B. G., and Garland, J. L.: Influence of elevated CO2 on the fungal community in a coastal scrub oak forest soil investigated with terminal-restriction fragment length polymorphism analysis, Appl. Environ. Microbiol., 68, 4370-4376, 2002.

Klironomos, J. N., Rillig, M. C., Allen, M. F., Zak, D. R., Pregitzer, K. S., and Kubiske, M. E.: Increased levels of airborne fungal spores in response to *Populus tremuloides* grown under elevated atmospheric CO_2, Can. J. Bot., 75, 1670-1673, 1997.

Kurup, V. P., Shen, H.-D., and Banerjee, B.: Respiratory fungal allergy, Microbes Infect., 2, 1101-1110, 2000.

Kurup, V. P., Shen, H. D., and Vijay, H.: Immunobiology of fungal allergens, Int. Arch. Allergy Immunol., 129, 181-188, 2002.

Kuske, C. R., Barns, S. M., Grow, C. C., Merrill, L., Dunbar, M. S., and Dunbar, J.: Environmental survey for four pathogenic bacteria and closely related species using phylogenetic and functional genes, J. Forensic. Sci., 51, 548-558, 2006.

Lee, T., Grinshpun, S. A., Kim, K. Y., Iossifova, Y., Adhikari, A., and Reponen, T.: Relationship between indoor and outdoor airborne fungal spores, pollen, and (1→3)-beta-D-glucan in homes without visible mold growth, Aerobiologia, 22, 227-236, 2006a.

Lee, T., Grinshpun, S. A., Martuzevicius, D., Adhikari, A., Crawford, C. M., and Reponen, T.: Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes, Atmos. Environ., 40, 2902-2910, 2006b.

Lehrer, S. B., Lopez, M., Butcher, B. T., Olson, J., Reed, M., and Salvaggio, J. E.: Basidiomycete mycelia and spore-allergen extracts: Skin test reactivity in adults with symptoms of respiratory allergy, J. Allergy Clin. Immunol., 78, 478-485, 1986.

Levetin, E.: An atlas of fungal spores, J. Allergy Clin. Immunol., 113, 366-368, 2004.

Madelin, T. M.: Fungal aerosols: A review, J. Aerosol Sci., 25, 1405-1412, 1994.

Maron, P.-A., Lejon, D. P. H., Carvalho, E., Bizet, K., Lemanceau, P., Ranjard, L., and Mougel, C.: Assessing genetic structure and diversity of airborne bacterial communities by DNA Fingerprinting and 16S rDNA clone library, Atmos. Environ., 39, 3687-3695, 2005.

Matthias-Maser, S. and Jaenicke, R.: Biological and biological contaminated aerosol particles in marine air, J. Aerosol Sci., 24, S539-S540, 1993.

McDevitt, J. J., Lees, P. S. J., Merz, W. G., and Schwab, K. J.: Development of a method to detect and quantify Aspergillus fumigatus conidia by quantitative PCR for environmental air samples, Mycopathologia, 158, 325-335, 2004.

Medrela-Kuder, E.: Seasonal variations in the occurrence of culturable airborne fungi in outdoor and indoor air in Cracow, Int. Biodeterior. Biodegradation, 52, 203-205, 2003.

Mielnichuk, N. and Lopez, S. E.: Interaction between Epicoccum purpurascens and xylophagous basidiomycetes on wood blocks, For. Pathol., 37, 236-242, 2007.

Mims, C. W. and Richardson, E. A.: Ultrastructure of sporodochium and conidium development in the anamorphic fungus Epicoccum nigrum, Can. J. Bot., 83, 1354-1363, 2005.

Mitakakis, T. Z., Barnes, C., and Tovey, E. R.: Spore germination increases allergen release from Alternaria, J. Allergy Clin. Immunol., 107, 388-390, 2001.

Moletta, M., Delgenes, J.-P., and Godon, J.-J.: Differences in the aerosolization behavior of microorganisms as revealed through their transport by biogas, Sci. Tot. Environ., 379, 75-88, 2007.

Newson, R., Strachan, D., Corden, J., and Millington, W.: Fungal and other spore counts as predictors of admissions for asthma in the Trent region, Occup. Environ. Med., 57, 786-792, 2000.

Nielsen, K. F.: Mycotoxin production by indoor molds, Fungal Genet. Biol, 39, 2003.

Niessen, L. and Steve, L. T.: PCR Based Diagnosis and Quantification of Mycotoxin Producing Fungi, in: Advances in Food and Nutrition Research, Taylor S. L. (ed.), Academic Press, Volume 54, 81-138, 2008.

O'Gorman, C. M. and Fuller, H. T.: Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air, Atmos. Environ., 42, 4355-4368, 2008.

Oliveira, M., Ribeiro, H., and Abreu, I.: Annual Variation of Fungal Spores in Atmosphere of Porto: 2003, Ann. Agric. Environ. Med., 12, 309-315, 2005.

Pitt, J. I. and Hocking, A. D.: Fungi and food spoilage, Second edition, Blackie Academic and Professional, London, 1997.

Porter, N. and Fox, F. M.: Diversity of Microbial Products - Discovery and Application, Pestic. Sci., 39, 161-168, 1993.

Pöschl, U.: Atmospheric Aerosols: Composition, Transformation, Climate and Health Effects, Angew. Chem. Int. Ed., 44, 7520-7540, 2005.

Pouleur, S., Richard, C., Martin, J. G., and Antoun, H.: Ice nucleation activity in Fusarium acuminatum and Fusarium avenaceum, Appl. Environ. Microbiol., 58, 2960–2964, 1992.

Radosevich, J. L., Wilson, W. J., Shinn, J. H., DeSantis, T. Z., and Andersen, G. L.: Development of a high-volume aerosol collection system for the identification of air-borne micro-organisms, Lett. Appl. Microbiol., 34, 162-167, 2002.

Read, D.: Mycorrhizal fungi - The ties that bind, Nature, 388, 517-518, 1997.

Reddy, S. R., Pindi, P. K., and Reddy, S. M.: Molecular methods for research on arbuscular mycorrhizal fungi in India: problems and prospects, Curr. Sci., 89, 1699-1709, 2005.

Samils, N., Olson, Å., and Stenlid, J.: The capacity in Heterobasidion annosum s.l. to resist overgrowth by the biocontrol agent Phlebiopsis gigantea is a heritable trait, Biolog. Control, 45, 419-426, 2008.

Santos, R. and Goossens, A.: An update on airborne contact dermatitis: 2001-2006, Contact Dermatitis, 57, 353-360, 2007.

Segal, B. H. and Walsh, T. J.: Current Approaches to Diagnosis and Treatment of Invasive Aspergillosis, Am. J. Respir. Crit. Care Med., 173, 707-717, 2006.

Seinfeld, J. H. and Pandis, S. N.: Atmospheric chemistry and physics – from air pollution to climate change, John Wiley & Sons, New York, 1998.

Shearer, C. A., Langsam, D. M., and Longcore, J. E.: Fungi in Freshwater Habitats, in: Biodiversity of Fungi, Mueller, G.M., Bills, G.F. and Foster, M.S.(eds.), Academic Press, Burlington, 513-531, 2004.

Shelton, B. G., Kirkland, K. H., Flanders, W. D., and Morris, G. K.: Profiles of airborne fungi in buildings and outdoor environments in the United States, Appl. Environ. Microbiol., 68, 2002.

Thorn, G. and Wellington, E.: The fungi in soil, in: Modern-soil-microbiology, Elsas, J.D. van, Trevors, J.T. (eds.), Marcel Dekker Inc., New York, USA., 63-127, 1997.

Thorn, R. G., Soil fungi, in Handbook of Soil Science, Sumner, M.E. (ed.), CRC Press Boca Raton, FL, C22-C37, 1999.

Torricelli, R., Johansson, S. G. O., and Wiithrich, B.: Ingestive and inhalative allergy to the mushroom Boletus edulis, Allergy, 52, 747-751, 1997.

Van Driesche, R. G., Bellows, T. S., Jr., Biological Control, 1-539, 1996.

Weissman, D. N., Halmepuro, L., Salvaggio, J. E., and Lehrer, S. B.: Antigenic/allergenic analysis of basidiomycete cap, mycelia, and spore extracts, Int. Arch. Allergy Appl. Immunol., 84, 56-61, 1987.

White, T.J., Bruns, T., Lee, S., and Taylor, J.: Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics, in PCR Protocols: A Guide to Methods and Applications, Innis M. A., Gelfand D. H., Sninsky J. J., White T. J. (eds.), Academic press, San Diego, 315–322, 1990.

Womiloju, T. O., Miller, J. D., Mayer, P. M., and Brook, J. R.: Methods to determine the biological composition of particulate matter collected from outdoor air, Atmos. Environ., 37, 4335-4344, 2003.

Wu, Y.-H., Chan, C.-C., Rao, C. Y., Lee, C.-T., Hsu, H.-H., Chiu, Y.-H., and Chao, H. J.: Characteristics, determinants, and spatial variations of ambient fungal levels in the subtropical Taipei metropolis, Atmos. Environ., 41, 2500-2509, 2007.

Appendix A: List of Abbreviations

AMC	Ascomycota
BMC	Basidiomycota
DNA	Deoxyribonucleic Acid
ITS	Internal Transcribed Spacer
PBA	Primary Biogenic Aerosol
PCR	Polymerase Chain Reaction
PM	Particulate Matter
rRNA	Ribosomal Ribonucleic Acid
T-RFLP	Terminal-Restriction Fragment Length Polymorphism

Appendix B: Personal List of Publications

Journal Articles

1) Després V.R., Nowoisky J.F., Klose M., Conrad R., Andreae M.O., Pöschl U.: Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes, Biogeosciences, 4, 1127–1141, 2007.

2) Vieler A., Scheidt H.A., Schmidt P., Montag C., Nowoisky J., Lohr M., Wilhelm C., Huster D., Goss R.: The Influence of Phase Transitions in Phosphatidylethanolamine Models on the Activity of Violaxanthin De-Epoxidase, Biochimica et Biophysica Acta – Biomembranes, 1778, 4, 1027-1034, 2008.

3) Georgakopoulos D.G., Després V., Fröhlich-Nowoisky J., Psenner R., Ariya P.A., Pósfai M., Ahern H.E., Moffett B.F., Hill T.C.J.: Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles, Biogeosciences Discussions, 5, 1469-1510, 2008.

4) Fröhlich-Nowoisky J., Després V.R., Pöschl U.: High diversity of fungi in air particulate matter, Proceedings of the National Academy of Sciences, under revision, 2009.

5) Fröhlich-Nowoisky J., Després V.R., Pöschl U.: Global atmospheric diversity of fungi: *Asco-* and *Basidiomycota* in continental and marine air, to be submitted, 2009.

6) Fröhlich-Nowoisky J., Conrad R., Pickergill D., Pöschl U. Després, V.R.: Atmospheric diversity of Archaea in continental boundary layer air, in preparation.

7) Fröhlich-Nowoisky J., Després V.R., Pöschl U.: Determination of airborne pathogens by DNA analysis: fungi and fungus-like water molds, in preparation.

8) Fröhlich-Nowoisky J., Després V.R., Pöschl, U.: Characterization of airborne fungi by terminal restriction fragment length polymorphism (T-RFLP) of ribosomal RNA genes, in preparation.

9) Germann I., Fröhlich-Nowoisky J., Pöschl U., Després V.R.: Allergenic *Asteraceae* in urban air: DNA analysis and relevance for human health, in preparation.

Oral Presentations

1) Nowoisky J.: DNA-based methods for genetical identification of biological particles in air, ESF LESC Exploratory Workshop on Microbiological Meteorology, Avignon, France, 2006.

2) Després V., Nowoisky J., Borrmann S., Andreae M.O., Paulsen H., Klose M., Conrad R., Pöschl U.: Biological Aerosol Particles in the Earth System: Molecular Genetic Characterization, Geocycles Symposium, Mainz, Germany, 2006.

3) Nowoisky J., Després V., Klose M., Conrad R., Andreae M.O., Paulsen H., Pöschl U.: Biological Aerosol Particles in the Earth System: Identification and Diversity of Fungi in the Air of Mainz, Geocycles Symposium, Mainz, Germany, 2007.

4) Després V., Nowoisky J., Klose M., Conrad R., Cimbal J.: Molecular genetics and diversity of bacteria and archaea in urban, rural and high alpine air, EAC, Salzburg, Austria, 2007.

Poster Presentations

1) Nowoisky J., Després V., Andreae M.O., Paulsen H., Pöschl U.: Molecular genetic analysis of biological aerosol particles, Autumn School on Measurement techniques in Atmospheric Chemistry, Oberwesel, Germany, 2006.

2) Zhang Y., Kanawati B., Nowoisky J., Winterhalter R., Moortgat G., Hoffmann T., Pöschl U.: Characterization and fragmentation of a synthetic oligonucleotide using Triple Quadrupole – TOF hybrid system coupled to Electrospray interface. 40. DGMS-Tagung, Bremen, Germany, 2007.

3) Després V., Nowoisky J., Klose M., Conrad R., Andreae M.O., Pöschl U.: Genetic analysis and diversity of primary biogenic aerosol particles, EGU, Wien, Austria, 2007.

4) Després V., Nowoisky J., Klose M., Conrad R., Andreae M.O., Pöschl U.: Genetic analysis and diversity of primary biogenic aerosol particles, IUGG, Perugia, Italia, 2007.

5) Nowoisky J., Després V., Cimbal J., Klose M., Conrad R., Andreae M.O., Pöschl U.: Biological Characterization of Atmospheric Aerosol Particles, IUGG, Perugia, Italia, 2007.

6) Knüfer J., Herwig S., Nowoisky J., Volz B., Lohr M.: Substrate Specificity of Native and Recombinant Xanthophyll De-epoxidases from Three Vascular Plants and a Diatom, Botanikertagung, Hamburg, Germany, 2007.

7) Després V., Nowoisky J., Klose M., Pfeifer C., Conrad R., Andreae M.O., Pöschl U.: Genetic analysis and diversity of primary biogenic aerosol particles, EAC, Salzburg, Austria, 2007.

8) Nowoisky J., Després V., Cimbal J., Klose M., Conrad R., Andreae M.O., Pöschl U.: Molecular genetic identification and diversity of fungi and archaea, EAC, Salzburg, Austria, 2007.

9) Nowoisky J., Després V., Cimbal J., Klose M., Conrad R., Andreae M.O., Pöschl U.: Molecular genetic identification and diversity of fungi and archaea, Geocycles Symposium, Mainz, Germany, 2008.

Appendix C: Selected Publications

C1) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes (Després et al., 2007).

C2) High diversity of fungi in air particulate matter (Fröhlich-Nowoisky et al., 2009).

C3) Global atmospheric diversity of fungi: *Asco-* and *Basidiomycota* in continental and marine air (Fröhlich-Nowoisky et al., to be submitted, 2009).

C1) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes

V. R. Després [1,3], J. F. Nowoisky[1,4], M. Klose[2], R. Conrad[2], M. O. Andreae[1], and U. Pöschl[1]

[1]Biogeochemistry Department, Max Planck Institute for Chemistry, Becherweg 27, 55128 Mainz, Germany

[2]Biogeochemistry Department, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, 35043 Marburg,Germany

[3]Department of General Botany, Johannes Gutenberg University, Saarstraße 1, 55099 Mainz, Germany

[4]Department of Geosciences, Johannes Gutenberg University, Becherweg 14, 55128 Mainz, Germany

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Authors Contribution

V.R.D. developed the concept, designed and performed experiments, supervised the project, analyzed the data, and prepared the manuscript. J.F.N performed many lysing and cloning reactions and prepared samples for sequencing reactions. J.F.N. also realized and performed the inhibition experiment and discussed the manuscript. M.K. extracted most filter samples and performed all T-RFLP experiments. U.P, .M.O.A., and R.C. provided aerosol samples, contributed to the project plan, and participated in the preparation of the manuscript.

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Biogeosciences

Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes

V. R. Després [1,3], J. F. Nowoisky[1,4], M. Klose[2], R. Conrad[2], M. O. Andreae[1], and U. Pöschl[1]

[1]Biogeochemistry Department, Max Planck Institute for Chemistry, Becherweg 27, 55128 Mainz, Germany
[2]Biogeochemistry Department, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, 35043 Marburg, Germany
[3]Department of General Botany, Johannes Gutenberg University, Saarstraße 1, 55099 Mainz, Germany
[4]Department of Geosciences, Johannes Gutenberg University, Becherweg 14, 55128 Mainz, Germany

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Abstract. This study explores the applicability of DNA analyses for the characterization of primary biogenic aerosol (PBA) particles in the atmosphere. Samples of fine particulate matter ($PM_{2.5}$) and total suspended particulates (TSP) have been collected on different types of filter materials at urban, rural, and high-alpine locations along an altitude transect in the south of Germany (Munich, Hohenpeissenberg, Mt. Zugspitze).

From filter segments loaded with about one milligram of air particulate matter, DNA could be extracted and DNA sequences could be determined for bacteria, fungi, plants and animals. Sequence analyses were used to determine the identity of biological organisms, and terminal restriction fragment length polymorphism analyses (T-RFLP) were applied to estimate diversities and relative abundances of bacteria. Investigations of blank and background samples showed that filter materials have to be decontaminated prior to use, and that the sampling and handling procedures have to be carefully controlled to avoid artifacts in the analyses.

Mass fractions of DNA in $PM_{2.5}$ were found to be around 0.05% in urban, rural, and high-alpine aerosols. The average concentration of DNA determined for urban air was on the order of $\sim 7\,\mathrm{ng\,m^{-3}}$, indicating that human adults may inhale about one microgram of DNA per day (corresponding to $\sim 10^8$ haploid bacterial genomes or $\sim 10^5$ haploid human genomes, respectively).

Most of the bacterial sequences found in $PM_{2.5}$ were from *Proteobacteria* (42) and some from *Actinobacteria* (10) and *Firmicutes* (1). The fungal sequences were characteristic for *Ascomycota* (3) and *Basidiomycota* (1), which are known to actively discharge spores into the atmosphere. The plant sequences could be attributed to green plants (2) and moss spores (2), while animal DNA was found only for one unicellular eukaryote (protist).

Over 80% of the 53 bacterial sequences could be matched to one of the 19 T-RF peaks found in the $PM_{2.5}$ samples, but only 40% of the T-RF peaks did correspond to one of the detected bacterial sequences. The results demonstrate that the T-RFLP analysis covered more of the bacterial diversity than the sequence analysis. Shannon-Weaver indices calculated from both sequence and T-RFLP data indicate that the bacterial diversity in the rural samples was higher than in the urban and alpine samples. Two of the bacterial sequences (*Gammaproteobacteria*) and five of the T-RF peaks were found at all sampling locations.

Correspondence to: V. R. Després
(despres@mpch-mainz.mpg.de)

1 Introduction

Biogenic aerosols are ubiquitous in the Earth's atmosphere, where they influence atmospheric chemistry and physics, the biosphere, climate, and public health. They play an important role in the spread of biological organisms and reproductive materials, and they can cause or enhance human, animal, and plant diseases. Moreover, they influence the Earth's energy budget by scattering and absorbing radiation, and they can initiate the formation of cloud droplets and precipitation as cloud condensation and ice nuclei (Dingle, 1966; Schnell and Vali, 1972; Cox and Wathes, 1995; Andreae and Crutzen, 1997; Pruppacher and Klett, 1997; Hamilton and Lenton, 1998; Andreae et al., 2002; Mikhailov et al., 2004; Taylor and Jonsson, 2004; Jaenicke, 2005; Kanakidou et al., 2005; Lohmann and Feichter, 2005; Pöschl, 2005; Dusek et al., 2006; Fuzzi et al., 2006; Hakola et al., 2006; Kloster et al.,

2006; McFiggans et al., 2006; Sun and Ariya, 2006; Möhler et al., 2007; Hock et al., 2007).

Primary biogenic aerosol (PBA) particles and components are emitted directly from the biosphere to the atmosphere. PBA particles range in size from millimeters down to tens of nanometers, and may thus be much smaller than originally thought (Jaenicke, 2005). Particles of biological origin, like pollen, bacteria, spores, viruses, plant and animal fragments (e.g., dandruffs, skin fragments), are all within this size range (Simoneit and Mazurek, 1982; Matthias-Maser and Jaenicke, 1994; Artaxo, 1995; Bauer et al., 2005; Ahern et al., 2007; Elster et al., 2007; Zhang et al., 2007). The actual abundance and origin of biogenic aerosol particles and components are, however, still poorly understood and quantified.

Compared to conventional methods of PBA analysis (microscopy, protein staining, cultivation of microorganisms, etc.), DNA analyses can provide much more information. They enable the identification and characterization of cultured and uncultured microorganisms (90 to 99% of fungi and bacteria presently cannot be cultured in the laboratory; Amann et al., 1995), of viable and dead cells, and of plant and animal fragments.

The polymerase chain reaction (PCR) enables very efficient amplification of characteristic regions of deoxyribonucleic acid (DNA), which can be analyzed, e.g., by sequencing and terminal restriction fragment length polymorphism analysis (T-RFLP), and identified by comparison with genetic databanks. Even minute amounts of DNA – as little as one molecule – are sufficient to identify biological organisms and materials. The high sensitivity of this technique, however, also bears the risk of amplifying trace amounts of DNA with which the investigated aerosol sample or the sampling material (filters, impaction foils, etc.) may have been contaminated in the course of material production, aerosol sampling, sample transport, storage, and analysis. Furthermore, long-term storage of samples can lead to chemical modification and degradation of DNA. Contamination and degradation effects can lead to substantial analytical artifacts and loss of information.

Depending on the genomic region that is sequenced, organisms can be identified to the domain, class, order, family, genus or even species level. Regions commonly used for taxonomic identification are the ribosomal RNA genes. These are the 16S ribosomal genes in the domains *Archaea* and *Bacteria*, and the 18S ribosomal genes in the domain *Eukarya* (animals and plants). For eukaryotic fungi usually the internal transcribed spacer (ITS) region, which is localized between the 18S, 5S and 28S regions, is used for the taxonomic identification. All these gene sequences are useful, because they exhibit both conserved regions for the binding of universal primer pairs and variable regions that are characteristic for different groups or individual species of biological organisms. For plants, the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (rbcL) in the chloroplast genome provides additional taxonomic information (Vilgalys and Hester, 1990; Weisburg et al., 1991; Gardes and Bruns, 1993; Whiting et al., 1997; Little and Barrington, 2003; Fierer et al., 2005). For bacteria and archaea, T-RFLP analysis provides information about the diversity and relative abundance, which is complementary to the information obtained by the analysis and blasting of DNA sequences (Liu et al., 1997; Lukow et al., 2000).

First applications of molecular genetic techniques for the analysis of PBA have been reported only recently. These studies had been focused on the sequence analysis of DNA from specific groups of organisms, mostly from bacteria or fungi collected at one or two sampling locations (Radosevich et al., 2002; Boreson et al., 2004; Hughes et al., 2004; Maron et al., 2005; Kuske et al., 2006; Brodie et al., 2007). In the present study we combine sequence analyses of different groups of organisms (bacteria, archaea, fungi, plants, animals) with T-RFLP investigations and DNA quantification in urban, rural, and high-alpine aerosol samples. Moreover, we investigate potential contamination problems, which have not been addressed in earlier publications. This pilot study has been limited to a set of exemplary samples, and the measurement results should not be over-interpreted with regard to the spatial and temporal variability of PBA. Nevertheless, they shall serve for orientation and planning of more comprehensive future investigations of PBA by DNA analysis.

2 Material and methods

2.1 Filter samples

Samples were collected on three different filter types (glass fiber, cellulose nitrate, and polypropylene) from urban, rural and high-alpine air at five different locations in Southern Germany, as detailed below and summarized in Table 1. Fine air particulate matter ($PM_{2.5}$; aerodynamic diameter $<2.5\,\mu$m) was collected on glass fiber filters (15 cm diameter, MN 85/90 binder-free, Macherey-Nagel, Düren, Germany) using a stand-alone high-volume filter sampler (HVFS, Digitel DHA-80, Riemer, Hausen, Germany; sample air flow $500\,\mathrm{L\,min^{-1}}$; sampling time 4 to 5 days). For decontamination (removal of organic compounds), the glass fiber filters were baked in a muffle furnace at 300°C for 12 h prior to use. The mass of particulate matter collected on each filter was determined by weighing the filter before and after sampling (Schauer et al., 2004) and the samples were stored at −20°C. Urban $PM_{2.5}$ samples were collected by the IWC (Institute of Hydrochemistry, Technical University of Munich, Munich-Grosshadern) on the campus of the University Hospital Grosshadern in the southwestern outskirts of Munich at 550 m above sea level (a.s.l.). The HVFS was positioned on a lawn in the front yard of the Institute building, about ten meters away from a minor on-campus road and several hundred meters away from the nearest parking lots and city roads. Rural $PM_{2.5}$ samples were collected at

Table 1. Investigated filter samples of fine particulate matter ($PM_{2.5}$). The sample ID specifies the sampling location: U=urban, Munich; R=rural, Hohenpeissenberg; HA=high-alpine, Zugspitze. The sampled air volume is normalized to standard temperature and pressure (STP; 0°C, 1013 hPa). The mass collected on the filter, the corresponding concentration in the sampled air volume, and mass fractions are given for particulate matter (PM) and DNA. In addition information on the total number of sequences, number of different measured sequences, the total number of detected T-RF peaks as well as the number and position of T-RF peaks corresponding to identified sequences per filter is given.

Sample ID	Sampling period	Air Volume (m^3)	PM mass (mg)	PM conc. ($\mu g/m^3$)	DNA mass (μg)	DNA conc. (ng/m^3)	DNA / PM (μg / mg)	Total Number of sequences (incl. plants etc.)	Number of different sequences	Total Number of T-RFs	Number of T-RFs corresp. to sequences
U-A1	18.02.05 – 22.02.05	2617.8	61.6	23.5	21.6	9.0	0.35	3	3	7	2 (158, 493)
U-A2	26.02.05 – 02.03.05	2651.8	62.2	23.4	20.8	8.0	0.33	6	5	6	3 (152, 493, 494)
U-A3	22.03.05 – 26.03.05	2582.4	64.8	25.1	24.8	9.8	0.39	9	6	6	2 (493, 494)
U-A4	17.05.05 – 21.05.05	2589.0	25.9	10.0	6.4	2.8	0.25	9	9	6	2 (493, 494)
U-A5	17.05.05	n.d.	n.d.	n.d.	1.6	n.d.	n.d.	6	6	4	2 (158, 489)
R-A10	02.06.04	n.d.	n.d.	n.d.	4.8	n.d.	n.d.	2	2	7	1 (58)
R-A11	02.06.04 – 07.06.04	3167.8	23.0	7.1	8.8	3.1	0.4	3	3	3	1 (493)
R-A12	17.06.04 – 22.06.04	3146.2	15.9	5.1	8.8	3.1	0.55	5	3	5	2 (71, 489)
R-A13	16.08.04 – 21.08.04	3028.8	15.1	5.0	4.8	1.7	0.32	2	2	6	1 (493)
R-A14	31.08.04 – 05.09.04	3126.4	30.1	9.6	12.0	4.2	0.4	4	3	7	1 (494)
HA-A6	13.09.03 – 17.09.03	2269.1	4.4	1.9	7.2	3.5	1.65	3	3	4	0
HA-A7	17.09.03 – 21.09.03	2243.3	13.0	5.8	6.4	3.5	0.5	3	3	4	1 (493)
HA-A8	21.09.03 – 25.09.03	2272.0	14.7	6.5	7.2	3.7	0.49	6	6	4	2 (493, 494)
HA-A9	21.09.03	n.d.	n.d.	n.d.	2.4	n.d.	n.d.	1	1	4	1 (493)

the Meteorological Observatory Hohenpeissenberg (MOHp), which is located on top of a small mountain (990 m a.s.l., 200–300 m higher than the surrounding terrain) about halfway between Munich and Mt. Zugspitze. The HVFS was positioned on an open platform on top of the meteorological observatory. High-alpine $PM_{2.5}$ samples were collected at the Environmental Research Station Schneefernerhaus, Zugspitze (UFS). The sampling site (2650 m a.s.l.) was located on the southern slope close to the summit of Mt. Zugspitze (2962 m a.s.l.) at the northern edge of the Alps. The HVFS was located on the south-western platform on top of the research station.

Samples of total suspended particles (TSP) on polypropylene filters ($44\times44\,cm^2$) were provided by the Institute of Radiation Protection, GSF-National Research Center for Environment and Health, Neuherberg. The samples were collected with a high-volume air sampler (ASS – 500 Central Laboratory for Radiological Protection, Warsaw, Poland, PTI Ulf Fischer, Erlangen) situated on the east side of the GSF Campus in Munich, next to one of the biggest dry lawns in southern Bavaria (sample air flow $700\,L\,h^{-1}$; sampling time 10 d). TSP samples on cellulose nitrate membrane filters (15 cm diameter, Schleicher and Schüll) were provided by the Forschungszentrum Karlsruhe GmbH, Institut für Meteorologie und Klimaforschung, IMK-IFU. They were collected with a filter sampler (Digitel DHA-80) at the summit of Mt. Zugspitze (2962 m a.s.l.; air sample volume 1181 L; sampling time 24 h). After sampling the filters were compressed into compact tablets and used for radionuclide measurements (^{7}Be and ^{210}Pb activity on Mt. Zugspitze TSP filter, while γ-spectrometry on Munich TSP filter). The TSP filter substrates had not been decontaminated prior to use, and the samples were stored at room temperature because they had originally not been foreseen for molecular genetic analysis.

2.2 DNA extraction and quantification

$PM_{2.5}$ filter sample pieces (0.5×1 cm, $\sim$0.2 g filter loaded with 0.5–8 mg $PM_{2.5}$) were lysed and extracted with a commercial soil DNA extraction kit (LysingMatrixE, Fast DNA Spin Kit, Biomedicals) according to the supplier's instructions with the following modifications: 10-min centrifugation step after the lysis, additional 900μl buffer, and repeated beating and centrifugation. Both generated supernatants were combined for the further extraction process. Finally, the DNA was dissolved in 100μl elution buffer, and the DNA concentration was measured by UV spectrophotometry at 260 nm.

The other filter samples were extracted with the same kit and procedures, except for extracting twice with 400μl sodium phosphate buffer.

2.3 DNA amplification

PCRs were performed for T-RFLP and sequence analyses (1–5μl sample extract used as template DNA). The reaction mixtures always contained ca. $18\,ng\,\mu l^{-1}$ template DNA, 1×PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate (dNTPs) (MP Biomedicals), 0.33μM of each primer (MWG-Biotech, Ebersberg, Germany), 10μg BSA and 5 U of Taq DNA polymerase (Invitrogen, Netherlands).

PCRs for sequence analyses of bacteria, archaea, fungi, plants and animals were performed with the primer pairs listed in Table 2 under the following experimental conditions: 50μl reaction volume (thermal cycler model 2400, PE Applied Biosystems or PTC 200/225, MJ Research); thermal profile: initial denaturing at 94°C for 3 min; 35 cycles with denaturing at 94°C for 30 s, annealing at primer pair specific temperature for 30 s, and elongation at 72°C for 30 s; final extension step at 72°C for 5 min. The specific annealing

Table 2. Polymerase chain reaction (PCR) primer information. Sequences of the forward and reverse primers are listed with target locations, product lengths, annealing temperatures, and literature references.

Organism	Forward primer	Reverse Primer	Gene	Product length	Annealing temperature	Reference
Bacteria	9/27f: 5′ gag ttt gat c(ac)t ggc tca g 3′	1492r: 5′ acg g(ct)t acc ttg tta cga ctt 3′	16S (nuclear)	1564 bp	57°C	(Weisburg et al., 1991)
Archaea	109f: 5′ ac (gt) gct cag taa cac gt 3′	934r: 5′ gtg ctc ccc cgc caa ttc ct 3′	16S (nuclear)	825 bp	52°C	(Großkopf, 1998)
Fungi	5.8s: 5′ cgc tgc gtt ctt cat cg 3′	ITSf: 5′ tcc gta ggt gaa cct gcg g 3′	ITS (nuclear)	∼300 bp	55°C	(Vilgalys and Hester, 1990; Gardes and Bruns, 1993; Fierer et al., 2005)
Plants	F1F: 5′ atg tca cca caa aca gaa act aaa gca agt 3′	F1379R: 5′ tca caa gca gca gct agt tca gga ctc 3′	rbcL (chloroplast)	1392 bp	57°C	(Little and Barrington, 2003)
Animals	18Sai_f: 5′ cct gag aaa cgg cta cca cat c 3′	18Sbi_r: 5′ gag tct cgt tcg tta tcg ga 3′	18S (nuclear)	1140 bp	55°C	(Whiting et al., 1997)

temperatures for the different primer pairs were determined in temperature gradient experiments (Table 2).

PCRs for T-RFLP were carried out using a carboxyfluorescein (fam)-labeled oligonucleotide primer and an unlabelled primer (MWG, Ebersberg). For bacteria we used 9 / 27f (fam) 5′-GAG TTT GAT C(AC)T GGC TCA G-3′ and 907 / 926r 5′-CCG TCA ATT C(AC)T TTR AGT TT-3′, which amplify 16S rRNA genes (Weisburg et al., 1991). For archaea we used 109f 5'-AC(GT) GCT CAG TAA CAC GT-3′ and 934r (fam) 5'-GTG CTC CCC CGC CAA TTC CT-3′ (Großkopf et al., 1998).

The thermal profile of the T-RFLP-PCR for bacteria was as follows: 94°C for 3 min; 35 cycles each with 45 s at 94°C, 30 s at 57°C, and 80 s at 72°C; 5 min at 72°C. PCR products were separated by electrophoresis (80V to 120V) on a 1 to 1.5% agarose gel and visualized by ethidium bromide staining. The amplification of archaea for the T-RFLP analysis was performed with the same thermal profile, except that the annealing step at 52°C lasted 45 s, elongation at 72°C 90 s and denaturing again 30 s at 94°C.

2.4 T-RFLP analysis

PCR products for T-RFLP were purified using the GenElute PCR clean up kit (Sigma-Aldrich Chemie GmbH, Taufkirchen). Approximately 100 ng of the amplicons were digested (3 h, 37°C, total volume 10μl) with 5 U of the restriction endonuclease MspI (bacteria) or TaqI (archaea) (Fermentas). Aliquots (1.25μl) of the digested amplicons were mixed with 0.85μl of formamide and 0.4μl of an internal lane standard (GeneScan-1000 ROX; PE Applied Biosystems, Darmstadt), denatured at 94°C for 2 min and then chilled on ice. Electrophoresis was performed on a polyacrylamide gel (automated DNA sequencer model 373; PE Applied Biosystems, Darmstadt) for 6 h at the following settings: 2500 V, 40 mA, and 27 W (24 cm gel length). After electrophoresis, the lengths (peak positions) of the terminal restriction fragments (T-RFs) and the intensities (peak areas) of their fluorescence emission signals were automatically calculated by the GeneScan Analysis software, version 2.1 (PE Applied Biosystems, Darmstadt).

For each of the 14 filter samples the sum of all measured peak heights of the T-RF fragments (50–928 bp) was calculated. Every peak was normalized by division through the peak-height sum of its sample and multiplication with the smallest peak-height sum of all samples. Normalized peak heights and normalized peak-height sums were then used to calculate relative abundances of the T-RF peaks for the different samples and locations (Lüdemann et al., 2000).

2.5 Cloning and sequencing

Amplification products for sequencing were cloned into *Escherichia coli* using the TOPO TA Cloning ^{TM}Kit (Invitrogen, Netherlands) following the supplier's instructions. The cells of *E.coli* were transformed by heat-shock for 30 s at 42°C and cultivated on LB plates containing ampicillin and X-Gal at 37°C for approximately 16 h. Colonies containing inserts were identified by blue-white selection and lysed in 20μl water for 10 min at 95°C. The inserts of 24 colonies were amplified using 3 μl lysate in a 40μl reaction (PCR master mix: 2.5 mM $MgCl_2$, 1×PCR Buffer, 250 nM of each primer, 250μM of each dNTP, 1.25 U Taq (Invitrogen, Netherlands)). The only primer pair used in these PCRs was M13F-40 and M13R, and the temperature programme was as follows: 94°C for 5 min; 40 cycles at 93°C for 30 s, 55°C for 1 min, and 72°C for 1 min; final extension step at 72°C for 15 min. DNA sequences were determined with ABI Prism 377, 3100, and 3730 sequencers (Applied Biosystems, Darmstadt) using BigDye-terminator v3.1 chemistry at the DNA Core Facility of the Max Planck Institute for Plant Breeding Research.

The measured sequences were aligned using the program BioEdit (BioEdit 7.05; http://www.mbio.ncsu.edu/BioEdit/bioedit.html). For comparison with known sequences, databank queries using the Basic Local Alignment Search Tool (BLAST) were performed via the website of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). For each of the measured sequences, the

first hit in the list of databank search results was taken as the "best-match sequence". The degree of accordance (identity) and accession number of the best-match sequences were recorded and are presented below.

2.6 Inhibition tests

To test inhibitory effects of components (extracted in association with the DNA) within the filter extract on the DNA amplification process, an artificial vector pET44a was used as a DNA template (20 ng) to amplify a 2004 bp long product with the forward primer T7 5′-TAA TAC GAC TCA TCA CTA TAG GG -3′ and the reverse primer pET44rev 5′-TAC GGC GTT TCA CTT CTG A-3′ (Nowoisky, 2005). The PCR (50μl) was performed as described above. The PCR program was: 94°C for 3 min, 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 1.5 min. The final elongation step was 72°C for 7 min.

The degree of inhibition a DNA extract causes was estimated by adding 1μl of this extract to a PCR master mix containing the pET44a vector. After PCR and gel electrophoresis, inhibition effects were detected by optical inspection of the gel and comparison to a positive vector control. Bands that were less bright than the vector control were assumed to indicate partial inhibition. Missing bands were assumed to indicate complete inhibition. This inhibition test was performed for one urban $PM_{2.5}$ DNA extract. The brightness of the band was reduced about 50% compared to the vector control.

Additonal tests were performed with a polypropylene filter TSP sample (TSP-U-5) and with a cellulose nitrate TSP sample (TSP-HA-12). For each sample, a 2–3 mm piece of the filter was added to the PCR mastermix, then the vector was added and the PCR and detection were performed as described above. The cellulose nitrate filter caused complete inhibition of the PCR reaction, and the polypropylene filter reduced the brightness of the amplified vector band by about 60% compared to the vector control.

2.7 Phylogenetic analysis

With the ARB programme (Ludwig et al., 2004) a phylogenetic analysis was performed for all bacterial sequences determined in this study. In this approach a parsimony tree was calculated using sequences provided by ARB. These sequences were calculated into a tree. Complete sequences, about 300, which were closely related, were extracted and used to calculate a Neighbor-Joining tree. Into this Neighbor-Joining tree the sequences from this study were included without changing the topology of the tree. All sequences that added no information to the tree were excluded again without changing the topology of the tree.

2.8 Statistical analysis

For each of the $PM_{2.5}$ sampling locations the sequence and T-RFLP data were used to calculate the Shannon-Weaver diversity index (H) and the evenness (e) as follows: $\overline{H} = (C/N)\left(N * \log N - \sum n_i \log n_i\right)$, where C is 2.3, N the total number of obtained sequences (peaks) at the sampling location, and n_i the number of sequences (peaks) belonging to a certain group of bacteria (e.g., *Gammaproteobacteria*); $e = \overline{H}/S$, where S is the number of bacterial groups (peaks) at the sampling location.

3 Results and discussion

3.1 DNA Detection

3.1.1 Atmospheric aerosol samples

A commercial soil DNA extraction kit was used to extract DNA from 28 filter samples of air particulate matter from urban, rural and high-alpine locations as detailed in Table 1. DNA was found in all extracts (2–38 ng μl^{-1} in 100 μl), which demonstrates that the extraction method is applicable for different aerosol filter types such as glass fiber, cellulose nitrate, and polypropylene filters (as well as a quartz fiber filter test sample).

The results show that DNA is present not only in coarse particles but also in fine particulate matter ($PM_{2.5}$, aerodynamic diameter $\leq 2.5 \mu m$), and that small sample aliquots with small amounts of air particulate matter (0.5–8 mg $PM_{2.5}$) are sufficient to extract measurable amounts of DNA. Moreover, they show that DNA can be extracted not only from fresh samples, but also from samples that have been taken for other purposes and stored for extended periods of time (in the present case three years at −20°C or one year at room temperature). DNA could be recovered even from the compact tablets into which the cellulose nitrate filters and polypropylene filters had been compressed and which had already been used for radionuclide measurements.

Nevertheless, DNA starts to degrade as soon as an organism dies. It breaks into smaller pieces and is chemically modified (Pääbo, 1989; Lindahl, 1993; Höss et al., 1996; Smith et al., 2001). Under cool, dark, and dry conditions DNA can be preserved for several thousand years (Pääbo et al., 2004), but the DNA amount decreases and DNA information is lost. Thus, air filter samples for molecular genetic analysis should be kept dry and frozen to slow down degradation processes.

From the DNA concentration in the sample extracts we calculated the equivalent mass of DNA per filter sample, the mass fraction of DNA in the sampled air particulate matter, and the concentration of DNA in the sampled air volume. Across sampling locations, the DNA concentrations in air varied in the range of 0.8–9.8 ng m^{-3}. Among the $PM_{2.5}$ glass fiber filters, the urban samples exhibited the highest DNA concentrations, with an average value of 7 ng m^{-3}. The

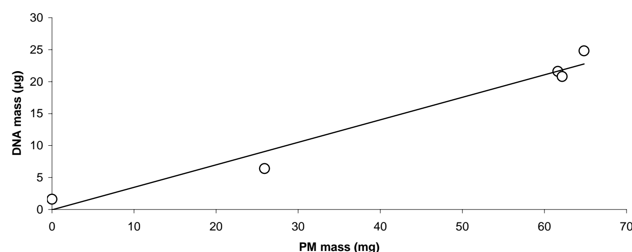

Fig. 1. DNA vs. PM mass in urban fine particulate matter ($PM_{2.5}$). Correlation between the mass of DNA and the total mass of particulate matter (PM) in samples collected in the city of Munich, Feb–May 2005; correlation coefficient 0.98.

DNA mass fraction in urban $PM_{2.5}$ was nearly constant at $\sim$0.03%, which is confirmed and illustrated by the plot and linear trend (r^2=0.98) of DNA vs. PM in Fig. 1. The observation of a high and near-constant relative abundance of DNA from biological particles in urban $PM_{2.5}$ is consistent with the high mass fraction of proteins (up to $\sim$5%) in the large set of samples, from which the filters investigated in this study were taken (Franze, 2004; Franze et al., 2005; Pöschl, 2005; Fehrenbach, 2006). The DNA/protein ratio of $\sim$1/100 is also consistent with the typical proportions of DNA and proteins in living organisms (e.g., 1/15 in prokaryotes in Voet and Voet, 1995).

To put the abundance of DNA in fine, and thus respirable, air particulate matter into perspective with bacterial and human genomes, we have performed the following back-of-the-envelope calculations: Under the assumption of an average DNA concentration of $\sim$7 ng m^{-3}, average DNA amounts of $\sim$4 fg per haploid bacterial genome (*E.coli*) or $\sim$3 pg DNA per haploid human genome, and adult human breathing rates between 5 and 120 L min^{-1} (sleep vs. sports), an adult person living in a city can be expected to inhale every day about 0.05–1.2 μg DNA, corresponding to 10^7–10^8 haploid bacterial genomes (*E.coli*) or 10^4–10^5 haploid human genomes, respectively. The rural and high-alpine $PM_{2.5}$ glass fiber filter samples exhibited lower PM and DNA concentrations, but similar or higher mass fractions of DNA (0.03–0.16%), which are again consistent with high mass fractions of proteins observed in these and related samples (Franze, 2004; Franze et al., 2005; Fehrenbach, 2006). These findings confirm that DNA is not rapidly degraded in frozen glass fiber filter samples of air particulate matter.

The absolute and relative concentrations of DNA determined for the urban and high-alpine TSP samples collected on cellulose nitrate and polypropylene filters were generally lower than those determined for the $PM_{2.5}$ glass fiber filters. This may be due to stronger inhibition effects (see Sect. 2.6) or faster degradation of DNA in the samples stored at room temperature. On the other hand, the TSP samples are likely to have been affected by elevated blank levels of DNA, because the filters had not been decontaminated prior to use (Sect. 3.1.2).

The above results are subject to uncertainties related to contamination, extraction efficiency, degradation, and photometric quantification of DNA. They certainly need to be corroborated by future investigations of larger sample numbers and complementary test experiments and measurement techniques. Nevertheless, we think that the observed trends, correlations, and orders of magnitude reported above provide a reasonable first estimate and basis for further comprehensive and systematic investigations of the abundance of DNA in air particulate matter. To our knowledge, no comparable measurement data on the abundance of DNA in air have been published yet.

3.1.2 Blank and background samples

In parallel to the filter samples of air particulate matter, four different types of blank and background samples have been investigated to check for possible sources of contamination: (1) six freshly pre-baked glass fiber filters ("glass fiber laboratory blank"); (2) a polypropylene filter freshly taken from the sealed original packing of the commercial supplier ("polypropylene supplier blank"); (3) a polypropylene filter pressed to a tablet but not exposed to air sampling ("polypropylene tablet blank"); (4) one pre-baked glass fiber filter treated exactly like air samples but exposed to the sampling flow for only 0.5 min ($\sim$0.25 m^3 of air; "glass fiber sampling background") at each of the $PM_{2.5}$ sampling locations (Munich, Hohenpeissenberg, Mt. Zugspitze). No blanks were available for the cellulose nitrate filter samples.

DNA was not detected on any of the glass fiber laboratory blanks, indicating that contamination could be excluded during the filter handling and extraction process in the laboratory. In contrast, up to 1 μg of DNA was detected on the polypropylene supplier and tablet blanks. The polypropylene filter blank DNA could be amplified and cloned using universal bacterial primer, indicating bacterial contaminations. The sequencing reaction, however, failed and thus the exact identity of the DNA could not bet determined. Nevertheless, these findings indicate that decontamination of sampling substrates by baking at high temperature or alternative methods such as autoclaving is a necessary prerequisite for reliable molecular genetic analyses of atmospheric aerosol samples. DNA was also found on each of the glass fiber sampling background filters: urban 1.6 μg, rural 4.8 μg, high-alpine 2.4 μg. The urban and high-alpine background filters were lower by factors of 3–4 than the lowest values determined for real PM samples. The rural background filter, however, was as high as the lowest PM sample, although the sampled volume was smaller by a factor of $\sim$1000. These findings indicate that the handling of filters at the beginning and end of the sampling period can be a major source of contamination and lead to high background DNA concentration levels. The composition and molecular signature of the biological

Table 3. DNA sequences found in fine particulate matter ($PM_{2.5}$). For each of the different measured sequences (bacteria S1–S34, fungi S35–S38, plants S39–S42, animals S43) information on the best-match sequences from the NCBI databank search (identity score and accession number) is given together with the organism and taxon determined by phylogenetic analysis. Additionally, the PCR primer pair used for their amplification (B=Bacteria, A=Animal, F=Fungi; Table 2) is given and how often and on which filter samples the different sequences were detected. For bacteria, the lengths (number of base pairs, bp) of tentatively assigned terminal-restriction fragments (T-RF) is presented. Note that the identified sequences may have originated from viable as well as from dead cells in the investigated aerosol samples, because DNA can persist in cells after they lose their viability.

Sequence ID	PCR Primer	Best match identity (%)	Best match accession number	Organism	Taxon	T-RF length (bp)	Number of occurences	Sample ID
S1	B	99	DQ163939	Bacteria	*γ-Proteobacteria*	493	10	U-A1, 2xU-A2, 4xU-A3, U-A-4, HA-A7, HA-A8
S2	B	99	AY168595	Bacteria	*Actinobacteria*	158	2	U-A1, U-A5
S3	B	99	DQ163939	Bacteria	*γ-Proteobacteria*	493	3	U-A1, R-A13, HA-A9
S4	B	99	AM055711	Bacteria	*γ-Proteobacteria*	494	4	U-A2, U-A3, R-A14, HA-A8
S5	B	99	U87763	Bacteria	*α-Proteobacteria*	152	1	U-A2
S6	B	100	AY642051	Bacteria	*Actinobacteria*		1	U-A3
S7	B	99	DQ163939	Bacteria	*γ-Proteobacteria*	493	1	U-A4
S8	B	99	AM055711	Bacteria	*γ-Proteobacteria*	494	1	U-A4
S9	B	99	AY053481	Bacteria	*Actinobacteria*		1	U-A4
S10	B	99	DQ279310	Bacteria	*γ-Proteobacteria*		1	U-A4
S11	B	99	DQ057384	Bacteria	*β-Proteobacteria*	489	1	U-A5
S12	B	99	AF509579	Bacteria	*β-Proteobacteria*	489	1	U-A5
S13	B	99	AF509579	Bacteria	*β-Proteobacteria*	489	4	U-A5, 3xR-A12
S14	B	99	AF509579	Bacteria	*β-Proteobacteria*	489	1	U-A5
S15	B	99	DQ057384	Bacteria	*β-Proteobacteria*	489	1	U-A5
S16	B	99	AY642054	Bacteria	*Actinobacteria*	58	1	R-A10
S17	B	99	DQ336995	Bacteria	*γ-Proteobacteria*		1	R-A10
S18	B	99	AB042289	Bacteria	*Actinobacteria*		1	R-A11
S19	B	99	AE017283	Bacteria	*Actinobacteria*		1	R-A11
S20	B	99	DQ163939	Bacteria	*γ-Proteobacteria*	493	1	R-A11
S21	B	99	AF509579	Bacteria	*β-Proteobacteria*	489	1	R-A12
S22	B	99	AY959147	Bacteria	*Firmicutes*	71	1	R-A12
S23	B	99	DQ163939	Bacteria	*γ-Proteobacteria*	493	1	R-A13
S24	B	100	AY168586	Bacteria	*Actinobacteria*		2	2xR-A14
S25	B	99	AM055711	Bacteria	*γ-Proteobacteria*	494	1	R-A14
S26	B	99	D14506	Bacteria	*α-Proteobacteria*		1	HA-A6
S27	B	99	AF385528	Bacteria	*β-Proteobacteria*		1	HA-A6
S28	B	99	AF385528	Bacteria	*β-Proteobacteria*		1	HA-A6
S29	B	99	AF509579	Bacteria	*β-Proteobacteria*		1	HA-A7
S30	B	99	AF385528	Bacteria	*β-Proteobacteria*		1	HA-A7
S31	B	99	AM055711	Bacteria	*γ-Proteobacteria*	494	1	HA-A8
S32	B	99	AM055711	Bacteria	*γ-Proteobacteria*	494	1	HA-A8
S33	B	100	AY642054	Bacteria	*Actinobacteria*		1	HA-A8
S34	B	99	DQ163939	Bacteria	*γ-Proteobacteria*	493	1	HA-A8
S35	A	99	AF530542	Fungi	*Basidiomycota*		1	U-A2
S36	A	99	AB108787	Fungi	*Ascomycota*		1	U-A3
S37	A	99	X69842	Fungi	*Ascomycota*		1	U-A3
S38	F	100	AY463365	Fungi	*Ascomycota*		1	U-A4
S39	A	99	AF206895	Plant	*Cucurbita*		1	U-A3
S40	F	94	D38246	Plant	*Pinaceae*		1	U-A4
S41	F	95	AY156588	Plant	*Bryophyta*		1	U-A4
S42	B	98	AY156592	Plant	*Bryophyta*		1	U-A4
S43	A	93	L31841	Animal	*Alveolata*		1	U-A2

material and DNA collected during this process may just be more concentrated but similar to that in the sampled air flow. On the other hand, it might also be characteristic for the sampling equipment rather than for the sampled air flow and investigated environment. In any case, analytical artifacts related to contamination of sampling materials prior to use or during sample collection and handling can strongly influence the molecular genetic analysis of atmospheric aerosols. In earlier studies, this aspect had not been addressed at all. Our results clearly demonstrate the necessity and effectiveness of material decontamination and parallel blank extractions.

3.2 Molecular genetic analyses

With the DNA extract from each of the filters listed in Table 1, five PCRs were performed to amplify DNA from bacteria, archaea, fungi, land plants, and animals for sequence analysis. PCR products were obtained for all categories except archaea. All PCR products were cloned, and from about 100 clones, 24 were randomly selected, lysed, and amplified separately again. Depending on the success rate of the second step of amplification, up to seven products of the 24 PCRs were sequenced. The obtained sequences were blasted in the databank of the National Center for Biotechnology

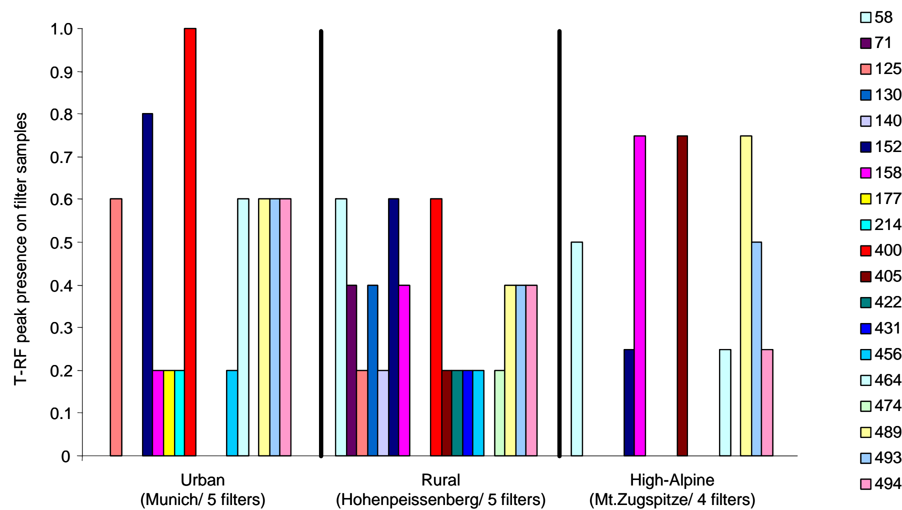

Fig. 2. Distribution of bacterial T-RF peaks among $PM_{2.5}$ filter samples and locations. The x-axis represents the different sampling locations and measured T-RF peak positions (number of base pairs, bp). The y-axis specifies the fraction of filter samples on which the T-RF peaks were found at each location. For example, the 125 bp fragment was found in 3 of 5 filters (0.6) from Munich, in 1 of 5 filters from Hohenpeissenberg and in none of Mt. Zugspitze.

Information (NCBI) to find the closest match and determine the taxonomy of the organisms from which the DNA on the filter samples had most likely originated.

Systematic sequence analyses and complementary T-RFLP investigations were performed only for the $PM_{2.5}$ samples, because the filters had been decontaminated prior to use. All sequences obtained for these samples are summarized in Table 3 and discussed in Sects. 3.2.1–3.2.3. As discussed above, the TSP samples were most likely influenced by filter contaminations. Nevertheless, some characteristic results obtained for the TSP samples will be outlined in Sect. 3.2.3.

3.2.1 Prokaryotes in $PM_{2.5}$

Sequences and phylogeny

As summarized in Table 3, 53 bacterial, but no archaeal, clone sequences could be retrieved from the investigated $PM_{2.5}$ samples. For 29 sequences the taxonomic identity was determined by a databank search on NCBI.

Three sequences exhibited 100% identity with *Actinobacteria,* the other sequences exhibited 99% identity with different *Gamma-* and *Betaproteobacteria*. Several of the clone sequences showed best match with the same databank sequence, possibly since the clone sequences were from bacteria whose sequence had not yet been determined and entered into database or because they have been partially degraded in the air or upon sampling and analysis (e.g., chemical modification of individual base pairs).

Twenty four sequences could not be resolved by comparison with NCBI sequences, since the taxonomy of the closest matches were unclear (categorized as "environmental samples" in the databank). For taxonomic identification of these 24 sequences we performed a phylogenetic analysis by calculating a neighbor-joining tree that consisted of all 53 sequences from this study and 74 sequences from the ARB databank (Ludwig et al., 2004).

The phylogenetic analysis yielded 27 *Gamma-*, 13 *Beta-,* and 2 *Alphaproteobacteria* sequences, 10 *Actinobacteria,* and 1 *Firmicute*. Unlike Maron et al. (Maron et al., 2005), we found no *Deltaproteobacteria* in the investigated aerosol samples. *Proteobacteria* are one of the largest groups in the domain *Bacteria*. They include many pathogens as well as nitrogen oxidizing bacteria living in soils and on plants. *Gammaproteobacteria* are known to be particularly flexible and adaptable to different environments. *Actinobacteria* and *Firmicutes* are mostly Gram positive bacteria, many of which form spores for aerial transport. Most *Actinobacteria* are found in soil, where they are involved in the decomposition of organic materials. The *Firmicutes* include common soil bacteria like the endospore-forming bacilli such as *Bacillus subtilis,* which is frequently detected in soil, water and air, but also prominent pathogens like *Bacillus anthracis* or *Bacillus thuringiensis*, which is used as an agricultural insecticide. Only recently Brodie et al., 2007, have shown that endemic pathogens do occur naturally in our environment. For *Gammaproteobacteria*, only 13 of the 27 clone sequences were different from each other, and for the 13 different clone sequences the databank search yielded only 4 different best-match sequences (DQ163939, AM055711, DQ279310, DQ336995). For *Betaproteobacteria,* 10 of the 13 sequences were different from each other and the databank search yielded 3 different best-match sequences

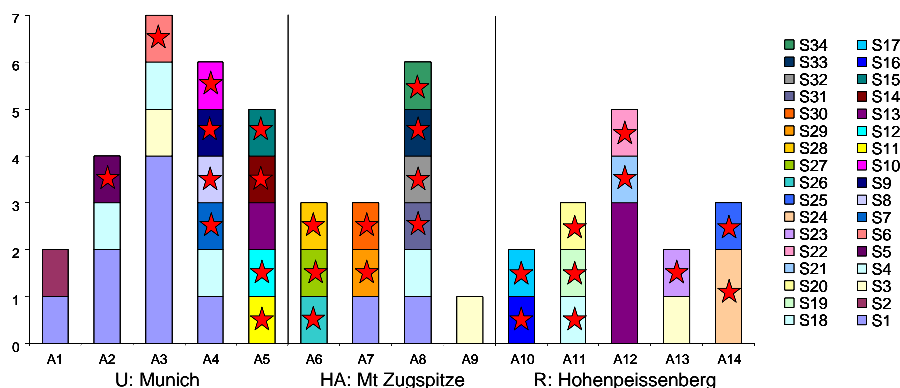

Fig. 3. Distribution of bacterial DNA sequences among $PM_{2.5}$ filter samples and locations. Number and identity of measured sequences per filter sample. Different sequences (S1–S34) are illustrated in different colors. Sequences that occurred uniquely on one filter sample are highlighted with a red star.

(DQ057384, AF385528, AF509579). For *Alphaproteobacteria*, on the other hand, both of the clone sequences were different and yielded different best-match sequences (U87763, D14506). Also for *Actinobacteria*, most of the determined sequences were different from each other (8 out of 10) and yielded different best-match sequences (7).

Diversity

For characterization of the bacterial diversity, T-RFLP analyses were performed with the DNA extracts from all $PM_{2.5}$ samples (Table 1). Figure 2 summarizes the number, length, and relative abundance of the 19 different T-RFs detected at the different sampling locations. The urban $PM_{2.5}$ samples from Munich filters exhibited in total 11 T-RF peaks (125–494 bp). One of the T-RF peaks (400 bp) was found in all five samples, and four were found only in one sample (Fig. 2). The rural samples from Hohenpeissenberg exhibited the highest bacterial diversity with 16 T-RF peaks (58–494 bp) in total; none of them in all five samples and seven only in one sample (Fig. 2). The high-alpine samples from Mt. Zugspitze exhibited the lowest diversity with eight peaks in total; none of them in all four samples and three only in one sample (Fig. 2).

To compare and connect the results of T-RFLP and clone sequence analyses, the number of base pairs until the first restriction site for *Msp I* "C-CGG" was determined for each of the clone sequences found in the $PM_{2.5}$ samples to obtain in-silico the size of the T-RF expected from this clone. When the calculated number coincided with the length of one of the observed T-RF peaks, the sequences and T-RF peaks were tentatively assigned to each other (Table 1).

As detailed in Table 3, all of the T-RFs with a length of 489 were matching multiple *Betaproteobacteria* and those with a length of 493 or 494 bp were matching multiple *Gammaproteobacteria* sequences. The T-RFs with a length of 152, 158, 58, or 71 bp were matching individual sequences of *Alphaproteobacteria*, *Actinobacteria (*TR-F 158 and 58), or *Firmicutes*, respectively. In total, 37 of the 44 bacterial sequences could be matched with one of the seven TR-F peaks specified above. For the other twelve T-RF peaks no tentative taxonomic assignment could be achieved, indicating that the T-RFLP analyses resolved fewer taxa than clone sequence analysis, but covered more of the bacterial diversity in the investigated samples.

As illustrated in Figs. 2 and 3, five T-RF peaks (152, 158, 489, 493, and 494 bp) and two of the clone sequences (S3, S4) were found at all sampling locations. Two T-RF peaks (177 and 214 bp) and 11 clone sequences (S2, S5–S13, S15) were found exclusively in the urban samples from Munich, six T-RF peaks (71, 130, 140, 422, 431, and 474 bp) and ten clone sequences (S16–S25) exclusively in the rural samples from Hohenpeissenberg. In the high-alpine samples from Mt. Zugspitze we found only T-RF peaks that were also detected in samples from the urban or rural locations. Nine of the measured sequences (S26–S34), however, were detected only in the high-alpine samples.

Shannon-Weaver diversity indices (H) were calculated for each sampling location from both the number of T-RF peaks observed and the number of clone sequences determined (Table 4). Both data sets indicate the highest bacterial diversity for the rural samples from Hohenpeissenberg. The H values based on the sequence data indicate similar diversities for the urban and high-alpine samples, whereas the H values based on the T-RFLP data indicate higher diversity for the urban samples than for the high-alpine samples. The results are consistent with earlier observations that the concentration of PBA particles in high-alpine regions is lower than in levels directly above the sea level (Jaenicke et al., 2000). Note, however, that we have calculated the Shannon-Weaver indices just as preliminary indicators for the diversities observed with the different techniques applied in this exploratory study. The calculated values should not be regarded

Table 4. Diversity indices for bacteria detected in $PM_{2.5}$ samples from different locations. Shannon-Weaver diversity indices (H) and evenness (e) of the data calculated from bacterial sequence and T-RFLP data. For the T-RFLP data also the range of the species richness (S, referring to number of T-RF peaks) for every location is given. All samples for one location were combined.

	Diversity (H)		Evenness (e)		Species Richness (S)
	H (seq)	H (T-RFLP)	e (seq)	e (T-RFLP)	S (T-RFLP)
Urban (Munich)	1.07	2.35	1.78	2.17	4-7
Rural (Hohenpeissenberg)	1.25	2.6	2.08	2.13	4-7
High-Alpine (Zugspitze)	1.09	1.98	1.82	2.19	4

as robust parameters for further statistical analysis and interpretation. As pointed out by Blackwood et al. (2007), diversity indices calculated from T-RFLP data can be biased, and we are planning to use and compare also other indices in follow-up studies with larger data sets.

Overall, the T-RFLP analysis indicates lower species richness for our $PM_{2.5}$ samples than for soil samples, for which the method had originally been developed and applied (Dunbar et al., 2000; Lüdemann et al., 2000; Lukow et al., 2000; Klamer and Hedlund, 2004; Noll et al., 2004). While in the air samples 4–7 T-RF peaks were observed per filter, in soil typically 20–30 TR-F peaks are found per sample. Note, however, that the apparent lower diversity may be partly due to chemical modification and degradation of DNA or inhibitory effects of atmospheric aerosol components on the amplification and cloning procedures. Control experiments with an urban $PM_{2.5}$ sample extract have confirmed the presence of inhibitors (reduced amplification efficiency, see also 2.6).

Abundances

The best ways of estimating relative abundances of bacteria are still under discussion (Hong et al., 2006 and references therein). For the $PM_{2.5}$ samples investigated in this study, the relative abundances of bacterial groups have been estimated by two approaches. One approach is based on the T-RFLP data, i.e., on normalized intensities of T-RF peaks assigned to different bacterial groups. The other approach is based on the sequence analysis data, i.e., on the numbers of sequences assigned to different bacterial groups. As detailed in Figure 4, both approaches indicate that *Proteobacteria* were the most abundant fraction of bacteria at all sampling locations (87–95% of T-RF peak intensities; 62–92% of sequence numbers), and that their abundance was dominated by *Beta-* and *Gammaproteobacteria*. These findings are consistent with the results of bacterial sequence analyses in air particulate matter at urban and rural locations in USA and France, respectively (Radosevich et al., 2002; Maron et al., 2005) and with most previous studies using culture-based methods (di Giorgio et al., 1996; Shaffer et al., 1997; Griffin et al., 2001). In a very recent study, where bacteria in the air of two cities in Texas have been investigated over several weeks with chip measurement technologies rather than full sequence analyses, however, *Proteobacteria* were found to be less frequent than *Actinomycetes*, *Bacteroides* and *Cyanobacteria* (Brodie et al., 2007). The variability of atmospheric aerosol sources and composition as well as the different measurement techniques may have contributed to the different findings. Further studies are needed to determine the extent to which each of these factors influence the results. For *Actinobacteria* and for the individual sub-groups of *Proteobacteria* at the different sampling locations of this study, however, the results based on T-RFLP and sequence data were substantially different. Although sequences from *Actinobacteria* were found relatively often (8–32%), the T-RFLP data indicate an abundance of only 1–3%. The latter observation is consistent with recent genetic analyses of bacteria at a rural location in France (12% in Maron et al., 2005).

We think that the abundance estimates derived from the T-RFLP data are more realistic than those from the sequence data, because the latter may be biased by the cloning procedure (v. Wintzingerode et al., 1997) and are statistically not well founded due to the limited number of measured sequences.

For samples of unknown diversity it has been suggested to use about 300 sequences or more for reliable estimation of relative abundances (Kemp and Aller, 2004), whereas only 13–24 sequences were available for the different sets of $PM_{2.5}$ samples investigated in this study. On the other hand, the T-RFLP analysis of 16S genes as applied in this study has been reported to provide good estimates for the abundance of bacteria in soil samples (Lueders and Friedrich, 2003).

3.2.2 Eukaryotes in $PM_{2.5}$

Fungal sequences

Four fungal sequences were detected in three of the five urban $PM_{2.5}$ samples (Table 3): three sequences from *Ascomycota* (*Cladosporium,* 100% identity with AY463365; *Saccharomycetes,* 99% identity with X69842; unspecified *Ascomycota*, 99% identity with AB108787) and one sequence

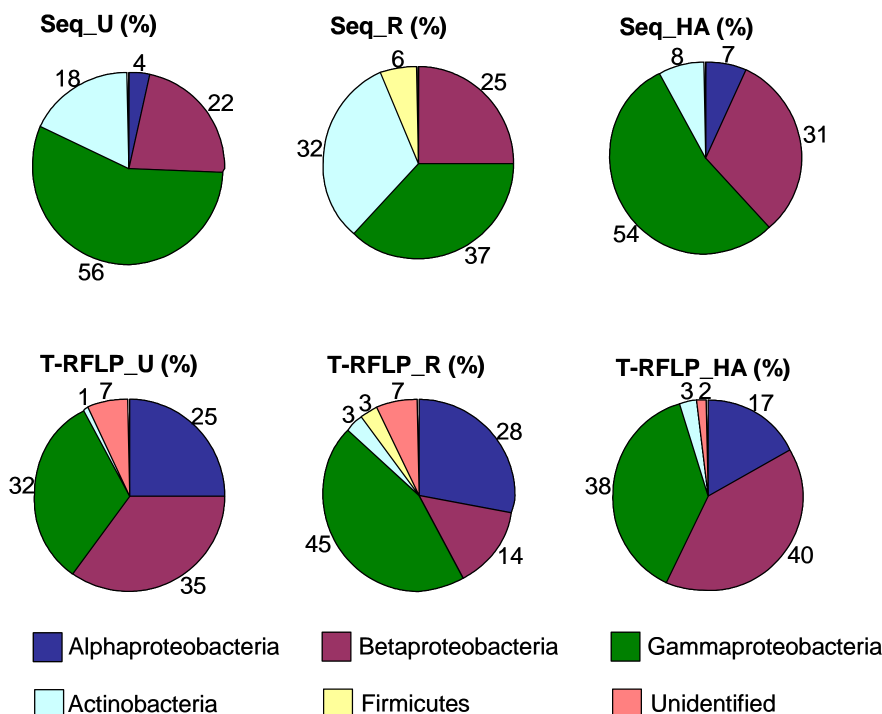

Fig. 4. Relative abundance of bacterial groups in fine particulate matter ($PM_{2.5}$). Percentage of different bacterial groups found in $PM_{2.5}$ samples from urban (U=urban/ Munich), rural (R=rural/ Hohenpeissenberg), and high-alpine air (HA=alpine/ Zugspitze) by sequence analyses (Seq) and T-RFLP investigations. The dark colors (blue, purple, green) represent the proteobacteria, while actinobacteria, firmicutes and unidentified sequences / T-RFLPs are illustrated in light colors.

from *Basidiomycota* (uncultured *Basidiomycetes*, 99% identity with AF530542). *Ascomycota* and *Basidiomycota* are known to actively discharge spores into the atmosphere, and their spores have been detected in a wide range of locations and concentrations (Garrison et al., 2003; Boreson et al., 2004; Griffin, 2004; Griffin and Kellogg, 2004; Elbert et al., 2006). In recent studies based on amplification of the 18S gene and cultivation of fungi, *Ascomycota* and *Basidiomycota* were found to be the most abundant group accounting for proportions of 40–100% of fungi detected in air particulate matter, (Wu et al., 2003; Boreson et al., 2004).

Spores are known to resist environmental stress and survive atmospheric transport (Griffin, 2004; Griffin and Kellogg, 2004), whereas DNA in fungal tissue fragments may be rapidly degraded by atmospheric photooxidants. No fungal DNA was detected in the investigated rural and high-alpine $PM_{2.5}$ samples. Since fungal spores are known to be ubiquitous components of atmospheric aerosols, we think that either the applied soil DNA extraction kit was not very efficient at extracting DNA from fungal spores (Brown and Hovmoller, 2002; Boreson et al., 2004; Griffin, 2004; Griffin and Kellogg, 2004) or the amplification was inhibited. Therefore, ongoing work is aimed at the optimization of DNA extraction and amplification for fungi as well as for other eukaryotes.

Plant sequences

Four plant sequences were detected in two of the five urban $PM_{2.5}$ samples, which had been collected in spring (Table 3): one sequence from a flowering plant (*Angiosperm*; 99% identity with AF206895), two from mosses (*Bryophytes*; 95% identity with AY156588 and 98% with AY156592), and one from the pine family (*Pinaceae*; 94% identity with D38246). The detected sequences had been amplified with the "animal" primer pair 18Sai_f / 18Sbi_r, which is specific for a region of the 18S gene in the eukaryotic nuclear genome. In contrast, the land plant primer pair F1F / F1379R, which amplifies a region of the chloroplast genome, did not give any positive PCR results, although its functionality had been tested positively.

The fact that plant sequences were found only in samples collected during the pollen season in spring time, suggests that the DNA was likely recovered from pollen (angiosperm, *Pinaceae*) or spores (*Bryophytes*) rather than tissue fragments. Pollen and spores are known and designed to resist environmental stress and survive atmospheric transport, whereas DNA in plant tissue fragments may be rapidly degraded by atmospheric photooxidants. The low identity values of the *Bryophyte* and *Pinaceae* sequences may indicate that the sequences of the detected organisms have not

yet been identified and entered into the NCBI database or that they have been partially degraded (e.g., chemical modification of individual base pairs).

All plant sequences identified in this study belong to the *Viridiplantae*, which were also found as the only plant category in a recent study by Boreson et al. (2004). They reported that plants accounted for 11% of the eukaryotic sequences found in a natural desert area and for 31% in urban air. In a couple of other recent studies, plants were only indirectly identified by the co-amplification of chloroplasts with bacterial primer pairs but no plant sequences were analyzed (Radosevich et al., 2002; Maron et al., 2005). In these studies the proportions of chloroplast clones were in the range of 3–32% (Radosevich et al., 2002; Maron et al., 2005). In our study, the plant sequences account for 44% of the eukaryotic sequences detected in urban $PM_{2.5}$ samples.

Animal sequences

Animal DNA was found only in an urban $PM_{2.5}$ sample, and the best-match databank sequence was from a protist (*Alveolata apicomplexa*, 93% identity with L31841). These spore forming unicellular eukaryotes are known as common parasites of insects and vertebrates. Animal DNA is usually not protected and degrades rapidly after the death of the organism. The detected protist DNA was most likely from a spore, which is resistant like bacterial and fungal spores.

Eukaryotic sequences of animals, plants or fungi were found only in the urban $PM_{2.5}$ samples from Munich although the DNA/PM ratio in these samples was smaller than in the rural and high-alpine samples (Table 3). Potential reasons are: (1) The absolute amount of DNA in the urban samples was higher. (2) The urban samples were only six months old when analyzed, whereas the rural and high-alpines samples were 2–3 y old. (3) The sampling season may have played a role especially with regard to plant sequences as discussed in Sect. 3.2.2. Plant sequences. (4) The relative abundance of inhibitors reducing the extraction or amplification efficiency might have been lower in the urban samples (see also 2.6).

3.2.3 Prokaryotes and Eukaryotes in TSP

As outlined above, the TSP samples may have been contaminated by DNA on the sampling materials. Nevertheless, we performed test experiments with these samples and obtained the following results. In the polypropylene tablet blank we found no bacterial PCR product, and the bacterial PCR product from the polypropylene supplier blank could be cloned but not sequenced. In the TSP samples from Munich we found only six bacterial sequences of *Firmicutes*, which were not found in the $PM_{2.5}$ samples, except for one rural sample (H-A12). In the TSP samples from Mt Zugspitze we measured 26 sequences of plant DNA from *Pinaceae*.

4 Conclusions

In this study we have demonstrated that DNA from bacteria, fungi, plants, and animals can be efficiently extracted from different types of atmospheric aerosol filter samples using a soil DNA extraction kit. The investigated samples were up to three years old and included fine and coarse air particulate matter ($PM_{2.5}$ and TSP) as well as glass fiber, cellulose nitrate, and polypropylene filter materials. Investigations of blank and background samples have shown that filter substrates have to be decontaminated prior to use. On the other hand, inhibitory effects of filter materials and of chemical components of the sampled particulate matter can reduce the efficiency of DNA amplification. In any case, the sampling, sample handling, and analytical procedures have to be carefully controlled to avoid artifacts in molecular genetic analyses of atmospheric aerosol samples.

We have combined sequencing and T-RFLP analyses and found the results to be highly complementary. The T-RFLP technique was found to resolve less, but cover more of the bacterial diversity than the sequence analysis. Both the sequence data and the T-RFLP data indicate that the bacterial diversity in the rural samples was higher than in the urban and high-alpine samples. *Proteobacteria* were found to be the most abundant group of bacteria in all samples. The measured sequences of fungi, plants, and animals were most likely from spores and pollen, which are known and designed to resist environmental stress and survive atmospheric transport.

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References

Ahern, H. E., Walsh, K. A., Hill, T. C. J., and Moffett, B. F.: Fluorescent pseudomonads isolated from Hebridean cloud and rain water produce biosurfactants but do not cause ice nucleation, Biogeosciences, 4, 115–124, 2007, http://www.biogeosciences.net/4/115/2007/.

Amann, R., Ludwig, W., and Schleifer, K.: Phylogenetic identification and in situ detection of individual microbial cells without cultivation, Microbiol. Rev., 59, 143–169, 1995.

Andreae, M. O., Artaxo, P., Brandao, C., Carswell, F. E., Ciccioli, P., da Costa, A. L., Culf, A. D., Esteves, J. L., Gash, J. H. C., Grace, J., Kabat, P., Lelieveld, J., Malhi, Y., Manzi, A. O.,

Meixner, F. X., Nobre, A. D., Ruivo, M. d. L. P., Silva-Dias, M. A., Stefani, P., Valentini, R., von Jouanne, J. and Waterloo, M. J.: Biogeochemical cycling of carbon, water, energy, trace gases, and aerosols in Amazonia: The LBA-EUSTACH experiments, J. Geophys. Res., 107(D20), 8066, doi:10.1029/2001JD000524, 2002.

Andreae, M. O. and Crutzen, P. J.: Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry, Science, 276, 1052–1058, 1997.

Artaxo, P.: Size distribution of biogenic aerosol particles from the amazon basin, Atmos. Environ., 29, 393–402, 1995.

Bauer, H., Weinke, G., Schueller, E., Berger, A., Kasper-Giebl, A., Puxbaum, H., Verneylen, R., Claeys, M., and Maenhaut, W.: Contribution of bioaerosols to organic carbon in urban-fringe PM10 aerosols, European Aerosol Conference, Ghent, 2005.

Blackwood, C. B., Hudleston, D., Zak, D. R., and Buyer, J. S.: Interpreting ecological diversity indices applied to terminal restriction fragment length polymorphism data: insights from simulated microbial communities, Appl. Environ. Microb., 73, 5276–5283, 2007

Boreson, J., Dillner, A. M., and Peccia, J.: Correlating bioaerosol load with $PM_{2.5}$ and PM10cf concentrations: a comparison between natural desert and urban-fringe aerosols, Atmos. Environ., 38, 6029–6041, 2004.

Brodie, E. L., DeSantis, T. Z., Moberg Parker, J. P., Zubietta, I. X., Piceno, Y. M., and Andersen, G. L.: Urban aerosols harbor diverse and dynamic bacterial populations, P. Natl. A. Sci., 104, 299–304, 2007.

Brown, J. K. M. and Hovmoller, M. S.: Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease, Science, 297, 537–541, 2002.

Cox, C. S. and Wathes, C. M.: Bioaerosol Handbook, 1st ed, CRS Lewis Publishers, Boca Raton, Florida, p. 656, 1995.

di Giorgio, C., Krempff, A., Guiraud, H., Binder, P., Tiret, C., and Dumenil, G.: Atmospheric pollution by airborne microorganisms in the city of Marsailles, Atmos. Environ., 30, 155–160, 1996.

Dingle, A. N.: Pollen as condensation nuclei, J. recherches atmosphériques, 2, 231–237, 1966.

Dunbar, J., Ticknor, L. O., and Kuske, C. R.: Assessment of microbial diversity in four southwestern united states soils by 16S rRNA gene terminal restriction fragment analysis, Appl. Environ. Microb., 66, 2943–2950, 2000.

Dusek, U., Frank, G. P., Hildebrandt, L., Curtius, J., Schneider, J., Walter, S., Chand, D., Drewnick, F., Hings, S., Jung, D., Borrmann, S., and Andreae, M. O.: Size matters more than chemistry for cloud-nucleating ability of aerosol particles, Science, 312, 1375–1378, 2006.

Elbert, W., Taylor, P. E., Andreae, M. O., and Pöschl, U.: Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions, Atmos. Chem. Phys., 7, 4569–4588, 2007, http://www.atmos-chem-phys.net/7/4569/2007/.

Elster, J., Delmas, R. J., Petit, J.-R., and eháková, K.: Composition of microbial communities in aerosol, snow and ice samples from remote glaciated areas (Antarctica, Alps, Andes), Biogeosciences Discuss., 4, 1779–1813, 2007, http://www.biogeosciences-discuss.net/4/1779/2007/.

Fehrenbach, T.: Analyse von Aminosäuren, Proteinen und Nitroderivaten in atmosphärischen Aerosolen und Straßenstaub, Ph.D., Technische Universität, München, 2006.

Fierer, N., Jackson, J. A., Vilgalys, R., and Jackson, R. B.: Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays, Appl. Environ. Microb., 71, 4117–4120, 2005.

Franze, T.: Analyse und Reaktivität von Proteinen in atmosphärischen Aerosolen und Entwicklung neuer Immunoassays zur Messung von Nitroproteinen, Ph.D, Technische Universität, München, 2004.

Franze, T., Weller, M. G., Niessner, R., and Pöschl, U.: Protein nitration by polluted air, Environ. Sci. Technol., 39, 1673–1678, 2005.

Fuzzi, S., M. O. Andreae, B. J. Huebert, M. Kulmala, T. C. Bond, M. Boy, S. J. Doherty, A. Guenther, M. Kanakidou, K. Kawamura, V.-M. Kerminen, U. Lohmann, L. M. Russell, and U. Pöschl: Critical assessment of the current state of scientific knowledge, terminology, and research needs concerning the role of organic aerosols in the atmosphere, climate, and global change, Atmos. Chem. Phys., 6, 2017–2038, 2006, http://www.atmos-chem-phys.net/6/2017/2006/.

Gardes, M. and Bruns, T. D.: ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts, Mol. Ecol., 2, 113–118, 1993.

Garrison, V. H., Shinn, E. A., Foreman, W. T., Griffin, D. W., Holmes, C. W., Kellogg, C. A., Majewski, M. S., Richardson, L. L., Ritchie, K. B., and Smith, G. W.: African and Asian dust: from desert soils to coral reefs, Bioscience, 53, 469–480, 2003.

Griffin, D. W.: Terrestrial microorganisms at an altitude of 20 000 m in Earth's atmosphere, Aerobiologia, 20, 135–140, 2004.

Griffin, D.W., Garrison, V.H., Hermann, J.R., and Shinn, E.A.: African desert dust in the Caribbean atmosphere: Microbiology and public health, Aerobiologia, 17, 203–213, 2001.

Griffin, D. W. and Kellogg, C. A.: Dust storms and their impact on ocean and human health: dust in Earth's atmosphere, Ecohealth, 1, 284–295, 2004.

Großkopf, R., Janssen, P. H., and Liesack, W.: Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval, Appl. Environ. Microb., 64, 960–969, 1998.

Hakola, H., Tarvainen, V., Bäck, J., Ranta, H., Bonn, B., Rinne, J., and Kulmala, M.: Seasonal variation of mono- and sesquiterpene emission rates of Scots pine, Biogeosciences, 3, 93–101, 2006, http://www.biogeosciences.net/3/93/2006/.

Hamilton, W. D. and Lenton, T. M.: Spora and Gaia: How microbes fly with their clouds, Ethol. Ecol. Evol., 10, 1–16, 1998.

Hock, B., J. Schneider, S. Borrmann, R. Römpp, G. Moortgat, T. Franze, C. Schauer, U. Pöschl, C. Plass-Dülmer, and H. Berresheim: Rural continental aerosol properties and processes observed during the Hohenpeissenberg Aerosol Characterization Experiment (HAZE2002) Atmos. Chem. Phys. Discuss., 8617–8662, 2007.

Hong, S., Bunge, J., Jeon, S., and Epstein, S.: Predicting microbial species richness, P. Natl. A. Sci., 103, 117–122, 2006.

Höss, M., Jaruga, P., Zastawny, T. H., Dizdaroglu, M., and Pääbo, S.: DNA damage and DNA sequence retrieval from ancient tissues, Nucleic. Acid. Res., 24, 1304–1307, 1996.

Hughes, K. A., McCartney, H. A., Lachlan-Cope, T. A., and Pearce,

D. A.: A preliminary study of airborne microbial biodiversity over Peninsular Antarctica, Cell. Mol. Biol., 50, 537–542, 2004.

Jaenicke, R.: Abundance of cellular material and proteins in the atmosphere, Science, 308, p. 73, 2005.

Jaenicke, R., Sprengard-Eichel, C., Matthias-Maser, S., and Reichert, K.: Measurements of primary biological aerosol and the soluble fraction of aerosol in remote continental area of Jungfraujoch, Switzerland, Eurotrac-2 Aerosol Annual Report, 45–49, 2000.

Kanakidou, M., Seinfeld, J. H., Pandis, S. N., Barnes, I., Dentener, F. J., Facchini, M. C., Van Dingenen, R., Ervens, B., Nenes, A., Nielsen, C. J., Swietlicki, E., Putaud, J. P., Balkanski, Y., Fuzzi, S., Horth, J., Moortgat, G. K., Winterhalter, R., Myhre, C. E. L., Tsigaridis, K., Vignati, E., Stephanou, E. G., and Wilson, J.: Organic aerosol and global climate modelling: a review, Atmos. Chem. Phys., 5, 1053–1123, 2005, http://www.atmos-chem-phys.net/5/1053/2005/.

Kemp, P. F. and Aller, J. Y.: Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us, FEMS Microb. Ecol., 47, 161–177, 2004.

Klamer, M. and Hedlund, K.: Fungal diversity in set-aide agricultural soil investigated using terminal-restriction fragment length polymorphism, Soil Biol. Biochem., 36, 983–988, 2004.

Kloster, S., Feichter, J., Maier-Reimer, E., Six, K. D., Stier, P., and Wetzel, P.: DMS cycle in the marine ocean-atmosphere system – a global model study, Biogeosciences, 3, 29–51, 2006, http://www.biogeosciences.net/3/29/2006/.

Kuske, C. R., Barns, S. M., Grow, C. C., Merrill, L., Dunbar, M. S., and Dunbar, J.: Environmental survey for four pathogenic bacteria and closely related species using phylogenetic and functional genes, J. Forensic. Sci., 51, 548–558, 2006.

Lindahl, T.: Instability and decay of the primary structure of DNA, Nature, 362, 709–715, 1993.

Little, D. P. and Barrington, D. S.: Major evolutionary events in the origin and diversification of the fern genus *Polystichum* (Dryopteridaceae), Am. J. Bot., 90, 508–514, 2003.

Liu, W. T., Marsh, T. L., Cheng, H., and Forney, L. J.: Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rDNA, Appl. Environ. Microb., 63, 4516–4522, 1997.

Lohmann, U. and Feichter, J.: Global indirect aerosol effects: A review, Atmos. Chem. Phys., 5, 715–737, 2005, http://www.atmos-chem-phys.net/5/715/2005/.

Lüdemann, H., Arth, I., and Liesack, W.: Spatial changes in the bacterial community structure along a vertical oxygen gradient in flooded paddy soil cores, Appl. Environ. Microb., 66, 754–762, 2000.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, B., A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüßmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., and Schleifer, K.: ARB: a software environment for sequence data, Nucleic. Acid. Res., 32, 1363–1371, 2004.

Lueders, T. and Friedrich, M.: Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and *mcrA* genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts, Appl. Environ. Microb., 69, 320–326, 2003.

Lukow, T., Dunfield, P. F., and Liesack, W.: Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants, FEMS Microb. Ecol., 32, 241–247, 2000.

Maron, P., Lejon, D. P. H., Carcalho, E., Bizet, K., Lemanceau, P., Ranjard, L., and Mougel, C.: Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library, Atmos. Env., 39, 3687–3695, 2005.

Matthias-Maser, S. and Jaenicke, R.: Examination of Atmospheric Bioaerosol Particles with Radii $>0.2\mu$m, J. Aerosol Sci., 25, 1605–1613, 1994.

McFiggans, G., Artaxo, P., Baltensperger, U., Coe, H., Faccini, M. C., Feingold, G., Fuzzi, S., Gysel, M., Laaksonen, A., Lohmann, U., Mentel, T. F., Murphy, D. M., O'Dowd, C. D., Snider, J. R., and Weingartner, E.: The effect of physical and chemical aerosol properties on warm cloud tropics, Atmos. Chem. Phys., 6, 2593–2649, 2006, http://www.atmos-chem-phys.net/6/2593/2006/.

Mikhailov, E., Vlasenko, S., Niessner, R., and Pöschl, U.: Interaction of aerosol particles composed of protein and salts with water vapor: hygroscopic growth and microstructural rearrangement, Atmos. Chem. Phys., 4, 323–350, 2004, http://www.atmos-chem-phys.net/4/323/2004/.

Möhler, O., DeMott, P. J., Vali, G., and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, Biogeosciences Discuss., 4, 2559–2591, 2007, http://www.biogeosciences-discuss.net/4/2559/2007/.

Noll, M., Matthles, D., Frenzel, P., Derakshan, M. and Liesack, W.: Succession of bacterial community structure and diversity in a paddy soil oxygen gradient, Environ. Microbiol., 7, 392–395, 2004.

Nowoisky, J.: Klonierung und heterologe Expression einer Xanthophyll-Deepoxidase aus der Kieselalge *Phaedactylum triconutum*, Diploma, Johannes-Gutenberg Universität, Mainz, 2005.

Pääbo, S.: Ancient DNA extraction, characterization, molecular cloning, and enzymatic amplification, P. Natl. A. Sci., 86, 1939–1943, 1989.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., and Hofreiter, M.: Genetic analysis from ancient DNA, Annu. Rev. Genet., 38, 645–679, 2004.

Pöschl, U.: Atmospheric aerosols: composition, transformation, climate and health effects, Angew. Chem. Int. Edit., 44, 7520–7540, 2005.

Pruppacher, H. R. and Klett, J. D.: Microphysics of clouds and precipitation, Kluwer, Dordrecht, 1997.

Radosevich, J. L., Wilson, W. J., Shinn, J. H., DeSantis, T. Z., and Andersen, G. L.: Development of a high-volume aerosol collection system for the identification of air-borne micro-organisms, Lett. Appl. Microbiol., 34, 162–167, 2002.

Schauer, C., Niessner, R., and Pöschl, U.: Analysis of nitrated polycyclic aromatic hydrocarbons by liquid chromatography with fluorescence and mass spectrometry detection: air particulate matter, soot, and reaction product studies, Anal. Bioanal. Chem., 378, 725–736, 2004.

Schnell, R. G. and Vali, G.: Atmospheric ice nuclei from decom-

posing vegetation, Nature, 236, 163–165, 1972.
Shaffer, B.T. and Lighthart, B.: Survey of culturable airborne bacteria at four diverse locations in Oregon: urban, rural, forest, and coastal, Microb. Ecol., 34, 167–177, 1997.
Simoneit, B. R. T. and Mazurek, M. A.: Organic-matter of the troposphere. 2. Natural background of biogenic lipid matter in aerosols over the rural western United-States, Atmos. Environ., 16, 2139–2159, 1982.
Smith, C. I., Chamberlain, A. T., Riley, M. S., Cooper, A., Stringer, C. B. and Collins, M. J.: Neanderthal DNA: not just old but old and cold?, Nature, 410, 771–772, 2001.
Sun, J. and Ariya, P. A.: Atmospheric organic and bio-aerosols as cloud condensation nuclei (CCN): A review, Atmos. Chem. Phys., 40, 795–820, 2006, http://www.atmos-chem-phys.net/40/795/2006/.
Taylor, P. E. and Jonsson, H.: Thunderstorm asthma, Curr. Allergy. Asthm. R., 4, 409–413, 2004.
Vilgalys, R. and Hester, M.: Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species, J. Bacteriol., 172, 4238–4246, 1990.
Voet, D. and Voet, J. G.: Biochemistry, John Wiley and Sons INC., New York, 1995.
von Wintzingerode, F., Göbel, U.B., Stackbrandt, E.: Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, FEMS Microbiol. Rev., 21, 213–229, 1997
Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J.: 16S ribosomal DNA amplification for phylogenetic study, J. Bacteriol., 173, 697–703, 1991.
Whiting, M. F., Carpenter, J. C., Wheeler, Q. D., and Wheeler, W. C.: The Stresiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology, Syst. Biol., 46, 1–68, 1997.
Wu, Z., Tsumura, Y., Blomquist, G., and Wang, X.: 18S rRNA gene variation among common airborne fungi, and development of specific oligonucleotide probes for the detection of fungal spores, Appl. Environ. Microbiol., 69, 5389–5397, 2003.
Zhang, S., Hou, S., Ma, X., Qin, D., and Chen, T.: Culturable bacteria in Himalayan glacial ice in response to atmospheric circulation, Biogeosciences, 4, 1–9, 2007, http://www.biogeosciences.net/4/1/2007/.

C2) High diversity of fungi in air particulate matter

Janine Fröhlich-Nowoisky[*†], Viviane R. Després[*‡§], and Ulrich Pöschl[*§]

[*]Biogeochemistry Department, Max Planck Institute for Chemistry, Joh.-Joachim-Becher-Weg 27, 55128 Mainz, Germany

[†]Institute of Geosciences, Johannes Gutenberg University, Joh.-Joachim-Becher-Weg 21, 55128 Mainz, Germany

[‡]Institute of General Botany, Johannes Gutenberg University, Johannes-von-Müller-Weg 6, 55128 Mainz, Germany

[§]To whom correspondence should be addressed. Email: despres@mpch-mainz.mpg.de (V.R.D.), poeschl@mpch-mainz.mpg.de (U.P.)

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J.F.N. conducted the experiments. All authors contributed collectively to designing the experiments, interpreting the data and writing the paper.

Abstract

Fungal spores account for large proportions of air particulate matter, and they influence the hydrological cycle and climate as nuclei for water droplets and ice crystals in clouds, fog, and precipitation. Moreover, some fungi are major pathogens and allergens. The diversity of airborne fungi is, however, hardly known. By DNA analysis we found pronounced differences in the relative abundance and seasonal cycles of various groups of fungi in coarse and fine particulate matter, with more plant pathogens in the coarse and more human pathogens and allergens in the respirable fine particle fraction (<3 µm). Moreover, the ratio of *Basidiomycota* to *Ascomycota* was found to be much higher than previously assumed, which might also apply to the biosphere.

Recent studies have shown that fungal spores and other biogenic aerosol particles account for a significant proportion of air particulate matter in pristine rainforest air as well as in rural and urban environments (1-5). Fungi have also been found in clouds, fog, and precipitation, where they act as nuclei for water droplets and ice crystals and can influence precipitation patterns and the Earth's energy budget [e.g., (6-13)]. On average, the number and mass concentrations of fungal spores in continental boundary layer air are on the order of 10^3-10^4 m^{-3} and ~1 µg m^{-3}, respectively, and the estimated global emissions of ~50 Tg yr^{-1} are among the largest sources of organic aerosol (1).

Some fungi are major pathogens or allergens for humans, animals, and plants, and air is the primary medium for their dispersal (14-16), but the diversity of fungi in air particulate matter is hardly known. The traditional cultivation, microscopy, and chemical tracer techniques applied in earlier studies were insufficient for a broad coverage of different fungal species, and recently reported first applications of molecular genetic techniques were very limited in scope and methodology as discussed below.

In this study, we have investigated and characterized the diversity and frequency of occurrence of fungi in air particulate matter by DNA extraction and sequence analysis of the internal transcribed spacer region (ITS).

Results and Discussion

Over a period of one year coarse and fine particle air filter samples were collected on glass fiber filters with a high-volume sampler and a nominal cut-off diameter of ~3 µm and average sampling times of ~7 days in central Europe. Fungal DNA was found in all air samples. As shown in Table 1, the number of different species (species richness, *S*) detected in the fine particle fraction was not much lower than in the coarse fraction. The Shannon index (*H'*), Shannon evenness (*E*), and Simpson's index (*D*) values calculated from the frequency of occurrence of the different species, i.e., from the number of samples in which each species had been detected, were nearly the same for coarse and fine particles (Table 1). These diversity parameter values are similar to the values commonly obtained for fungi in soil and on plants as well as for bacteria in soil (17-19).

The most frequent species (*Cladosporium sp.*) was detected in all air samples except one. On the other hand, 70% of the detected species were found only in one sample [supplementary information (SI) Table S3]. The high proportion of species that were found only once and the limited number of investigated samples and DNA amplification products imply that the actual diversity of fungi in the sampled air masses was higher than detected. As shown in Table 1, estimates of the actual species richness (*S**) based on the Chao-1 estimator approach (17, 20) are by a factor of ~3 higher than the measured species richness values (*S*), suggesting that the total number of fungal species in the investigated air samples was >1000.

As illustrated in Fig. 1a, nearly all detected fungal species were *Basidiomycota* (BMC, club fungi, 64%) or *Ascomycota* (AMC, sac fungi, 34%), many of which actively eject their spores with aqueous jets or droplets (1). Only ~2% of the measured sequences were from *Fungi Incertae Sedis*

(FIS, formerly called *Zygomycota*) or other fungi that could not be attributed to a phylum. This is consistent with the predominance of *Asco*- and *Basidiomycota* in the biosphere, where the subkingdom of *Dikarya* (AMC plus BMC) accounts for 98% of the known species in the biological kingdom of *Eumycota* (fungi) (21). The AMC species found in the air samples were distributed over four major classes (*Dothideomycetes, Eurotiomycetes, Leotiomycetes, Sordariomycetes*; Fig. 1b) and about one third could not be attributed to any class. In contrast, most of the detected BMC species belonged to a single class, the *Agaricomycetes* (Fig. 1c). More information about the taxonomic classes and families of the detected fungi is available online (*SI Methods*) (22).

Fig. 2 shows seasonal variations in the normalized species richness (S_n: number of detected species divided by number of investigated samples), and in the ratio of S_n between coarse and fine particles ($S_{n,c}/S_{n,f}$). With regard to all fungal species, both S_n and $S_{n,c}/S_{n,f}$ were nearly constant throughout the year (relative deviations <30%). AMC and BMC, however, exhibited pronouncedly different values and seasonal cycles of S_n and $S_{n,c}/S_{n,f}$. For AMC, S_n was highest in spring and lowest in summer and $S_{n,c}/S_{n,f}$ was lowest in fall, whereas for BMC both S_n and $S_{n,c}/S_{n,f}$ were highest in fall and lowest in winter. Throughout all seasons, S_n remained higher for BMC than for AMC (Fig. 2a) and $S_{n,c}/S_{n,f}$ remained >1 for BMC but <1 for AMC (Fig. 2b). In other words, most BMC occurred in the coarse particle fraction during summer/fall, whereas most AMC were found in the fine particle fraction during winter/spring. This seems to reflect a predominance of BMC among the mushrooms that form fruiting bodies in summer and fall, and a high proportion of AMC among the molds, endophytes, and epiphytes spreading in winter and spring (23, 24).

Several allergens and pathogens were frequently found in both fine and coarse particle samples (e.g., *Cladosporium sp., Alternaria spp., Penicillium spp., Aspergillus spp.*). Some plant pathogens, however, were found only or mostly in coarse particle samples (e.g., *Blumeria graminis, Puccinia spp.*), whereas some human pathogens were detected only in the fine particle samples

(e.g., *Candida tropicalis, Wallemia spp.*). Overall, the species richness and cumulative frequency of occurrence of plant pathogens was found to be higher in the coarse particle fraction, whereas those of human pathogens and allergens were similar or higher in the fine particle fraction. More information about the differences between coarse and fine particle samples and about fungal spore size is available online (*SI Methods* and Table S3).

Fig. 3 illustrates that the relative frequency of occurrence (*RFO*) of individual species, i.e., the proportion of samples in which these species were detected, is independent of the seasonal variability of AMC and BMC species richness. For example, the prominent allergenic AMC species *Cladosporium sp.* and *Alternaria spp*. occurred most frequently in summer and fall, whereas the mold *Penicillium spp.* and the plant pathogen *Blumeria graminis* (mildew) were mostly found in winter and spring. With regard to BMC, the seasonal cycle of S_n was not matched by the *RFO* of any frequently detected species. The fall maximum of BMC species richness is primarily due to species that were found only once and can be attributed to mushroom fruiting and enhanced activity of plant decomposers during that season (23).

Earlier studies of airborne fungi have mostly not evaluated the diversity of AMC vs. BMC, or they have reported a predominance of AMC [e.g., (25, 26)]. Most of these studies were based on cultivation techniques and have detected much fewer species (typically <<100). Lugauskas et al. (27) reported a high number of different fungal species (~430), but for this they had analyzed over 900 aerosol samples collected at multiple locations over several years, corresponding to a normalized species richness of only ~0.5 as opposed to ~10 from our DNA-based investigations. Over 80% of all known fungi are not cultivable on synthetic media, and fast growing fungi can mask slower growing fungi (28). BMC are often out-competed by the more rapidly growing mold fungi (29), and some AMC like *Cladosporium* can actively inhibit the growth of others by production of inhibitory substances (30). Thus, cultivation-based studies of airborne fungi are likely biased against the detection of BMC (especially by the high abundance of *Cladosporium*).

To our knowledge, only three studies have reported DNA analyses of fungi in atmospheric aerosol samples up to now. Boreson et al. (31) found 18 species in urban air (40 % AMC, 6% BMC) and 31 species in desert air (65% AMC, 17% BMC), Despres et al. (32) found only 3 AMC and 1 BMC species in several dozens of filter samples of urban, rural, and high-alpine air, and Fierer et al. (33) measured over 1500 fungal DNA sequences from 5 urban air samples and attributed 97% to AMC but did not specify the species richness. We have tested and applied multiple polymerase chain reaction (PCR) primer pairs; these and other experimental details were identified as key elements for efficient amplification of DNA from AMC, BMC and other fungi (*SI Methods*). Consequently, we have been able to detect a wide range of species that are well-known from cultivation-based studies (e.g., *Cladosporium spp., Penicillium spp., Alternaria spp.*, *Candida spp.*) as well as non cultivable species (e.g., *Blumeria graminis, Puccinia spp.*) and previously unknown species as indicated by 136 operational taxonomic units (OTUs) with low NCBI similarity scores (<97%). Some of these might also stem from degraded DNA (32), but at least the 21 OTUs that were found in multiple samples are much more likely to represent unknown species (*SI Discussion*). The other DNA-based studies of airborne fungi have neither found the expected high abundance of *Cladosporium* nor a high species richness of BMC, which we suppose to be due to limitations of the applied methods.

Ongoing investigations of aerosol samples from several locations around the world suggest that the species richness of fungi in the atmosphere is generally higher for BMC than for AMC. In contrast, the number and proportion of all fungal species known from the biosphere are higher for AMC (~60000, ~60-70%) than for BMC (25000-30000, ~30-40%) (21, 34). Recently, however, Hunt et al. (35) compared culture- and DNA-based analyses of fungi in grassland soil samples and found that 54% of the fungal sequences were from BMC whereas no BMC species could be isolated by plate culturing. Thus, the high proportion of BMC in air as observed in our study (64%) may be explained by one or both of the following hypotheses:

(1) BMC may be enriched in the atmosphere relative to the biosphere, because their proportion at the atmosphere-biosphere interface (i.e., on vegetation and soil surfaces) or their efficiency in dispersing spores may be enhanced relative to AMC (dry release vs. active wet discharge with aqueous jets or droplets) (1).

(2) The ratio of BMC to AMC in the biosphere may have been underestimated in earlier studies for the reasons outlined above (bias of culture-based techniques, primer selection in DNA-based techniques). In this case, our atmospheric measurement results might reflect the biospheric proportions.

Clearly, our results will need to be complemented by further investigations to confirm or discard the above hypotheses. In any case, however, our data show that the diversity of airborne fungi - and in particular the species richness of BMC - is much higher than indicated by earlier studies.

As many fungal species in the biosphere are still unknown, the detection and characterization of fungi in atmospheric aerosol samples by DNA analysis can help to elucidate the regional and global spread and diversity of fungi. Moreover, DNA analyses also enables efficient and unambiguous identification of other primary biogenic aerosol particles and components (bacteria, animal, and plant fragments, etc.)(32).

Information about the diversity and abundance of airborne fungi and other bioaerosol particles is relevant for many areas of research such as biogeosciences, climate and ecology, human and veterinary medicine, industrial and environmental hygiene, agriculture, bioengineering and security. Two of the key perspectives for future investigations of air particulate matter by DNA analysis are the spread of genetically modified organisms (36) and climate-related changes in the diversity and abundance of fungi and other organisms on local, regional, and global scales (37).

Materials and Methods

We investigated the diversity of fungi in atmospheric aerosol samples by DNA extraction and sequence analysis of the internal transcribed spacer region (ITS). The air filter samples were collected with a dichotomous high-volume sampler over a period of one year in central Europe (Mainz, Germany; March 2006 – April 2007): 42 pairs of fine and coarse particle samples with a nominal cut-off diameter of ~3 µm and average sampling times of ~7 days. A comprehensive description of the investigated samples and applied methods is given in *SI Methods*.

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References

1. Elbert W, Taylor PE, Andreae MO, Pöschl U (2007) Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. *Atmos Chem Phys* 7: 4569-4588.
2. Bauer H*, et al.* (2008) Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol. *Atmos Environ*, 42: 588-593, 2008.
3. Womiloju TO, Miller JD, Mayer PM, Brook JR (2003) Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmos Environ* 37: 4335-4344.
4. Held A*, et al.* (2008) Aerosol size distributions measured in urban, rural and high-alpine air with an electrical low pressure impactor (ELPI). *Atmos Environ* 42: 8502-8512.

5. Hock N, *et al.* (2008) Rural continental aerosol properties and processes observed during the Hohenpeissenberg Aerosol Characterization Experiment (HAZE2002). *Atmos Chem Phys* 8: 603-623.

6. Christner BC, *et al.* (2008) Ubiquity of biological ice nucleators in snowfall. *Science* 319: 1214-1214.

7. Bauer H, *et al.* (2002) The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. *Atmos Res* 64: 109-119.

8. Pouleur S, Richard C, Martin JG, Antoun H (1992) Ice nucleation activity in Fusarium acuminatum and Fusarium avenaceum. *Appl Environ Microbiol* 58: 2960–2964.

9. Andreae MO, Crutzen PJ (1997) Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry. *Science* 276: 1052-1058.

10. Jaenicke R (2005) Abundance of cellular material and proteins in the atmosphere. *Science* 308: 73-73.

11. Pöschl U (2005) Atmospheric Aerosols: Composition, Transformation, Climate and Health Effects. *Angew Chem Int Ed* 44: 7520-7540.

12. Andreae MO, Rosenfeld D (2008) Aerosol-cloud-precipitation interactions. Part 1. The nature and sources of cloud-active aerosols. *Earth Sci Rev* 89: 13-41.

13. Rosenfeld D, *et al.* (2008) Flood or drought: How do aerosols affect precipitation? *Science* 321: 1309-1313.

14. Kurup VP, Shen H-D, Banerjee B (2000) Respiratory fungal allergy. *Microbes Infect.* 2: 1101-1110.

15. Adhikari A, Sen MM, Gupta-Bhattacharya S, Chanda S (2004) Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. *Sci Total Environ* 326: 123-141.

16. Brown JKM, Hovmøller MS (2002) Epidemiology - Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297: 537-541.

17. Hill TCJ, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol Ecol* 43: 1-11.

18. Richard F, Moreau PA, Selosse MA, Gardes M (2004) Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by Quercus ilex L. *Can J Bot* 82: 1711-1729.

19. Maria GL, Sridhar KR (2002) Richness and diversity of filamentous fungi on woody litter of mangroves along the west coast of India. *Curr Sci* 83: 1573-1580.

20. Chao A (1984) Nonparametric-Estimation of the Number of Classes in a Population. *Scand J Stat* 11: 265-270.

21. James TY*, et al.* (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443: 818-822.

22. Hibbett DS*, et al.* (2007) A higher-level phylogenetic classification of the Fungi. *Mycol Res* 111: 509-547.

23. Levetin E (1990) Studies on airborne basidiospores. *Aerobiologia* 6: 177-180.

24. Guo LD, Huang GR, Wang Y (2008) Seasonal and Tissue Age Influences on Endophytic Fungi of Pinus tabulaeformis (Pinaceae) in the Dongling Mountains, Beijing. *J Integr Plant Biol* 50: 997-1003.

25. Lee T*, et al.* (2006) Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes. *Atmos Environ* 40: 2902-2910.

26. Adhikari A*, et al.* (2006) Correlation of ambient inhalable bioaerosols with particulate matter and ozone: A two-year study. *Environ Pollut* 140: 16-28.

27. Lugauskas A, Sveistyte L, Ulevicius V (2003) Concentration and species diversity of airborne fungi near busy streets in Lithuanian urban areas. *Ann Agric Environ Med* 10: 233-239.

28. Bridge P, Spooner B (2001) Soil fungi: diversity and detection. *Plant Soil* 232: 147-154.

29. Thorn RG (1999) Soil fungi. In*: Handbook of Soil Science,* eds Summer ME (CRC Press Boca Raton)*,* pp C22-C37.

30. Scott PM, Vanwalbe.W,Maclean WM (1971) Cladosporin, a New Antifungal Metabolite from Cladosporium-Cladosporioides. *J. Antibiot.* 24: 747-755.

31. Boreson J, Dillner AM, Peccia J (2004) Correlating bioaerosol load with PM2.5 and PM10cf concentrations: a comparison between natural desert and urban-fringe aerosols. *Atmos Environ* 38: 6029-6041.

32. Després VR*, et al.* (2007) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. *Biogeosciences* 4: 1127-1141.

33. Fierer N*, et al.* (2008) Short-term temporal variability in airborne bacterial and fungal populations. *Appl Environ Microbiol* 74: 200-207.

34. de Meeus T, Renaud F (2002) Parasites within the new phylogeny of eukaryotes. *Trends Parasitol* 18: 247-251.

35. Hunt J, Boddy L, Randerson PF, Rogers HJ (2004) An evaluation of 18S rDNA approaches for the study of fungal diversity in grassland soils. *Microbial Ecol* 47: 385-395.

36. Wang C, St Leger RJ (2007) A scorpion neurotoxin increases the potency of a fungal insecticide. *Nat Biotech* 25: 1455-1456.

37. Klironomos JN*, et al.* (1997) Increased levels of airborne fungal spores in response to *Populus tremuloides* grown under elevated atmospheric CO2. *Can J Bot* 75: 1670-1673.

Table 1. Diversity parameters for all fungi, *Basidiomycota* (BMC), *Ascomycota* (AMC), and *Fungi Incertae Sedis* (FIS) found in the investigated air samples (total, coarse and fine PM). Species richness (*S* measured, *S** estimated), Shannon index (*H'*), Shannon Evenness (*E*), and Simpson's index (*D*).

	Fungi			**BMC**			**AMC**			**FIS**		
	total	coarse	fine	total	coarse	fine	total	coarse	fine	total	coarse	fine
S	368	227	203	238	160	124	124	62	78	4	3	1
S*	1136	713	647	658	448	377	500	327	267	9	5	-
H´	5.2	4.8	4.7	4.9	4.5	4.2	4.1	3.3	3.7	1.3	1	-
E	0.88	0.88	0.88	0.88	0.89	0.87	0.84	0.80	0.85	0.96	0.94	-
D	0.01	0.015	0.018	0.015	0.017	0.027	0.033	0.066	0.054	0.1	0.167	-

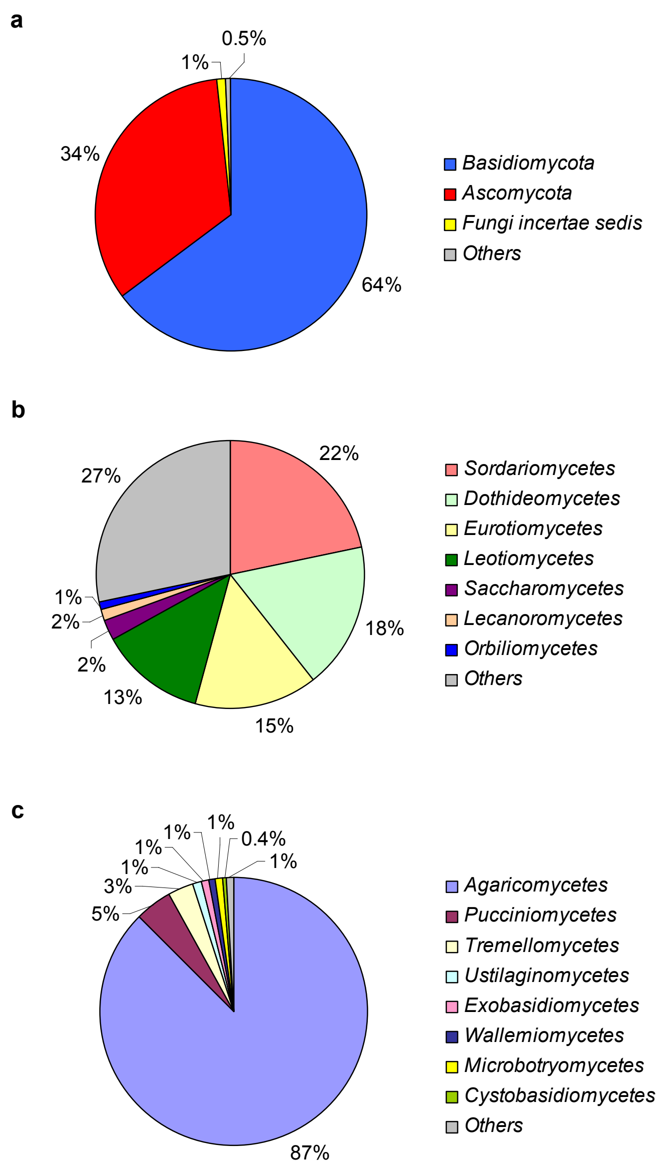

Fig. 1. Species richness of airborne fungi: relative proportions of (**a**) different phyla, (**b**) different classes of *Ascomycota*, and (**c**) different classes of *Basidiomycota*.

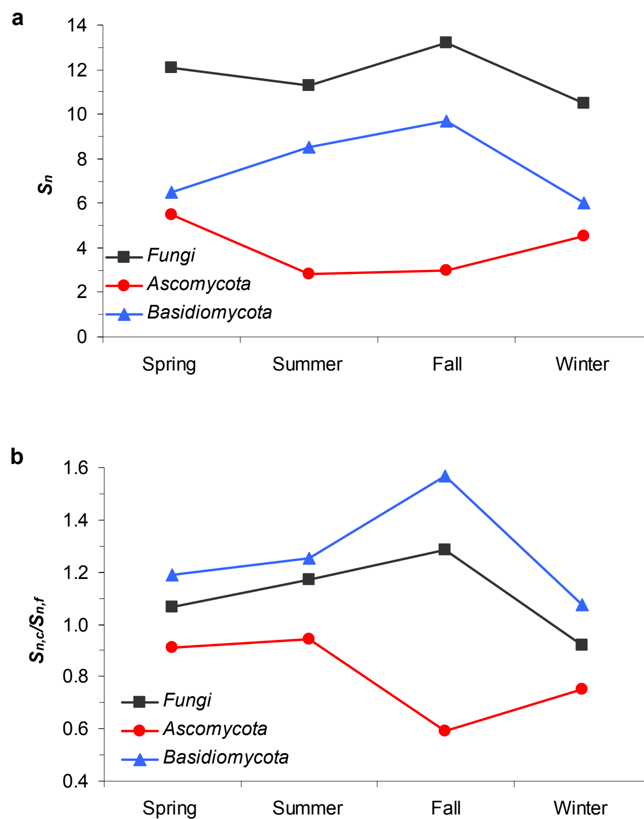

Fig. 2. Seasonal variations in (**a**) the species richness normalized by the number of investigated air samples (S_n) and (**b**) the ratio of normalized species richness between coarse and fine particles ($S_{n,c}/S_{n,f}$).

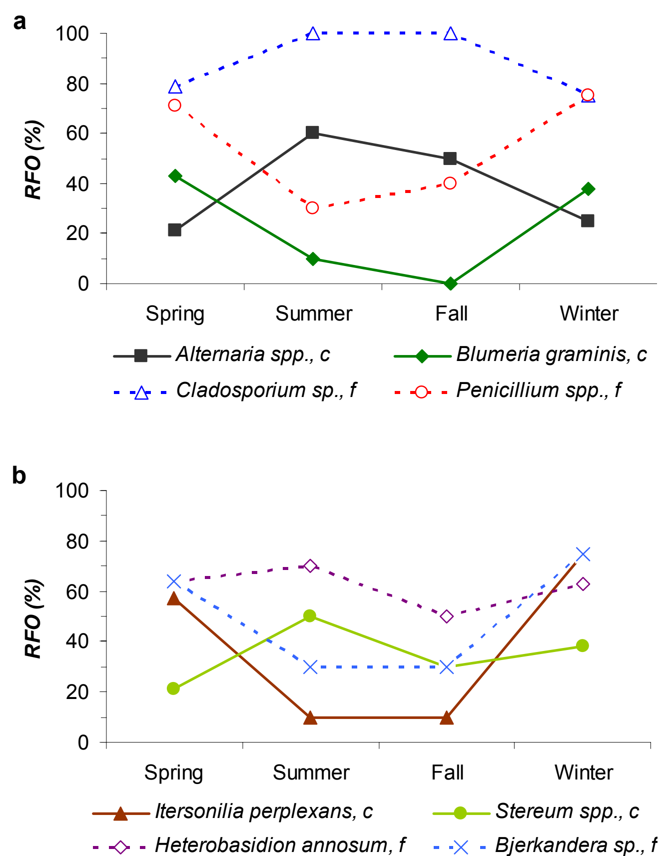

Fig. 3. Seasonal variations in the relative frequency of occurrence (*RFO*) of selected species of *Ascomycota* (**a**) and *Basidiomycota* (**b**) in coarse and fine air particulate matter (c/f).

Supplementary Information for the Manuscript “High Diversity of Fungi in Air Particulate Matter”

To complement the information given in the main manuscript, the following sections provide Supplementary Methods, Discussion and References, Supplementary Tables S1-S8, and Supplementary Figure S1.

Supplementary Methods and Discussion

Aerosol sampling. Aerosol samples were collected on glass fiber filters (Pall Corporation, Type A/A, 102 mm diameter) over one year in Mainz, Germany (130 m a.s.l., March 2006 - April 2007). The sampling station was positioned on a mast at the top of the Max Planck Institute for Chemistry (MPIC, about 5 m above the flat roof of the 3-story building) on the campus of the University of Mainz (49°59'31.36''N 8°14'15.22''E). The air masses sampled at MPIC represent a mix of urban and rural continental boundary layer air in central Europe. A high-volume dichotomous sampler [self-built based on Solomon et al., (1)] was used to separate and collect coarse and fine aerosol particles on a pair of glass fiber filters. The sampler was operated with a rotary vane pump (Becker VT 4.25) at a total flow rate of ~300 L min^{-1}, corresponding to a nominal cut-off diameter of ~3 μm. Coarse particles with aerodynamic diameters larger than the cut-off were collected through a virtual impactor operated in line with the inlet (~30 L min^{-1}), and fine particles with aerodynamic diameters smaller than the cut-off were collected from the main gas flow perpendicular to the inlet (~270 L min^{-1}).

The sampling period was generally ~7 days, corresponding to a sampled air volume of ~3000 m^3. A few samples were collected over shorter periods (1-5 days, ~400-2000 m^3). A listing of all investigated pairs of air filter samples (42 coarse and 42 fine particle samples) is given in Table S1. Prior to use, all glass fiber filters were decontaminated by baking at 500°C over night (2). Loaded filters were packed in aluminum foil (also prebaked at 500°C), and stored in a freezer at -80°C until DNA extraction. To detect possible contaminations from the sampler and sample handling, blank samples were taken at regular intervals (~4 weeks). The filters were mounted in the sampler like for regular sampling, but the pump was turned on either not at all ("mounting blanks") or for only 5 s ("start-up blank").

DNA extraction and amplification. Filter sample aliquots (⅛ - ¼) were extracted with a commercial soil extraction kit (LysingMatrixE, Fast DNA Spin Kit for Soil, MP Biomedicals) according to the supplier's instructions with the following modifications: 15-min-centrifugation step after the lysis, partly additional 900 µl buffer and repeated beating and centrifugation. Both generated supernatants were combined for the further extraction process. Finally, the DNA was dissolved in 100 µl elution buffer. Decontaminated filters were included during the extractions as extraction blanks.

With the DNA extract from each of the filters listed in Table S1, several PCRs were performed to amplify DNA from fungi for sequence analysis. The 50 µl reaction mixture always contained the template DNA (0.5-2 µl sample extract), 1×PCR buffer, 0.2 mM each dNTP (Roth), 0.33 µM of each primer (Sigma-Aldrich), and 2.5 units of JumpStart™ REDTaq DNA polymerase (Sigma-Aldrich). A negative control was included in all PCR runs.

PCR reactions were performed with the primer pairs listed in Table S2 and Fig. S1, respectively. The thermal profile (DNA Engine, Bio-Rad Laboratories) was as follows: initial denaturing at 94°C for 3 min; 35 cycles with denaturing at 94°C for 30 s, annealing at primer pair specific temperature for 30 s (Table S2), elongation at 72°C for 90 s, and a final extension step at 72°C for 5 min.

Fungal DNA was detected in all of the 84 investigated air filter samples, and 200 PCR products were obtained with different primer pairs as specified in Tables S1 and S2. No DNA was detected in the mounting, start-up, extraction, and PCR blanks, indicating that no contaminations occurred during sample handling and analysis in the laboratory.

PCR products were obtained with all primer combinations except for the *Zygomycota*-specific primer pair D (Table S1 and S2, Fig. S1). The primer pairs A and F amplified *Basidiomycota* (BMC) as well as *Ascomycota* (AMC) and the primer pairs that were designed to be specific for BMC (B) and AMC (C) indeed amplified only BMC and AMC, respectively. The sequences obtained with the primer pair E which should be specific for *Chytridiomycota*

matched with members of the *Pucciniomycetes* (within the BMC) and *Fungi incertae sedis* (formerly *Zygomycota*).

Fungal DNA was also co-amplified with primer pairs (G-I) that were supposed to be specific for animal, insect, and archaea 18S or 16SrDNA (Table S1 and S2, Fig. S1). Table S1 gives information on obtained sequences resulting from co-amplification reactions in coarse particle filter extracts. The primer pairs G and H amplified BMC whereas the primer pair I amplified AMC. This primer behavior calls for reevaluation of the primer performance and requires attention before the primer pair is further used in samples that harbor material from different groups of organism. The obtained fungal 18S sequences confirm knowledge from other studies that the ITS region is a better target region and 18S rDNA analysis often is not able to discriminate between species in environmental samples (3).

Cloning and restriction fragment length polymorphism. Amplification products for sequencing were cloned using the TOPO TA Cloning® Kit (Invitrogen) following the supplier's instructions. Colonies containing inserts were identified by blue-white selection and lysed in 20 µl water for 10 min at 95°C. The inserts of 12-24 colonies were amplified ("colony PCRs") using 3 µl lysate in a 40 µl reaction. The PCR reaction mixture always contained: 1×PCR Buffer, 2.5 mM $MgCl_2$, 0.25 mM each dNTP (Roth), 0.25 µM of each primer (Sigma-Aldrich), 1.25 units *Taq* DNA Polymerase (Invitrogen). PCR reactions were performed with the primer pair M13F-40 and M13R, and the thermal profile was as follows: initial denaturing at 94°C for 5 min; 40 cycles with 94°C for 30 s, annealing at 55°C for 1 min, elongation at 72°C for 1 min, and a final extension step at 72°C for 15 min.

The colony PCR was followed by a restriction fragment length polymorphism (RFLP) analysis to select as many as possible different clones for sequencing. 2 µl of the PCR-products were digested without further purification with 5 units of the enzymes *TaqI*, *HinfI*, or *AluI* (Fermentas). Restriction fragments were separated by gel electrophoresis in a 3% agarose

gel stained with ethidium bromide and the images were documented with the Gel Doc XR system and analyzed with Quantity One software (Bio-Rad Laboratories). On the basis of the resulting restriction fragment patterns, representative colony PCR products with different numbers and sizes of fragments were selected for sequencing.

DNA sequence analysis, taxonomic attribution, and statistical parameters. DNA sequences were determined with ABI Prism 377, 3100, and 3730 sequencers (Applied Biosystems) using BigDye-terminator v3.1 chemistry at the DNA Core Facility of the Max Planck Institute for Plant Breeding Research, Cologne. For comparison with known sequences, databank queries using the Basic Local Alignment Search Tool (BLAST) were performed via the website of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Each sequence was identified to the lowest taxonomic rank common to the top BLAST hits (up to ~100 data base sequences with highest similarity and total scores). For 17 of the 1513 sequences determined in this study, the ITS1 and ITS2 regions matched in different genera. These 17 sequences were assumed to be chimeric results of PCR recombination and were excluded from further analysis.

For each aerosol filter sample, sequences that produced the same BLAST results were pairwise aligned using the program BioEdit (BioEdit 7.05; http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The similarity between them was calculated using the PAM250 Matrix. Sequences with similarity scores ≥97% were clustered into an operational taxonomic unit (OTU). The different OTUs can be regarded as different fungal species, because the sequence variability due to intraspecific ITS divergence is usually <3% (4). PCR errors can also introduce some sequence variability (5). The ~100 PCR cycles typically applied in our study (amplification, colony, and sequencing PCRs) are, however, unlikely to cause substitutions of more than 1% of the base pairs in the measured sequences, because the total error rates of *Taq* polymerase are typically in the range of 10^{-5}-10^{-4} errors per base pair (6, 7) When sequences

obtained by co-amplification with non-fungal primer pairs (18S; G, H, I; Table S2) yielded the same best match results as ITS sequences from the same sample, they were attributed to the same OTU, because they are likely to have the same origin (see Fig. S1).

Table S3 gives an overview of the 368 detected OTUs with corresponding characteristic NCBI sequence accession numbers, taxonomic class and species names, and frequencies of occurrence, i.e., numbers of air samples and coarse or fine particle filters, respectively, in which they were detected. Note that some OTUs exhibited high similarity (98-100%) with multiple NCBI sequences attributed to different orders, families, genera or species, which may be due to data base entries with incorrect/inconsistent taxonomic information or high interspecific ITS similarity (e.g., BMC2: *Bjerkandera/Thanatephorus*; BMC3: *Trametes/Tricholoma/Phellinus sp.*). About one third of the OTUs exhibited less than 97% similarity with NCBI sequences (<90% for 37 OTUs, 90-96% for 99 OTUs) and may represent species that are unknown or at least not listed in the NCBI data base. Alternatively, the measured DNA may have been partially degraded in the air or upon sampling and analysis (8-11). This is, however, not very likely for fungal spores that are known and designed to resist environmental stress and survive atmospheric transport (2). Moreover, 21 OTUs with low best match similarity scores were found in more than one sample, which appears unlikely for damaged DNA. Thus, we assume that most if not all OTUs represent different fungal species, and that about 30% of these have not yet been characterized by DNA analysis and listing in the NCBI data base.

The detection and apparent frequency of occurrence of different species can also be affected by technical factors like extraction efficiency of DNA, varying rDNA copy number in the species, primer matching and performance, amplification efficiency of the target region, and cloning success. As discussed in the main manuscript, the high proportion of fungal species that were detected only once indicates, that a higher number of samples and clones would have to be investigated for a complete coverage of species diversity.

Thus, the detected and reported numbers and frequencies of occurrence of species, families, and classes should be regarded as a lower limit for the actual diversity and frequency of occurrence of airborne fungi.

To characterize and compare the diversity of fungal species (OTUs) in the investigated air masses, we have calculated the parameters defined in Table S4 for the whole data set and for different subsets (phyla, seasons, etc.). The results are summarized in Tables S5-S7, key findings are presented in the main manuscript, and additional information is given in the following sections.

Taxonomic classes and families. According to the best match sequences and taxonomy used in the NCBI database, the 368 OTUs or fungal species detected in this study have been attributed to 3 phyla, 15 classes, and 61 families. The species richness and proportions of the different phyla and classes are described and discussed in the main manuscript. Additional information about the taxonomic classes and families is given below.

Basidiomycotic classes and families. Out of the 15 accepted classes of BMC (12), 8 classes were found in the investigated aerosol samples (Tables S3 and S5). 208 of the 238 detected BMC species can be attributed to the most species rich class of BMC, the *Agaricomycetes*. The proportion of *Agaricomycetes* among the different species of BMC found in the atmospheric samples (~87%) was even higher than in the biosphere, where *Agaricomycetes* (about 16000-20000 species) account for ~50% of the known BMC species (13).They act as decomposer (decompose lignin and cellulose), pathogens, parasites, and symbionts of both plants (trees, shrubs, grasses) and animals (e.g., insects) (14-16). Some species of *Agaricomycetes* are edible whereas others produce secondary metabolites that make them hallucinogenic or toxic (13). A diverse array of fruiting bodies like gilled mushrooms, chanterelles, stinkhorns, false truffles, puffballs, corticoid fungi and coral fungi are produced from members of this class (17). *Agaricomycetes* were found in all coarse particles samples (134 species) and in all fine particles samples (112 species), i.e., the *RFO* of

this class was 100% throughout all seasons (Table S5). 38 species of the *Agaricomycetes* occurred in both coarse and fine particle samples.

Compared to the *Agaricomycetes*, all other classes of BMC exhibited much less species richness and lower *RFO* values (Table S5). Two classes were found only in coarse or only in fine particle samples. *Pucciniomycetes* were detected only in the coarse particle samples (*RFO* 14%). In contrast to the *Agaricomycetes*, the proportion of *Pucciniomycetes* among the different species of BMC found in the atmospheric samples (~5 %) was substantially lower than in the biosphere, where *Pucciniomycetes* account for ~30% of the known BMC species (18). They are parasites of animals and fungi as well as obligate pathogens of ferns, conifers, flowering plants, cereal grains, legumes, and trees such as apple, where they can cause extensive reduction in yield and even host death (18-20). Most *Pucciniomycetes* (~7000 species or ~90%) belong to a single order, the *Pucciniales* or plant parasitic rust fungi (18), which was indeed the only type of *Pucciniomycetes* found in the investigated air samples. *Wallemiomycetes* could be detected only in fine particle samples (*RFO* 7 %). This class includes only three species of xerophilic molds from the genus *Wallemia*, two of which we found (*W. sebi, W. muriae*). They are mutualists of plants, green algae, bryophytes and cyanobacteria, and they can act as decomposers and pathogens of plants, animals, and other fungi (21-23).

Out of the 38 detected BMC families, 33 belonged to the class of *Agaricomycetes*. As shown in Table S6, the most species rich BMC family was *Corticiaceae* with 33 different species (mostly wood decaying fungi). Species from this and 19 other families occurred in coarse as well as in fine particle samples (*Hymenochaetaceae, Lachnocladiaceae*, *Tricholomataceae,* etc.). 16 families were found only in coarse particle samples (*Bolbitiaceae, Pleurotaceae, Suillaceae*, etc.), and two families were found only in fine particle samples (*Lycoperdaceae, Meruliaceae*). With regard to families, the taxonomic attribution according to the NCBI database applied in this study is often not in agreement with the Mycobank taxonomy. For example, only two of the 33 species attributed to the *Corticiaceae* according to NCBI would be

attributed to this family according to Mycobank. In view of the taxonomic uncertainties, we have not calculated *RFO* values for the families.

Ascomycotic classes and families. Out of the 15 accepted classes of AMC (12), 7 classes were found in the investigated aerosol samples (Tables S3 and S5). The most species rich class of AMC were the *Sordariomycetes* (27 of 124 detected AMC species), which include plant pathogens and endophytes, animal pathogens, and mycoparasites (24). Sordariomycetes were found in 19 fine particle samples (21 species) and in 7 coarse particle samples (8 species), corresponding to *RFO* values of 45% and 17%, respectively (Table S5). The AMC class with the highest *RFO* (98%) were the *Dothideomycetes* (22 species). This class contains several allergenic fungi and plant pathogens, endophytes or epiphytes of living plants, and also saprobes degrading cellulose and other complex carbohydrates in dead or partially digested plant matter, in leaf litter, or dung (25).

Two more AMC classes with high species richness and frequency of occurrence were the *Leotiomycetes* (16 species, *RFO* 78%), which are the largest non-lichen-forming group of fungi (26), and the *Eurotiomycetes* (18 species, *RFO* 74%), which include well-known molds like *Penicillum spp.*. No taxonomic class attribution was available for 35 (partly mitosporic) AMC.

Out of the 23 detected AMC families, 13 were found in both coarse and fine particle samples (e.g., *Davidiellaceae, Sclerotiniaceae, Trichocomaceae*), six were found only in fine particle samples (e.g., *Orbiliaceae, Saccharomycetaceae*) and four were found only in coarse particle samples (e.g., E*rysiphaceae*, Table S6). The most species rich AMC family were the *Trichocomaceae* (16 species), which include the molds *Aspergillus* and *Penicillium.*

Coarse vs. fine particle samples and fungal spore size. Fungal DNA was found in both the coarse and the fine aerosol particle samples that had been collected with a nominal cut-off diameter of ~3 µm as detailed above. Particle size is one of the main parameters that govern the effects of aerosols on climate and human health. Fine particles have longer residence times in the atmosphere (multiple days to weeks) and upon inhalation they can reach the alveolar region of human lungs, whereas coarse particles are rapidly removed from the atmosphere (sedimentation, scavenging, and precipitation) and are deposited in the upper airways when inhaled. Thus, the scientific investigation and public discussion of climate and health effects are mostly focused on fine aerosol particles (27). Overall, the species richness and relative frequency of occurrence of fungal species in coarse and fine particle samples were of similar magnitude. 165 fungi were found only in coarse particle samples, and 141 were found only in fine particle samples; 62 species were detected in both types of samples (Tables S3 and S7).

As discussed by Després et al. (2), fungal DNA detected in aerosol filter samples is most likely to originate from spores which are known to resist environmental stress and survive atmospheric transport (28, 29), whereas DNA in fungal tissue fragments may be rapidly degraded by atmospheric photooxidants. Therefore, the following discussion will be focused on interpreting the observed differences and similarities between coarse and fine particles samples with regard to the size of fungal spores. Nevertheless, it should be kept in mind that not only fungal spores but also hyphae and tissue fragments can be present in coarse and fine air particulate matter (30-32). Moreover, the disruption of fungal spores due to environmental stress may lead to the formation and sampling of smaller fragments, and the association of spores with each other or with other particulate matter may lead to the formation and sampling of larger particles. For example, it is likely that spores, unicellular fungi (like endophytic fungi) and plant pathogens (rust fungi, powdery mildew) are associated with plant fragments (33-35).

Fungal spores are usually in the size range of 1-50 μm (36) and have been found in coarse as well as in fine aerosol particle samples (37-40). The aerodynamic behavior of a particle depends on its size, density, shape, and structure, and the aerodynamic diameter is defined as the diameter of an equivalent spherical particle with a density of 10^3 kg m^{-3} that has the same aerodynamic behavior as the investigated particle. The shape of fungal spores, however, is often non-spherical and their density can be lower than the reference density. Therefore, the aerodynamic diameter of fungal spores is often smaller than the size of their geometric envelope. Exemplary properties and aerodynamic diameters of spores from different fungal species are given in Table S8.

The aerodynamic diameter of spores, however, does not only depend on the fungal species from which they are emitted but also on their biological age and on ambient conditions. For example, the diameter of *Penicillium* spores typically increases with age (2.3-2.6 μm) (41), freshly released spores are usually larger than spores that have been airborne for a longer time (42), and the size of spores can increase with the ambient relative humidity (43, 44). The effects outlined above can lead to the deposition of DNA from fungi with spore diameters >3 μm in the investigated fine particle samples as well as to the deposition of DNA from fungi with spore diameters <3 μm in the coarse particle samples.

Besides the uncertainties and variability related to the type and size of aerosol particles carrying fungal DNA, the distinction between coarse and fine particles is also affected by imperfections of the aerosol sampling techniques. The nominal cut-off diameter of the applied high-volume sampler cannot be regarded as a sharp limit; it is just the midpoint of the collection efficiency curve of the virtual impactor which extends over several micrometers (1). Thus, particles that are a couple of micrometers smaller or larger than the nominal cut-off diameter can be deposited on both the coarse and the fine particle filters. Moreover, the operating principles of the applied high-volume sampler imply that ~10% of the fine particulate matter in the sampled air mass

are deposited on the coarse particle sample. As a consequence, fungi which occur with high frequency in the fine particle fraction are likely to be detected also in the coarse particle samples and the following points should be taken as a guideline for the interpretation of the measurement results reported above:

(1) Zero or very low *RFO* values in the fine particle fraction indicate that the detected fungal species had been present in the sampled air masses as particles (spores) with aerodynamic diameters larger than ~3 µm. Examples from Table S7 are *Blumeria graminis* and *Itersonilia perplexans*. Moreover, members of the order *Pucciniales* (rust fungi) are known to have large spores (up to >20 µm) and were found only in coarse particle samples (20).

(2) High *RFO* values in the fine and in the coarse particle fraction or high/medium *RFO* values in the fine and lower *RFO* values in the coarse particle fraction indicate that the detected fungal species had been present in the sampled air masses as particles (spores) with aerodynamic diameters of ~3 µm or less. Examples from Table S7 are *Cladosporium sp., Penicillium spp.* and *Heterobasidion annosum*.

(3) Medium *RFO* values of similar magnitude in the fine and in the coarse particle fraction indicate that the detected fungal species had been present in the sampled air masses as particles (spores) with aerodynamic diameters around ~3 µm (or as larger and smaller particles with similar abundance). Examples from Table S7 are *Eurotium sp*. and *Stereum spp.*

The species richness and the relative proportion of BMC detected in this study was higher in coarse than in fine particle samples, whereas the species richness and the relative proportion of AMC was higher in fine than on coarse particle samples. This is consistent with the following basic features of BMC and AMC (12, 17, 45, 46): Smut spores and other BMC spores are known to have large (aerodynamic) diameters (Table S8), and BMC produce most mushrooms with large fruiting bodies that may fragment and release large tissue fragments. In contrast, AMC are mostly single-celled (yeasts) or filamentous (hyphal), and some of the most prominent AMC species like

Cladosporium sp. and *Penicillium spp.* have spores with small aerodynamic diameters (Table S8).

References

1. Solomon PA, Moyers JL, Fletcher RA (1983) High-Volume Dichotomous Virtual Impactor for the Fractionation and Collection of Particles According to Aerodynamic Size. *Aerosol Sci Tech* 2: 455-464.
2. Després VR*, et al.* (2007) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. *Biogeosciences* 4: 1127-1141.
3. Reddy SR, Pindi PK, Reddy SM (2005) Molecular methods for research on arbuscular mycorrhizal fungi in India: problems and prospects. *Curr Sci* 89: 1699-1709.
4. O'Brien HE*, et al.* (2005) Fungal community analysis by large-scale sequencing of environmental samples. *Appl Environ Microbiol* 71: 5544-5550.
5. v. Wintzingerode F, Göbel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* 21: 213-229.
6. Keohavong P, Thilly WG (1989) Fidelity of DNA-Polymerases in DNA Amplification. *P Natl Acad Sci US A* 86: 9253-9257.
7. Ling LL, Keohavong P, Dias C, Thilly WG (1991) Optimization of the polymerase chain reaction with regard to fidelity: modified T7, Taq, and vent DNA polymerases. *PCR Meth Appl* 1: 63-69.
8. Höss M*, et al.* (1996) DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acid Res* 24: 1304-1307.
9. Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature* 362: 709-715.
10. Pääbo S (1989) Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *P Natl Acad Sci USA* 86: 1939-1943.
11. Sinha RP, Hader DP (2002) UV-induced DNA damage and repair: a review. *Photochem Photobiol Sci* 1: 225-236.
12. Hibbett DS*, et al.* (2007) A higher-level phylogenetic classification of the Fungi. *Mycol Res* 111: 509-547.
13. Hibbett DS (2006) A phylogenetic overview of the Agaricomycotina. *Mycologia* 98: 917-925.

14. Aanen DK*, et al.* (2002) The Evolution of Fungus-Growing Termites and Their Mutualistic Fungal Symbionts. *P Natl Acad Sci USA* 99: 14887-14892.

15. Read D (1997) Mycorrhizal fungi - The ties that bind. *Nature* 388: 517-518.

16. Smith SE, Read DJ (1997) Mycorrhizal Symbiosis, (Academic Press, London).

17. Hibbett DS (2007) After the gold rush, or before the flood? Evolutionary morphology of mushroom-forming fungi (Agaricomycetes) in the early 21st century. *Mycol Res* 111: 1001-1018.

18. Aime MC*, et al.* (2006) An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia* 98: 896-905.

19. Frieders EM, McLaughlin, David J. a, Szabo LJ (2008) Pucciniomycotina (formerly Urediniomycetes). A diverse group of fungi, including rusts, yeasts, smut-like and jelly-like fungi. http://tolweb.org/Pucciniomycotina/20528/2008.02.21 in The Tree of Life Web Project, http://tolweb.org/.

20. Anikster Y, Eilam T, Bushnell WR, Kosman E (2005) Spore dimensions of Puccinia species of cereal hosts as determined by image analysis. *Mycologia* 97: 474-484.

21. Matheny PB*, et al.* (2007) Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Mol Phylogenet Evol* 43: 430-451.

22. Wood GM*, et al.* (1990) Studies on a Toxic Metabolite from the Mold Wallemia. *Food Addit Contam* 7: 69-77.

23. Zalar P, de Hoog GS, Schroers HJ, Frank, J.M., , Gunde-Cimerman N, . (2005) Taxonomy and phylogeny of the xerophilic genus Wallemia (Wallemiomycetes and Wallemiales, cl. et ord. nov.). *Ant Leeuw Int J G* 87: 311-328.

24. Zhang N, Sung G-H (2008) Sordariomycetes. http://tolweb.org/ Sordario-mycetes/29050/2008.01.14 in The Tree of Life Web Project, http://tolweb.org/.

25. Schoch CL*, et al.* (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041-1052.

26. Wang Z*, et al.* (2006) Toward a phylogenetic classification of the Leotiomycetes based on rDNA data. *Mycologia* 98: 1065-1075.

27. Pöschl U (2005) Atmospheric Aerosols: Composition, Transformation, Climate and Health Effects. *Angew Chem Int Ed* 44: 7520-7540.

28. Griffin DW (2004) Terrestrial microorganisms at an altitude of 20,000 m in Earth's atmosphere. *Aerobiologia* 20: 135-140.

29. Griffin DW, Kellogg CA (2004) Dust storms and their impact on ocean and human health: dust in Earth’s atmosphere. *Ecohealth* 1: 284–295.

30. Gorny RL*, et al.* (2002) Fungal fragments as indoor air biocontaminants. *Appl Environ Microbiol* 68: 3522-3531.

31. Green BJ*, et al.* (2006) Airborne fungal fragments and allergenicity. *Med Mycol* 44: S245-S255.

32. Marshall WA (1997) Seasonality in Antarctic airborne fungal spores. *Appl Environ Microbiol* 63: 2240-2245.

33. Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontier's. *Fungal Biol Rev* 21: 51-66.

34. Eichmann R, Hückelhoven R (2008) Accommodation of powdery mildew fungi in intact plant cells. *J Plant Physiol* 165: 5-18.

35. Hata K, Atari R, Sone K (2002) Isolation of endophytic fungi from leaves of Pasania edulis and their within-leaf distributions. *Mycoscience* 43: 369-373.

36. Gregory PH (1973) *The microbiology of the atmosphere* (Leonard Hill Books, Aylesbury).

37. Bauer H*, et al.* (2002) The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. *Atmos Res* 64: 109-119.

38. Elbert W, Taylor PE, Andreae MO, Pöschl U (2007) Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. *Atmos Chem Phys* 7: 4569-4588.

39. Battarbee JL, Rose NL, Long XZ (1997) A continuous, high resolution record of urban airborne particulates suitable for retrospective microscopical analysis. *Atmos Environ* 31: 171-181.

40. Womiloju TO, Miller JD, Mayer PM, Brook JR (2003) Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmos Environ* 37: 4335-4344.

41. Kanaani H, Hargreaves M, Ristovski Z, Morawska L (2007) Performance assessment of UVAPS: Influence of fungal spore age and air exposure. *J Aerosol Sci* 38: 83-96.

42. Reponen T*, et al.* (1994) Comparison of Concentrations and Size Distributions of Fungal Spores in Buildings with and without Mold Problems. *J Aerosol Sci* 25: 1595-1603.

43. Reponen T*, et al.* (1996) Effect of relative humidity on the aerodynamic diameter and respiratory deposition of fungal spores. *Atmos Environ* 30: 3967-3974.

44. Liao CM*, et al.* (2004) Temporal/seasonal variations of size-dependent airborne fungi indoor/outdoor relationships for a wind-induced naturally ventilated airspace. *Atmos Environ* 38: 4415-4419.

45. Swann E, Hibbett DS (2007) Basidiomycota. The Club Fungi. http://tolweb.org/Basidiomycota/20520/2007.04.20 in The Tree of Life Web Project, http://tolweb.org/.

46. Taylor JW, Spatafora J, Berbee M, (2006) Ascomycota. Sac Fungi. http://tolweb.org/Ascomycota/20521/2006.10.09 in The Tree of Life Web Project, http://tolweb.org/.

47. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. in *PCR Protocols: A Guide to Methods and Applications,* eds *Innis M A*, *Gelfand D H*, *Sninsky J J*, *White T J* (Academic*,* San Diego), pp 315–322.

48. Nikolcheva LG, Bärlocher F (2004) Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycol Prog* 3: 41-49.

49. Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl Environ Microbiol* 71: 4117-4120.

50. Whiting MF*, et al.* (1997) The Stresiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst Biol* 46: 1-68.

51. Heraty J, D. Hawks, J. S. Kostecki, Carmichael A (2004) Phylogeny and behaviour of the Gollumiellinae, a new subfamily of the ant-parasitic Eucharitidae (Hymenoptera: Chalcidoidea). *Syst Entomol* 29: 544-559.

52. Grosskopf R, Janssen PH, Liesack W (1998) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl Environ Microbiol* 64: 960-969.

53. Hill, TCJ, Walsh, KA, Harris, JA, Moffett, BF (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbio. Ecol* 43, 1-11.

54. Chao, A. (1984) Nonparametric-Estimation of the Number of Classes in a Population. *Scand J Stat* 11, 265-270 (1984).

55. Adhikari A*, et al.* (2006) Correlation of ambient inhalable bioaerosols with particulate matter and ozone: A two-year study. *Environ Pollut* 140: 16-28.

56. Zalar P*, et al.* (2007) Phylogeny and ecology of the ubiquitous saprobe Cladosporium sphaerospermum, with descriptions of seven new species from hypersaline environments. *Stud Mycol* 58: 157-183.

57. Grinshpun SA, *et al.* (2005) Collection of airborne spores by circular single-stage impactors with small jet-to-plate distance. *J Aerosol Sci* 36: 575-591.

58. Helbling A, Brander KA, Horner WE, Lehrer SB (2002) in *Fungal Allergy and Pathogenicity*, eds. Breitenbach M, Crameri R & Lehrer S B (Karger, Basel), Vol. 81, pp. 28-47.

59. Ingold CT (1971) *Fungal Spores. Their Liberation and Dispersal* (Clarendon Press, Oxford).

60. Sowell G, Korf RP (1960) An Emendation of the Genus Itersonilia Based on Studies of Morphology and Pathogenicity. *Mycologia* 52: 934-945.

61. Teodoro NG, Gomez ET (1926) Coffee diseases and their control. *Philippine Agric Rev* 19: 249-257.

Table S1. Overview of air samples (aerosol filter pairs). Sample ID (running number); sampling period and time; sampled air volume; number of DNA sequences obtained with different primer pairs (A-I, Supplementary Table S2) and all together from the coarse and fine particle filter samples, respectively (coarse/fine); number of different species (OTU) of all fungi and of *Ascomycota* (AMC), *Basidiomycota* (BMC), and *Fungi Incertae Sedis* or others (FIS) detected in the whole air sample and in the coarse or fine particle filters, respectively (total/coarse/fine).

Sample ID	Sampling Period	Sampling time (days)	Sampled air volume (m^3)	Sequences									Species			
				A	B	C	E	F	G	H	I	All	Fungi	AMC	BMC	FIS
MZ 1	24.03.06 -31.03.06	7.02	3019	10/11	-/12	-/12		8/-	3/-			21/35	29/11/20	7/3/5	22/8/15	
MZ 2	31.03.06- 07.04.06	6.95	3057	12/11	-/11	-/9	2/-					14/31	23/8/18	9/4/7	14/4/11	
MZ 4	07.04.06- 12.04.06	4.97	2202	12/11	-/10	-/4						12/25	18/8/12	13/6/8	5/2/4	
MZ 9	20.04.06- 27.04.06	7.00	3026	37/12	-/11				11/-			48/23	41/34/9	16/14/2	25/20/7	
MZ 10	27.04.06- 02.05.06	4.92	2077	11/11	-/11	-/11						11/33	22/10/15	11/5/7	11/5/8	
MZ 11	02.05.06-03.05.06	1.01	444	18/12	-/12				4/-		1/-	23/24	24/16/11	12/6/7	12/10/4	
MZ 15	04.05.06-09.05.06	4.91	2064		6/6	4/7						10/13	12/7/6	6/3/3	6/4/3	
MZ 18	12.05.06-15.05.06	3.02	1321		6/11	7/6						13/17	16/11/8	8/6/4	8/5/4	
MZ 25	22.05.06-23.05.06	0.97	425	10/-	7/9	12/11			3/-			32/20	23/17/10	10/7/6	13/10/4	
MZ 26	23.05.06-30.05.06	6.98	3100	10/-	12/8	4/12			2/-			28/20	31/15/17	12/4/9	18/10/8	1/1/-
MZ 31	01.06.06-06.06.06	4.95	2199		8/9	3/6						11/15	17/10/11	7/3/5	10/7/6	
MZ 35	14.06.06-21.06.06	6.97	2999	31/-	7/8	-/4						38/12	15/12/6	5/5/1	10/7/5	
MZ 36	21.06.06-22.06.06	0.97	422	11/-	12/7	-/3	3/-		1/-			27/10	25/20/8	4/4/1	21/16/7	
MZ 40	27.06.06-04.07.06	6.96	3045	8/-	12/8	3/4			1/-			24/12	18/13/7	5/4/2	13/9/5	
MZ 41	04.07.06-11.07.06	6.96	3019	6/-	8/8	5/5			4/-			23/13	20/13/9	6/5/2	14/8/7	

MZ 45	19.07.06-21.07.06	1.98	872		6/9	4/7					10/16	17/8/11	5/4/3	12/4/8	
MZ 47	26.07.06-02.08.06	6.97	3025	8/-	12/12	-/3					20/15	19/10/12	5/4/2	14/6/10	
MZ 50	02.08.06-09.08.06	6.94	3025	7/-	12/6	4/4		3/-			26/10	24/18/10	7/5/4	17/13/6	
MZ 51	09.08.06-16.08.06	7.01	3060	12/-	8/8	-/5		4/-			24/13	26/15/12	8/4/5	18/11/7	
MZ 52	16.08.06-23.08.06	6.95	3081	8/27	8/-	-/4				2/-	18/31	22/11/12	7/3/5	15/8/7	
MZ 54	30.08.06-06.09.06	7.00	3091	16/-	-/8	-/5					16/13	22/12/12	9/5/5	13/7/7	
MZ 59	11.09.06-18.09.06	6.86	2977	13/7	12/12	-/3	3/-			1/-	29/22	27/18/11	8/5/4	19/13/7	
MZ 60	18.09.06-25.09.06	7.02	3033		9/9	4/3					13/12	14/10/6	3/2/2	11/8/4	
MZ 62	02.10.06-09.10.06	6.99	3026	12/-	12/9	-/4	2/-	5/-			31/13	26/18/9	5/2/4	20/15/5	1/1/-
MZ 63	09.10.06-16.10.06	6.98	3079	11/12	10/-		6/-				27/12	24/20/5	4/3/2	16/13/3	4/4/-
MZ 66	16.10.06-23.10.06	6.96	2995	9/10	11/5	-/7					20/22	19/12/12	7/3/6	12/9/6	
MZ 67	23.10.06-30.10.06	6.97	3028	6/-	12/8	5/4					23/12	25/15/11	7/5/3	18/10/8	
MZ 69	02.11.06-09.11.06	7.00	3034	12/11							12/11	16/8/9	6/5/2	10/3/7	
MZ 71	16.11.06-23.11.06	6.98	3063	11/11							11/11	12/6/8	6/3/4	6/3/4	
MZ 74	23.11.06-30.11.06	6.95	2808		10/11	8/4					18/15	25/14/13	7/5/3	17/9/9	1/-/1
MZ 75	30.11.06-07.12.06	6.95	2992	12/10	-/9	-/3			4/-		16/22	22/10/13	4/1/4	18/9/9	
MZ 77	14.12.06-21.12.06	6.98	3011	-/12	9/-	2/-					11/12	14/7/8	5/2/3	9/5/5	
MZ 81	28.12.06-04.01.07	7.03	3005	17/10	12/-				2/-		31/10	16/10/6	2/0/2	14/10/4	
MZ 82	04.01.07-11.01.07	6.99	3056		6/9	6/7					12/16	23/10/14	9/4/5	14/6/9	
MZ 84	18.01.07-25.01.07	7.01	3121		8/10	6/6					14/16	23/10/15	14/6/9	9/4/6	
MZ 88	01.02.07-08.02.07	6.99	3056		8/10	7/2					15/12	16/12/7	8/6/2	8/6/5	
MZ 90	15.02.07-22.02.07	7.02	2998		8/11	6/6					14/17	22/10/13	15/6/10	7/4/3	
MZ 93	22.02.07-01.03.07	6.93	3023		3/4	6/5					9/9	12/7/8	8/4/5	4/3/3	
MZ 95	08.03.07-15.03.07	7.00	3025		6/6	6/5					12/11	16/10/8	11/6/5	5/4/3	
MZ 97	22.03.07-29.03.07	6.97	3025		8/4	9/5					17/9	21/16/8	11/9/4	10/7/4	
MZ 101	05.04.07-12.04.07	7.01	3061	-/1	7/8	4/4					11/13	14/9/9	8/4/6	6/5/3	
MZ 103	19.04.07-26.04.07	7.05	3078		7/5	7/5					14/10	12/8/8	5/3/4	7/5/4	

Table S2. PCR primer combinations (A-I). Forward and reverse primer names, amplified region/gene, approx. length of the PCR product (number of base pairs, bp), annealing temperature, and references.

Primer pair	Forward	Reverse	Amplified region	Product (bp)	Temperature (°C)	References
A	ITS5	ITS4	ITS1-5.8S-ITS2	500 - 700	54	(47)
B	ITS5	ITS4B	ITS1-5.8S-ITS2	~900	58	(47, 48)
C	ITS5	ITS4A	ITS1-5.8S-ITS2	~600	55	(47, 48)
D	ITS5	ITS4Z	ITS1-5.8S-ITS2	~700	45	(47, 48)
E	ITS5	ITS4C	ITS1-5.8S-ITS2	~1000	53	(47, 48)
F	ITS1	5.8S	ITS1-5.8S	~300	55	(47, 49)
G	18Sai	18Sbi	18S	1140	55	(50)
H	18S-H17F	18S-H35R	18S	900	58	(51)
I	109f	934r	16S	800	52	(52)

Table S3. Operational taxonomic units (OTUs) attributed to Ascomycota (A) and Basidiomycota (B). Operational ID (AMC/BMC and running number); frequency of occurrence (number of air samples and coarse/fine particle filters in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence			NCBI accession numbers and similarity scores	Class, species
	total	coarse	fine		
AMC1	41	35	37	EU167591 (99-100%), AF455517 (99-100%), AF393712 (99-100%), AF393707 (99-100%)	*Dothideomycetes, Cladosporium sp.*
AMC2	24	20	8	EF207415 (98-100%), AF455526 (98-100%), EU563125 (99%), EF153017 (98%)	*Leotiomycetes, Botryotinia fuckeliana* *
AMC3	18	18	0	EU552134 (100%), AJ279463 (100%)	*Dothideomycetes, Epicoccum sp.* *
AMC4	13	7	7	AF455536 (100%), AF455528 (100%)	*Eurotiomycetes, Eurotium sp.*
AMC5	12	11	1	EF432293 (99%), AY154712 (99%)	*Dothideomycetes, Alternaria sp.*
AMC6	12	0	12	FJ008997 (99%)	*Eurotiomycetes, Penicillium expansum*
AMC7	11	9	3	AF520642 (99%)	*mitosporic AMC, Ascochyta sp.*
AMC8	11	3	10	DQ426522 (100%), AY373898 (100%), AM158223 (100%)	*Eurotiomycetes, Penicillium sp.*
AMC9	10	10	0	AB273542 (99%), AB273566 (99%)	*Leotiomycetes, Blumeria graminis*
AMC10	9	8	2	AY154692 (99%), EF432282 (99%), AY154695 (98%)	*Dothideomycetes, Alternaria sp.*
AMC11	6	5	4	EU998929 (95%), EF601602 (95%)	
AMC12	6	5	1	EU167608 (99%), AM160630 (99%)	*Dothideomycetes, Aureobasidium sp.* *
AMC13	6	2	4	AY805550 (100%),	*Leotiomycetes, Cistella acuum*
AMC14	5	1	4	AM901991 (99%), AF501262 (99%), AF435827 (98%), AF501259 (98%), EU167591 (97%)	
AMC15	5	0	5	EF652038 (99%), EF652039 (98%)	*Eurotiomycetes, Aspergillus sp.*
AMC16	4	1	3	AY373862 (95%), AF540513 (95%)	
AMC17	4	0	4	AY373851 (100%),	*Eurotiomycetes, Aspergillus fumigatus*
AMC18	4	0	4	EU288196 (99%)	*Saccharomycetes, Candida tropicalis*
AMC19	3	3	0	DQ006952 (95%), AF455427 (94%)	*Sordariomycetes*
AMC20	3	1	2	AY853228 (99%),	*Leotiomycetes, Naevala minutissima*
AMC21	3	0	3	DQ195780 (100%),	*Dothideomycetes, Leptospora rubella*
AMC22	3	0	3	EF434058 (99%), AJ560686 (99%)	*Sordariomycetes, Beauveria bassiana*
AMC23	2	2	0	DQ530453 (96%), U04207 (94%)	
AMC24	2	2	0	AM901702 (99%), DQ778909 (99%), AJ488260 (99%), AF459728 (99%), EF652065 (99%)	
AMC25	2	2	0	AJ293875 (100%), AJ888477 (99%),	
AMC26	2	1	2	AY183369 (96%)	*Sordariomycetes*
AMC27	2	1	1	AM084454 (99%), AF439488 (99%)	*Dothideomycetes, Phaeosphaeria juncophila*

AMC28	2	1	1	DQ006952 (98%)	*Sordariomycetes, Diatrype sp.*
AMC29	2	0	2	EU019285 (99%), AB109183 (99%), AY607019 (99%), AY152593 (99%)	*Dothideomycetes, Ramularia sp.* *
AMC30	2	0	2	AF439504 (96%), AF383951 (91%)	*Dothideomycetes*
AMC31	2	0	2	EU687059 (92%), AY754945 (91%)	*Sordariomycetes*
AMC32	2	0	2	EF026143 (99%)	*Sordariomycetes, Hypoxylon rubiginosum*
AMC33	2	0	2	AM749928 (99%)	*Sordariomycetes, Hypoxylon howeanum*
AMC34	1	1	0	EU292653 (93%), U57089 (93%), DQ182427 (93%)	*Leotiomycetes*
AMC35	1	1	0	AY634167 (98%), AF486133 (97%), DQ249994 (96%)	
AMC36	1	1	0	DQ679486 (91%), DQ182451 (91%), AY864822 (90%)	
AMC37	1	1	0	EU826481 (99%), EF024754 (99%), EU167600 (99%)	
AMC38	1	1	0	DQ683973 (95%)	
AMC39	1	1	0	DQ683973 (94%)	
AMC40	1	1	0	DQ979719 (92%), AF013225 (91%)	
AMC41	1	1	0	EF159443 (97%), EU552134 (92%), AJ279463 (92%)	
AMC42	1	1	0	AY969725 (92%), AF050278 (83%)	
AMC43	1	1	0	EF521252 (99%), EU035412 (85%)	
AMC44	1	1	0	EU754979 (97%), EU883432 (96%), EU883430 (96%)	*mitosporic AMC, Tetracladium sp.*
AMC45	1	1	0	EU687056 (93%), EU326188 (89%), EF577238 (89%)	
AMC46	1	1	0	AJ244263 (92%), AY969659 (91%)	
AMC47	1	1	0	EU529999 (95%), EF207415 (89%)	
AMC48	1	1	0	EU167569 (98%), AY357266 (98%), AF279583 (98%)	
AMC49	1	1	0	AB085929 (98%),	*mitosporic AMC, Thermomyces lanuginosus*
AMC50	1	1	0	EU019284 (97%), AJ301970 (97%), AY154692 (96%), EF432286 (96%)	
AMC51	1	1	0	FJ000399 (98%)	*mitosporic AMC, Tumularia aquatica*
AMC52	1	1	0	AY387657 (94%), EU848215 (88%)	*Dothideomycetes*
AMC53	1	1	0	AY961406 (91%), EU885415 (91%)	*Dothideomycetes*
AMC54	1	1	0	EF420004 (96%), AM262366 (95%), DQ092527 (93%)	*Dothideomycetes*
AMC55	1	1	0	DQ273287 (89%), EU167596 (86%), EU707892 (85%)	*Dothideomycetes*
AMC56	1	1	0	AF383957 (100%)	*Dothideomycetes, Massarina corticola*
AMC57	1	1	0	AM902015 (100%), DQ384571 (99%)	*Dothideomycetes, Leptosphaerulina chartarum*
AMC58	1	1	0	AY739861 (99%)	*Dothideomycetes, Pyrenophora tritici-repentis*
AMC59	1	1	0	AF203450 (99%), EF452450 (99%)	*Dothideomycetes, Stemphylium sp.*
AMC60	1	1	0	EF432300 (100%), AY196988 (100%)	*Dothideomycetes, Phaeosphaeria sp.*
AMC61	1	1	0	AY681194 (99%), AY681193 (99%)	*Sordariomycetes, Neurospora sp.*
AMC62	1	1	0	EU918705 (99%), AY681180 (99%)	*Sordariomycetes, Sordaria sp.*
AMC63	1	1	0	DQ323535 (99%)	*Sordariomycetes, Gnomonia leptostyla*
AMC64	1	1	0	AF000567 (97%),	*Sordariomycetes, Diaporthe sp.*
AMC65	1	1	0	U57673 (93%), AB067714 (92%)	*Sordariomycetes*
AMC66	1	1	0	AY484909 (96%)	*Eurotiomycetes*
AMC67	1	1	0	AF489434 (98%),	*Eurotiomycetes, Penicillium sp.*
AMC68	1	1	0	EU035422 (99%)	*Eurotiomycetes, Exophiala sp.*
AMC69	1	1	0	EF191234 (86%), EF191241 (85%)	*Leotiomycetes*

AMC70	1	1	0	Z73791 (99%), Z73771 (98%)	*Leotiomycetes, Monilinia padi*
AMC71	1	1	0	AB193380 (99%)	*Leotiomycetes, Sawadaea bicornis*
AMC72	1	1	0	EF029230 (96%), AJ430397 (96%)	
AMC73	1	1	0	AY541277 (93%), AJ457152 (89%)	*Lecanoromycetes*
AMC74	1	0	1	AM262380 (95%), AY075113 (93%)	
AMC75	1	0	1	DQ093661 (99%), AM292050 (99%)	
AMC76	1	0	1	EF029228 (93%), EU639650 (92%)	
AMC77	1	0	1	EU041789 (99%)	*mitosporic AMC, Radulidium subulatum*
AMC78	1	0	1	AY961406 (92%), EU040234 (91%), EU040233 (91%)	
AMC79	1	0	1	AY345346 (90%), AJ244276 (89%), DQ402525 (89%)	
AMC80	1	0	1	EU852369 (92%), AJ244276 (90%), DQ402525 (89%)	
AMC81	1	0	1	AY354273 (99%), DQ273341 (97%)	
AMC82	1	0	1	EU543253 (96%), AY773457 (95%)	
AMC83	1	0	1	EU707899 (96%), EU167569 (96%), AJ301970 (96%)	
AMC84	1	0	1	AJ496632 (97%)	*Dothideomycetes, Phaeosphaeria pontiformis*
AMC85	1	0	1	EU040243 (98%), AM176696 (97%)	*Dothideomycetes, Toxicocladosporium irritans*
AMC86	1	0	1	AJ608969 (99%)	*Dothideomycetes, Leptosphaeria sp.*
AMC87	1	0	1	AM262352 (96%), AJ244276 (90%)	*Dothideomycetes*
AMC88	1	0	1	EF060804 (91%)	*Sordariomycetes*
AMC89	1	0	1	EU686961 (89%), EU164404 (86%), EU552101 (85%)	*Sordariomycetes*
AMC90	1	0	1	EF212848 (96%), EU167609 (95%)	*Sordariomycetes*
AMC91	1	0	1	AJ557830 (99%), EU557361 (99%), AY138846 (99%)	*Sordariomycetes, Nectria sp.* *
AMC92	1	0	1	EU686848 (90%), EU552098 (88%), EF423534 (90%)	*Sordariomycetes*
AMC93	1	0	1	AY908999 (98%),	*Sordariomycetes, Rosellinia corticium*
AMC94	1	0	1	AB017661 (95%), AY805591 (94%)	*Sordariomycetes*
AMC95	1	0	1	AY908993 (99%)	*Sordariomycetes, Dicyma pulvinata*
AMC96	1	0	1	EU326205 (93%),	*Sordariomycetes*
AMC97	1	0	1	EF212848 (99%)	*Sordariomycetes, Gnomonia rostellata*
AMC98	1	0	1	EF447416 (98%), EF447401 (98%)	*Sordariomycetes, Valsa sp.* *
AMC99	1	0	1	DQ402530 (94%), AB208110 (93%)	*Sordariomycetes*
AMC100	1	0	1	DQ459870 (99%), DQ459868 (98%)	*Sordariomycetes, Fusarium sp.*
AMC101	1	0	1	AB027382 (99%),	*Sordariomycetes, Beauveria bassiana*
AMC102	1	0	1	AF455478 (100%)	*Sordariomycetes, Arthrinium sp.*
AMC103	1	0	1	AY373910 (100%)	*Eurotiomycetes, Penicillium digitatum*
AMC104	1	0	1	EF634441 (99%)	*Eurotiomycetes, Penicillium brevicompactum*
AMC105	1	0	1	DQ681321 (99%), DQ681325 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC106	1	0	1	AF033491 (98%), AF033490 (98%), EF669708 (98%)	*Eurotiomycetes, Penicillium sp.* *
AMC107	1	0	1	EU833220 (95%), AF125937 (96%)	*Eurotiomycetes*
AMC108	1	0	1	AF033453 (98%), AF033452 (98%)	*Eurotiomycetes, Penicillium sp.*
AMC109	1	0	1	AB263742 (97%), AB086631 (97%)	*Eurotiomycetes, Paecilomyces sp.*
AMC110	1	0	1	EF029810 (92%), AB175237 (86%)	*Eurotiomycetes*
AMC111	1	0	1	AF033387 (99%), AF033386 (99%)	*Eurotiomycetes, Geosmithia*
AMC112	1	0	1	AJ131688 (97%)	*Eurotiomycetes, Chrysosporium lobatum*
AMC113	1	0	1	AY781221 (99%),	*Orbiliomycetes, Dactylaria sp.*
AMC114	1	0	1	EU040232 (91%), EF093148 (91%)	*Leotiomycetes*

AMC115	1	0	1	EU292504 (95%), DQ093752 (95%)	*Leotiomycetes*
AMC116	1	0	1	AY590782 (99%)	*Leotiomycetes, Chalara microchona*
AMC117	1	0	1	EF207423 (97%), EF153017 (97%)	*Leotiomycetes, Monilinia sp.*
AMC118	1	0	1	AY787711 (99%)	*Leotiomycetes, Lachnum sp.*
AMC119	1	0	1	AJ430221 (98%)	*Leotiomycetes, Lachnum virgineum*
AMC120	1	0	1	AJ430398 (98%)	*Leotiomycetes, Pezizella amenti*
AMC121	1	0	1	AY266169 (100%), AY266145 (100%)	*Leotiomycetes, Oculimacula sp.*
AMC122	1	0	1	AF410838 (99%)	*Lecanoromycetes, Parmelia sulcata*
AMC123	1	0	1	AY923248 (95%), AM850055 (94%)	*Saccharomycetes*
AMC124	1	0	1	AM279255 (99%)	*Saccharomycetes, Candida deformans*

[*] Some of the characteristic NCBI accession numbers correspond to different genera.

(**B**)

OTU	Frequency of occurrence			NCBI accsession numbers and similarity scores	Class, species
	total	coarse	fine		
BMC1	30	12	26	AF455496 (99-100%), X70027 (99%), DQ384592 (99%)	*Agaricomycetes, Heterobasidion annosum*
BMC2	29	22	21	AJ006672 (99%), AF455463 (99%), AF455461 (99%), DQ060096 (99%), EF155506 (99%), AY633927 (99%), AF455421 (99%), AF455454 (99%), AJ279471 (99%), AJ279465 (99%)	*Agaricomycetes, Bjerkandera sp.* [*]
BMC3	25	16	19	AY309018 (99%), AF139961 (99%), AB158314 (99%), AF139961 (99%), AY089738 (99%), AF455529 (99%), DQ311085 (99%)	*Agaricomycetes, Trametes sp.* [*]
BMC4	22	14	9	AY787677 (98-99%), AY208796 (98%), AF210821 (97%-98%)	*Agaricomycetes, Peniophora sp.*
BMC5	19	14	9	AY854063 (99%), AM269810 (99%), DQ000294 (99%)	*Agaricomycetes, Stereum sp.*
BMC6	17	16	1	AB072233 (99%)	*Tremellomycetes, Itersonilia perplexans*
BMC7	13	13	0	DQ398959 (97%, 99%)	*Agaricomycetes, Vuilleminia comedens*
BMC8	12	6	7	AY271810 (98%), AY854087 (98%), AF455458 (97%)	*Agaricomycetes, Phlebia sp.* [*]
BMC9	8	7	3	AY015438 (100%), AB030351 (100%), AY070003 (100%)	*Microbotryomycetes, Sporobolomyces roseus*
BMC10	8	3	5	AY840572 (100%), AF516556 (99%)	*Agaricomycetes, Trametes sp.*
BMC11	7	5	4	DQ200924 (99%)	*Agaricomycetes, Botryobasidium subcoronatum*
BMC12	6	3	3	DQ093653 (99%), DQ093737 (99%), AB301610 (99%), AF345820 (99%), DQ899094 (99%), AB286935 (99%)	*Agaricomycetes, Sistotrema sp.* [*]
BMC13	6	3	3	AM084814 (99%), EU918698 (94%), DQ118417 (94%)	*Agaricomycetes*
BMC14	5	4	1	AM160645 (99%),	*Cystobasidiomycetes, Sporobolomyces coprosmae*
BMC15	5	2	3	EU047805 (99%)	*Agaricomycetes, Phanerochaete sordida*
BMC16	5	1	4	EU118668 (98%)	*Agaricomycetes, Steccherinum fimbriatum*
BMC17	4	4	0	AF026586 (99%), AF334912 (98%)	*Agaricomycetes, Peniophora sp.* [*]
BMC18	4	4	0	EU118651 (95%), AF210821 (95%)	*Agaricomycetes*
BMC19	4	3	2	DQ347490 (99%), AY558617 (97%)	*Agaricomycetes, Fuscoporia ferrea*
BMC20	4	3	1	AF347080 (88%), AY641458 (84%), AF347092 (83%)	*Agaricomycetes*
BMC21	4	3	1	AJ437602 (99%)	*Agaricomycetes, Serpula himantioides*
BMC22	4	3	1	AB072232 (99%)	*Tremellomycetes, Udeniomyces pannonicus*

BMC23	4	2	2	DQ340338 (99%)	*Agaricomycetes, Hyphodontia alutacea*
BMC24	4	0	4	DQ384584 (98%)	*Agaricomycetes, Phlebia tremellosa*
BMC25	3	3	0	AY633927 (88%), AY089741 (88%), AF455461(87%), EU118655 (84%)	*Agaricomycetes*
BMC26	3	3	0	DQ868345 (99%), DQ868346 (98%)	*Agaricomycetes, Baeospora sp.*
BMC27	3	2	1	AY271810 (85%), AY219390 (83%), EU118665 (83%)	*Agaricomycetes*
BMC28	3	2	1	DQ097370 (98%), AY354227 (98%)	*Agaricomycetes, Strobilurus sp.* [*]
BMC29	3	2	1	AM076640 (99%), AM712271 (98%)	*Agaricomycetes, Psathyrella sp.*
BMC30	3	2	1	AM269811 (100%), AF533962 (99%)	*Agaricomycetes, Stereum sp.*
BMC31	3	2	1	EU326210 (90%), AF516524 (89%)	*Agaricomycetes*
BMC32	3	2	1	AY781266 (100%)	*Agaricomycetes, Phanerochaete sordida*
BMC33	3	2	1	AY558616 (97%)	*Agaricomycetes, Fuscoporia ferruginosa*
BMC34	3	2	1	AB032638 (99%), AY242817 (98%)	*Tremellomycetes, Dioszegia sp.*
BMC35	3	1	3	AB084621 (98%), AY787676 (98%)	*Agaricomycetes, Phlebia uda*
BMC36	3	1	2	DQ822818 (97%), AF335450 (85%), EU669216 (84%)	*Agaricomycetes*
BMC37	3	1	2	AM902093 (99%), EU877759 (98%), EU436684 (98%)	*Agaricomycetes, Coprinellus sp.* [*]
BMC38	3	1	2	AJ006684 (99%)	*Agaricomycetes, Ceriporiopsis gilvescens*
BMC39	3	1	2	EU118659 (97%), AY805620 (97%)	*Agaricomycetes, Phlebiella sp.*
BMC40	3	0	3	AY302514 (100%), AY302534 (99%)	*Wallemiomycetes, Wallemia muriae*
BMC41	3	0	3	AY969566 (86%), AY641458 (84%), DQ411529 (83%)	*Agaricomycetes*
BMC42	3	0	3	AY854075 (99%), DQ112557(99%)	*Agaricomycetes, Morganella pyriformis*
BMC43	2	2	1	DQ873601 (99%)	*Agaricomycetes, Hyphodontia abieticola*
BMC44	2	2	0	AM901851 (91%), DQ340326 (89%)	*Agaricomycetes*
BMC45	2	2	0	DQ367906 (94%), AY634136 (80%), AF335450 (80%), AY669602 (80%)	*Agaricomycetes*
BMC46	2	2	0	AF518588 (99%), AF334899.1 (98%)	*Agaricomycetes, Resinicium sp.* [*]
BMC47	2	2	0	AB074655 (99%), DQ851582 (99%), DQ459375 (99%), AF026622 (99%)	*Agaricomycetes*
BMC48	2	2	0	DQ444863 (99%), AY787215 (99%)	*Agaricomycetes, Nematoloma sp.* [*]
BMC49	2	2	0	AY293129 (99%), DQ520099 (99%), AY293130 (99%)	*Agaricomycetes, Exidia sp.* [*]
BMC50	2	2	0	DQ520099 (99%),	*Agaricomycetes, Auricularia auricula-judae*
BMC51	2	2	0	EU158830 (98%), AY336782 (98%), AY336765 (98%)	*Agaricomycetes, Antrodia sp.* [*]
BMC52	2	2	0	AM901975 (99%), AY781273 (98%), AM269816 (98%)	*Agaricomycetes, Trichaptum sp.*
BMC53	2	2	0	DQ365617 (99%), AJ408372 (99%)	*Agaricomycetes, Ramaria stricta*
BMC54	2	2	0	AF444418 (99%), AF444417 (99%)	*Tremellomycetes*
BMC55	2	2	0	AF468041 (99%), AF468040 (99%)	*Pucciniomycetes, Puccinia sp.*
BMC56	2	1	2	DQ093750 (94%), DQ873618 (93%), DQ384575 (93%), AB084616 (91%)	*Agaricomycetes*
BMC57	2	1	2	AM902071 (99%), AY781263 (98%), AF210821 (97%)	*Agaricomycetes, Peniophora sp.*
BMC58	2	1	2	AF444402 (99%)	*Tremellomycetes, Udeniomyces pyricola*
BMC59	2	1	1	U85790 (97%), U85794 (97%)	*Agaricomycetes, Fibulorhizoctonia sp.*
BMC60	2	1	1	AJ890441 (83%), EU113196 (82%), AM901850 (81%),	*Agaricomycetes*
BMC61	2	1	1	AF310096 (99%), AF310095 (99%)	*Agaricomycetes, Gloeocystidiellum porosum*
BMC62	2	1	1	AY219361 (99%)	*Agaricomycetes, Ceriporiopsis pannocincta*

BMC63	2	1	1	DQ411529 (99%)	*Agaricomycetes, Trechispora alnicola*
BMC64	2	1	1	AF145571 (99%)	*Agaricomycetes, Schizopora paradoxa*
BMC65	2	1	1	EF068136 (88%), EU826014 (86%)	*Agaricomycetes*
BMC66	2	1	1	AM922292 (82%), AM410637 (82%)	*Microbotryomycetes*
BMC67	2	1	1	DQ157700 (99%)	*Ustilaginomycetes, Ustilago maydis*
BMC68	2	0	2	AF455461 (91%), AY271810 (91%)	*Agaricomycetes*
BMC69	2	0	2	AF518763 (99%), AF533963 (99%)	*Agaricomycetes, Polyporus sp.* *
BMC70	2	0	2	EF174452 (99%), AY265847 (99%)	*Agaricomycetes, Sarcomyxa sp.* *
BMC71	2	0	2	AF087487 (99%)	*Agaricomycetes, Phlebiopsis gigantea*
BMC72	2	0	2	EU326210 (92%), EU661877 (91%)	*Agaricomycetes*
BMC73	2	0	2	AF347089 (98%)	*Agaricomycetes, Trechispora farinacea*
BMC74	2	0	2	EU118665 (99%)	*Agaricomycetes, Scopuloides hydnoides*
BMC75	1	1	0	AF026586 (97%), DQ915464 (97%)	*Agaricomycetes, Peniophora sp.* *
BMC76	1	1	0	AM922290 (87%), AY841862 (85%), AF444322 (85%), EU218894 (84%),	
BMC77	1	1	0	AM084814 (99%), EU918698 (94%), DQ118417 (93%)	*Agaricomycetes*
BMC78	1	1	0	AY633927 (90%), EU118654 (89%), EU118653 (89%)	*Agaricomycetes*
BMC79	1	1	0	AM901974 (91%), DQ398959 (91%), AF139961 (84%)	*Agaricomycetes*
BMC80	1	1	0	DQ367906 (90%), AY174809 (79%), DQ112621 (84%)	*Agaricomycetes*
BMC81	1	1	0	EU003034 (84%), DQ411529 (83%)	*Agaricomycetes*
BMC82	1	1	0	AY969526 (94%), AY641458 (84%), AF347080 (84%)	*Agaricomycetes*
BMC83	1	1	0	AY969397 (96%), U66450 (94%), EU669216 (86%)	*Agaricomycetes*
BMC84	1	1	0	AY288099 87%), AF440673 (86%), AJ606042 (85%)	*Agaricomycetes*
BMC85	1	1	0	DQ278949 (95%), EU622841 (87%), DQ672336 (86%)	*Agaricomycetes*
BMC86	1	1	0	DQ915469 (90%), DQ899095 (84%)	*Agaricomycetes*
BMC87	1	1	0	DQ273371 (91%), DQ309138 (91%), AY805624 (88%)	*Agaricomycetes*
BMC88	1	1	0	DQ490645 (89%), DQ026258 (89%), U66435 (82%), AM999704 (81%)	*Agaricomycetes*
BMC89	1	1	0	AM999723 (100%), AF394919 (90%), AJ534710 (88%)	*Agaricomycetes*
BMC90	1	1	0	DQ851579 (98%), DQ115781 (98%), DQ115779 (98%)	*Agaricomycetes*
BMC91	1	1	0	AY219355 (91%), EU118655 (87%), EU118648 (85%),	*Agaricomycetes*
BMC92	1	1	0	AY219392 (83%), EU118656 (83%), EU118662 (82%)	*Agaricomycetes*
BMC93	1	1	0	AM902085 (99%), EU118655 (91%), DQ912694 (91%), EU118648 (88%)	*Agaricomycetes*
BMC94	1	1	0	AB210074 (88%), EF577058 (87%), AY633927 (87%), AY463486 (86%)	*Agaricomycetes*
BMC95	1	1	0	AY805625 (92%), EF687930 (91%)	*Agaricomycetes*
BMC96	1	1	0	DQ241782 (94%), U66438 (93%), EU118621 (88%)	*Agaricomycetes*
BMC97	1	1	0	AY382822 (99%), DQ234537 (99%)	*Agaricomycetes, Tomentella sp.* *
BMC98	1	1	0	EU301643 (86%), EU118655 (86%), EU918713 (86%)	*Agaricomycetes*
BMC99	1	1	0	D85631 (95%), EF025042 (95%), EF024029 (95%), DQ873594 (95%)	*Agaricomycetes*
BMC100	1	1	0	DQ444856 (98%), AY916753 (98%), AY916719 (98%)	*Agaricomycetes*
BMC101	1	1	0	AY219404 (99%), AB084592 (99%),	*Agaricomycetes*

				EU670846 (99%), AF334913 (99%)	
BMC102	1	1	0	AB267393 (92%), EU669216 87%)	*Agaricomycetes*
BMC103	1	1	0	DQ873655 (91%), AY228345 (83%), AM901941 82%), EU118621 (82%),	*Agaricomycetes*
BMC104	1	1	0	DQ117964 (86%), U66430 (85%), EU669363 (85%)	*Agaricomycetes*
BMC105	1	1	0	AY558594 (90%), AY251309 (89%)	*Agaricomycetes*
BMC106	1	1	0	DQ309229 (88%), DQ481971 (88%), EU846251 (87%)	*Agaricomycetes*
BMC107	1	1	0	AB084488 (100%), AF289069 (100%)	*Agaricomycetes, Panellus stypticus*
BMC108	1	1	0	AY771606 (99%), DQ437681 (98%)	*Agaricomycetes, Collybia sp.* *
BMC109	1	1	0	EU669216 (96%), AM902078 (95%), EU486440 (95%),	*Agaricomycetes*
BMC110	1	1	0	DQ093734 (100%), AM113456 (97%), AF335444 (95%)	*Agaricomycetes*
BMC111	1	1	0	AF377194 (99%)	*Agaricomycetes, Tricholoma sp.*
BMC112	1	1	0	AF345811 (97%)	*Agaricomycetes, Lacrymaria velutina*
BMC113	1	1	0	DQ403253 (99%)	*Agaricomycetes, Panaeolus sp.*
BMC114	1	1	0	AY228340 (90%), EF530937 (88%)	*Agaricomycetes*
BMC115	1	1	0	AY540327 (99%), AY540332 (99%)	*Agaricomycetes, Pleurotus sp.*
BMC116	1	1	0	EF218784 (97%), DQ474576 (96%)	*Agaricomycetes, Lactarius sp.*
BMC117	1	1	0	DQ384592 (96%), X70027 (96%)	*Agaricomycetes*
BMC118	1	1	0	AF533962 (95%), AM269811 (94%)	*Agaricomycetes*
BMC119	1	1	0	AY805604 (99%), AY781246 (98%)	*Agaricomycetes, Amylostereum sp.*
BMC120	1	1	0	EU118638 (98%)	*Agaricomycetes, Junghuhnia nitida*
BMC121	1	1	0	EU084665 (100%), DQ647827 (99%)	*Agaricomycetes, Paxillus sp.*
BMC122	1	1	0	EF218744 (99%), AJ889934 (99%), DQ066404 (99%)	*Agaricomycetes, Xerocomus sp.*
BMC123	1	1	0	AJ272404 (99%)	*Agaricomycetes, Suillus bovinus*
BMC124	1	1	0	AJ437601 (99%)	*Agaricomycetes, Serpula lacrymans*
BMC125	1	1	0	AJ420946 (100%)	*Agaricomycetes, Gloeophyllum sepiarium*
BMC126	1	1	0	AJ889982 (99%), AY874382 (99%)	*Agaricomycetes, Tomentella sp.*
BMC127	1	1	0	AJ966746 (98%), DQ979014 (95%)	*Agaricomycetes, Geastrum sp.* *
BMC128	1	1	0	AF291279 (99%)	*Agaricomycetes, Exidia truncata*
BMC129	1	1	0	AY805609 (99%), AY509555 (98%)	*Agaricomycetes, Exidia sp.*
BMC130	1	1	0	AM901944 (100%), AF516524 (96%), EU661877 (96%)	*Agaricomycetes*
BMC131	1	1	0	AF126896 (99%), AF126884 (99%)	*Agaricomycetes, Antrodiella sp.*
BMC132	1	1	0	EF088657 (100%), EU840622 (99%)	*Agaricomycetes, Laetiporus sp.*
BMC133	1	1	0	AY336772 (99%), AF255198 (99%), AF255199 (99%)	*Agaricomycetes, Coriolopsis sp.* *
BMC134	1	1	0	EU294161 (99%)	*Agaricomycetes, Piptoporus betulinus*
BMC135	1	1	0	EU118630 (99%)	*Agaricomycetes, Hyphodermella corrugata*
BMC136	1	1	0	AY513227 (97%)	*Agaricomycetes, Lentinellus cochleatus*
BMC137	1	1	0	EU118651 (97%), AF210821 (94%)	*Agaricomycetes, Peniophora sp.*
BMC138	1	1	0	AF506470 (99%)	*Agaricomycetes, Scytinostroma portentosum*
BMC139	1	1	0	AF518571 (96%), AF026607 (96%), DQ457625 (95%)	*Agaricomycetes*
BMC140	1	1	0	EF060012 (99%), EF060011 (99%)	*Agaricomycetes, Ganoderma sp.*
BMC141	1	1	0	AF347080 (93%), AF347081 (91%)	*Agaricomycetes*
BMC142	1	1	0	AB210074 (99%)	*Agaricomycetes, Phlebia acanthocystis*
BMC143	1	1	0	EU872426 (94%), AY219365 (87%)	*Agaricomycetes*
BMC144	1	1	0	DQ340340 (96%)	*Agaricomycetes*
BMC145	1	1	0	DQ340310 (99%)	*Agaricomycetes, Hyphodontia nespori*

BMC146	1	1	0	DQ340311 (99%)	*Agaricomycetes, Hyphodontia hastata*
BMC147	1	1	0	DQ340341 (99%)	*Agaricomycetes, Hyphodontia subaluctata*
BMC148	1	1	0	EU118631 (99%)	*Agaricomycetes, Hyphodontia alutaria*
BMC149	1	1	0	EU118653 (97%), AY219351 (97%)	*Agaricomycetes, Phanerochaete sp.*
BMC150	1	1	0	EU622846 99%)	*Agaricomycetes, Limonomyces roseipellis*
BMC151	1	1	0	DQ677498 (98%)	*Agaricomycetes, Hyphoderma mutatum*
BMC152	1	1	0	AJ534294 (99%)	*Agaricomycetes, Hyphoderma setigerum*
BMC153	1	1	0	EU118609 (99%)	*Agaricomycetes, Candelabrochaete septocystidia*
BMC154	1	1	0	EU811510 (99%), EF024029 (99%)	*Agaricomycetes*
BMC155	1	1	0	AY558599 (95%), AY624992 (95%)	*Agaricomycetes*
BMC156	1	1	0	AY558616 (98%)	*Agaricomycetes, Fuscoporia ferruginosa*
BMC157	1	1	0	AB105437 (99%), AY821994 (99%)	*Tremellomycetes*
BMC158	1	1	0	DQ317359 (99%), AB105438 (99%)	*Tremellomycetes, Cryptococcus sp.*
BMC159	1	1	0	DQ460724 (93%), AY956552 (93%, AY956563 (93%)	*Pucciniomycetes*
BMC160	1	1	0	EU014070 (89%), EU014062 (89%)	*Pucciniomycetes*
BMC161	1	1	0	DQ417401 (93%), AY956560 (93%)	*Pucciniomycetes*
BMC162	1	1	0	AB192988 (99%)	*Pucciniomycetes, Puccinia tanaceti*
BMC163	1	1	0	EF635898 (99%)	*Pucciniomycetes, Puccinia rupestris*
BMC164	1	1	0	U57351 (90%), DQ250744 (88%)	*Pucciniomycetes*
BMC165	1	1	0	EU014070 (97%),	*Pucciniomycetes, Uromyces trifolii-repentis*
BMC166	1	1	0	AF180189 (99%),	*Pucciniomycetes, Uromyces pisi*
BMC167	1	1	0	DQ087231 (99%)	*Pucciniomycetes, Thekopsora areolata*
BMC168	1	1	0	AF125178 (99%)	*Pucciniomycetes, Melampsoridium hiratsukanum*
BMC169	1	1	0	AY854976 (99%), DQ025481 (99%)	*Exobasidiomycetes, Tilletiopsis sp.*
BMC170	1	1	0	U00973 (99%), DQ831012 (99%)	*Ustilaginomycetes*
BMC171	1	1	0	DQ875355 (96%)	*Ustilaginomycetes*
BMC172	1	0	1	AJ866976 (88%), AM901979 (88%), DQ317357 (88%)	
BMC173	1	0	1	AY328913 (99%)	*Wallemiomycetes, Wallemia sebi*
BMC174	1	0	1	EU118659 (83%), EU118658 (83%), AY605709 (81%)	*Agaricomycetes*
BMC175	1	0	1	AY254876 (94%), AF347080 (92%)	*Agaricomycetes*
BMC176	1	0	1	AY641458 (91%), DQ411529 (86%), EU003034 (85%)	*Agaricomycetes*
BMC177	1	0	1	AY969535 (87%), DQ411529 (85%), AY641458 (84%)	*Agaricomycetes*
BMC178	1	0	1	AY641458 (84%), DQ411529 (84%), AM902006 (82%)	*Agaricomycetes*
BMC179	1	0	1	DQ060097 (91%), AF455461 (91%)	*Agaricomycetes*
BMC180	1	0	1	AF455461 (91%), DQ060096 (90%)	*Agaricomycetes*
BMC181	1	0	1	EU003034 (93%), DQ411529 (85%), AY969566 (84%)	*Agaricomycetes*
BMC182	1	0	1	DQ309120 (99%), AM084802 (95%), DQ397337 (93%)	*Agaricomycetes*
BMC183	1	0	1	EF381671 (99%), DQ102418 (82%)	*Agaricomycetes*
BMC184	1	0	1	AY254874 (88%), AF394919 (86%)	*Agaricomycetes*
BMC185	1	0	1	EU113196 (82%), AY461837 (82%), AM076643 (82%), AF462637 (80%)	*Agaricomycetes*
BMC186	1	0	1	L43380 (84%), AY433812 (83%), EU118648 (82%)	*Agaricomycetes*
BMC187	1	0	1	AY219393 (92%), AF087487 (91%), EU118657 (90%)	*Agaricomycetes*

BMC188	1	0	1	AY513231 (89%), AY534580 (86%), AF454422 (84%)	*Agaricomycetes*
BMC189	1	0	1	AF310090 (95%), AY513224 (83%), AF336150 (81%)	*Agaricomycetes*
BMC190	1	0	1	EU819413 (90%), DQ149727 (90%), DQ097876 (88%)	*Agaricomycetes*
BMC191	1	0	1	DQ672292 (90%), AY456375 (88%), DQ979014 (84%),	*Agaricomycetes*
BMC192	1	0	1	EU292660 (87%), EU118617 (85%)	*Agaricomycetes*
BMC193	1	0	1	AJ006683 (84%), AB210075 (84%)	*Agaricomycetes*
BMC194	1	0	1	DQ493754 (98%), U85798 (97%)	*Agaricomycetes, Fibulorhizoctonia sp.*
BMC195	1	0	1	DQ276871 (97%)	*Agaricomycetes, Fibulorhizoctonia sp.*
BMC196	1	0	1	DQ276871 (88%), U85798 (87%)	*Agaricomycetes*
BMC197	1	0	1	AM922223 (88%), EF493272 (92%)	*Agaricomycetes*
BMC198	1	0	1	AM712272 (96%), EU292654 (93%)	*Agaricomycetes*
BMC199	1	0	1	DQ534576 (100%), EU661888 (99%)	*Agaricomycetes, Plicaturopsis sp.* *
BMC200	1	0	1	AY461832 (97%), EU622272 (97%)	*Agaricomycetes, Coprinellus sp.* *
BMC201	1	0	1	EU819413 (88%), DQ149728 (88%), EU669216 (87%)	*Agaricomycetes*
BMC202	1	0	1	AY176379 (99%)	*Agaricomycetes, Lepiota lilacea*
BMC203	1	0	1	EU669311 (89%), AY228340 (88%)	*Agaricomycetes*
BMC204	1	0	1	AY781254 (95%), AF533962 (89%)	*Agaricomycetes*
BMC205	1	0	1	EU301643 (99%), EU273517 (99%)	*Agaricomycetes, Irpex lacteus*
BMC206	1	0	1	EU118669 (97%)	*Agaricomycetes, Steccherinum ochraceum*
BMC207	1	0	1	DQ112556 (99%)	*Agaricomycetes, Vascellum sp.*
BMC208	1	0	1	AJ617490 (94%), AB067724 (92%)	*Agaricomycetes*
BMC209	1	0	1	EF218819 (99%), TTU83486 (98%)	*Agaricomycetes, Thelephora sp.*
BMC210	1	0	1	AY382822 (98%)	*Agaricomycetes,Tomentella sp.*
BMC211	1	0	1	AY382822 (96%)	*Agaricomycetes*
BMC212	1	0	1	EU661877 (99%), AF516525 (99%)	*Agaricomycetes*
BMC213	1	0	1	EF011121 (98%)	*Agaricomycetes, Oxyporus populinus*
BMC214	1	0	1	AJ006673 (99%), DQ060097 (99%)	*Agaricomycetes, Bjerkandera sp.*
BMC215	1	0	1	AF454430 (98%)	*Agaricomycetes, Auriscalpium vulgare*
BMC216	1	0	1	AF347104 (100%), AM269816 (98%)	*Agaricomycetes, Trichaptum sp.*
BMC217	1	0	1	AJ006679 (99%)	*Agaricomycetes, Skeletocutis nivea*
BMC218	1	0	1	AF516528 (99%), EU661877 (99%), AY494980 (98%)	*Agaricomycetes, Polyporus sp.* *
BMC219	1	0	1	AF347080 (97%), AF347081 (97%)	*Agaricomycetes, Trechispora sp.*
BMC220	1	0	1	AF347080 (87%), AY254876 (85%)	*Agaricomycetes*
BMC221	1	0	1	DQ411529 (94%)	*Agaricomycetes*
BMC222	1	0	1	AF347093 (100%)	*Agaricomycetes, Porpomyces mucidus*
BMC223	1	0	1	DQ365649 (91%), EU624408 (88%)	*Agaricomycetes*
BMC224	1	0	1	AJ408375 (98%), AJ408374 (97%)	*Agaricomycetes, Ramaria decurrens*
BMC225	1	0	1	AB210074 (95%), EU118656 (93%), EU118665 (90%)	*Agaricomycetes*
BMC226	1	0	1	DQ873609 (98%)	*Agaricomycetes, Hyphodontia barba-jovis*
BMC227	1	0	1	DQ873618 (99%)	*Agaricomycetes, Hyphodontia flocossa*
BMC228	1	0	1	DQ873614 (99%)	*Agaricomycetes, Hyphodontia crustosa*
BMC229	1	0	1	AF475150 (79%), AF475147 (78%)	*Agaricomycetes*
BMC230	1	0	1	AB361645 (100%)	*Agaricomycetes, Phanerochaete chrysosporium*
BMC231	1	0	1	DQ911612 (97%)	*Agaricomycetes, Haplotrichum conspersum*
BMC232	1	0	1	AF429426 (99%)	*Agaricomycetes, Hypochnicium geogenium*

BMC233	1	0	1	DQ647507 (100%), DQ873599 (99%)	*Agaricomycetes, Hyphoderma puberum*
BMC234	1	0	1	AM747290 (100%), DQ822795 (99%)	*Agaricomycetes*
BMC235	1	0	1	DQ200924 (96%)	*Agaricomycetes*
BMC236	1	0	1	DQ267124 (97%)	*Agaricomycetes, Botryobasidium botryosum*
BMC237	1	0	1	AY081045 (93%), AY854976 (93%)	*Exobasidiomycetes*
BMC238	1	0	1	AF505782 (99%), DQ480098 (99%)	*Agaricomycetes, Gymnopus sp.*

[*] Some of the characteristic NCBI accession numbers correspond to different genera.

Table S4. Statistical parameters.

Symbol	Quantity/Definition
D	Simpson's index[†], $D = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N-1)}$
E	Shannon evenness, $E = H'/\ln S$
H'	Shannon index, $H' = \sum_{i=1}^{S} P_i \ln P_i$
n	Number of investigated samples
n_i	Frequency of occurrence of an individual species *i* (number of samples in which species *i* was detected)
N	Cumulative frequency of occurrence of investigated species, $N = \sum_{i=1}^{S} n_i$
P_i	Relative proportion of an individual species *i*, $P_i = n_i / N$
RFO	Relative frequency of occurrence of a species or group of species
S	Species richness measured (number of detected species)
*S**	Species richness estimated with the Chao-1 approach, [‡] $S^* = S + a^2 / (2\,b)$, *a* = number of species detected only once (singletons), *b* = number of species detected twice (doubletons)
S_n	Species richness normalized by the number of investigated samples, $S_n = S / n$

[†] (53), [‡](53,54)

Table S5. Taxonomic classes of Ascomycota (A) and Basidiomycota (B). Relative frequency occurrence (*RFO*; proportion of total/coarse/fine particle samples in which the class was detected) for different seasons and overall; number of different families and species (total/coarse/fine; n.a. = not available; n.d. = not determined). The number of air samples (filter pairs of fine and coarse particles) collected in spring (Mar-May), summer (Jun-Aug), fall (Sep-Nov) and winter (Dec-Feb) were 14, 10, 10, and 8, respectively.

(**A**)

AMC class	*RFO* (%)					Families	Species
	Spring	Summer	Fall	Winter	Overall		
Dothideomycetes	100/100/93	100/100/100	100/100/100	88/63/75	98/93/93	6/6/5	22/14/12
Eurotiomycetes	79/36/79	50/10/40	70/20/60	100/38/75	74/26/64	2/2/1	18/5/15
Lecanoromycetes	14/7/7	0/0/0	0/0/0	0/0/0	5/2/2	1/n.d./1	2/1/1
Leotiomycetes	78/71/57	70/50/20	90/90/20	75/75/50	78/71/38	4/4/3	16/8/11
Orbiliomycetes	7/0/7	0/0/0	0/0/0	0/0/0	2/0/2	1/0/1	1/0/1
Saccharomycetes	14/0/14	0/0/0	10/0/10	25/0/25	12/0/12	1/0/1	3/0/3
Sordariomycetes	71/36/43	30/10/30	50/0/50	63/13/63	55/17/45	8/5/7	27/8/21
Unknown	86/71/57	80/50/50	80/40/50	75/63/38	81/57/50	n.a./n.d	35/24/14

(**B**)

BMC class	*RFO* (%)					Families	Species
	Spring	Summer	Fall	Winter	Overall		
Agaricomycetes	100/100/100	100/100/100	100/100/100	100/100/100	100/100/100	33/31/20	208/134/112
Cystobasidiomycetes	7/7/0	30/20/10	10/10/0	0/0/0	12/10/2	n.a.	1/1/1
Exobasidiomycetes	0/0/0	0/0/0	0/0/0	25/13/13	5/2/2	1/1/1	2/1/1
Microbotryomycetes	14/14/0	50/50/20	0/0/0	25/0/25	21/17/10	n.d.	2/2/2
Pucciniomycetes	14/14/0	10/10/0	30/30/0	0/0/0	14/14/0	2/2/0	11/11/0
Tremellomycetes	71/64/7	40/30/20	20/10/10	88/88/0	55/48/10	n.a.	7/7/4
Ustilaginomycetes	7/7/0	30/20/10	0/0/0	0/0/0	10/7/2	2/2/1	3/3/1
Wallemiomycetes	0/0/0	0/0/0	20/0/20	13/0/13	7/0/7	n.a.	2/0/2
Unknown	0/0/0	10/10/0	10/0/10	0/0/0	5/2/2	n.d.	2/1/1

Table S6. Taxonomic families of Ascomycota (A) and Basidiomycota (B). Number of species (OTUs) detected in the air samples and coarse or fine particle filters, respectively (total/coarse/fine). Note that with regard to the definition of families the NCBI taxonomy as applied here may deviate from that of other data bases (e.g., Mycobank).

(A)

AMC Family	Species
Apiosporaceae	1/0/1
Chaetomiaceae	1/0/1
Cordycipitaceae	2/0/2
Davidiellaceae	1/1/1
Dermateaceae	2/1/2
Diatrypaceae	2/2/1
Dothioraceae	2/2/1
Erysiphaceae	2/2/0
Gnomoniaceae	2/1/1
Herpotrichiellaceae	1/1/0
Hyaloscyphaceae	4/1/4
Leptosphaeriaceae	2/1/1
Massarinaceae	1/1/0
Orbiliaceae	1/0/1
Parmeliaceae	1/0/1
Phaeosphaeriaceae	4/2/3
Pleosporaceae	5/5/2
Saccharomycetaceae	1/0/1
Sclerotiniaceae.	3/2/2
Sordariaceae	2/2/0
Trichocomaceae	16/4/14
Valsaceae	2/1/1
Xylariaceae	6/1/6

(B)

BMC Family	Species
Agaricaceae	3/2/2
Atheliaceae	5/1/5
Auriculariaceae	1/1/0
Auriscalpiaceae	2/1/1
Bolbitiaceae	1/1/0
Boletaceae	1/1/0
Bondarzewiaceae	2/2/1
Botryobasidiaceae	3/1/3
Corticiaceae	33/22/19
Entolomataceae	2/1/1
Entylomataceae	2/1/1
Exidiaceae	2/2/0
Ganodermataceae	1/1/0
Geastraceae	1/1/0
Gloeocystidiellaceae	1/1/1
Gloeophyllaceae	1/1/0
Gomphaceae	3/1/2
Hymenochaetaceae	5/5/3
Lachnocladiaceae	8/8/3
Lycoperdaceae	3/0/3
Meruliaceae	1/0/1
Phanerochaetaceae	1/1/0
Paxillaceae	1/1/0
Pleurotaceae	1/1/0
Polyporaceae	4/1/3
Pucciniaceae	7/7/0
Pucciniastraceae	2/2/0
Russulaceae	1/1/0
Serpulaceae	2/2/1
Steccherinaceae	4/2/3
Stereaceae	5/4/3
Strophariaceae	1/1/0
Suillaceae	1/1/0
Thelephoraceae	4/1/3
Trechisporaceae	7/2/6
Tricholomataceae	10/7/4
Urocystaceae	1/1/0
Ustilaginaceae	2/2/1

Table S7. Relative frequency occurrence (*RFO*) of selected species from *Ascomycota* (AMC) and *Basidiomycota* (BMC; proportion of total/coarse/fine particle samples in which the species were detected) for different seasons and overall.

Species	*RFO* (%)				
	Spring	Summer	Fall	Winter	Overall
AMC					
Alternaria spp.	21/21/0	70/60/20	50/50/0	38/25/13	43/38/7
Blumeria graminis.	43/43/0	10/10/0	0/0/0	38/38/0	24/24/0
Cladosporium sp.	100/93/79	100/100/100	100/90/100	88/38/75	98/83/88
Penicillium spp.	71/21/71	30/0/30	50/10/40	75/0/75	55/10/55
Eurotium sp.	36/21/21	0/0/0	20/10/10	75/38/38	31/17/17
BMC					
Heterobasidion annosum	79/36/64	70/10/70	70/40/50	63/25/63	71/28/62
Itersonilia perplexans	57/57/0	10/10/0	10/10/10	75/75/0	40/38/2
Stereum spp.	43/21/21	50/50/20	40/30/30	63/38/25	48/33/24

Table S8. Exemplary geometric and aerodynamic diameters and shapes of fungal spores.

Species	Geometric diameter (µm), shape	Aerodynamic diameter (µm)	References
Aspergillus spp.	2-3.5, near-spherical	2.5-3.7	(43, 55)
Cladosporium spp.	3-8 × 2-5, ovoid, lemon-shaped, oblong, near-spherical	1.8-2.3	(30, 43, 56, 57)
Penicillium spp.	3-6, near-spherical	3.0-3.7	(30, 55)
Basidiospores	5-15, elliptic, oblong	6.8	(58, 59)
Itersonilia perplexans	10-12 × 10-13		(60)
Smut spores		9.7	(55)
Rust spores (*Puccinia spp.*)	> 20		(20, 61)

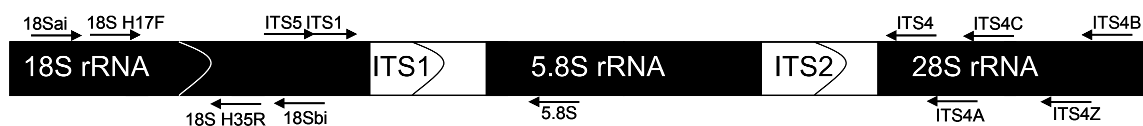

Figure S1. Binding sites of the applied primers on the fungal rRNA gene: schematic illustration of the ITS regions and the 18S, 5.8S, and 28S rRNA region (not completely shown and not to scale).

C3) Global atmospheric diversity of fungi: *Asco-* and *Basidiomycota* in continental and marine air

Janine Fröhlich-Nowoisky[*†], Viviane R. Després[*‡], and Ulrich Pöschl[*]

[*]Biogeochemistry Department, Max Planck Institute for Chemistry, Joh.-Joachim-Becher-Weg 27, 55128 Mainz, Germany

[†]Institute of Geosciences, Johannes Gutenberg University, Joh.-Joachim-Becher-Weg 21, 55128 Mainz, Germany

[‡]Institute of General Botany, Johannes Gutenberg University, Johannes-von-Müller-Weg 6, 55128 Mainz, Germany

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Authors Contribution

J.F.N. conducted the experiments. All authors contributed collectively to designing the experiments, interpreting the data and writing the paper.

Abstract

Fungal spores are ubiquitous in the Earth's atmosphere, but their diversity is hardly known. In this study, we performed DNA analyses of air filter samples collected at different locations and climatic zones around the world (tropical, mid-latitude, sub-polar; continental, marine), and we obtained over 2500 fungal DNA sequences. Nearly all sequences could be attributed to the phyla of *Ascomycota* and *Basidiomycota*. With regard to species richness, the ratio of *Basidiomycota* to *Ascomycota* was much higher in continental air samples (~60:40) than in marine air samples (~30:70), which can be explained by smaller spore sizes and larger atmospheric residence times of *Ascomycota*. The basidiomycotic species belonged mostly to the class *Agaricomycetes* (mushroom forming fungi), while the ascomycotic species were mainly distributed over four classes (*Dothideomycetes, Eurotiomycetes, Leotio-mycetes, Sordariomycetes*). Allergenic molds and plant pathogens were found everywhere. Some of the detected fungi are also known as ice nuclei and may thus be important for the hydrological cycle and climate.

1 Introduction

Recent studies have shown that fungal spores and other biogenic aerosol particles account for a significant fraction of atmospheric aerosols in pristine rainforest air as well as in rural and urban environments (Womiloju et al., 2003; Elbert et al., 2007; Bauer et al., 2008; Held et al., 2008; Hock et al., 2008). On average, the number and mass concentrations of fungal spores in continental boundary layer air are on the order of 10^3-10^4 m^{-3} and ~1 μg m^{-3}, respectively, the estimated global emissions of ~50 Tg yr^{-1} are among the largest sources of organic aerosol (Elbert et al., 2007).

Fungi (e.g., *Fusarium spp.*) have also been found in clouds, fog, and precipitation, where they act as nuclei for water droplets and ice crystals and can influence precipitation patterns and the Earth's energy budget (e.g.,

Pouleur et al., 1992; Andreae and Crutzen, 1997; Bauer et al., 2002; Jaenicke, 2005; Pöschl, 2005; Möhler et al., 2007; Andreae and Rosenfeld, 2008; Christner et al., 2008; Rosenfeld et al., 2008). Some fungi are major pathogens or allergens (e.g., *Aspergilllus spp., Cladosporium spp.*) for humans, animals, and plants, and air is the primary medium for their dispersal (Kurup et al., 2000; Brown, 2002; Adhikari et al., 2004). Still, the diversity of fungi in air particulate matter is hardly known. The detection and characterization of fungi in atmospheric aerosol samples by DNA analysis can help to elucidate the regional and global spread, and diversity of fungi.

Earlier studies, investigating samples of continental boundary layer air from central Europe indicated a high diversity of fungi, including a range of plant pathogens and allergenic fungi. The diversity of *Basidiomycota* (BMC) was much higher than previously assumed (Fröhlich-Nowoisky et al., 2009). The question remained if the species richness of fungi in the atmosphere is generally higher for BMC than for *Ascomycota* (AMC) or if there are differences in regions with e.g., different climate or habitats. In soil, AMC are thought to be more abundant than BMC both in biomass and in the number of different species. The known soil fungi comprise 15000 AMC (~60%) and 10000 BMC (~40%) as described in Thorn, 1999. From aquatic habitats about 3000 fungal species, comprising also mostly AMC have been reported (Shearer et al., 2007).

In this study, we have investigated and compared the diversity of fungi in air particulate matter in continental and marine environments, ranging from the tropics to high latitudes, by DNA extraction and sequence analysis of the internal transcribed spacer region (ITS).

2 Material and Methods

2.1 Aerosol sampling

Samples were collected at several locations around the world, as detailed below and summarized in Table 1.

2.1.1 Austria

PM10 samples on quartz fiber filters (Tissuequartz 2500QAT-UP, 150mm diameter, Pall, USA) were provided by the Institute for Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria. The samples were taken using a high-volume filter sampler (sample air flow ~500 L min^{-1}; sampling time 24 h) in parallel at two sampling sites in Vienna in July 2005 (Table S1). The suburban site (48°14’09’’N, 16°18’10’’E) is situated in a park-like residential area in the northwest of the city next to a park bordered by woodland. The urban site (48°11’05’’N, 16°24’28’’E) is situated in a mixed residential/industrial area on a grassy strip with trees and bushes between a sidewalk and a street. A major urban freeway passes within around 200 m. The sampling height was 4 m above ground (Bauer at al., 2008).

2.1.2 China

Samples of total suspended particles (TSP) were collected with a high-volume filter sampler (Anderson Instruments, Smyrna, GA; 1.5 m above the ground, sample air flow 1000 L min^{-1}; sampling time 2-26 h) during the Program of Regional Integrated Experiments of Pearl River Delta Region (PRIDE-PRD) Campaign in July 2006 in Backgarden (23.5°N, 113°E, South China) (Table S2). It was a small village in a rural farming environment ~60 km northwest of the mega city Guangzhou on the outskirts of the densely populated center of the PRD. The sampling site was situated on the edge of the highly populated PRD region, though the area itself was mostly a farming area. Due to the prevailing monsoon circulation, at this time of year, the air masses came mainly from the south/southeast, making this site a rural receptor site for the

regional pollution resulting from the outflow of the city cluster around Guangzhou (Garland et al., 2008; Rose et al. 2008). Prior to use, all quartz fiber filters were decontaminated by baking at 500°C for at least 12 h.

2.1.3 Germany

Air filter samples were collected with a dichotomous high-volume sampler over a period of one year in central Europe (Mainz, Germany; March 2006 – April 2007): 42 pairs of fine and coarse particle samples with a nominal cut-off diameter of ~3 µm and average sampling times of ~7 days. A comprehensive description of the investigated samples is given in Fröhlich-Nowoisky et al., (2009).

2.1.4 Puerto Rico

Air samples on quartz fiber filters (<1.7 µm) and nucleopore filters (>1.7 µm) were collected by the Institute for Tropical Ecosystem Studies (ITES), University of Puerto Rico, USA at three different locations in Puerto Rico (Table S3). The Sampling stations were the Lighthouse in Fajardo (marine site), University of Puerto Rico-Río Piedras (urban site) and the Caribbean National Forest (forest site). The sample air flow was 50 L min^{-1} and the sampling time 48-72 h. Prior to use, all quartz fiber filters were decontaminated by baking at 450°C for 24 h.

2.1.5 Ship sampling

TSP samples of tropical, mid-latitude, and sub-polar marine boundary layer air were collected during the 24th China Antarctic Research Expedition (October 2007 to April 2008) on glass fiber filters (23 cm × 18 cm) using a high-volume filter sampler (sample air flow 1005 L min^{-1}; sampling time 24-72 h) (Table S4). The sampler was positioned on the platform of the Icebreaker Xuoelong (30 m a.s.l.). The cruise covered regions somewhere from China, Australia, Antarctic, and Argentina, including East China Sea, South China Sea, South Pacific Ocean, East Indian Ocean, and Southern Ocean. Prior to use, all glass

fiber filters were decontaminated by baking at 500°C over night. To avoid ship emission contamination, a wind controller for the sampler was designed which stopped automatically when the velocity of the wind from the front of the ship was lower than 5 m s^{-1}. The samples were stored at -20°C.

2.1.6 Taiwan

PM2.5 and TSP samples on quartz fiber filters (20 cm × 25 cm) were provided by the Research Center for Environmental Changes, Taiwan. The samples were collected using a high-volume filter sampler (Ecotech HVS-3000, sample air flow 1130 L min^{-1}; sampling time 12-24 h) at several locations in Taiwan including suburban and remote sites (mountains, 750 m a.s.l.; Table S5). Prior to use, all quartz fiber filters were decontaminated by baking at 500°C for at least 8 h.

2.1.7 United Kingdom

Samples on glass fiber filters were provided by the School of Earth, Atmospheric, and Environmental Sciences, University of Manchester, United Kingdom (UK). The samples were collected as parts of the Tropospheric ORganic CHemistry (TORCH) field campaigns during summer 2003 and spring 2004 (Table S6).

The TORCH1 sampling site was located at Writtle Agricultural College, near Chelmsford, Essex, UK, (51.74° N, 0.42° E), ~50 km northeast of London. The site was a ~1.5 ha grass field situated to the south east of the main college buildings, and was not influenced by any significant local vehicular, domestic or industrial sources. The air masses were dominated by prevailing winds from the Atlantic, with air mainly arriving at the measurement site from a westerly or south-westerly direction (Ireland, Southern UK) thus giving the opportunity to sample air recently out flowing from the London area (Johnson et al., 2006, Cubison et al., 2006).

TORCH2 took place at the Weybourne Atmospheric Observatory (WAO, 52°57’02’’N, 1°07’19’’E), which is located on the North Norfolk coastline

near Weybourne, UK. Norfolk is a sparsely populated rural region without large population centres or industrial areas. Like described in Gysel et al., (2007) the air masses encountered at this station represent aged polluted outflow from London, the West Midlands or the European continent, or relatively clean air masses transported across the North Sea region by northerly wind.

2.2 DNA extraction and amplification

Filter sample aliquots (30-150 mg) were extracted with a commercial soil extraction kit (LysingMatrixE, Fast DNA Spin Kit for Soil, MP Biomedicals) according to the supplier's instructions with the following modifications: 15-min-centrifugation step after the lysis, partly additional 900 µl buffer and repeated beating and centrifugation. Both generated supernatants were combined for the further extraction process. Finally, the DNA was dissolved in 100 µl elution buffer. Decontaminated Filter or LysingMatrixE reaction tubes without a filter were included during the extractions as extraction blanks.

With the DNA extract from each of the filters listed in Tables S1-S6, several PCRs were performed to amplify DNA from fungi for sequence analysis. The 50 µl reaction mixture always contained the template DNA (0.5-5 µl sample extract), 1×PCR buffer, 0.2 mM each dNTP (Roth), 0.33 µM of each primer (Sigma-Aldrich), and 2.5 units of JumpStartTM REDTaq DNA polymerase (Sigma-Aldrich). A negative control was included in all PCR runs.

PCR reactions of were performed with the primer pairs listed in Table 2, except for the samples collected in Mainz, Germany where more primer pairs were used (see Fröhlich-Nowoisky et al., 2009). For the first PCR primer pairs A and B and for the second PCR the nested primer pairs C, D, and E were used. The thermal profile (DNA Engine, Bio-Rad Laboratories) was as follows: initial denaturing at 94°C for 3 min; 35 cycles with denaturing at 94°C for 30 s, annealing at primer pair specific temperature for 30 s (Table 2), elongation at 72°C for 90 s, and a final extension step at 72°C for 5 min.

Fungal DNA was detected in 4% of the extraction or PCR blanks, indicating that contaminations occurred rarely during analysis in the laboratory. No DNA could be detected in the provided baking filter blanks. The obtained PCR products from blank samples were cloned and sequenced, whereas PCR products of filter extracts obtained in these PCRs were completely excluded from the cloning reactions (see 2.5).

2.3 Cloning and restriction fragment length polymorphism

Amplification products for sequencing were cloned using the TOPO TA Cloning® Kit (Invitrogen) following the supplier's instructions. Colonies containing inserts were identified by blue-white selection and lysed in 20 µl water for 10 min at 95°C. The inserts of 12-24 colonies were amplified ("colony PCRs") using 3 µl lysate in a 40 µl reaction. The PCR reaction mixture always contained: 1×PCR Buffer, 0.25 mM each dNTP (Roth), 0.25 µM of each primer (Sigma-Aldrich), 1.25 units *Taq* DNA Polymerase (NEB). PCR reactions were performed with the primer pair M13F-40 and M13R, and the thermal profile was as follows: initial denaturing at 94°C for 5 min; 40 cycles with 94°C for 30 s, annealing at 55°C for 1 min, elongation at 72°C for 1 min, and a final extension step at 72°C for 15 min.

The colony PCR was followed by a restriction fragment length polymorphism (RFLP) analysis to select as many as possible different clones for sequencing. 2 µl of the PCR-products were digested without further purification with 5 units of the enzyme *TaqI* (Fermentas). Restriction fragments were separated by gel electrophoresis in a 3% agarose gel stained with ethidium bromide and the images were documented with the Gel Doc XR system and analyzed with Quantity One software (Bio-Rad Laboratories). On the basis of the resulting restriction fragment patterns, representative colony PCR products with different numbers and sizes of fragments were selected for sequencing.

2.4 DNA sequence analysis, taxonomic attribution, and statistical parameters

DNA sequences were determined with ABI Prism 377, 3100, and 3730 sequencers (Applied Biosystems) using BigDye-terminator v3.1 chemistry at the DNA Core Facility of the Max Planck Institute for Plant Breeding Research, Cologne. For comparison with known sequences, databank queries using the Basic Local Alignment Search Tool (BLAST) were performed via the website of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Each of the 2780 obtained sequences was identified to the lowest taxonomic rank common to the top BLAST hits (up to ~100 data base sequences with highest similarity and total scores). Sequences, for which the ITS1 and ITS2 regions matched in different genera were assumed to be chimeric results of PCR recombination and were excluded from further analysis. Sequences, which were obtained from field, extraction or PCR blanks and identical sequences obtained from the air filter samples and filter blank samples were also excluded from further analysis.

For each aerosol filter sample, sequences that produced the same BLAST results were pairwise aligned using the program BioEdit (BioEdit 7.05; http://www.mbio.ncsu.edu/BioEdit/ bioedit.html). The similarity between them was calculated using the PAM250 Matrix. Sequences with similarity scores ≥97% were clustered into an operational taxonomic unit (OTU).

To characterize and compare the diversity of fungal species (OTUs) in the investigated air masses, we have calculated the parameters defined in Table 3.

2.5 Blank and Background samples

Whereas no DNA could be detected in the provided baking filter blanks, fungal DNA was detected in 4% of the extraction or PCR blanks, indicating that contaminations occurred rarely during analysis in the laboratory. The obtained PCR products from blank samples were cloned and sequenced, whereas PCR products of filter extracts obtained in these PCRs were excluded completely

from the cloning reactions. However, the obtained sequences of blank samples and identical sequences obtained from the air filter samples were excluded from further analysis.

PCR is a powerful technique and the high sensitivity of this technique bears the risk of amplifying trace amounts of DNA with which the analysis material (e.g., reagents) may have been contaminated in the course of material production and analysis. Several studies described bacterial and fungal DNA contaminations in *Taq* Polymerase and commercially available reagents like lysis enzymes (Meier et al., 1993, Hughes et al., 1994, Löffler et al., 1999, Rimeck at al., 1999). In our study the contaminations in the PCR blanks could not be detected anymore after performing the experiments with new reagents (new lot number). The detection of DNA in extraction blanks could partly attributed to contaminations originating from the PCR. Six species e.g., *Brettanomyces bruxellenis* and *Candida tropicalis* were only detected in extraction blanks. Maybe they are laboratory contaminations of the extracts or contaminations occurred during extraction or PCR preparation in the laboratory (material used, extraction procedure). In total 11 different OTUs (mostly yeast species, e.g., *Candida sake, Candida deformans, Candida tropicalis, Cryptococcus longus*) were identified as possible contaminations.

As described in Fröhlich-Nowoisky et al., (2009), no DNA was detected in the mounting, start-up, extraction, and PCR blanks of the samples from Mainz, Germany, indicating that no contaminations occurred during sample handling and analysis in the laboratory. Like described above during the analysis of all other sample sets DNA was rarely detected in PCR and extraction blanks. Four of the OTUs identified as possible contaminations had also been detected in the samples from Mainz. To avoid any bias in the comparison with other sample

sets, we excluded the possible contaminations also from the statistical analysis of the Mainz samples.

2.6 Impact of different sampling methods and conditions

As described in Table 1, the samples from different locations were collected with different types of samplers, cut-off diameters, and filter substrates. Also the sampled air volumes and the sampling years and seasons were different. These differences may have influenced the results from individual measurement locations as discussed below, but they are not likely to have influenced the main conclusions of this study such as the different ratios of AMC to BMC in continental and marine air masses.

Depending on sampler type and cut-off diameter, large spores or fungal tissue fragments are likely to be discriminated in certain types of samples (e.g., PM2.5 samples from Taiwan). The sampling height influences the impact of the surrounding area and vegetation. Larger particles as well as particles from fungi growing near the sampler may be preferentially collected by samplers at ground level, whereas sampling on elevated platforms, masts or towers are likely to be less influenced by local sources. Rare species are less likely to be found in case of short sampling times and low air volumes.

The detection and apparent frequency of occurrence of different species can also be affected by the efficiency of DNA extraction from different kinds of filter material. Further investigations will be required to quantify such effects. Nevertheless, this study confirms that a wide range of filter materials can be used for DNA analysis of air samples (Després et al. 2007).

Different climates might influence degradation processes of DNA. DNA starts to degrade as soon as an organism dies in the air or upon sampling. Spores are known to resist environmental stress and survive atmospheric transport (Griffin, 2004; Griffin and Kellogg, 2004), whereas DNA in fungal tissue fragments may be rapidly degraded by atmospheric photooxidants. The DNA breaks into smaller pieces and is chemically modified (Pääbo, 1989; Lindahl,

1993; Höss et al., 1996; Smith et al., 2001).These degradation effects can lead to a decreasing DNA amount, substantial analytical artifacts, and loss of information (Despres et al., 2007). Tropical climate may result in more information loss than sub-polar climate. It is known, that under cool conditions DNA can be preserved for longer times (Pääbo et al., 2004). Furthermore, the investigated samples were up to five years old. Different storage times and conditions can also lead to chemical modification and degradation of DNA.

Air filter samples may also contain inhibitory substances like humic like acids, soot, or salt particles affecting the amplification efficiency of DNA (Moreira, 1998; Graber and Rudich, 2006, Després et al., 2007). Among other things they influence e.g., the pH or the salt concentration of the PCR reaction mixture. These changes can result in a less, absent, or unspecific amplification product. For example, salt particles may be more present in marine environments than in continental environments which may be more influenced by soot particles (Yin et al., 2005). Beside these environmental conditions, air pollution might also have an affect on the live time of the particles and DNA during the transport like discussed above. Certain types of spores may be more resistant against air pollution and e.g., salt particle influence.

In any case, different sampling and storage procedures should be kept in mind with regard to the comparison of the different sets of filter samples. Nevertheless, we think that the presented measurement methods and results provide a basis for further comprehensive and systematic investigations of the global atmospheric diversity of fungi.

3 Results and Discussion

3.1 Species richness

Fungal DNA was found in all sampling locations. Air masses from marine (tropical, mid-latitude, sub-polar) and continental mid-latitude locations were studied. Table 1 summarizes the number of obtained sequences and OTU of each location. Detailed lists of species detected in the samples are given in Tables S7-S12 and in Fröhlich-Nowoisky et al., (2009).

Continental mid-latitude air samples collected in central Europe (Mainz, Germany) over a period of one year exhibited the greatest species richness. With 42 pairs of fine and coarse particle samples and average sampling times of ~7 days, this sample set represents the largest data set with 364 detected species. All other sample sets had a much lower number of air filter samples and the species richness was also much lower (up to maximal 124 species in tropical marine samples of Puerto Rico). In other words, the more filters per sample site are studied the more likely it is to observe a higher species richness.

Overall, the investigated sample sets indicate high fungal species richness. A high proportion of species (~70-90%) were found only once per sampling location and the limited number of investigated samples and DNA amplification products imply that the actual diversity of fungi in the sampled air masses was higher than detected. As described in Table 1 the samples were collected under different sampling conditions. This - like discussed under 2.6 might have an impact on the relative amount of total species richness. To get a better comparison of species richness of airborne fungi in different locations and climatic zones our results will need to be complemented by further investigations of samples collected in the same way.

The Shannon index (H'), Shannon evenness (E), and Simpson's index (D) values were calculated from the frequency of occurrence of the different species, i.e., from the number of samples in which each species had been

detected, were similar in all regions. These diversity parameter values are similar to the values commonly obtained for fungi in soil and on plants as well as for bacteria in soil (Maria et al., 2002, Hill et al., 2003; Richard et al., 2004, Satish et al., 2007). As also shown in Table 1, estimates of the actual species richness (S*) based on the Chao-1 estimator approach (Chao, 1984; Hill et al., 2003) are by factors of ~3-9 higher than the measured species richness values (S), suggesting that the total number of fungal species in the investigated air samples was >1000 in continental mid-latitude samples (Mainz, Germany), ~1000 in tropical marine samples (Puerto Rico), and up to ~250 in all other sample sets. A detailed comparison of these values was not performed because of the different sampling methods. Interestingly, the median values of S, S*, H`, E and D (Table 1d) are similar for the tropical and mid-latitude samples but the focus in this study was on the species richness and relative abundance of phyla and classes.

3.2 Phyla and classes

As illustrated in Fig. 1, nearly all detected fungal species were *Ascomycota* (AMC, sac fungi) or *Basidiomycota* (BMC, club fungi), many of which actively eject their spores with aqueous jets or droplets in the atmosphere (Elbert et al., 2007).

Besides sampling on continental and coastal locations, and on a ship the samples were taken in different climatic zones (Fig. 1). All samples collected in tropical and sub-polar regions also represent marine environments (coastal and ship) and except for Puerto Rico we found a higher species richness of AMC. Maybe this exception can be explained by the influence of the tropical rainforest in Puerto Rico and with sampling of ~⅓ of the filters directly in the rainforest. Samples were also collected at a coastal site of Puerto Rico. The forest site samples show that 70% of the detected species were BMC whereas the coastal samples exhibited only ~40% BMC (Fig. S1).

Two types of mid-latitude samples where analyzed: samples from continental (Austria, Germany) and coastal (United Kingdom, UK) locations in Europe as well as marine samples collected on a ship. The continental samples exhibited more species of BMC (~64%) than of AMC (~34%) whereas samples from the UK exhibited more AMC species (~54%). These samples were from two sampling locations in the UK which are geographically near the coast and actually, both represent marine environments. Separating them, the TORCH2 samples, collected in Weybourne directly at the coast, were more species rich for AMC (~70%) and the TORCH1 samples were more diverse for BMC (Fig. S1). In contrast to Weybourne, where the air masses sampled during TORCH2 originated mostly from the North Sea (Gysel et al., 2007), the air masses encountered at the sampling station in Writtle during TORCH1 were transported over the Atlantic, Ireland, and Southern UK (Cubison et al., 2006).

The other type of mid-latitude samples was taken on a ship in coastal regions of South America (Argentinia) and in the Indian Ocean. The samples show a predominance of AMC species (~70%).

In total, the ratio of AMC to BMC was higher in marine ship (~70:30) and coastal (~60:40) samples whereas in continental samples the ratio of BMC to AMC was found to be much higher (~65:35; Fig. 2).

The high proportion of BMC species in continental and tropical forest site samples as observed in our study, and the predominance of AMC species in marine and coastal environments may be explained by the following basic features of BMC and AMC.

The number and proportion of all fungal species known from the biosphere are higher for AMC (~60000, ~60-70%) than for BMC (25000-30000, ~30-40%; De Meus and Renaud, 2002; James et al., 2006). The vast majority of these fungi are terrestrial; a few (~3000 species) are aquatic (Shearer et al., 2007). Thus, land contact of air masses before sampling is the main source of fungal particulate matter whereas the oceans appear to be a small source of fungal particles. A rough estimate of potential emissions of fungal spores/cells of

~10 t yr^{-1} is six orders of magnitude smaller than the land surface emission estimate of ~50 Tg yr^{-1} (Elbert et al., 2007). Thus, the air mass origin is an important factor and has to be regarded in comparable analysis of different locations, because air masses which were transported without land contact to the sampling stations might have a different species richness and composition than air masses which were transported over land.

In marine environments AMC particulate matter might be sampled more than BMC matter because the greatest numbers of aquatic taxa comprise the AMC (e.g., aquatic lichen). They play an important role in nutrient cycling in water ecosystems. In one study, 75% of the fungal biomass on underwater substrates was made up of AMC (Nikolcheva and Bärlocher, 2004; Wang et al., 2005; Shearer et al., 2007). Besides the occurrence of AMC in marine environments, the particle sizes might also influence the ratio of AMC and BMC. Many BMC spores are known to have larger aerodynamic diameters (~5-10 µm) than AMC spores (~2-5 µm; Fröhlich-Nowoisky et al., 2009 and references therein). BMC are the mushroom forming fungi which produce a diverse array of fruiting bodies that may fragment and release great portions of tissue particles. In contrast, AMC are mostly single-celled (yeasts) or filamentous (hyphal), and some of the most prominent AMC species found in the samples like *Cladosporium sp.* and *Penicillium spp.* have spores with small aerodynamic diameters. Smaller AMC spores could have longer residence times in the atmosphere (multiple days to weeks) and may be transported over greater distances than larger BMC spores. BMC spores und tissue fragments may be more rapidly removed from the atmosphere because of their size. Pathogen species and decomposer may also be associated with fragments of e.g., plants which might also be hindering long distance transports.

These features might also explain why samples which were collected on the ship had more BMC species when they were collected near the coast whereas the samples taken in greater distances of land masses had only a few BMC species. The air masses had no directly land contact before sampling and the

results may simply reflect long distance transports of mainly small AMC particulate matter. The results of the filter samples collected at two stations in the UK also confirm this. The land contact of air masses measured at the inland station (TORCH1) might have been a source of BMC matter whereas the air masses at the coastal station originating from the North Sea without land contact for a few days present mainly long distance transport of AMC particulate matter.

Like already discussed under 2.6, the vegetation around the sampling locations (forests, flat land, water etc.), e.g., in combination with sampling height could have influence on the species proportion of AMC compared to BMC. More diverse regions and regions with larger numbers of plants (larger leave surface) provide e.g., more host material etc. for certain fungal species. The rainforest in Puerto Rico might be an important source of growth material and host plants, animals, or insects for basidiomycotic fungi that are known to decompose organic material or growing on rotten wood, live in symbiotic relationships or as parasites and pathogens (Read, 1997; Aanen et al. 2002). In general tropical forests are known to be an intense source of primary biogenic aerosol particles (Artaxo and Hanson, 1995). Puerto Rico also exhibited the greatest fraction (7%) of species that could not be attributed to any phylum (Fig. 1). Tropical forests are likely a source of many undescribed fungi (Hawksworth and Rossmann, 1997; Hawksworth, 2004). Thus, they may possibly represent fungi that are undescribed or not represented in the NCBI database.

3.2.1 Ascomycotic classes

The AMC species found in the air samples were distributed over nine major classes (*Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes*; *Orbiliomycetes*, *Pezzizomycetes, Saccharomycetes, Sordariomycetes, and Taphrinomycetes*; Figs. 3 and 4).

The *Dothideomycetes, Eurotiomycetes,* and *Sordariomycetes* were present everywhere. In every sampling location between two and four main classes (>10%) were present. The class *Sordariomycetes* includes plant pathogens and endophytes, animal pathogens, and mycoparasites (Zhang and Sung, 2008). Furthermore, this class includes ice nuclei active fungi like *Microdochium nivale* and *Fusarium spp*. (Pouleur et al, 1992, Snider et al., 2000), which were detected in this study. Ice nucleation is a significant atmospheric process, which leads to the formation of clouds. In marine tropical samples (Puerto Rico, Taiwan) and in continental mid-latitude samples (Germany, Austria) the *Sordariomycetes* represent the most diverse class.

The class *Eurotiomycetes*, which includes well-known molds like *Penicillium spp.* and *Aspergillus spp.* account for ~50% of the AMC in the sub-polar marine samples. Also in tropical marine samples a great portion (~30%) of members of this class was detected whereas the other sample sets had maximal 14% members of this class.

The *Dothideomycetes* were the most diverse class in mid-latitude marine samples and in tropical marine samples from China. Species of this class account for ~40-60% of the AMC species in these samples. This class contains several allergenic molds and plant pathogens, endophytes or epiphytes of living plants and also saprobes degrading cellulose and other complex carbohydrates in dead or partially digested plant matter, in leaf litter, or dung (Schoch et al., 2006).

Additionally, in continental and coastal mid-latitude samples the *Leotiomycetes* represent one of the main classes. Plant pathogens, endophytes, symbionts, fungal parasites and saprobes belong to this class (Wang and Zeng, 2007). They are only with a few species present in tropical marine samples from China and Taiwan.

In total, in continental and coastal samples the proportions of the classes are similar, with predominance of *Sordariomycetes* and *Dothideomycetes*, whereas marine samples are dominated by members of the *Eurotiomycetes*.

3.2.2 Basidiomycotic classes

The BMC species found in the air samples were distributed over eight classes (*Agaricomycetes, Agaricostilbomycetes, Cystobasidiomycetes, Exobasidiomycetes, Microbotryomycetes, Pucciniomycetes, Tremellomycetes, and Ustilaginomycetes*; Figs. 5 and 6).

In contrast to the distribution of the AMC classes, the distribution of BMC classes is similar everywhere. One single class, the *Agaricomycetes*, represent always the biggest fraction. The *Agaricomycetes* (about 16000-20000 species) account for ~50% of the known BMC species (Hibbet, 2006, Hibbet, 2007, Matheny et al., 2007). The proportion of *Agaricomycetes* among the different species of BMC found in the atmospheric samples was everywhere higher than 50%, indicating that species of this class may be enriched in the atmosphere relative to the biosphere. Their proportion at the atmosphere-biosphere interface (i.e., on vegetation and soil surfaces) or their efficiency in dispersing spores may be enhanced relative to the other classes of BMC.

Compared to the *Agaricomycetes*, all other classes of BMC exhibited much less species richness. Except for the *Agaricostilbomycetes*, which were only found in tropical samples from China, the other classes were detected only in the continental and coastal mid-latitude samples.

In other words, in continental and coastal samples several classes were present, whereas in marine samples the species belonged to one class, the *Agaricomycetes*.

4 Conclusions

From air filter samples collected at different locations and climatic zones around the world (tropical, mid-latitude, sub-polar; continental, marine) we obtained over ~2500 fungal DNA sequences. Nearly all of them could be attributed to AMC and BMC. The ratio of BMC to AMC was much higher in continental air samples (~60:40) than in marine air samples (~30:70) which can

be explained by smaller spore sizes and longer atmospheric residence times of AMC. Marine samples also exhibited lower numbers of classes within AMC and BMC than continental and coastal samples. The BMC belonged mostly to the class *Agaricomycetes* (mushroom forming fungi), while the AMC were mainly distributed over four classes (*Dothideomycetes, Eurotiomycetes, Leotiomycetes, Sordariomycetes*). Allergenic molds and plant pathogens were found everywhere. Some of the detected fungi are also known as ice nuclei and may thus be important for the hydrological cycle and climate. To our knowledge, this is the first study characterizing the global atmospheric diversity of fungi. The presented analytical techniques and measurement results should provide a basis for further comprehensive and systematic investigations. Information about the diversity and abundance of airborne fungi is relevant for many areas of research, including biogeosciences, climate, and ecology.

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References

Aanen, D. K., Eggleton, P., Rouland-Lefére, C., Guldberg-Frøslev, T., Rosendahl, S., and Boomsma, J. J.: The Evolution of Fungus-Growing Termites and Their Mutualistic Fungal Symbionts, P. Natl. Acad. Sci. USA, 99, 14887-14892, 2002.

Adhikari, A., Sen, M. M., Gupta-Bhattacharya, S., and Chanda, S.: Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations, Sci. Total Environ., 326, 123-141, 2004.

Andreae, M. O. and Crutzen, P. J.: Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry, Science, 276, 1052–1058, 1997.

Andreae, M. O. and Rosenfeld, D.: Aerosol-cloud-precipitation interactions. Part 1. The nature and sources of cloud-active aerosols, Earth Sci. Rev., 89, 13-41, 2008.

Artaxo, P. and Hansson, H.-C.: Size distribution of biogenic aerosol particles from the Amazon basin, Atmos. Environ., 29, 393-402, 1995.

Bauer, H., Kasper-Giebl, A., Loflund, M., Giebl, H., Hitzenberger, R., Zibuschka, F., and Puxbaum, H.: The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols, Atmos. Res., 64, 109-119, 2002.

Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitzenberger, R., Marr, I. L., and Puxbaum, H.: Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol, Atmos. Environ., 42, 588, 2008.

Brown, J. K. M. and Hovmoller, M. S.: Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease, Science, 297, 537–541, 2002.

Chao, A.: Nonparametric-Estimation of the Number of Classes in a Population, Scand. J. Stat., 11, 265-270, 1984.

Christner, B. C., Morris, C. E., Foreman, C. M., Cai, R. M., and Sands, D. C.: Ubiquity of biological ice nucleators in snowfall, Science, 319, 1214-1214, 2008.

Cubison, M. J., Alfarra, M. R., Allan, J., Bower, K. N., Coe, H., McFiggans, G. B., Whitehead, J. D., Williams, P. I., Zhang, Q., Jimenez, J. L., Hopkins, J., and Lee, J.: The characterisation of pollution aerosol in a changing photochemical environment, Atmos. Chem. Phys., 6, 5573-5588, 2006.

de Meeus, T. and Renaud, F.: Parasites within the new phylogeny of eukaryotes, Trends Parasitol., 18, 247-251, 2002.

Després, V. R., Nowoisky, J. F., Klose, M., Conrad, R., Andreae, M. O., and Pöschl, U.: Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes, Biogeosciences, 4, 1127-1141, 2007.

Elbert, W., Taylor, P. E., Andreae, M. O., and Pöschl, U.: Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions, Atmos. Chem. Phys., 7, 4569–4588, 2007.

Fröhlich-Nowoisky, J. Després, V.R., and Pöschl, U.: High diversity of fungi in air particulate matter, 2009, submitted.

Garland, R. M., Yang, H., Schmid, O., Rose, D., Nowak, A., Achtert, P., Wiedensohler, A., Takegawa, N., Kita, K., Miyazaki, Y., Kondo, Y., Hu, M., Shao, M., Zeng, L. M., Zhang, Y. H., Andreae, M. O., and Pöschl, U.: Aerosol optical properties in a rural environment near the mega-city Guangzhou, China: implications for regional air pollution, radiative forcing and remote sensing, Atmos. Chem. Phys., 8, 5161-5186, 2008.

Graber, E. R. and Rudich, Y.: Atmospheric HULIS: How humic-like are they? A comprehensive and critical review, Atmos. Chem. Phys., 6, 729-753, 2006.

Griffin, D. W.: Terrestrial microorganisms at an altitude of 20,000 m in Earth's atmosphere, Aerobiologia, 20, 135-140, 2004.

Griffin, D. W. and Kellogg, C. A.: Dust storms and their impact on ocean and human health: dust in Earth’s atmosphere, Ecohealth, 1, 284–295, 2004.

Gysel, M., Crosier, J., Topping, D. O., Whitehead, J. D., Bower, K. N., Cubison, M. J., Williams, P. I., Flynn, M. J., McFiggans, G. B., and Coe, H.: Closure study between chemical composition and hygroscopic growth of aerosol particles during TORCH2, Atmos. Chem. Phys., 7, 6131-6144, 2007.

Hawksworth, D. L.: Fungal diversity and its implications for genetic resource collections, Stud. Mycol., 9-17, 2004.

Hawksworth, D. L. and Rossman, A. Y.: Where Are All the Undescribed Fungi?, Phytopathology, 87, 888-891, 1997.

Held, A., Zerrath, A., McKeon, U., Fehrenbach, T., Niessner, R., Plass-Dülmer, C., Kaminski, U., Berresheim, H., and Pöschl, U.: Aerosol size distributions measured in urban, rural and high-alpine air with an electrical low pressure impactor (ELPI), Atmos Environ, 42, 8502-8512, 2008.

Hibbett, D. S.: A phylogenetic overview of the Agaricomycotina, Mycologia, 98, 917-925, 2006.

Hibbett, D. S. 2007. Agaricomycetes. Mushroom-Forming Fungi. Version 20 April 2007. http://tolweb.org/Agaricomycetes/20535/2007.04.20 *in* The Tree of Life Web Project, http://tolweb.org/

Hill, T. C. J., Walsh, K. A., Harris, J. A., and Moffett, B. F.: Using ecological diversity measures with bacterial communities, FEMS Microbiol. Ecol., 43, 1-11, 2003.

Hock, N., Schneider, J., Borrmann, S., Rompp, A., Moortgat, G., Franze, T., Schauer, C., Pöschl, U., Plass-Dulmer, C., and Berresheim, H.: Rural continental aerosol properties and processes observed during the Hohenpeissenberg Aerosol Characterization Experiment (HAZE2002), Atmos Chem Phys, 8, 603-623, 2008.

Höss, M., Jaruga, P., Zastawny, T. H., Dizdaroglu, M., and Pääbo, S.: DNA damage and DNA sequence retrieval from ancient tissues,Nucleic. Acid. Res., 24, 1304–1307, 1996.

Hughes, M. S., Beck, L. A., and Skuce, R. A.: Identification and elimination of DNA sequences in Taq DNA polymerase. J. Clin. Microbiol. 32, 1994

James, T. Y., et al.: Reconstructing the early evolution of Fungi using a six-gene phylogeny, Nature, 443, 818-822, 2006.

Jaenicke, R.: Abundance of cellular material and proteins in the atmosphere, Science, 308, 73, 2005.

Johnson, D., Utembe, S. R., Jenkin, M. E., Derwent, R. G., Hayman, G. D., Alfarra, M. R., Coe, H., and McFiggans, G.: Simulating regional scale secondary organic aerosol formation during the TORCH 2003 campaign in the southern UK, Atmos. Chem. Phys., 6, 403-418, 2006.

Kurup, V. P., Shen, H.-D. and Banerjee, B.: Respiratory fungal allergy, Microbes Infect., 2, 1101-1110, 2000.

Lindahl, T.: Instability and decay of the primary structure of DNA, Nature, 362, 709–715, 1993.

Löffler, J., Hebart, H., Bialek, R., Hagmeyer, L., Schmidt, D., Serey, F., Hartmann, M., Eucker J., and Einsele H.: Contaminations occurring in fungal PCR assays. J. Clin. Microbiol. 37:1200-1202, 1999.

Maria, G. L. and Sridhar, K. R.: Richness and diversity of filamentous fungi on woody litter of mangroves along the west coast of India, Curr. Sci., 83, 1573-1580, 2002.

Matheny, P. B. etal.: Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi), Mol. Phylogenet. Evol., 43, 430-451, 2007.

Meier, A., Persing, D. H., Finken, M., and Bottger, E. C.: Elimination of contaminating DNA within polymerase chain reaction reagents: implications for a general approach to detection of uncultured pathogens, J. Clin. Microbiol., 31, 646-652, 1993.

Möhler, O., DeMott, P. J., Vali, G., and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, Biogeosciences, 4, 1059-1071, 2007.

Moreira, D.: Efficient removal of PCR inhibitors using agarose-embedded DNA preparations, Nucl. Acids Res., 26, 3309-3310, 1998.

Nikolcheva, L. G. and Bärlocher, F.: Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream, Mycol. Prog., 3, 41-49, 2004.

Pääbo, S.: Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification., P. Natl. Acad. Sci. USA, 86, 1939-1943, 1989.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., and Hofreiter, M.: Genetic analysis from ancient DNA, Annu. Rev. Genet., 38, 645–679, 2004.

Pöschl, U.: Atmospheric aerosols: composition, transformation, climate and health effects, Angew. Chem. Int. Edit., 44, 7520–7540, 2005.

Pouleur, S., Richard, C., Martin, J. G., and Antoun, H.: Ice nucleation activity in Fusarium acuminatum and Fusarium avenaceum., Appl. Environ. Microbiol., 58, 2960–2964, 1992.

Richard, F., Moreau, P. A., Selosse, M. A., and Gardes, M.: Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by Quercus ilex L, Can. J. Bot., 82, 1711-1729, 2004.

Rimek, D., Garg, A. P., Haas W. H. and Kappe R.:. Identification of contaminating fungal DNA sequences in Zymolyase. J. Clin. Microbiol. 37, 830-831, 1999.

Read, D.: Mycorrhizal fungi - The ties that bind, Nature, 388, 517-518, 1997.

Rose, D., Nowak, A., Achtert, P., Wiedensohler, A., Hu, M., Shao, M., Zhang, Y., Andreae, M. O., and Pöschl, U.: Cloud condensation nuclei in polluted air and biomass burning smoke near the mega-city Guangzhou, China – Part 1: Size-resolved measurements and implications for the modeling of aerosol particle hygroscopicity and CCN activity, Atmos. Chem. Phys. Discuss., 8, 17343-17392, 2008.

Rosenfeld, D., Lohmann, U., Raga, G. B., O'Dowd, C. D., Kulmala, M., Fuzzi, S., Reissell, A., and Andreae, M. O.: Flood or drought: How do aerosols affect precipitation?, Science, 321, 1309-1313, 2008.

Satish, N., Sultana, S., and Nanjundiah, V.: Diversity of soil fungi in a tropical deciduous forest in Mudumalai, southern India, Curr. Sci., 93, 669-677, 2007.

Schoch, C. L., Shoemaker, R. A., Seifert, K. A., Hambleton, S., Spatafora, J. W., and Crous, P. W.: A multigene phylogeny of the Dothideomycetes using four nuclear loci, Mycologia, 98, 1041-1052, 2006.

Shearer, C. A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanova, L., Padgett, D., Porter, D., Raja, H. A., Schmit, J. P., Thorton, H.A., and Voglymayr, D.: Fungal biodiversity in aquatic habitats. Biodiversity and Conservation, 16(1):49-67, 2007

Smith, C. I., Chamberlain, A. T., Riley, M. S., Cooper, A., Stringer, C. B., and Collins, M. J.: Neanderthal DNA: not just old but old and cold?, Nature, 410, 771–772, 2001.

Snider, C.S., Hsiang, T., Zhao, G.Y., and Griffith, M.: Role of ice nucleation and antifreeze activities in pathogenesis and growth of snow molds. Phytopathology 90: 354–361, 2000.

Wang, Z., Binder, M., and Hibbett, D. S.: Life history and systematics of the aquatic discomycete Mitrula (Helotiales, Ascomycota) based on cultural, morphological, and molecular studies, Am. J. Bot., 92, 1565-1574, 2005.

Wang, Z. 2007. Leotiomycetes. Version 26 September 2007. http://tolweb.org/ Leotiomycetes/29048/2007.09.26 in The Tree of Life Web Project, http://tolweb.org/

Womiloju, T. O., Miller, J. D., Mayer, P. M., and Brook, J. R.: Methods to determine the biological composition of particulate matter collected from outdoor air, Atmos. Environ., 37, 4335-4344, 2003.

Yin, J., Allen, A. G., Harrison, R. M., Jennings, S. G., Wright, E., Fitzpatrick, M., Healy, T., Barry, E., Ceburnis, D., and McCusker, D. Major component composition of urban PM10 and PM2.5 in Ireland, Atmos. Res., 78, 149– 165, 2005.

Zhang, N. and Sung, G.-H.: Sordariomycetes. Version 14 January 2008. http://tolweb.org/ Sordariomycetes/29050/2008.01.14, in The Tree of Life Web Project, http://tolweb.org/, 2008.

Table 1. Overview and diversity parameters of aerosol filter samples. Sampling site; air masses; filter type; size range; sampling period and time; air flow; sampled air volume; number of filter and blank (blk) samples; obtained DNA sequences; sum of obtained OTU of all samples; OTU per sample; species richness (S measured, *S** estimated), Shannon index (*H'*), Shannon Evenness (*E*), and Simpson's index (*D*) for all fungi (**a**), *Ascomycota* (AMC) (**b**), *Basidiomycota* (BMC) (**c**), and the average and median values (average/median) for (sub-)tropical, mid-latitude and (sub-)polar samples (**d**).

a)

	(Sub-)Tropical, 0°-25°				**Mid-Latitude, 25°-55°**				**(Sub-)Polar, 55°-70°**	**Global marine**
	Guangzhou	**Taiwan**	**Puerto Rico**	**Ship**	**Mainz**	**Vienna**	**UK**	**Ship**	**Ship**	**Ship**
Sampling sites	coastal	coastal	coastal	marine	continental	continental	coastal	marine	marine	marine
Air masses	sea/land	sea/land	sea/land	sea	land	land	sea/land	sea	sea	sea
Filter type	glass fiber	quartz fiber	quartz fiber, nucleopore	glass fiber	glass fiber	quartz fiber	glass fiber	glass fiber	glass fiber	glass fiber
Size range	TSP	PM2.5, TSP	coarse + fine	TSP	coarse + fine	PM10	-	TSP	TSP	TSP
Period	7/2006	10/2006-03/2008	Summer 2007	11/2007, 3-4/2008	3/2006-5/2007	07/2005	07-08/2003, 05/2004	1-3/2008	12/2007-3/2008	11/2007-4/2008
Time (h)	2-26	12-24	48-72	24	24-170	24	21-35	24	24-72	12-54
Flow (L min^{-1})	1000	1130	50	1005	300	520	-	1005	1005	1005
Air volume (m^3)	~150 - 1600	~550 - 3000	~60 - 270	~1500	400-2000	~730 - 750	-	~1450	~770-3300	~770-3300
Number of air filter samples	14	17	16	5	96	4	13	2	10	17
Number of blk samples	3	3	5	0	12	0	4	1	1	2
Number of obtained sequences	272	149	199	81	1316	90	156	32	85	198
OTU all samples	142	77	150	34	908	73	87	22	37	90
OTU/sample	6	3	8	6	4	15	4	9	3	4
S	90	56	124	31	364	60	57	18	33	75
*S**	255	268	988	162	1120	239	183	163	259	1264
H'	4.2	3.8	4.7	3.4	5.2	4	3.9	2.9	3.4	4.2
E	0.93	0.96	0.98	0.99	0.89	0.98	0.96	0.99	0.99	0.97
D	0.017	0.015	0.004	0.005	0.009	0.02	0.015	0.006	0.008	0.009

b)

					Ascomycota					
S	62	40	43	21	120	21	26	12	28	54
*S**	139	185	442	75	480	64	97	73	185	654
H´	3.8	3.5	3.7	3.0	4.1	2.9	3.2	2.5	3.3	3.8
E	0.90	0.95	0.97	0.99	0.85	0.97	0.93	0.99	0.98	0.96
D	0.028	0.022	0.025	0.011	0.031	0.024	0.021	0.013	0.010	0.015

c)

					Basidiomycota					
S	28	16	72	10	238	37	31	6	5	21
*S**	184	88	433	-	658	173	86	-	-	-
H´	3.3	2.6	4.1	2.3	4.9	3.6	3.1	1.8	1.6	3
E	0.98	0.94	0.96	1	0.88	0.99	0.94	1.0	1.0	1
D	0.010	0.047	0.010	0	0.015	0.005	0.035	0	0	0

d)

Average/Median	**(Sub-)Tropical, 0°-25°**	**Mid-Latitude, 25°-55°**	**(Sub-)Polar, 55°-70°**	**Global marine**
OTU all samples	101/110	273/80	37	31/34
OTU/sample	6/6	8/7	3	6/6
S	75/73	125/59	33	27/31
*S**	418/262	426/211	259	195/163
H´	4.0/4.0	4.0/4.0	3.4	3.2/3.4
E	0.97/0.97	0.96/0.97	0.99	0.99/0.99
D	0.010/0.010	0.013/0.012	0.008	0.006/0.006

Table 2. PCR primer combinations. Forward and reverse primer names, annealing temperature, and references. The amplified region was the 5.8S rRNA gene and both internal transcribed spacer regions (ITS1, ITS2).

Primer pair	Forward	Reverse	Temperature (°C)	References
First PCR				
A	ITS5	ITS4A	55	White et al., 1990; Nicolcheva and Bärlocher, 2004
B	ITS5	ITS4B	58	White et al., 1990; Nicolcheva and Bärlocher, 2004
Second PCR				
C	ITS5	ITS4	54	White et al., 1990
D	ITS1	ITS4B	58	Fierer et al., 2005; Nicolcheva and Bärlocher, 2004
E	ITS1	ITS4A	55	Fierer et al., 2005; Nicolcheva and Bärlocher, 2004

Table 3. Statistical parameters.

Symbol	Quantity/Definition
D	Simpson's index[†], $D = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N-1)}$
E	Shannon evenness, $E = H' / \ln S$
H'	Shannon index, $H' = \sum_{i=1}^{S} P_i \ln P_i$
n_i	Frequency of occurrence of an individual species i (number of samples in which species i was detected)
N	Cumulative frequency of occurrence of investigated species, $N = \sum_{i=1}^{S} n_i$
P_i	Relative proportion of an individual species i, $P_i = n_i / N$
S	Species richness measured (number of detected species)
S^*	Species richness estimated with the Chao-1 approach, [‡] $S^* = S + a^2 / (2\,b)$, a = number of species detected only once (singletons), b = number of species detected twice (doubletons)

[†] (Hill et al., 2003), [‡](Hill et al., 2003; Chao, 1984)

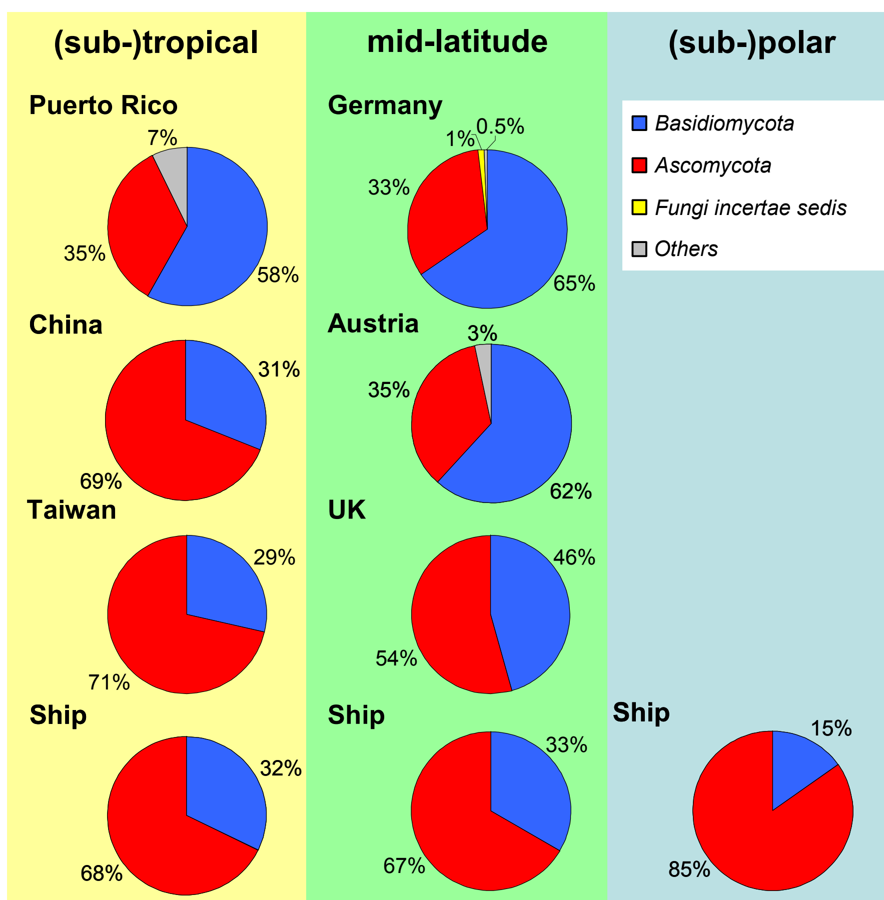

Fig. 1. Species richness of airborne fungi: relative proportions of different phyla in (sub-)tropical, mid-latitude, and (sub-)polar samples.

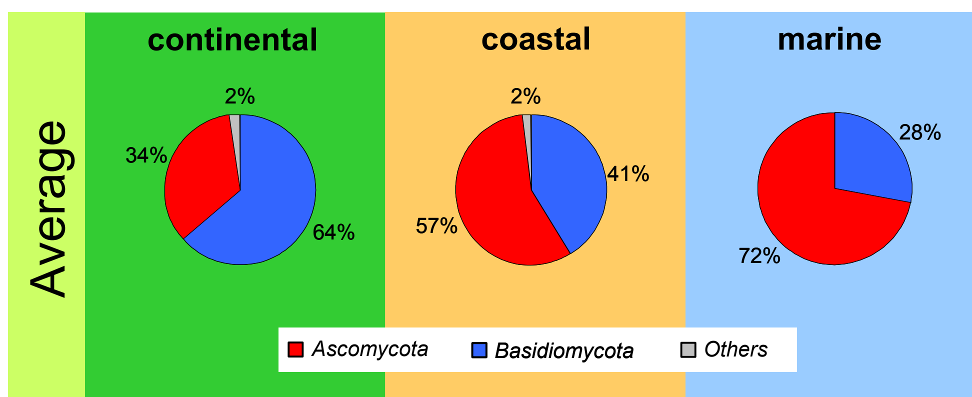

Fig. 2. Species richness of airborne fungi: relative proportions of different phyla in continental (Austria, Germany), coastal (China, Taiwan, Puerto Rico, UK), and in marine (ship) samples.

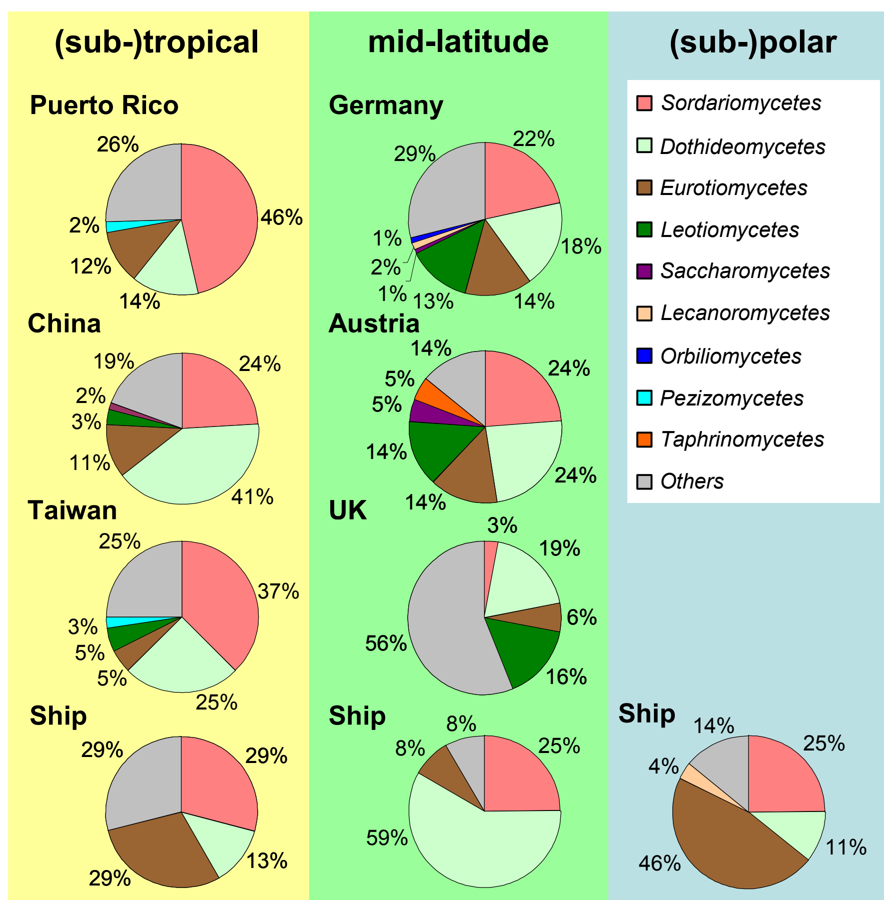

Fig. 3. Species richness of airborne fungi: relative proportions of different classes of *Ascomycota* in (sub-)tropical, mid-latitude, and in (sub-)polar samples.

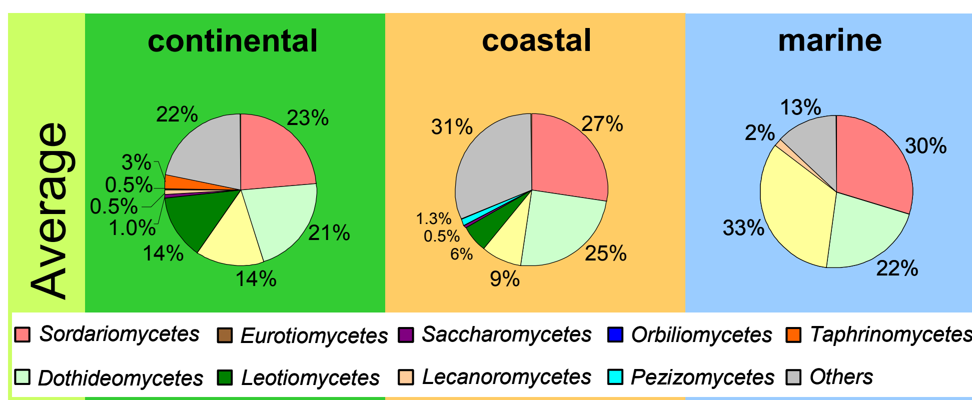

Fig. 4. Species richness of airborne fungi: relative proportions of different classes of *Ascomycota* in continental (Austria, Germany), coastal (China, Taiwan, Puerto Rico, UK), and in marine (ship) samples.

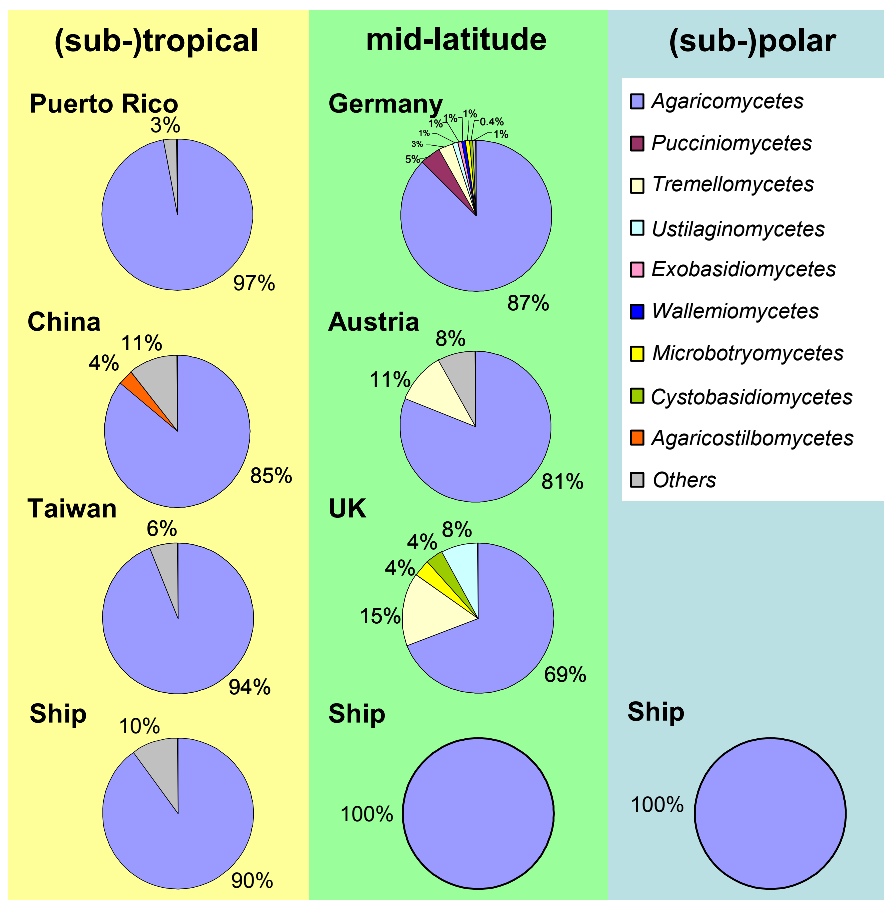

Fig. 5. Species richness of airborne fungi: relative proportions of different classes of *Basidiomycota* in (sub-)tropical, mid-latitude, and in (sub-)polar samples.

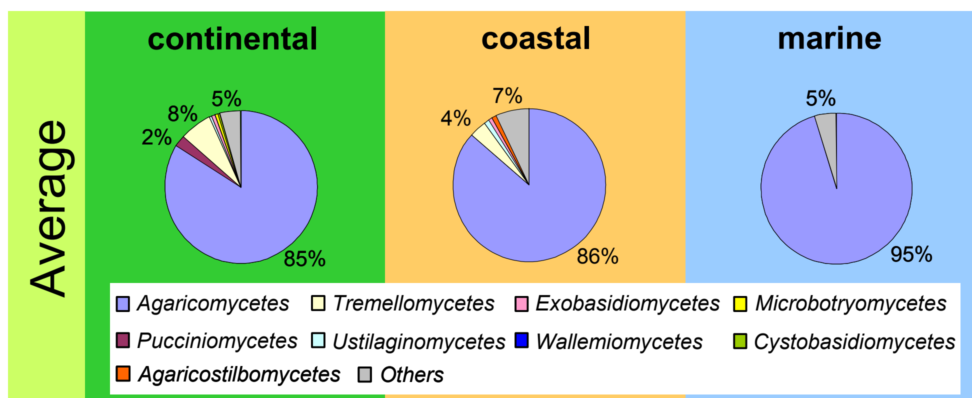

Fig. 6. Species richness of airborne fungi: relative proportions of different classes of *Basidiomycota* in continental (Austria, Germany), coastal (China, Taiwan, Puerto Rico, United Kingdom), and in marine (ship) samples.

Supplementary Information for the Manuscript

"Global atmospheric diversity of fungi: *Asco*- and *Basidiomycota* in continental and marine air"

To complement the information given in the main manuscript, the following sections provide Supplementary Tables S1-S12 and Supplementary Figures S1-S3.

Table S1. Austria. Overview of air samples. Sample ID (running number); sampling period; sampled air volume; number of DNA sequences; number of different species (OTU) of all fungi and of *Ascomycota* (AMC), and *Basidiomycota* (BMC), detected in the air sample.

Sample ID	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
				Fungi	AMC	BMC
Vienna 1	07.07.2005	730	14	12	9	2
Vienna 2	07.07.2005	752	33	24	4	19
Vienna 3	19.07.2005	739	21	18	6	11
Vienna 4	19.07.2005	747	22	19	9	9

Table S2. China. Overview of air samples. Sample ID (running number); sampling period; sampled air volume; number of DNA sequences; number of different species (OTU) of all fungi and of *Ascomycota* (AMC), and *Basidiomycota* (BMC), detected in the air sample.

Sample ID	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
				Fungi	AMC	BMC
China1	4.7.2006	334	40	27	13	14
China2	7.7.2006	800	13	6	6	0
China3	9.7.2006 - 10.7.2006	1158	8	6	4	2
China4	10.7.2006 - 11.7.2006	n.a.	15	7	7	0
China5	11.7.2006 - 12.7.2006	1158	12	3	2	1
China6	12.7.2006 - 13.7.2006	1435	25	20	14	6
China7	13.7.2006 - 14.7.2006	1431	23	13	8	5
China8	14.7.2006 - 15.7.2006	1431	18	6	6	0
China9	15.7.2006 - 16.7.2006	1598	15	6	6	0
China10	16.7.2006 - 17.7.2006	1506	8	5	5	0
China11	17.7.2006 - 18.7.2006	1197	34	9	6	3
China12	25.7.2006	146	18	12	12	0
China13	28.7.2006 - 29.7.2006	1424	22	12	11	1
China14	29.7.2006 - 30.7.2006	1363	16	10	10	0

Table S3. Puerto Rico. Overview of air samples. Sample ID (running number; Puerto1-4 = forest, Puerto 5-7 = urban, Puerto 8-11 = marine, nuc = nucleopore, FQ = quartz fiber filter); sampling period; sampled air volume; number of DNA sequences; number of different species (OTU) of all fungi and of *Ascomycota* (AMC), and *Basidiomycota* (BMC), detected in the air sample.

Sample ID	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
				Fungi	AMC	BMC
Puerto1nuc	Summer 2007	129	21	17	5	11
Puerto2FQ	Summer 2007	129	32	22	1	20
Puerto3nuc	Summer 2007	129	37	30	5	25
Puerto4nuc	Summer 2007	129	24	21	10	8
Puerto5nuc	Summer 2007	269	4	3	2	1
Puerto6FQ	Summer 2007	269	2	1	1	0
Puerto7nuc	Summer 2007	269	20	15	8	7
Puerto8FQ	Summer 2007	269	13	2	0	2
Puerto9nuc	Summer 2007	269	10	10	6	4
Puerto10nuc	Summer 2007	269	21	17	3	11
Puerto11nuc	Summer 2007	269	15	12	6	5

Table S4. Ship. Overview of air samples. Sample ID (running number; t = tropical, m = mid-latitude, s = (sub-)polar); sampling period; sampled air volume (n.a. = not available); number of DNA sequences; number of different species (OTU) of all fungi and of *Ascomycota* (AMC), and *Basidiomycota* (BMC), detected in the air sample.

Sample ID	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
				Fungi	AMC	BMC
Ship1t	20.11.2007 – 21.11.2007	1489	16	10	5	5
Ship2t	23.11.2007 – 24.11.2007	1430	23	9	9	0
Ship3s	05.12.2007 – 06.12.2007	1346	37	18	15	3
Ship4s	08.12.2007 – 11.12.2007	1495	3	1	1	0
Ship5s	24.12.2007 – 25.12.2007	771	1	1	0	1
Ship 6s	25.12.2007 – 25.12.2007	-	11	3	2	1
Ship7s	30.12.2007 – 31.12.2007	1506	0	0	0	0
Ship8s	08.01.2008 – 11.01.2008	2762	19	8	8	0
Ship9s	14.01.2008 – 17.01.2008	2498	0	0	0	0
Ship10m	30.01.2008 – 31.01.2008	1457	26	17	11	6
Ship11s	05.02.2008 – 08.02.2008	2349	9	4	4	0
Ship12s	15.02.2008 – 16.02.2008	1471	2	1	1	0
Ship13s	05.03.2008 – 10.03.2008	3343	3	1	1	0
Ship14m	19.03.2008 – 20.03.2008	1453	6	2	2	0
Ship15t	31.03.2008 – 01.04.2008	1509	13	3	3	0
Ship16t	04.04.2008 – 05.04.2008	1508	29	12	8	5
Ship17t	07.04.2008 – 08.04.2008	1512	0	0	0	0

Table S5. Taiwan. Overview of air samples. Sample ID (running number); sampling period; sampled air volume (n.a. = not available); number of DNA sequences; number of different species (OTU) of all fungi and of *Ascomycota* (AMC), and *Basidiomycota* (BMC), detected in the air sample.

Sample ID	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
				Fungi	AMC	BMC
Taiwan1	27.03.2008 - 27.03.2008	575	0	0	0	0
Taiwan2	20.04.2007 - 21.04.2007	753	7	4	1	3
Taiwan3	23.05.2007 - 24.05.2007	748	11	5	2	3
Taiwan4	26.02.2008 - 26.02.2008	552	15	7	7	0
Taiwan5	28.03.2007 - 29.03.2007	n.a	20	10	10	0
Taiwan6	11.12.2007 - 12.12.2007	741	6	3	2	1
Taiwan7	29.11.2007 - 29.11.2007	752	0	0	0	0
Taiwan8	11.06.2008 - 11.06.2008	655	24	11	8	3
Taiwan9	06.10.2006 - 08.10.2006	3109	18	6	6	0
Taiwan10	26.03.2008 - 27.03.2008	766	0	0	0	0
Taiwan11	20.06.2007 - 20.06.2007	9	6	3	2	1
Taiwan12	24.08.2007 - 25.08.2007	673	24	18	10	8
Taiwan13	26.09.2007 - 27.09.2007	703	11	8	3	5

Table S6. United Kingdom. Overview of air samples. Sample ID (running number, UK1-9 TORCH2, UK10-12 TORCH1); sampling period; sampled air volume (n.a. = not available); number of DNA sequences; number of different species (OTU) of all fungi and of *Ascomycota* (AMC), and *Basidiomycota* (BMC), detected in the air sample.

Sample ID	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
				Fungi	AMC	BMC
UK1	06.05.2004 - 13.05.2004	n.a.	32	15	5	10
UK2	06.05.2004 - 13.05.2004	n.a.	10	7	5	2
UK3	06.05.2004 - 13.05.2004	n.a.	17	12	8	4
UK4	15.05.2004 - 21.05.2004	n.a.	10	10	9	1
UK5	15.05.2004 - 21.05.2004	n.a.	15	5	3	2
UK6	06.05.2004 - 13.05.2004	n.a.	29	11	9	2
UK7	06.05.2004 - 13.05.2004	n.a.	2	1	1	0
UK8	06.05.2004 - 13.05.2004	n.a.	5	3	3	0
UK9	23.05.2004 - 25.05.2004	n.a.	0	0	0	0
UK10	30.02.2003 – 01.08.2003	n.a.	10	9	3	6
UK11	30.02.2003 – 01.08.2003	n.a.	10	8	0	8
UK12	30.02.2003 – 01.08.2003	n.a.	16	6	0	6

Table S7. Austria. Operational taxonomic units (OTUs) attributed to Ascomycota (A), Basidiomycota (B), and Fungi (C). Operational ID (AMC/BMC and running number); frequency of occurrence (number of air samples in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
AMC1	3	EU167608 (99%), AJ876481 (99%)	*Dothideomycetes*
AMC2	3	AY616705 (97-98%)	*Sordariomycetes, Hypoxylon macrocarpum*
AMC3	2	DQ339557 (100%), DQ339570 (100%)	*Eurotiomycetes, Penicillium sp.*
AMC4	2	EU552134 (99%), EF432273 (99%), EU552164 (99%)	*Dothideomycetes*
AMC5	2	AY251074 (99%), EF432298 (99%)	*Dothideomycetes, Cladosporium sp.*
AMC6	1	AY154692 (99%), AY154695 (99%)	*Dothideomycetes, Alternaria sp.*
AMC7	1	EF452492 (99%)	*Dothideomycetes, Pyrenophora tritici-repentis*
AMC8	1	AB193391 (99%), EU247884 (99%)	*Leotiomycetes, Sawadaea sp.*
AMC9	1	AM901810 (99%), AM262391 (97%)	*Sordariomycetes*
AMC10	1	AM901808 (89%), EU852366 (89%), AY239218 (89%)	*Taphrinomycetes*
AMC11	1	AF138814 (89%), EU041790 (80%)	
AMC12	1	EU918705 (99%), AY681180 (99%), AY681197 (98%)	*Sordariomycetes*
AMC13	1	EF187912 (99%), AM902031 (99%)	*Sordariomycetes, Microdochium nivale*
AMC14	1	EF207415 (99-100%), AJ279480 (99-100%), EF153017 (98%)	*Leotiomycetes*
AMC15	1	EU273521 (99%), EU167566 (99%)	
AMC16	1	EU272488 (99%)	*Sordariomycetes, Nigrospora oryzae*
AMC17	1	EF634441 (100%)	*Eurotiomycetes, Penicillium brevicompactum*
AMC18	1	AF298539 (99%), AF298541 (99%)	*Leotiomycetes, Microsphaera sp.*
AMC19	1	AF455536 (99%), AF455528 (99%)	*Eurotiomycetes, Eurotium sp.*
AMC20	1	EU594572 (93%), DQ100416 (92%)	

AMC21	1	AM850055 (94%)	*Saccharomycetes*

(**B**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
BMC1	2	AY840572 (100%), AF516556 (99%)	*Agaricomycetes, Trametes sp.*
BMC2	2	AF455463 (99-100%), AJ006672 (99%)	*Agaricomycetes*
BMC3	2	AY781266 (99%), AM901886 (99%)	*Agaricomycetes, Phanerochaete sordida olrim*
BMC4	2	EU872426 (98%)	*Agaricomycetes, Phanerochaete chrysosporium*
BMC5	1	AY081229 (100%)	*Agaricomycetes, Chlorophyllum brunneum*
BMC6	1	DQ309181 (99%), EU645602 (95%), DQ061931 (93%)	
BMC7	1	EU871492 (85%), EU108787 (85%)	
BMC8	1	AB071802 (93%), AB071613 (93%)	*Agaricomycetes*
BMC9	1	AF455496 (100%), X70025 (99%)	*Agaricomycetes, Heterobasidion annosum*
BMC10	1	AM901952 (99%), EU118665 (98%)	*Agaricomycetes, Scopuloides hydnoides*
BMC11	1	AB072233 (99%), AM901971 (99%)	*Tremellomycetes, Itersonilia perplexans*
BMC12	1	EU046047 (99%)	*Tremellomycetes*
BMC13	1	EU047805 (99%), AY219382 (99%)	*Agaricomycetes, Phanerochaete sordida*
BMC14	1	AY558613 (92%), DQ340338 (90%)	*Agaricomycetes*
BMC15	1	EU546104 (95%), AB084618 (94%)	*Agaricomycetes*
BMC16	1	AF126891 (99%)	*Agaricomycetes*
BMC17	1	U52886 (98%), DQ398959 (95%)	*Agaricomycetes, Vuilleminia sp.*
BMC18	1	AF324171 (90%), AF324161 (86%)	*Agaricomycetes*
BMC19	1	DQ273355 (94%)	*Agaricomycetes*
BMC20	1	FJ010207 (91-92%), U85799 (91%)	*Agaricomycetes*
BMC21	1	EU301643 (99%), EU273517 (99%)	*Agaricomycetes, Irpex lacteus*
BMC22	1	FJ553297 (93%), DQ093649 (93%)	*Agaricomycetes*
BMC23	1	AF444435 (97%)	*Tremellomycetes, Udeniomyces puniceus*
BMC24	1	DQ278949 (95%), EU622841 (86%)	*Agaricomycetes*
BMC25	1	AB210076 (88%), AF455458 (88%)	*Agaricomycetes*
BMC26	1	EU118638 (99%)	*Agaricomycetes, Junghuhnia nitida*

BMC27	1	AJ419931 (89%), EU645602 (87%), DQ061931 (87%)	
BMC28	1	FJ010207 (98%)	*Agaricomycetes, Flavodon flavus*
BMC29	1	U85799 (93%), FJ478126 (92%)	*Agaricomycetes*
BMC30	1	AY969535 (88%), DQ411529 (86%)	*Agaricomycetes*
BMC31	1	AF310096 (99%)	*Agaricomycetes, Gloeocystidiellum porosum*
BMC32	1	EF060520 (94%)	*Agaricomycetes*
BMC33	1	AY558613 (86%), AY558617 (85%)	*Agaricomycetes*
BMC34	1	FJ478126 (86%), FJ481018 (85%), U85799 (85%)	*Agaricomycetes*
BMC35	1	EU673086 (91%), AM269811 (90%)	*Agaricomycetes*
BMC36	1	FJ481048 (99%)	*Agaricomycetes, Trametes gibbosa*
BMC37	1	AB049614 (99%), AF314987 (99%)	*Tremellomycetes, Dioszegia hungarica*

(**C**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores
F1	3	EU823316 (97-98%)
F2	1	EU035443 (86%), EF585657 (85%)

Table S8. China. Operational taxonomic units (OTUs) attributed to Ascomycota (A) and Basidiomycota (B). Operational ID (AMC/BMC and running number); frequency of occurrence (number of air samples in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
AMC1	12	AY251074 (99%), AJ876482 (99%), AJ300331 (99%)	*Dothideomycetes, Cladosporium sp.*
AMC2	10	FJ478134 (97100%), EU714386 (97-99%), EF687956 (97-100%), EU918714 (97-99%),	*Sordariomycetes, Nigrospora sp.*
AMC3	8	AM076665 (98-99%), DQ680839 (98-99%), EU167608 (98-99%),	
AMC4	4	AF071327 (97-98%), AF212308 (97%)	*Dothideomycetes, Cochliobolus sp.*
AMC5	3	EU301633 (100%), DQ123663 (100%), DQ267827 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC6	3	EU497958 (99%), EU076930 (98%)	*Eurotiomycetes, Penicillium sp.*
AMC7	3	EU167575 (99%), EU167600 (97%)	
AMC8	2	AB220272 (98-100%), AB220246 (99%)	*Sordariomycetes, Arthrinium phaeospermum*
AMC9	2	EU707889 (90%), DQ885901 (89%), EU707898 (89%)	*Dothideomycetes*
AMC10	2	AY561209 (85%), AY999135 (82%)	*Sordariomycetes*
AMC11	2	DQ885894 (99%), AF439470 (99%)	
AMC12	2	AB255248 (98-99%), EU781663 (98%), AB255238 (97%), EU918714 (95%)	*Sordariomycetes, Xylaria sp.*
AMC13	2	FJ040177 (100%), EU828350 (99%)	*Dothideomycetes, Cochliobolus sp.*
AMC14	2	EU514294 (98%)	*Dothideomycetes, Stenella musicola*
AMC15	2	AF071352 (97%)	*Dothideomycetes, Cochliobolus kusanoi*
AMC16	2	DQ092521 (95%), DQ092505 (94%), EU680536 (93%)	*Dothideomycetes*
AMC17	2	DQ184477 (99%), DQ885903 (99%)	*Dothideomycetes*

AMC18	2	EF577236 (99-100%), AY625063 (99-100%)	*Dothideomycetes, Cladosporium sp.*
AMC19	2	EU272517 (99%)	*Sordariomycetes, Annulohypoxylon stygium*
AMC20	1	EU326200 (99%)	*Sordariomycetes, Arthrinium phaeospermum*
AMC21	1	NW001849579 (99%), EU301638 (99%), AY373848 (99%)	*Eurotiomycetes, Aspergillus sp.*
AMC22	1	AY339348 (99%), AY339330 (99%)	*Sordariomycetes*
AMC23	1	EU781672 (99%), AF502780 (99%)	
AMC24	1	AY428782 (100%), U17212 (99%)	*Sordariomycetes, Gaeumannomyces graminis*
AMC25	1	EU680530 (100%), DQ993652 (99%)	*Dothideomycetes, Curvularia sp.*
AMC26	1	FJ478093 (98%)	
AMC27	1	EU041784 (94%), EU552148 (94%), AF064779 (94%)	*Sordariomycetes*
AMC28	1	AB040308 (99%), AF011319 (99%), AB040330 (99%)	*Leotiomycetes, Podosphaera sp.*
AMC29	1	DQ923535 (88%), EU019297 (86%), EU552103 (85%)	*Dothideomycetes*
AMC30	1	AY244417 (99%), EU019290 (98%)	*Dothideomycetes*
AMC31	1	AY373915 (99%), AY373934 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC32	1	FJ037756 (99%)	*Dothideomycetes, Cochliobolus sp.*
AMC33	1	AY907347 (98%), AB286211 (97%), FJ478124 (97%)	*Sordariomycetes, Phomopsis sp.*
AMC34	1	EF060651 (96%), EF619689 (95%), AF422976 (94%)	*Dothideomycetes*
AMC35	1	EF159355 (90%), AM901717 (89%)	
AMC36	1	EU009993 (97%)	*Sordariomycetes*
AMC37	1	FJ037772 (99%), EU167576 (94%)	
AMC38	1	AM176696 (97%), FJ372403 (97%)	
AMC39	1	EF669715 (99%), AF163084 (99%)	
AMC40	1	AY509761 (99%), FJ425204 (99%)	*Dothideomycetes*
AMC41	1	DQ223749 (99-100%), AB449093 (98-99%), EU365344 (98-99%)	*Sordariomycetes*
AMC42	1	EU052786 (90%), AJ005480 (81%)	
AMC43	1	DQ420802 (91%), EF060504 (90%), AJ301967 (88%)	*Sordariomycetes*
AMC44	1	AY840527 (99%), EU167596 (98%)	*Dothideomycetes*
AMC45	1	EU668993 (99%), EU339371 (99%)	*Dothideomycetes*

AMC46	1	EF467438 (93%), EU295634 (93%)	
AMC47	1	AM176695 (100%), DQ993647 (99%)	*Leotiomycetes, Articulospora sp.*
AMC48	1	EF060527 (97%), FJ040177 (97%)	*Dothideomycetes*
AMC49	1	EU179868 (98%)	*Sordariomycetes, Xylaria nigripes*
AMC50	1	EU490102 (93%), EU707898 (90%), FJ372398 (90%)	*Dothideomycetes*
AMC51	1	EU833205 (100%), EU440776 (99%)	*Eurotiomycetes, Aspergillus sp.*
AMC52	1	EU490102 (90%), FJ415477 (89%), EU019278 (89%)	*Dothideomycetes*
AMC53	1	DQ480350 (100%)	*Dothideomycetes, Curvularia sp.*
AMC54	1	EF026143 (99%)	*Sordariomycetes, Hypoxylon rubiginosum*
AMC55	1	EU687122 (84%), EU750691 (78%)	
AMC56	1	DQ123648 (99%)	*Eurotiomycetes, Penicillium cecidicola*
AMC57	1	AF071336 (98%), AF163077 (98%)	*Dothideomycetes, Curvularia sp.*
AMC58	1	FJ037732 (99%),	*Eurotiomycetes, Paecilomyces sp.*
AMC59	1	EU232716 (89%), EU552134 (89%)	*Dothideomycetes*
AMC60	1	EU552134 (98%), EF432273 (98%)	*Dothideomycetes*
AMC61	1	AY394668 (91%), FJ008691 (85%), DQ923535 (84%)	*Dothideomycetes*
AMC62	1	AM850055 (94%)	*Saccharomycetes*

(**B**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
BMC1	3	EU872426 (98%)	*Agaricomycetes, Phanerochaete chrysosporium*
BMC2	2	FJ010207 (98-99%)	*Agaricomycetes, Flavodon flavus*
BMC3	2	DQ912697 (100%), AY219341 (97%)	*Agaricomycetes, Phanerochaete sp.*
BMC4	1	AB361645 (98-99%)	*Agaricomycetes, Phanerochaete chrysosporium*
BMC5	1	FJ481018 (93%), AB361645 (91%)	*Agaricomycetes*
BMC6	1	AB369909 (100%),	*Agaricomycetes, Schizophyllum commune*
BMC7	1	AY969789 (87%), DQ672292 (85%)	
BMC8	1	AB084615 (99%), AB084616 (98%)	*Agaricomycetes, Phlebia brevispora*
BMC9	1	AY456375 (88%), DQ672292 (84%), DQ979014 (83%)	*Agaricomycetes*

BMC10	1	EU118656 (88%), AM902085 (87%), AY271810 (86%)	*Agaricomycetes*
BMC11	1	AY969789 (96%), DQ979014 (84%)	*Agaricomycetes*
BMC12	1	AF475150 (99%)	*Agaricomycetes, Phanerochaete sordida*
BMC13	1	AB099900 (98%)	*Agaricomycetes, Scleroderma sp.*
BMC14	1	EU661879 (98-99%), FJ372671 (98-99%)	*Agaricomycetes*
BMC15	1	FJ462746 (91%), AY730555 (91%), EU918701 (89%)	*Agaricomycetes*
BMC16	1	AB097563 (99%), AY461825 (99%)	*Agaricomycetes, Coprinopsis cinerea*
BMC17	1	EU047803 (88%), EU872426 (88%)	*Agaricomycetes*
BMC18	1	AB210074 (90%), EU118656 (88%), AY605710 (87%)	*Agaricomycetes*
BMC19	1	U85799 (89%), AY216474 (88%), FJ481018 (87%)	*Agaricomycetes*
BMC20	1	U85799 (94%), U65623 (91%), FJ478126 (90%)	*Agaricomycetes*
BMC21	1	EF060520 (94%), AF283726 (89%)	*Agaricomycetes*
BMC22	1	EU872426 (92%), FJ481018 (91%), U85799 (91%)	*Agaricomycetes*
BMC23	1	AF261656 (83%), FJ475570 (78%)	
BMC24	1	EU273512 (97%)	*Agaricomycetes, Clitopilus prunulus*
BMC25	1	AM269816 (99%), AY781273 (99%)	*Agaricomycetes, Trichaptum sp.*
BMC26	1	AY969526 (87%), AY641458 (82%)	
BMC27	1	AF475147 (95%), AB210078 (94%)	*Agaricomycetes*
BMC28	1	AF444556 (99%)	*Agaricostilbomycetes*

Table S9. Puerto Rico. Operational taxonomic units (OTUs) attributed to Ascomycota (A), Basidiomycota (B), and Fungi (C). Operational ID (AMC/BMC/F and running number); frequency of occurrence (number of air samples in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
AMC1	3	EF405864 (100%), AY251074 (99%), AJ300331 (99%)	*Dothideomycetes, Cladosporium sp.*
AMC2	2	EF488380 (98%), EU436688 (97%)	*Sordariomycetes, Eutypella sp.*
AMC3	2	AF510496 (98%), AB353909 (97-98%)	*Eurotiomycetes, Penicillium sp.*
AMC4	1	EU687075 (91%), AY754945 (87%)	*Sordariomycetes*
AMC5	1	DQ480357 (95%), EF026123 (88%)	*Sordariomycetes*
AMC6	1	EU750695 (99%), EU595566 (99%)	*Sordariomycetes, Fusarium sp*
AMC7	1	EU436688 (89%), EF488380 (88%)	*Sordariomycetes*
AMC8	1	AY667576 (85%), DQ784689 (84%)	*Dothideomycetes*
AMC9	1	EF152423 (91%), EU595566 (91%)	*Sordariomycetes*
AMC10	1	AB449093 (99%), DQ223749 (99%), EU365344 (99%)	*Sordariomycetes*
AMC11	1	AF368810 (99%)	
AMC12	1	EU002920 (99%), AF001025 (98%)	*Sordariomycetes*
AMC13	1	AY373849 (100%), EF669614 (99%)	*Eurotiomycetes*
AMC14	1	EU490102 (93%), FJ372400 (90%)	*Dothideomycetes*
AMC15	1	FJ196609 (95%), DQ235697 (92%)	
AMC16	1	DQ421181 (99%), FJ439584 (99%)	*Dothideomycetes*
AMC17	1	EU837206 (90%), DQ979687 (89%)	
AMC18	1	AJ271577 (100%)	*Sordariomycetes, Thielavia microspora*
AMC19	1	FJ037769 (98%), EU547495 (94%), DQ680839 (93%)	

AMC20	1	AY373925 (99%)	*Eurotiomycetes, Penicillium olsonii*
AMC21	1	FJ025204 (99%), DQ889176 (99%)	*Sordariomycetes*
AMC22	1	EU686817 (99%), EU678667 (99%), EU272517 (99%)	*Sordariomycetes*
AMC23	1	AY554211 (98%)	*Sordariomycetes*
AMC24	1	AY999121 (82%), AY587912 (82%)	*Sordariomycetes*
AMC25	1	EU002909 (99%)	
AMC26	1	AF178555 (89%), AB278178 (87%)	
AMC27	1	AM410609 (98%), DQ682585 (98%)	*Sordariomycetes*
AMC28	1	AF033426 (97%)	*Eurotiomycetes, Penicillium paxilli*
AMC29	1	DQ421060 (82%), EU139146 (81%)	
AMC30	1	EU041792 (99%), EF627452 (99%)	*Dothideomycetes*
AMC31	1	EU552114 (86%), EU837209 (85%)	*Pezizomycetes*
AMC32	1	EU687030 (97%), DQ227290 (95%)	*Sordariomycetes*
AMC33	1	AF201756 (99%)	*Sordariomycetes, Nodulisporium sp.*
AMC34	1	EF394859 (98%), AF297226 (97%)	*Dothideomycetes*
AMC35	1	FJ172283 (99%), AF373064 (98%)	*Sordariomycetes, Eutypella sp.*
AMC36	1	EU436681 (96%)	*Sordariomycetes*
AMC37	1	AY373930 (99%)	*Eurotiomycetes, Penicillium sclerotiorum*
AMC38	1	AY862570 (85%), EF157664 (84%)	*Sordariomycetes*
AMC39	1	EF434060 (90%), FJ475656 (84%)	
AMC40	1	EU489996 (92%), FJ415477 (92%)	
AMC41	1	EU826887 (91%), AJ558114 (91%), EU557361 (91%)	*Sordariomycetes*
AMC42	1	EF197817 (99%), EF060638 (99%), DQ680839 (98%)	
AMC43	1	DQ421072 (88%), FJ554031 (87%)	

(**B**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
BMC1	7	AM901952 (92%), EU118665 (91%), AY219349 (91%)	*Agaricomycetes*
BMC2	4	AY216475 (99%)	*Agaricomycetes, Marasmius cladophyllus*
BMC3	3	AY605710 (98-99%), AB210077 (95%)	*Agaricomycetes*
BMC4	3	AF506490 (98-99%)	*Agaricomycetes, Wrightoporia tropicalis*
BMC5	3	AY443531 (97-98%)	*mitosporic Agaricomycotina, Rhizoctonia sp*
BMC6	3	AY884175 (87%), EU030180 (87%), AM988622 (87%)	*Agaricomycetes*
BMC7	2	EU118668 (82%), AF533965 (81%), AF126893 (79%)	*Agaricomycetes*
BMC8	2	AY219367 (93%), AB210074 (90%)	*Agaricomycetes*
BMC9	2	AF200240 (98%), AY558649 (98%)	*Agaricomycetes*
BMC10	2	AY641458 (90%), DQ411529 (85%)	*Agaricomycetes*
BMC11	2	AB158315 (93%), AY089738 (93%)	*Agaricomycetes*
BMC12	1	EU735845 (99%)	*Agaricomycetes, Ganoderma sp.*
BMC13	1	AY254874 (85%), AJ606041 (85%), AF440673 (79%)	*Agaricomycetes*
BMC14	1	AY969532 (86%), EU003034 (84%), DQ411529 (83%),	*Agaricomycetes*
BMC15	1	AY216475 (92%), EF060532 (90%), AY884175 (89%)	*Agaricomycetes*
BMC16	1	EU118668 (83%), AF126893 (81%)	*Agaricomycetes*
BMC17	1	AY641458 (89%), EU003034 (89%), DQ411529 (83%)	*Agaricomycetes*
BMC18	1	EU003034 (85%), DQ411529 (83%)	*Agaricomycetes*
BMC19	1	EU292528 (89%), AM113458 (87%), DQ309116 (83%)	*Agaricomycetes*
BMC20	1	AM902035 (92%), DQ200924 (91%)	*Agaricomycetes*
BMC21	1	AY509555 (95%), AY969449 (94%), AF291275 (94%)	*Agaricomycetes*
BMC22	1	AY969535 (87%), EU003034 (85%), DQ411529 (85%)	*Agaricomycetes*
BMC23	1	AY571042 (90%), DQ097355 (90%), EU668272 (88%)	*Agaricomycetes*
BMC24	1	EU085019 (96%), AY787680 (88%)	*Agaricomycetes*

BMC25	1	EU326212 (94%), AJ279465 (94%), EU918694 (93%)	*Agaricomycetes*
BMC26	1	DQ411529 (85%), AY969535 (85%)	*Agaricomycetes*
BMC27	1	AY641458 (83%), DQ411529 (83%)	*Agaricomycetes*
BMC28	1	AB084616 (99%), AB084614 (98%)	*Agaricomycetes, Phlebia brevispora*
BMC29	1	AM269811 (91%), EU273556 (90%), AY618666 (90%)	*Agaricomycetes*
BMC30	1	AY781265 (94%), EU118665 (93%)	*Agaricomycetes*
BMC31	1	DQ241775 (88%), EU818696 (85%), AF210828 (84%)	*Agaricomycetes*
BMC32	1	AB298703 (94%), FJ010207 (91%), U85799 (90%)	*Agaricomycetes*
BMC33	1	DQ826548 (100%), DQ826549 (99%)	*Agaricomycetes, Resinicium saccharicola*
BMC34	1	AY509554 (86%)	*Agaricomycetes*
BMC35	1	FJ236850 (84%), AJ606043 (84%)	*Agaricomycetes*
BMC36	1	AB071802 (93%)	*Agaricomycetes*
BMC37	1	AB210074 (97%	*Agaricomycetes, Phlebia acanthocystis*
BMC38	1	AF291284 (99%)	*Agaricomycetes, Heterochaete shearii*
BMC39	1	AY558620 (99%)	*Agaricomycetes, Fuscoporia gilva*
BMC40	1	DQ873633(81%), DQ873651 (80%)	*Agaricomycetes*
BMC41	1	AY787680 (84%), AB361645 (79%)	*Agaricomycetes*
BMC42	1	AJ006671 (85%), AB361645 (83%)	*Agaricomycetes*
BMC43	1	AB361645 (79%), EU326212 (79%)	*Agaricomycetes*
BMC44	1	AY787680 (83%), EU326212 (79%)	*Agaricomycetes*
BMC45	1	DQ411529 (87%), AY641458 (81%), AY969566 (80%)	*Agaricomycetes*
BMC46	1	AB210074 (90%), EU118665 (89%), AJ006683 (88%)	*Agaricomycetes*
BMC47	1	EU003034 (88-89%), DQ411529 (88-89%), AY641458 (87-88%)	*Agaricomycetes*
BMC48	1	DQ340333 (99%)	*Agaricomycetes, Hyphodontia palmae*
BMC49	1	AF347088 (99%)	*Agaricomycetes, Trechispora sp.*
BMC50	1	EU118662 (95%), EF174448 (94%)	*Agaricomycetes*
BMC51	1	AY968077 (96%), EU009979 (95%)	*Agaricomycetes*
BMC52	1	AY089740 (91%), AY219393 (87%)	*Agaricomycetes*
BMC53	1	DQ873641 (90%)	*Agaricomycetes*

BMC54	1	AY633927 (95%), AF141624 (86%)	*Agaricomycetes*
BMC55	1	AY089738 (94%), AB158314 (94%)	*Agaricomycetes*
BMC56	1	AY216474 (90%), EF174448 (90%)	*Agaricomycetes*
BMC57	1	AF508346 (84%), AM901912 (79%)	*Agaricomycetes*
BMC58	1	DQ249274 (90%), AY969526 (85%)	*Agaricomycetes*
BMC59	1	AY558647 (86%), AM269803 (83%)	*Agaricomycetes*
BMC60	1	DQ873618 (87%), DQ873601 (87%)	*Agaricomycetes*
BMC61	1	AY558635 (85%), AY558613 (85%)	*Agaricomycetes*
BMC62	1	FJ475775 (87%), FJ475704 (87%)	*Agaricomycetes*
BMC63	1	EF174448 (89%), EU118662 (88%)	*Agaricomycetes*
BMC64	1	FJ475775 (88%), FJ475704 (88%)	*Agaricomycetes*
BMC65	1	FJ475775 (84%), FJ475704 (84%)	*Agaricomycetes*
BMC66	1	FJ475775 (87%), FJ475704 (87%)	*Agaricomycetes*
BMC67	1	AY969526 (96%)	
BMC68	1	AY263444 (96%), DQ450042 (94%)	*Agaricomycetes*
BMC69	1	EU790491 (84%), FJ478098 (84%)	*Agaricomycetes*
BMC70	1	AY254874 (91%), FM866336 (91%)	*Agaricomycetes*
BMC71	1	DQ340323 (96%)	*Agaricomycetes*
BMC72	1	AB210074 (87%), AJ006683 (85%)	*Agaricomycetes*

(**C**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores
F1	1	EU490118 (90%), EU490099 (89%), AY641458 (88%)
F2	1	EU490118 (89%), AY641458 (85%)
F3	1	EU490099 (90%), EU490118 (90%)
F4	1	EU490118 (84%), EU490099 (82%)
F5	1	AY035664 (88%), AF455468 (88%)
F6	1	EU490099 (90%), EU490118 (88%)
F7	1	EU490118 (84%), EU003034 (84%)
F8	1	EU003034 (90%), EU490180 (84%), EU490118 (84%)
F9	1	AF064595 (91%), AY593868 (87%)

Table S10. Ship. Operational taxonomic units (OTUs) attributed to Ascomycota (A) and Basidiomycota (B). Operational ID (AMC/BMC and running number); frequency of occurrence (number of air samples in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
AMC1	7	EU833212 (99-100%), DQ339568 (99%), DQ339549 (99%), DQ339561 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC2	4	AM883156 (99%), AF455505 (99%), AY373869 (99%)	*Eurotiomycetes, Aspergillus sp.*
AMC3	3	EF060638 (99%), EF197817 (99%), DQ680839 (99%)	
AMC4	3	AY251074 (100%), AJ876482 (100%), EF432298 (98%)	*Dothideomycetes, Cladosporium sp.*
AMC5	2	AF455536 (100%), AF455528 (100%), AF459728 (99%)	*Eurotiomycetes, Eurotium sp.*
AMC6	2	EU439264 (97-100%), DQ480361 (97-100%), EU918714 (96-99%), EU002898 (97%)	*Sordariomycetes*
AMC7	1	EU035417 (84%), EU035415 (84%), EU019265 (83%)	
AMC8	1	EU497960 (94%), EU497942 (94%)	*Eurotiomycetes*
AMC9	1	AF540513 (97%)	*Lecanoromycetes, Dirinaria confluens*
AMC10	1	EF417482 (100%)	*Sordariomycetes, Trichoderma atroviride*
AMC11	1	EU497942 (100%), EF198663 (100%)	*Eurotiomycetes, Penicillium sp.*
AMC12	1	EU085019 (97-99%%), FJ213547 (85-86%)	*Eurotiomycetes*
AMC13	1	EF652084 (95%), EF652082 (95%)	*Eurotiomycetes*
AMC14	1	EU678658 (96%), EU552100 (95%)	*Sordariomycetes*
AMC15	1	AY373862 (96%)	*Eurotiomycetes*
AMC16	1	EU552105 (96%), DQ885897 (96%)	*Dothideomycetes*
AMC17	1	EU918714 (99%)	*Sordariomycetes, Nigrospora oryzae*

AMC18	1	AF368810 (94%), DQ681344 (93%)	
AMC19	1	AJ271575 (97%), AJ271576 (96%)	*Sordariomycetes, Thielavia sp.*
AMC20	1	EU167576 (86%), EU547495 (86%)	*Dothideomycetes*
AMC21	1	EU707879 (99%)	*Dothideomycetes, Teratosphaeria parva*
AMC22	1	EF619689 (95%), AY208791 (94%)	*Dothideomycetes*
AMC23	1	AY251071 (99%), AY251070 (99%)	*Dothideomycetes, Cladosporium sp.*
AMC24	1	FJ176470 (99%), AY681194 (99%), AY681193 (99%)	*Sordariomycetes*
AMC25	1	AY971709 (98%), DQ270247 (95%)	
AMC26	1	DQ420990 (95%), AJ246158 (94%)	*Sordariomycetes*
AMC27	1	AF201754 (98%)	*Sordariomycetes, Nodulisporium sp.*
AMC28	1	AY373941 (100%), EU037053 (100%)	*Eurotiomycetes, Paecilomyces sp.*
AMC29	1	AY213678 (100%), EF198531 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC30	1	AB080725 (99-100%), EU262660 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC31	1	EU009985 (94%), AB041994(93%), EF026122 (93%)	*Sordariomycetes*
AMC32	1	AY373886 (93%), AY373885 (93%), EF652082 (93%)	*Eurotiomycetes*
AMC33	1	DQ336713 (93%), AJ246158 (92%), DQ420990 (91%)	*Sordariomycetes*
AMC34	1	AM901741 (86%), AF050278 (84%), FJ150695 (82%)	
AMC35	1	AB449093 (99%), DQ223748 (99%)	*Sordariomycetes*
AMC36	1	AY373904 (99%), EU497951 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC37	1	FJ360521 (95%), AY681194 (95%), EU918705 (94%)	*Sordariomycetes*
AMC38	1	EU041797 (94%), DQ421272 (87%)	*Dothideomycetes*
AMC39	1	EU707887 (98%)	*Dothideomycetes*
AMC40	1	DQ468027 (98-100%)	*Dothideomycetes*
AMC41	1	AF461673 (89%), FJ360521 (88%), EU918705 (88%)	*Sordariomycetes*
AMC42	1	FJ176474 (95%), EU833212 (95%)	*Eurotiomycetes, Penicillium sp.*
AMC43	1	AY373930 (99%), EF634374 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC44	1	EU552113 (85%), AY843195 (84%), AM901723 (83%)	
AMC45	1	AJ390411 (99%)	*Sordariomycetes, Biscogniauxia atropunctata*

AMC46	1	AB105954 (99%),	*Sordariomycetes, Ustilaginoidea virens*
AMC47	1	EU040243 (95%), EU167574 (91%)	*Dothideomycetes*
AMC48	1	DQ631933 (84%), AF153729 (83%)	*Sordariomycetes*
AMC49	1	FJ025341 (97%), EU683044 (94%)	*Dothideomycetes*
AMC50	1	AY373930 (94%), EU833220 (93%)	*Eurotiomycetes, Penicillium sp.*
AMC51	1	AF033401 (99%), U72594 (98%)	
AMC52	1	AY373915 (100%), DQ681325 (99%)	*Eurotiomycetes, Penicillium sp.*
AMC53	1	EU833211 (99%)	*Eurotiomycetes, Penicillium brevicompactum*
AMC54	1	EU552134 (99%), EF432273 (99%), EU552164 (99%)	*Dothideomycetes*

(**B**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
BMC1	1	AB084617 (91%), AB210074 (89%), AJ006683 (88%)	*Agaricomycetes*
BMC2	1	AJ555514 (93%), AF516569 (89%)	*Agaricomycetes*
BMC3	1	U85795 (94-95%)	*Agaricomycetes*
BMC4	1	EF672293 (95%, 97%), EU918698 (92-93%)	*Agaricomycetes*
BMC5	1	EU818699 (96%), EF529621 (95%)	*Agaricomycetes*
BMC6	1	AM901849 (99%), AF210825 (97%)	*Agaricomycetes*
BMC7	1	AB210075 (92%), EU118665 (90%)	*Agaricomycetes*
BMC8	1	EF060520 (94-95%)	*Agaricomycetes*
BMC9	1	EU118662 (95%), EF174448 (95%)	*Agaricomycetes*
BMC10	1	AB210074 (88%), AJ006683 (85%), EU118665 (85%)	*Agaricomycetes*
BMC11	1	AM946630 (94%), DQ989497 (94%), AJ419204 (94%)	*Agaricomycetes*
BMC12	1	AM901849 (98%), DQ350127 (97%), DQ411533 (97%)	*Agaricomycetes*
BMC13	1	U85799 (94%), U65623 (92%)	*Agaricomycetes*
BMC14	1	AY219341 (97%), EU118662 (95%), EF174448 (94%),	*Agaricomycetes*
BMC15	1	DQ900975 (87%), DQ900958 (87%), AF347092 (84%)	*Agaricomycetes*

BMC16	1	EU661879 (98%), FJ372671 (98%)	*Agaricomycetes*
BMC17	1	AY484692 (99%)	*Agaricomycetes, Agaricus bisporus*
BMC18	1	FJ010208 (99%)	*Agaricomycetes, Cerrena sp.*
BMC19	1	EU918699 (97%)	*Agaricomycetes, Coprinopsis strossmayeri*
BMC20	1	FJ010207 (98-99%)	*Agaricomycetes, Flavodon flavus*
BMC21	1	AY627794 (97%), AY969566 (97%)	

Table S11. Taiwan. Operational taxonomic units (OTUs) attributed to Ascomycota (A) and Basidiomycota (B). Operational ID (AMC/BMC and running number); frequency of occurrence (number of air samples in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
AMC1	7	AY251074 (99%), EF432298 (98%)	*Dothideomycetes, Cladosporium sp.*
AMC2	4	EU687125 (99%), EU918714 (97-99%)	*Sordariomycetes, Nigrospora oryzae*
AMC3	2	AF153741 (99%), AF153742 (99%)	*Sordariomycetes*
AMC4	2	EF207415 (99%), AJ279480 (99%), EF153017 (98%)	*Leotiomycetes*
AMC5	2	AF203451 (100%), AY372679 (100%)	*Dothideomycetes, Stemphylium sp.*
AMC6	2	EU436763 (99%)	*Dothideomycetes, Devriesia strelitziae*
AMC7	1	AB073533 (99%)	*Sordariomycetes, Xylaria sp.*
AMC8	1	FJ172283 (99%), DQ480354 (99%)	*Sordariomycetes, Eutypella sp.*
AMC9	1	AB027380 (100%), AB189444 (100%)	*Sordariomycetes*
AMC10	1	AB360370 (93-94%), AB360367 (93%), EF555097 (93%)	*Sordariomycetes*
AMC11	1	AB284189 (98-99%), EU363038 (98-99%), AF280628 (98-99%)	*Sordariomycetes*

AMC12	1	EF417483 (100%), EU401558 (99%), X93933 (99%), X93964 (99%)	*Sordariomycetes*
AMC13	1	DQ318204 (99%), AF275488 (99%)	*Sordariomycetes*
AMC14	1	AY999133 (98%), AY999135 (98%)	*Sordariomycetes*
AMC15	1	DQ322087 (98%), FJ205454 (98%)	*Sordariomycetes*
AMC16	1	EU690794 (96%), AB297798 (94%), AF502793 (91%)	*Sordariomycetes*
AMC17	1	FJ176470 (88%), AY681195 (88%)	*Sordariomycetes*
AMC18	1	AY254161 (88%)	*Sordariomycetes*
AMC19	1	DQ420990 (91%), AJ246158 (90%)	*Sordariomycetes*
AMC20	1	DQ491504 (99%)	*Pezizomycetes, Ascobolus crenulatus*
AMC21	1	AF505516 (98%)	*Leotiomycetes, Lachnum hyalopus*
AMC22	1	EU833210 (99%), AY373868 (99%), AF455499 (99%)	*Eurotiomycetes, Aspergillus sp.*
AMC23	1	EU085019 (99%%), FJ213547 (87%)	*Eurotiomycetes*
AMC24	1	EU707900 (85%), EU019278 (84%), EU343297 (84%)	*Dothideomycetes*
AMC25	1	EU552134 (100%), AJ279463 (100%), EU552164 (100%)	*Dothideomycetes*
AMC26	1	EF619925 (99%), EU700365 (99%)	*Dothideomycetes*
AMC27	1	AJ300331 (90%), FJ213502 (90%)	*Dothideomycetes*
AMC28	1	EU935608 (96%), FJ378087 (96%)	*Dothideomycetes*
AMC29	1	EU041803 (99%)	*Dothideomycetes*
AMC30	1	DQ863675 (89%), DQ974708 (88%),	*Dothideomycetes*
AMC31	1	FJ025285 (98-99%), AF502787 (98%)	
AMC32	1	EU167575 (99%), AY131203 (99%)	
AMC33	1	DQ388849 (92%), AB255289 (91%)	
AMC34	1	DQ885895 (92%), AJ582956 (92%)	
AMC35	1	AJ877178 (100%), AF502803 (98%)	
AMC36	1	EU167608 (98%), AF455533 (98%), DQ680839 (98%)	
AMC37	1	AB085929 (99%), EF550970 (99%)	
AMC38	1	DQ420962 (90%), EU049289 (97%)	
AMC39	1	EU750691(90%), EU040239 (89%)	
AMC40	1	AY238606 (91%)	

(**B**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
BMC1	4	AM901952 (92%), EU118665 (91-92%), AY219349 (91-92%)	*Agaricomycetes*
BMC2	3	FJ235147 (88%), DQ873599 (81%), EU826082 (80%)	*Agaricomycetes*
BMC3	3	AY627794 (97%), AY969566 (97%)	
BMC4	2	AB210077 (99%)	*Agaricomycetes, Phlebia sp.*
BMC5	1	EF687930 (97%), AY805625 (93%)	*Agaricomycetes*
BMC6	1	FJ010208 (99%), AY433810 (99%), EF060457 (99%)	*Agaricomycetes*
BMC7	1	AB361644 (91%), AY216474 (90%), U85799 (90%)	*Agaricomycetes*
BMC8	1	FJ010207 (98%)	*Agaricomycetes, Flavodon flavus*
BMC9	1	AB210074 (99%)	*Agaricomycetes, Phlebia acanthocystis*
BMC10	1	DQ404386 (98%), AY558596 (97%)	*Agaricomycetes, Hydnochaete sp.*
BMC11	1	DQ411529 (87%), AY641458 (85%)	*Agaricomycetes*
BMC12	1	AM901952 (92%), EU118665 (92%), AY219349 (91%)	*Agaricomycetes*
BMC13	1	AB084617 (99%)	*Agaricomycetes, Phlebia chrysocreas*
BMC14	1	AF347080 (92%), EU003034 (92%), AY969566 (90%)	*Agaricomycetes*
BMC15	1	AY089740 (93%), AY271810 (92%)	*Agaricomycetes*
BMC16	1	AY219343 (99%), AB210078 (98%)	*Agaricomycetes*

Table S12. United Kingdom. Operational taxonomic units (OTUs) attributed to Ascomycota (A) and Basidiomycota (B). Operational ID (AMC/BMC and running number); frequency of occurrence (number of air samples in which the species was detected); most characteristic NCBI accession numbers sorted by similarity score; taxonomic class and species name according to NCBI data base (if determined). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

(**A**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
AMC1	5	AY251074 (99%), AJ876482 (99%), AJ300331 (99%)	*Dothideomycetes, Cladosporium sp.*
AMC2	4	EF207415 (99%), AJ279480 (99%)	*Leotiomycetes*
AMC3	3	AY154692 (98-99%), AY278834 (98%)	*Dothideomycetes, Alternaria sp.*
AMC4	3	FM875938 (99-100%), AF520641 (99%), EU167564 (97%)	AMC
AMC5	2	AB273527 (99%)	*Leotiomycetes, Blumeria graminis*
AMC6	2	AM901991 (99%), AF435827 (97-98%), AJ300336 (97-98%)	AMC
AMC7	2	EF197817 (99%), DQ680839 (99%), DQ680839 (99%),	AMC
AMC8	2	AF459728 (100%), U18357 (99%)	*Eurotiomycetes, Eurotium sp.*
AMC9	1	AY373862 (97%)	*Eurotiomycetes, Aspergillus penicillioides*
AMC10	1	U77358 (98%), U77361 (97%)	*Dothideomycetes, Phaeosphaeria sp.*
AMC11	1	FJ553913 (98%)	AMC
AMC12	1	AF049088 (95%), DQ435529 (94%)	AMC
AMC13	1	DQ420814 (99%), AF383953 (97%), AB433276 (97%)	*Dothideomycetes, Lophiostoma sp.*
AMC14	1	AF138814 (89%), EU490056 (86%)	AMC
AMC15	1	AY590793 (99%)	AMC
AMC16	1	DQ683973 (93%), DQ420990 (89%)	AMC
AMC17	1	FJ553513 (86%), DQ351724 (85%)	AMC
AMC18	1	EU490073 (89%), EU489976 (86%)	AMC
AMC19	1	EU041799 (99%)	*Dothideomycetes, Ramichloridium indicum*
AMC20	1	EF187912 (99%)	*Sordariomycetes, Microdochium nivale*

AMC21	1	EU490089 (98%), Z81442 (98%)	*Leotiomycetes, Poculum henningsianum*
AMC22	1	DQ683973 (94%), AM084451 (93%)	AMC
AMC23	1	DQ683973 (95%), AM084451 (94%)	AMC
AMC24	1	AJ244263 (92%), AJ507323 (88%)	AMC
AMC25	1	DQ404349 (95%), AB434662 (94%)	AMC
AMC26	1	EU490000 (92%), AY916492 (89%)	AMC
AMC27	1	AM262373 (94%), AY265336 (83%)	AMC
AMC28	1	EU998929 (95%), EF601602 (95%)	AMC
AMC29	1	EU041813 (85%), EU750692 (81%)	AMC
AMC30	1	EU035433 (95%), EU035448 (94%)	*Dothideomycetes*
AMC31	1	EF207415 (92%), AJ279480 (92%)	*Leotiomycetes*

(**B**)

OTU	Frequency of occurrence	NCBI accession numbers and similarity scores	Class, species
BMC1	5	FJ481021 (96%)	*Agaricomycetes, Vuilleminia comedens*
BMC2	5	AB072233 (99%)	*Tremellomycetes, Itersonilia perplexans*
BMC3	3	AY309018 (99%), AF455480 (98%)	*Agaricomycetes*
BMC4	3	AM160645 (100%), AF444546 (99%)	*Cystobasidiomycetes, Sporobolomyces sp.*
BMC5	2	AY015438 (100%)	*Microbotryomycetes, Sporobolomyces roseus*
BMC6	2	AY740066 (99%), AY740169 (99%)	*Ustilaginomycetes, Ustilago sp.*
BMC7	2	EU622849 (98%)	*Agaricomycetes, Limonomyces culmigenus*
BMC8	1	DQ398959 (99%), FJ481021 (98%)	*Agaricomycetes, Vuilleminia comedens*
BMC9	1	FJ228210 (99%)	*Agaricomycetes, Phanerochaete sordida*
BMC10	1	AB361644 (99-100%)	*Agaricomycetes, Phanerochaete chrysosporium*
BMC11	1	AY781266 (99%)	*Agaricomycetes, Phanerochaete sordida olrim*
BMC12	1	AB072232 (99%)	*Tremellomycetes, Udeniomyces pannonicus*
BMC13	1	DQ200924 (99%)	*Agaricomycetes, Botryobasidium subcoronatum*
BMC14	1	AY787677 (100%), DQ411533 (99%)	*Agaricomycetes, Peniophora sp.*
BMC15	1	DQ182503 (99%)	*Agaricomycetes, Panaeolus sphinctrinus*
BMC16	1	DQ403253 (97%)	*Agaricomycetes, Panaeolus sp.*

BMC17	1	AF444322 (85%), AY038835 (85%)	*Tremellomycetes*
BMC18	1	AF444448 (99%), AY052490 (99%)	*Tremellomycetes, Cryptococcus sp.*
BMC19	1	DQ157700 (99%)	*Ustilaginomycetes, Ustilago maydis*
BMC20	1	AY618243 (98%), AF210821 (96%)	*Agaricomycetes, Peniophora sp.*
BMC21	1	EF174437 (99%)	*Agaricomycetes, Phlebiopsis gigantea*
BMC22	1	AY854063 (99%), DQ000294 (99%)	*Agaricomycetes, Stereum sp.*
BMC23	1	EU622846 (98%)	*Agaricomycetes, Limonomyces roseipellis*
BMC24	1	AY254874 (89%), AF394919 (86%)	*Agaricomycetes*
BMC25	1	DQ672316 (89%), EU622849 (85%)	*Agaricomycetes*
BMC26	1	DQ873599 (99%)	*Agaricomycetes, Hyphoderma puberum*

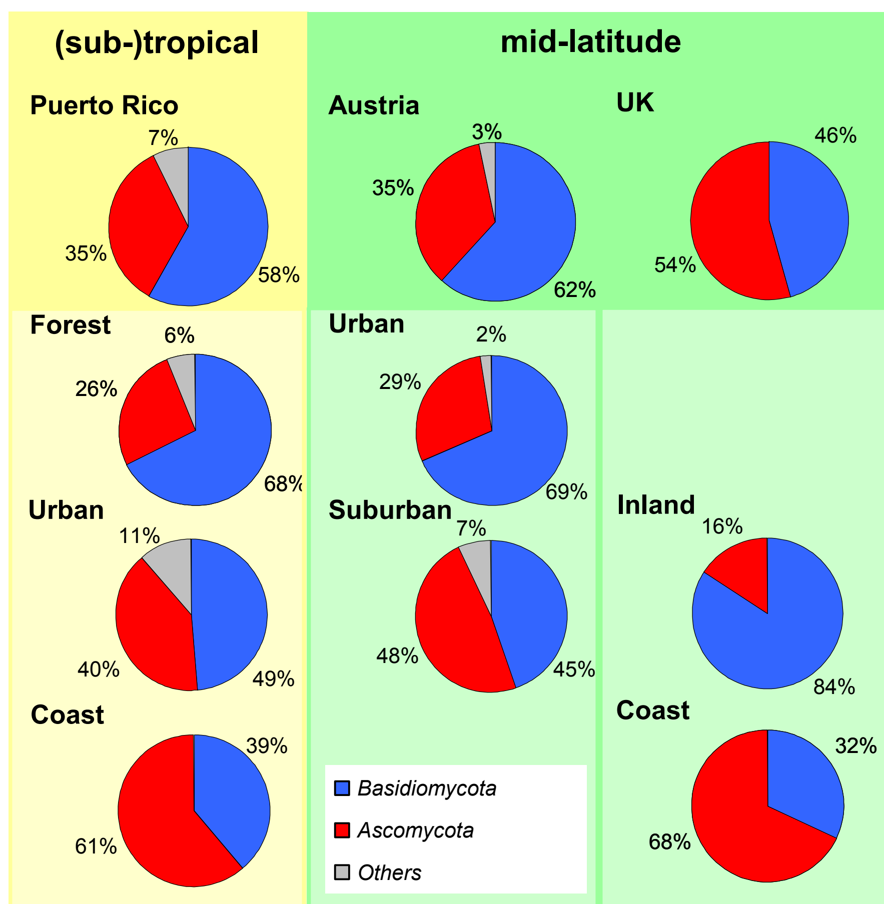

Fig. S1. Species richness of airborne fungi: relative proportions of different phyla at different sampling sites in Puerto Rico, Austria, and United Kingdom (UK; Inland = TORCH1, Coast = TORCH2).

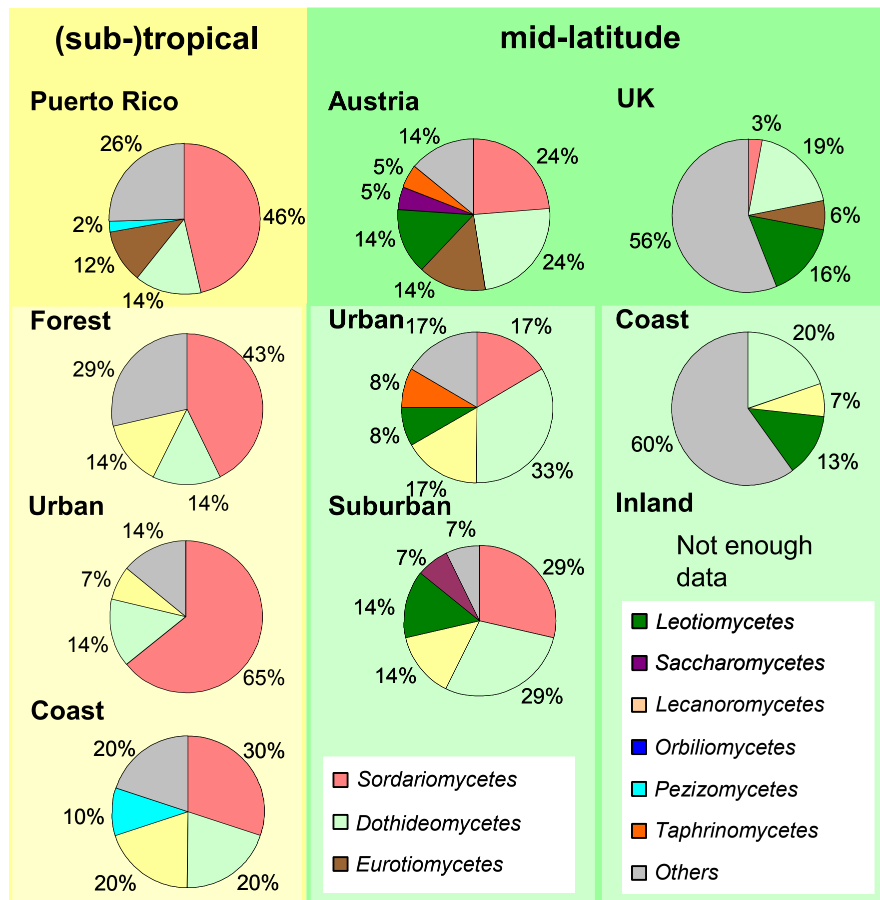

Fig. S2. Species richness of airborne fungi: relative proportions of different classes of *Ascomycota* at different sampling sites in Puerto Rico, Austria, and United Kingdom (UK; Inland = TORCH1, Coast = TORCH2).

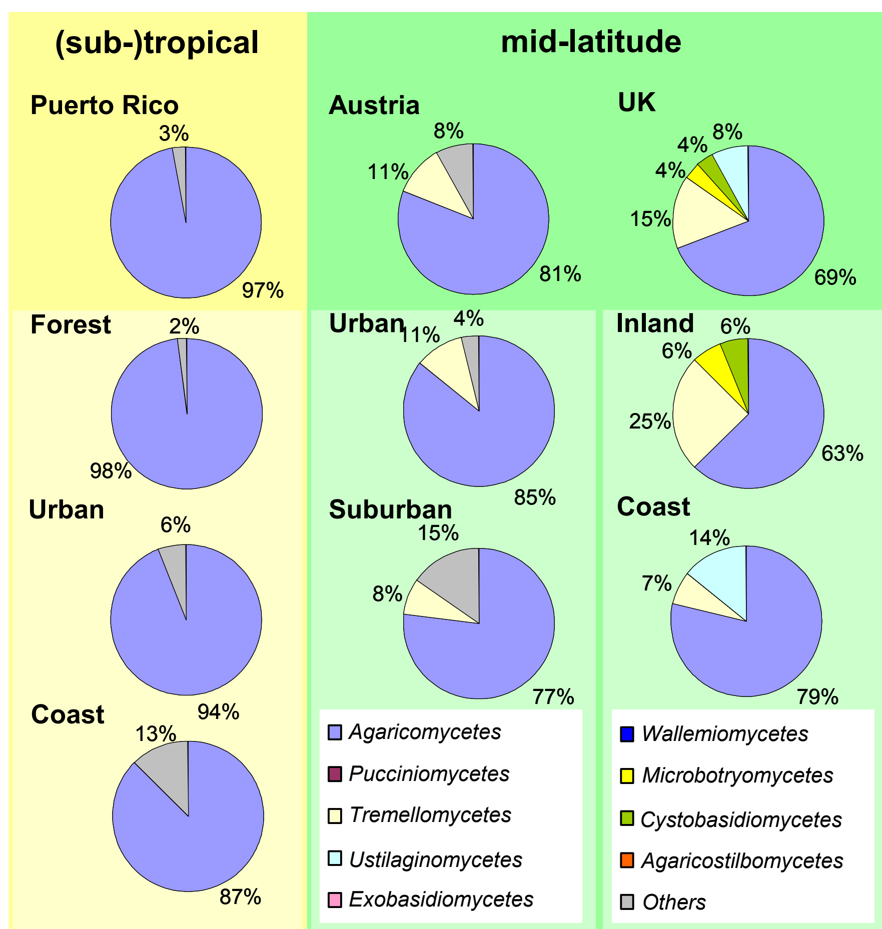

Fig. S3. Species richness of airborne fungi: relative proportions of different classes of *Basidiomycota* at different sampling sites in Puerto Rico, Austria, and United Kingdom (UK; Inland = TORCH1, Coast = TORCH2).