Supplementary Material for the manuscript:

Biogeography in the air: fungal diversity over land and oceans

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To complement the information given in the main manuscript, the following sections provide Supplementary Text, Supplementary Figures S1-S4 and Legends, Supplementary Tables S1-S12, and Supplementary References.

Supplementary Text

Blank and Background samples. Whereas no DNA could be detected in the filter blanks (baked and unbaked), fungal DNA was detected in 4% of the extraction or PCR blank reactions, indicating that contaminations occurred rarely during analysis in the laboratory. The PCR products obtained 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 (Hughes et al., 1994; Loeffler et al., 1999; Meier et al., 1993). In our study the initially observed 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 was likely due to contaminations originating from the PCR. Six species, e.g., *Brettanomyces bruxellenis* and *Candida tropicalis* were only detected in extraction blanks. Possibly, they were 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 contaminants.

As described in Fröhlich-Nowoisky et al. (2009), no DNA was detected in the blank samples from Mainz, Germany, indicating that no contaminations occurred during sample handling and analysis in the laboratory. However, four of the OTUs identified as possible contaminants in the other sets of filter samples were detected in the samples from Mainz **(***Candida deformans, Candida tropicalis, Nectria sp., Alternaria sp.*). 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.

Impact of different sampling methods and conditions. As described under materials and methods, the samples from different locations were collected with different types of samplers, cut-off diameters, and filter substrates. In addition, the sampled air volumes, sampling periods (year, season) and sample storage conditions were different (Tabs. S2-S9, material and method section and references therein). These differences may have influenced the results obtained for different measurement locations as follows. 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) and in others the inlet cut-off is wind speed dependant possibly varying from ~30 to 100 μm. The sampling height can influence 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 also influence recovery of DNA from air samples, because DNA starts to degrade as soon as an organism dies. Spores resist environmental stress and atmospheric transport and are thus unlikely to degrade during sampling (Griffin, 2004, Griffin and Kellog, 2004). Fungal tissue fragments, however, may be more rapidly degraded in tropical climates because DNA is best preserved under dry and cool conditions (Després et al., 2007; Pääbo at al., 2004). Furthermore, different storage times and conditions might have led to different degrees of DNA degradation in the investigated sets of samples. Thus, different sampling and storage conditions should be kept in mind when comparing the different sets of filter samples investigated in this study. The comparability of absolute values of species richness determined for different sampling locations and regions is also limited by the different numbers of investigated samples. Nevertheless, the experimental results do not indicate any bias of the applied methods with regard to the relative proportions between AMC and BMC. The consistency of major trends and similarities observed over all types of samples suggests that the main findings and conclusions of this study (gross

differences AMC/BMC in continental and marine air, major classes of AMC and BMC, etc.) are not significantly affected by the uncertainties outlined above.

Emisson and transport of fungal spores. Air is the primary medium for the dispersal of fungal spores, which can be transported over long distances depending on spore properties and atmospheric conditions (Brown et al., 2002; Hirst et al., 1967*)*. An essential condition for widespread dispersal is that the spores have to leave the laminar boundary layer at the surface and enter into turbulent air flow in the atmosphere (Gregory, 1973; Lacey, 1996; Madelin, 1994). This can be achieved through a range of active and passive discharge and liberation processes of spores (Lacey, 1996; Ingold, 1971; Elbert et al., 2007). The residence time of fungal spores in air depends on their size and decreases with increasing aerodynamic diameter (Gregory, 1973; Lacey, 1996). Because the spores of many BMC (~5-10 μm) are typically larger than those of prominent airborne AMC (~2-5 μm) (Fröhlich-Nowoisky et al., 2009; Ingold, 2001; Lacey, 1996; Muilenberg, 1995; Stenlid, 2008), they are expected to be deposited more rapidly and are less likely to undergo long-range transport, as also indicated by global atmospheric transport model results (Fig. S4).

Atmospheric loss processes can be conceptualized in an idealized manner as an exponential decay over time of the total particle mass remaining in the atmosphere. For particles emitted instantaneously at time t=0, the remaining mass M is given by $M(t) = M_0 \exp(-t/\tau)$, with M_0 the initial mass and τ the size-dependent atmospheric residence time. In Figure S4, we show how this idealized conceptualization of atmospheric loss processes would result in an increase in the abundance of the smaller AMC relative to the larger BMC over time, in an air parcel that had lost contact with the source.

Due to varying discharge mechanisms and meteorological conditions, the concentration and diversity of airborne fungal spores are likely to vary with the time of day and altitude above ground. These effects should have no influence on the systematic differences between continental and marine sampling sites observed in this study. To develop a comprehensive understanding of temporal and spatial variability of the atmospheric biogeography and transport of fungi, however,

we propose and intend to pursue further studies and experiments including air samples collected at different altitudes and distances from the coast.

Supplementary Figures S1-S4

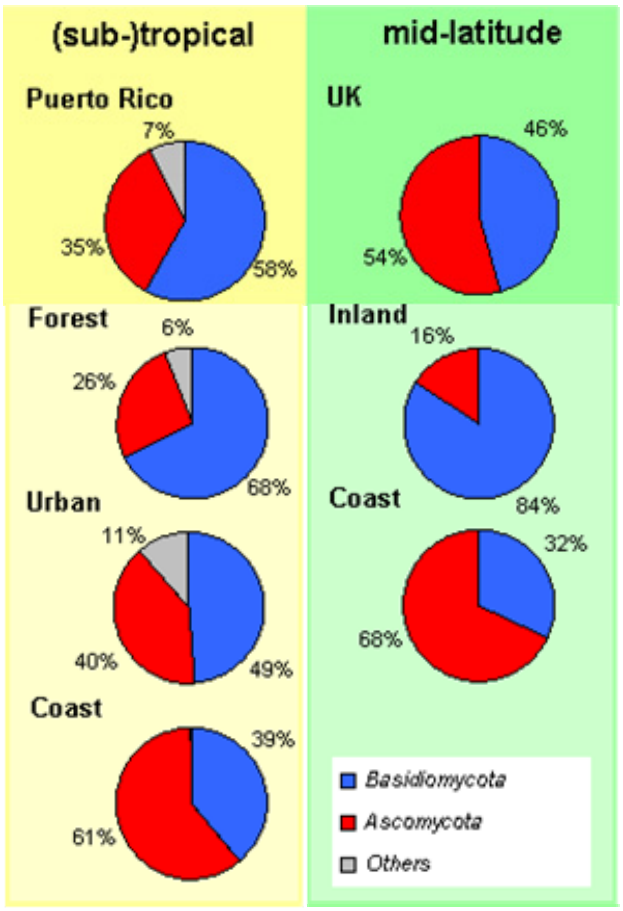

Figure S1. Species richness of airborne fungi: relative proportions of different phyla at different sampling sites in Puerto Rico (forest, urban, coast) and United Kingdom (UK; Inland = Chelmsford, Essex, Coast = Weybourne).

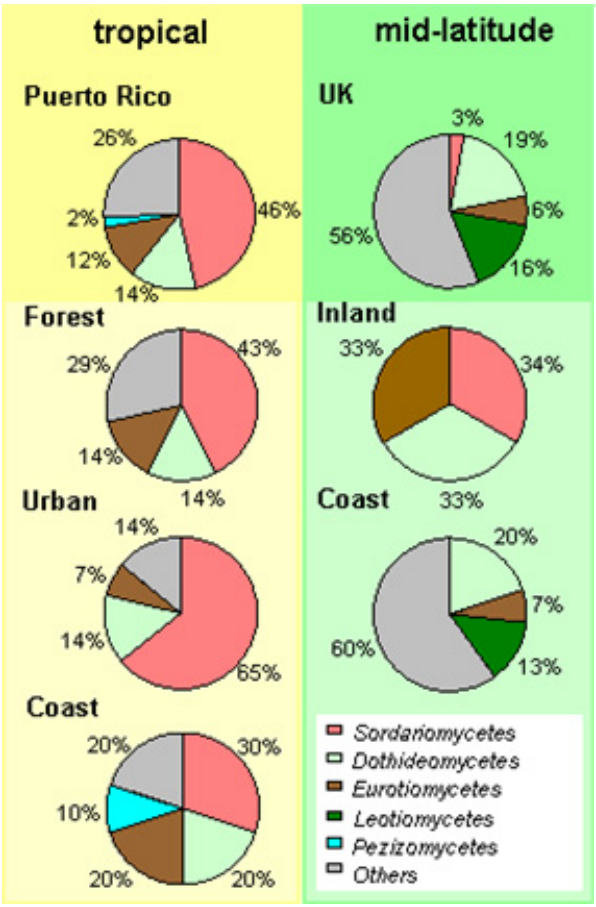

Figure S2. Species richness of airborne fungi: relative proportions of different classes of *Ascomycota* at different sampling sites in Puerto Rico (forest, urban, coast) and United Kingdom (UK; Inland = Chelmsford, Essex, Coast = Weybourne).

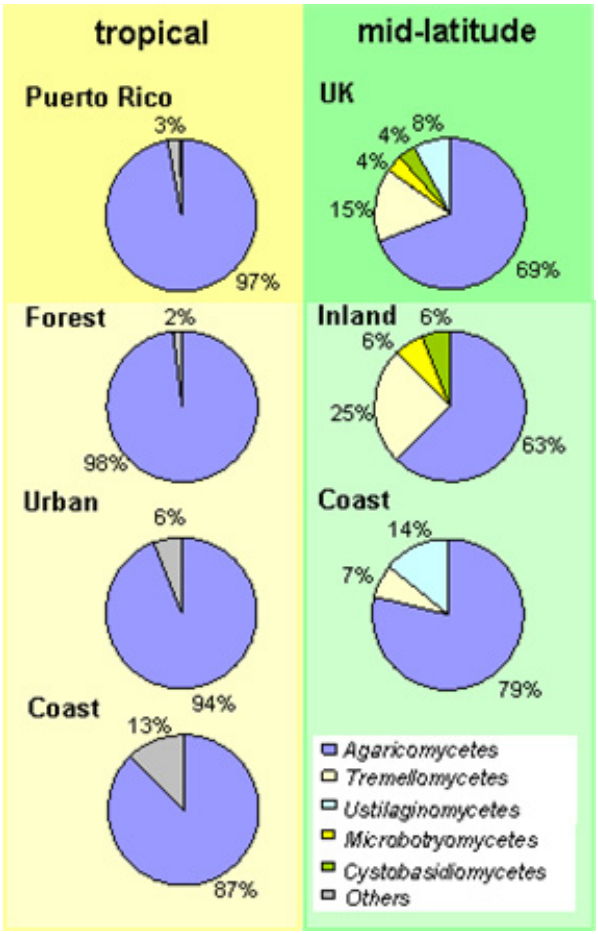

Figure S3. Species richness of airborne fungi: relative proportions of different classes of *Basidiomycota* at different sampling sites in Puerto Rico (forest, urban, coast) and United Kingdom (UK; Inland = Chelmsford, Essex, Coast = Weybourne).

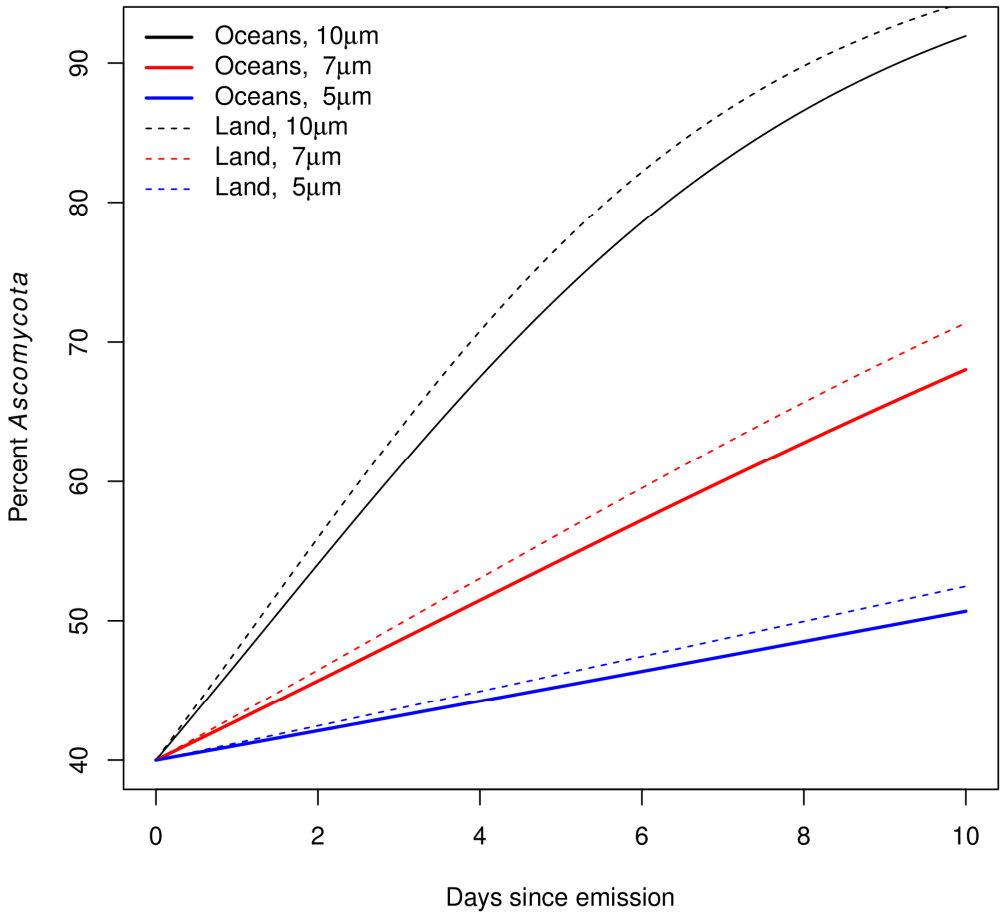

Figure S4. Increase in fraction of *Ascomycota* due to size-differentiated loss processes during idealized transport from a source over land and oceans.

Ascomycota were assumed to have an aerodynamic diameter of 3 µm, while *Basidiomycota* aerodynamic diameter was assumed to be 5 µm, 7 µm or 10 µm, as noted in the figure legend.

Supplementary Tables S1-S12

Table S1. Overview and diversity parameters of aerosol filter samples and detected fungi. Tropical (0-25°), mid-latitude (25-55°) and sub-polar (55-70°) sampling regions (continental, coastal – within 100 km of shoreline, marine); number of air samples and obtained DNA sequences; species richness (S measured, S* estimated), Shannon index (H'), Shannon Evenness (E), and Simpson's index (D) **(a)**, and the median and mean values for continental, coastal, and marine samples **(b)**.

a)

	Tropical, 0°-25°						**Mid-Latitude, 25°-55°**				**Sub-Polar, 55°-70°**
Sampling Region	**Arizona**	**Brazil**	**China**	**Taiwan**	**Puerto Rico**	**Pacific/Indian Ocean**	**Austria**	**Germany**	**UK**	**Indian/Atlantic Ocean**	**Southern Ocean**
Location	continental	continental	coastal	coastal	coastal	marine	continental	continental	coastal	marine	marine
Samples	10	13	14	13	11	5	4	42	12	2	10
Sequences	93	193	267	142	199	81	90	1316	156	32	85
S	31	100	90	56	124	31	60	364	57	18	33
*S**	135	436	255	268	988	162	239	1120	183	163	259
H´	3.2	4.4	4.2	3.8	4.7	3.4	4	5.2	3.9	2.9	3.4
E	0.93	0.95	0.93	0.96	0.98	0.99	0.98	0.89	0.96	0.99	0.99
D	0.039	0.010	0.017	0.015	0.004	0.005	0.02	0.009	0.015	0.006	0.008

b)

Median/Mean	continental	coastal	marine
Samples	12/17	13/13	5/6
Sequences	143/423	162/166	81/66
S	80/139	74/82	31/27
*S**	338/483	262/424	163/195
H´	4.2/4.2	4.1/4.15	3.4/3.2
E	0.94/0.94	0.96/0.96	0.99/0.99
D	0.015/0.020	0.015/0.013	0.006/0.006

Table S2. Austria. Overview of air samples. Sample ID (running number, AT1,2: urban, AT3,4: suburban); 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
AT1	07.07.2005	730	14	12	9	2
AT2	07.07.2005	752	33	24	4	19
AT3	19.07.2005	739	21	18	6	11
AT4	19.07.2005	747	22	19	9	9

Table S3. Arizona. Overview of air samples. Sample ID (running number; c = coarse, f = fine); 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
ARZ1c	18.02.2009 - 19.02.2009	1296	8	6	5	1
ARZ2c	18.02.2009 - 19.02.2009	1296	14	8	2	6
ARZ3f	18.02.2009 - 19.02.2009	1296	11	3	3	0
ARZ4f	18.02.2009 - 19.02.2009	1296	15	4	3	1
ARZ5f	18.02.2009 - 19.02.2009	1296	11	5	4	1
ARZ6c	24.02.2009	6.3	0	0	0	0
ARZ7c	24.02.2009 - 25.02.2009	1296	9	8	3	5
ARZ8f	24.02.2009 - 25.02.2009	1296	9	6	3	3
ARZ9c	02.03.2009	6.3	3	2	2	0
ARZ10f	02.03.2009 - 03.03.2009	1296	13	8	3	5

Table S4. Brazil. 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
BR1	16.09.2002 - 17.09.2002	19	20	13	2	11
BR2	22.09.2002 – 22.09.2002	17	6	4	4	0
BR3	22.09.2002 – 23.09.2002	21	14	10	7	3
BR4	28.09.2002 – 28.09.2002	16	23	14	7	6
BR5	02.10.2002 – 03.10.2002	21	19	11	1	10
BR6	06.10.2002 – 07.10.2002	20	14	8	3	5
BR7	07.10.2002 – 07.10.2002	16	9	10	10	0
BR8	09.10.2002 – 10.10.2002	38	17	9	2	7
BR9	18.10.2002 – 19.10.2002	38	12	9	0	9
BR10	01.11.2002 – 04.11.2002	64	12	12	4	8
BR11	01.11.2002 – 04.11.2002	80	23	19	5	14
BR12	10.11.2002 – 12.11.2002	66	24	22	3	18

Table S5. China. 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
CN1	4.07.2006	334	40	27	13	14
CN2	7.07.2006	800	13	6	6	0
CN3	9.07.2006 - 10.07.2006	1158	8	6	4	2
CN4	10.07.2006 - 11.07.2006	n.a.	15	7	7	0
CN5	11.07.2006 - 12.07.2006	1158	12	3	2	1
CN6	12.07.2006 - 13.07.2006	1435	25	20	14	6
CN7	13.07.2006 - 14.07.2006	1431	23	13	8	5
CN8	14.07.2006 - 15.07.2006	1431	18	6	6	0
CN9	15.07.2006 - 16.07.2006	1598	15	6	6	0
CN10	16.07.2006 - 17.07.2006	1506	8	5	5	0
CN11	17.07.2006 - 18.07.2006	1197	34	9	6	3
CN12	25.07.2006	146	18	12	12	0
CN13	28.07.2006 - 29.07.2006	1424	22	12	11	1
CN14	29.07.2006 - 30.07.2006	1363	16	10	10	0

Table S6. Puerto Rico. Overview of air samples. Sample ID (running number; PR1-4 = forest, PR5-7 = urban, PR 8-11 = coast, N = Nuclepore, Q = 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
PR1N	06.07.2007 - 09.07.2007	160	21	17	5	11
PR2Q	25.07.2007 – 27.07.2007	110	32	22	1	20
PR3N	25.07.2007 – 27.07.2007	110	37	30	5	25
PR4N	31.07.2007 - 02.08.2007	78	24	21	10	8
PR5N	01.07. 2007 - 03.07.2007	269	4	3	2	1
PR6Q	27.07.2007 – 29.07.2007	269	2	1	1	0
PR7N	29.07.2007 – 31.07.2007	269	20	15	8	7
PR8Q	02.07.2007 – 03.07. 2007	56	13	2	0	2
PR9N	02.07.2007 – 03.07. 2007	56	10	10	6	4
PR10N	31.07.2007 - 02.08.2007	101	21	17	3	11
PR11N	01.07. 2007 - 02.07.2007	58	15	12	6	5

Table S7. Taiwan. Overview of air samples. Sample ID (running number, TW1-2: Yunlin County, TSP; TW3-11: Taipei (Nangang), PM2.5; TW12,13: Liouguei Shanping, PM2.5); 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
TW1	06.10.2006 - 08.10.2006	3109	18	6	6	0
TW2	29.03.2007 - 30.03.2007	1533	20	10	10	0
TW3	20.04.2007 - 21.04.2007	753	7	4	1	3
TW4	23.05.2007 - 24.05.2007	748	11	5	2	3
TW5	20.06.2007 - 20.06.2007	9	6	3	2	1
TW6	24.08.2007 - 25.08.2007	673	24	18	10	8
TW7	26.09.2007 - 27.09.2007	703	11	8	3	5
TW8	11.12.2007 - 12.12.2007	741	6	3	2	1
TW9	29.11.2007 - 29.11.2007	752	0	0	0	0
TW10	26.03.2008 - 27.03.2008	766	0	0	0	0
TW11	11.06.2008 - 11.06.2008	655	24	11	8	3
TW12	26.02.2008 - 26.02.2008	552	15	7	7	0
TW13	27.03.2008 - 27.03.2008	575	0	0	0	0

Table S8. United Kingdom. Overview of air samples. Sample ID (running number, UK1-9: Weybourne; UK10-12: Chelmsford, Essex); 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	2168	32	15	5	10
UK2	06.05.2004 - 13.05.2004	2168	10	7	5	2
UK3	06.05.2004 - 13.05.2004	2168	17	12	8	4
UK4	15.05.2004 - 21.05.2004	2372	10	10	9	1
UK5	15.05.2004 - 21.05.2004	2372	15	5	3	2
UK6	06.05.2004 - 13.05.2004	2168	29	11	9	2
UK7	06.05.2004 - 13.05.2004	2168	2	1	1	0
UK8	06.05.2004 - 13.05.2004	2168	5	3	3	0
UK9	23.05.2004 - 25.05.2004	1868	0	0	0	0
UK10	31.07.2003 – 01.08.2003	932	10	9	3	6
UK11	31.07.2003 – 01.08.2003	932	10	8	0	8
UK12	31.07.2003 – 01.08.2003	932	16	6	0	6

Table S9. Ocean. Overview of air samples. Sample ID (running number; t = tropical, m = mid-latitude, s = sub-polar); 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	Latitude and Longitude (Start, Stop)	Sampling Period	Sampled air volume (m^3)	Sequences	Species		
					Fungi	AMC	BMC
Ocean1t	6° 01.834'N 126°08.646'E 2° 09.753'N 121°23.469'E	20.11.2007 – 21.11.2007	1489	16	10	5	5
Ocean2t	7° 28.502'S 116°12.211'E 12° 55.520'S 115°02.886'E	23.11.2007 – 24.11.2007	1430	23	9	9	0
Ocean3s	58° 54.304'S 118°22.719'E 61° 59.844'S 108°27.573'E	05.12.2007 – 06.12.2007	1346	37	18	15	3
Ocean4s	62° 08.983'S 84° 24.899'E 69° 10.874'S 76° 27.475'E	08.12.2007 – 11.12.2007	1495	3	1	1	0
Ocean5s	69° 09.422'S 74° 28.306'E 65° 17.598'S 66° 31.963'E	24.12.2007 – 25.12.2007	771	1	1	0	1

Ocean6s	65° 17.598'S 66° 31.963'E 65° 17.598'S 66° 31.963'E	25.12.2007 – 25.12.2007	0.08	11	3	2	1
Ocean7s	60° 47.493'S 3° 54.103'E 60° 25.741'S 8° 28.707'W	30.12.2007 – 31.12.2007	1506	0	0	0	0
Ocean8s	62° 12.696'S 58° 50.063'W 62° 13.681'S 58° 55.845'W	08.01.2008 – 11.01.2008	2762	19	8	8	0
Ocean9s	62° 13.727'S 58° 55.634'W 62° 13.727'S 58° 55.634'W	14.01.2008 – 17.01.2008	2498	0	0	0	0
Ocean10m	35° 10.156'S 56° 43.431'W 39° 55.270'S 56° 48.748'W	30.01.2008 – 31.01.2008	1457	26	17	11	6
Ocean11s	62° 12.711'S 58° 49.786'W 62° 12.998'S 58° 47.837'W	05.02.2008 – 08.02.2008	2349	9	4	4	0
Ocean12s	67° 15.738'S 5° 59.665'E 68° 44.103'S	15.02.2008 – 16.02.2008	1471	2	1	1	0

	16° 23.771’E						
Ocean13s	68° 32.667’S 74° 09.847’E 69° 19.562’S 76° 24.163’E	05.03.2008 – 10.03.2008	3343	3	1	1	0
Ocean14m	38° 02.528’S 102°30.047’E 34° 59.579’S 108°58.767’E	19.03.2008 – 20.03.2008	1453	6	2	2	0
Ocean15t	20° 33.840’S 113°49.027’E 14° 18.054’S 114°50.985’E	31.03.2008 – 01.04.2008	1509	13	3	3	0
Ocean16t	2° 45.389’N 122°07.267’E 6° 30.222’N 126°34.222’E	04.04.2008 – 05.04.2008	1508	29	12	8	5
Ocean17t	17° 49.631’N 125°59.808’E 24° 14.915’N 126°00.153’E	07.04.2008 – 08.04.2008	1512	0	0	0	0

Table S10. Selected species. Class attribution and relative frequency of occurrence of species that can act as ice nuclei for different sampling regions (*RFO* = Proportion of samples in which the species were detected).

species	class	RFO (%)								
		Austria	Arizona	Brazil	China	Germany	Puerto Rico	Taiwan	UK	Ocean
Cladosporium spp.	*Dothideomycetes*	50	60	15	86	98	27	54	42	18
Fusarium spp.	*Sordariomycetes*	-	-	-	-	2	9	-	-	-
Microdochium spp.	*Sordariomycetes*	25	-	8	-	-	-	-	8	-
Penicillium spp.	*Eurotiomycetes*	50	-	-	50	57	36	-	-	59

Table S11. 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	Nikolcheva and Bärlocher., 2004; White et al., 1990
B	ITS5	ITS4B	58	Nikolcheva and Bärlocher., 2004; White et al., 1990
C	Glom1	Glom2	54	Renker et al., 2003
Second PCR				
D	ITS5	ITS4	54	White et al., 1990
E	ITS1	ITS4B	58	Nikolcheva and Bärlocher., 2004; Fierer et al., 2005
F	ITS1	ITS4A	55	Nikolcheva and Bärlocher., 2004; Fierer et al., 2005

Table S12. Statistical parameters.

Symbol	Quantity/Definition
D	Simpson's index (Hill et al., 2003), $D = \sum_{i=1}^{S} \frac{n_i(n_i-1)}{N(N-1)}$
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 (Chao et al., 1984; Hill et al., 2003), $S^* = S + a^2 / (2\,b)$, a = number of species detected only once (singletons), b = number of species detected twice (doubletons)

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