

1 **Supplementary Material for the manuscript:**

2 **Biogeography in the air: fungal diversity over land and**  
3 **oceans**

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

## 1 **Supplementary Text**

2 **Blank and Background samples.** Whereas no DNA could be detected in the filter blanks (baked  
3 and unbaked), fungal DNA was detected in 4% of the extraction or PCR blank reactions,  
4 indicating that contaminations occurred rarely during analysis in the laboratory. The PCR  
5 products obtained from blank samples were cloned and sequenced, whereas PCR products of  
6 filter extracts obtained in these PCRs were excluded completely from the cloning reactions.  
7 However, the obtained sequences of blank samples and identical sequences obtained from the air  
8 filter samples were excluded from further analysis.

9 PCR is a powerful technique and the high sensitivity of this technique bears the risk of  
10 amplifying trace amounts of DNA with which the analysis material (e.g., reagents) may have  
11 been contaminated in the course of material production and analysis. Several studies described  
12 bacterial and fungal DNA contaminations in *Taq* Polymerase and commercially available  
13 reagents like lysis enzymes (Meier et al., 1993; Hughes et al., 1994; Loeffler et al., 1999). In our  
14 study the initially observed contaminations in the PCR blanks could not be detected anymore  
15 after performing the experiments with new reagents (new lot number). The detection of DNA in  
16 extraction blanks was likely due to contaminations originating from the PCR. Six species, e.g.,  
17 *Brettanomyces bruxellensis* and *Candida tropicalis* were only detected in extraction blanks.  
18 Possibly, they were laboratory contaminations of the extracts or contaminations occurred during  
19 extraction or PCR preparation in the laboratory (material used, extraction procedure). In total 11  
20 different OTUs (mostly yeast species, e.g., *Candida sake*, *Candida deformans*, *Candida*  
21 *tropicalis*, *Cryptococcus longus*) were identified as possible contaminants.

22 As described in Fröhlich-Nowoisky et al. (2009), no DNA was detected in the blank samples  
23 from Mainz, Germany, indicating that no contaminations occurred during sample handling and  
24 analysis in the laboratory. However, four of the OTUs identified as possible contaminants in the  
25 other sets of filter samples were detected in the samples from Mainz (*Candida deformans*,  
26 *Candida tropicalis*, *Nectria sp.*, *Alternaria sp.*). To avoid any bias in the comparison with other  
27 sample sets, we excluded the possible contaminations also from the statistical analysis of the  
28 Mainz samples.

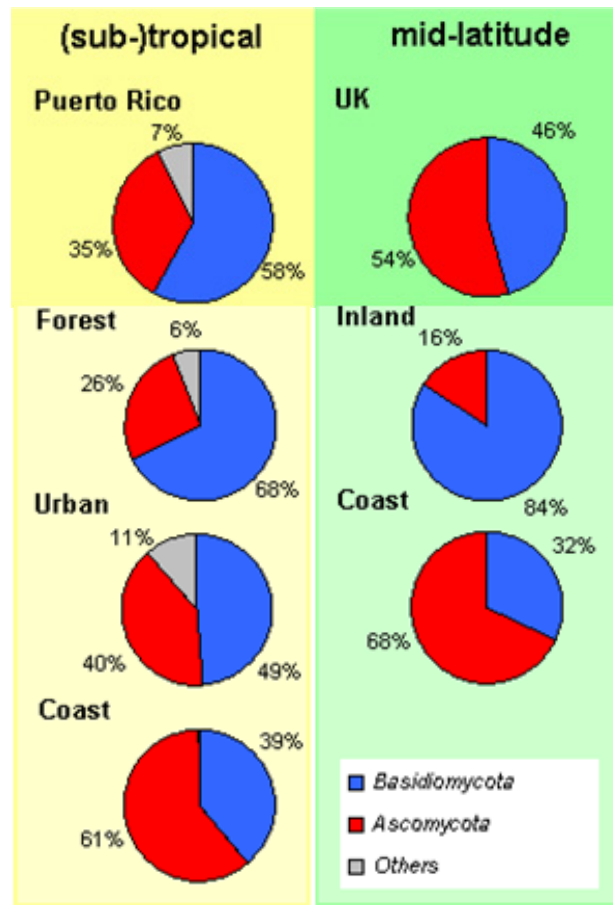
29

1 **Emission and transport of fungal spores.** Air is the primary medium for the dispersal of fungal  
2 spores, which can be transported over long distances depending on spore properties and atmos-  
3 pheric conditions (Hirst et al., 1967; Brown et al., 2002). An essential condition for widespread  
4 dispersal is that the spores have to leave the laminar boundary layer at the surface and enter into  
5 turbulent air flow in the atmosphere (Gregory, 1973; Madelin, 1994; Lacey, 1996). This can be  
6 achieved through a range of active and passive discharge and liberation processes of spores (In-  
7 gold, 1971; Lacey, 1996; Elbert et al., 2007). The residence time of fungal spores in air depends  
8 on their size and decreases with increasing aerodynamic diameter (Gregory, 1973; Lacey, 1996).  
9 Because the spores of many BMC (~5-10  $\mu\text{m}$ ) are typically larger than those of prominent air-  
10 borne AMC (~2-5  $\mu\text{m}$ ) (Muilenberg, 1995; Lacey, 1996; Ingold, 2001; Stenlid, 2008; Fröhlich-  
11 Nowoisky et al., 2009), they are expected to be deposited more rapidly and are less likely to un-  
12 dergo long-range transport, as also indicated by global atmospheric transport model results (Fig.  
13 S4, Wilkinson et al. 2011).

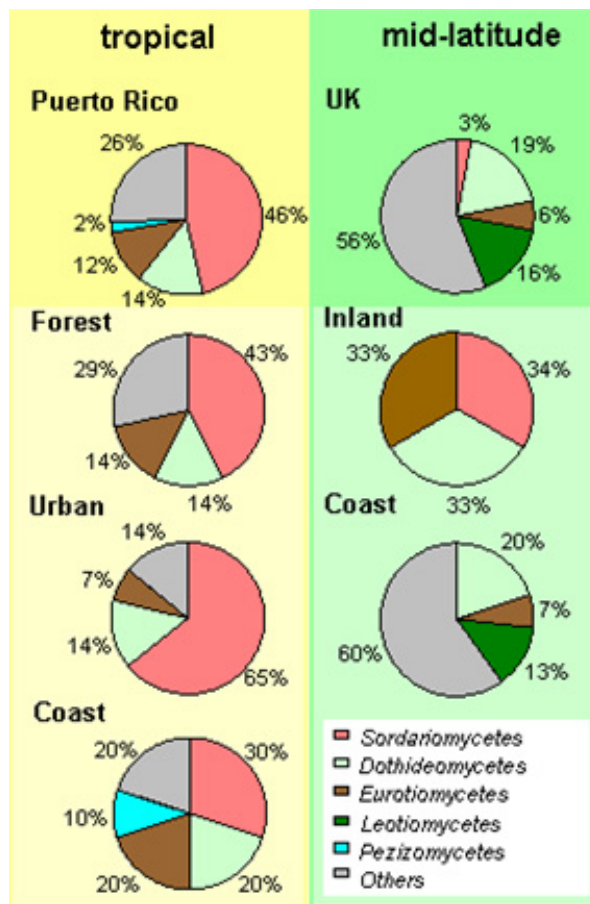
14 Atmospheric loss processes can be conceptualized in an idealized manner as an exponential de-  
15 cay over time of the total particle mass remaining in the atmosphere. For particles emitted instan-  
16 taneously at time  $t=0$ , the remaining mass  $M$  is given by  $M(t) = M_0 \exp(-t/\tau)$ ,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

21 Due to varying discharge mechanisms and meteorological conditions, the concentration and di-  
22 versity of airborne fungal spores are likely to vary with the time of day and altitude above  
23 ground. These effects should have no influence on the systematic differences between continental  
24 and marine sampling sites observed in this study. To develop a comprehensive understanding of  
25 temporal and spatial variability of the atmospheric biogeography and transport of fungi, however,  
26 we propose and intend to pursue further studies and experiments including air samples collected  
27 at different altitudes and distances from the coast.

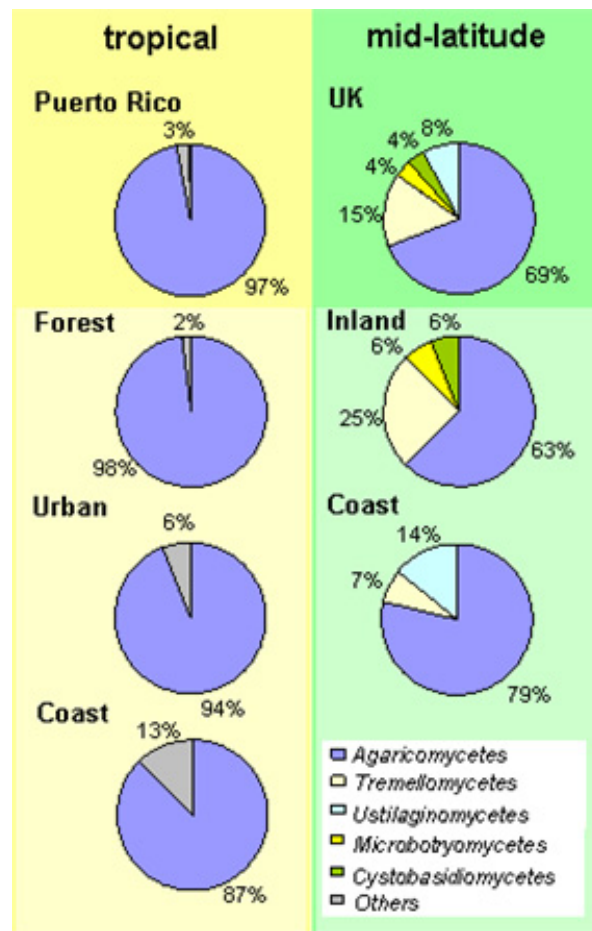
## Supplementary Figures S1-S4



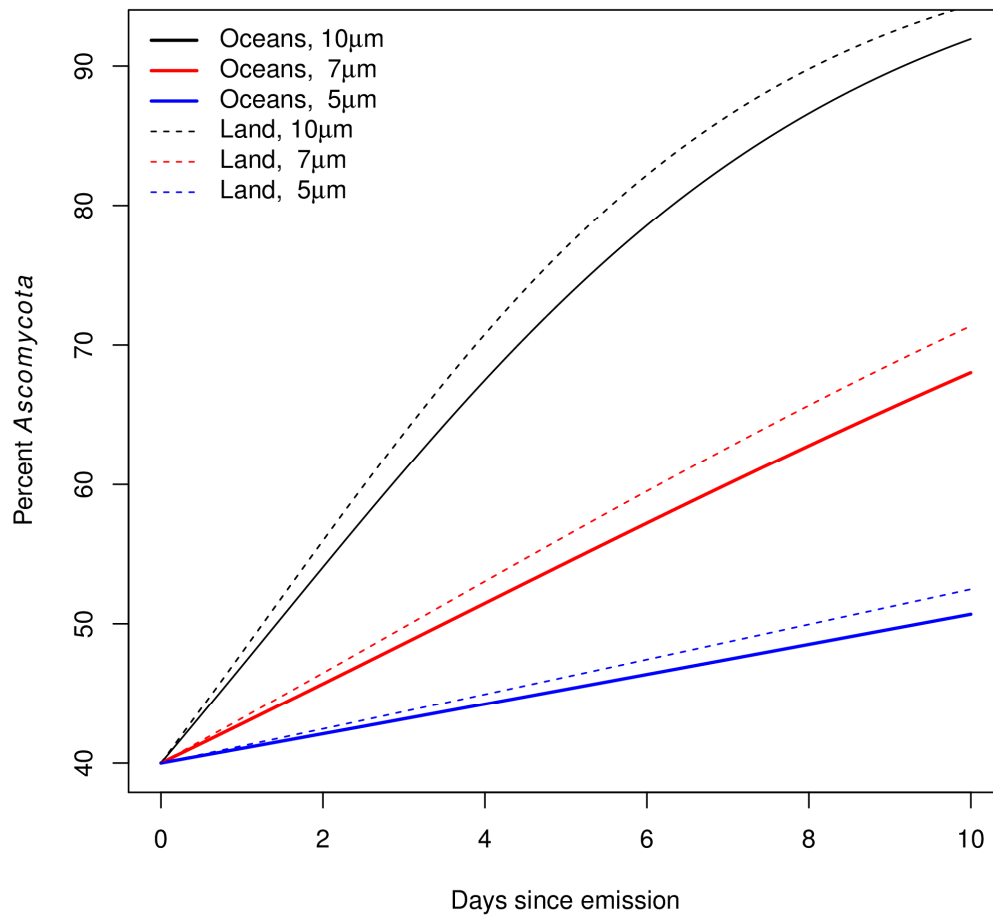
**Figure S1.** Species richness of airborne fungi: relative proportions of different phyla at different sampling sites in Puerto Rico (forest, urban, coast) and United Kingdom (UK; Inland = Chelmsford, Essex, Coast = Weybourne).



**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 the *Basidiomycota* aerodynamic diameter was assumed to be 5 μm, 7 μm or 10 μm.



## 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, sampler, size range, total volume of air, and obtained DNA sequences; species richness (S measured, S\* estimated), Shannon index (H'), Shannon Evenness (E), and Simpson's index (D).

	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
Sampler	Hi- Vol	Hi-Vol	Hi-Vol	Hi-Vol	SFU	Hi-Vol	Hi-Vol	Hi-Vol	Hi-Vol	Hi-Vol	Hi-Vol
Size range	PM10	>3 µm	TSP	TSP, PM2.5	<1.7 µm, >1.7 µm	TSP	PM10	<3 µm, >3 µm	<4.49 µm	TSP	TSP
Total volume of air (m <sup>3</sup> )	10381	416	14981	11569	1536	7448	2968	112063	22416	2910	17541
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

**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 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	Sequences	Species		
					Fungi	AMC	BMC
Ocean1t	6° 01.834'N 126°08.646'E	20.11.2007 - 21.11.2007	1489	16	10	5	5
	2° 09.753'N 121°23.469'E						
Ocean2t	7° 28.502'S 116°12.211'E	23.11.2007 - 24.11.2007	1430	23	9	9	0
	12° 55.520'S 115°02.886'E						
Ocean3s	58° 54.304'S 118°22.719'E	05.12.2007 - 06.12.2007	1346	37	18	15	3
	61° 59.844'S 108°27.573'E						
Ocean4s	62° 08.983'S 84° 24.899'E	08.12.2007 - 11.12.2007	1495	3	1	1	0
	69° 10.874'S 76° 27.475'E						
Ocean5s	69° 09.422'S 74° 28.306'E	24.12.2007 - 25.12.2007	771	1	1	0	1
	65° 17.598'S 66° 31.963'E						
Ocean6s	65° 17.598'S 66° 31.963'E	25.12.2007 - 25.12.2007	0.08	11	3	2	1
	65° 17.598'S 66° 31.963'E						
Ocean7s	60° 47.493'S 3° 54.103'E	30.12.2007 - 31.12.2007	1506	0	0	0	0
	60° 25.741'S 8° 28.707'W						
Ocean8s	62° 12.696'S 58° 50.063'W	08.01.2008 - 11.01.2008	2762	19	8	8	0
	62° 13.681'S						

	58° 55.845'W						
Ocean9s	62° 13.727'S 58° 55.634'W	14.01.2008 - 17.01.2008	2498	0	0	0	0
	62° 13.727'S 58° 55.634'W						
Ocean10m	35° 10.156'S 56° 43.431'W	30.01.2008 - 31.01.2008	1457	26	17	11	6
	39° 55.270'S 56° 48.748'W						
Ocean11s	62° 12.711'S 58° 49.786'W	05.02.2008 - 08.02.2008	2349	9	4	4	0
	62° 12.998'S 58° 47.837'W						
Ocean12s	67° 15.738'S 5° 59.665'E	15.02.2008 - 16.02.2008	1471	2	1	1	0
	68° 44.103'S 16° 23.771'E						
Ocean13s	68° 32.667'S 74° 09.847'E	05.03.2008 - 10.03.2008	3343	3	1	1	0
	69° 19.562'S 76° 24.163'E						
Ocean14m	38° 02.528'S 102°30.047'E	19.03.2008 - 20.03.2008	1453	6	2	2	0
	34° 59.579'S 108°58.767'E						
Ocean15t	20° 33.840'S 113°49.027'E	31.03.2008 - 01.04.2008	1509	13	3	3	0
	14° 18.054'S 114°50.985'E						
Ocean16t	2° 45.389'N 122°07.267'E	04.04.2008 - 05.04.2008	1508	29	12	8	5
	6° 30.222'N 126°34.222'E						
Ocean17t	17° 49.631'N 125°59.808'E	07.04.2008 - 08.04.2008	1512	0	0	0	0
	24° 14.915'N 126°00.153'E						

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**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
<i>Cladosporium spp.</i>	<i>Dothideomycetes</i>	50	60	15	86	98	27	54	42	18
<i>Fusarium spp.</i>	<i>Sordariomycetes</i>	-	-	-	-	2	9	-	-	-
<i>Microdochium spp.</i>	<i>Sordariomycetes</i>	25	-	8	-	-	-	-	8	-
<i>Penicillium spp.</i>	<i>Eurotiomycetes</i>	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), $S^* = S + a^2 / (2b)$ , $a$ = number of species detected only once (singletons), $b$ = number of species detected twice (doubletons)

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