

1 **Supplementary Online Information (SOM)**

3 **Title: High concentrations of biological aerosol particles and ice nuclei during and** 4 **after rain**

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29 **Supplementary Materials:**

30 S1 Materials and Methods

31 Figures S1-S3

32

33 **S1 Materials and Methods**

34 ***S1.1 Site and Campaign Description***

35 The BEACHON-RoMBAS (Bio-hydro-atmosphere interactions of Energy, Aerosols, Carbon,
36 H₂O, Organics and Nitrogen – Rocky Mountain Biogenic Aerosol Study) campaign was a
37 component of the greater BEACHON project sponsored by the National Center for Atmospheric
38 Research (<http://cires.colorado.edu/jimenez-group/wiki/index.php/BEACHON-RoMBAS>;
39 <http://web3.acd.ucar.edu/beachon/>). BEACHON-RoMBAS brought together a large,
40 interdisciplinary set of scientists to address issues surrounding the biogeochemical cycling of
41 carbon and water at a location representative of the semi-arid Western U.S. The measurements
42 were located in a part of the Manitou Experimental Forest in a semi-arid, montane ponderosa
43 pine zone in the Central Rocky Mountains 35 km northwest of Colorado Springs, Colorado and
44 15 km north of Woodland Park, CO (2370 m elevation, lat. 39°6'0" N, long. 105°5'30" W). All
45 instruments and samplers were sampled from a height of 1 – 4 m above ground from fixed inlets,
46 with the exception of the WIBS, high-volume sampler, and biosamplers (details listed below).

47

48 ***S1.2 Meteorological and Leaf Moisture Measurements***

49 Precipitation occurrence, rate and microphysical state (i.e., rain versus hail) were measured using
50 a laser-optical disdrometer (PARTicle Size and VELOCITY ‘PARSIVEL’ sensor; OTT Hydromet
51 GmbH, Kempton, Germany). The instrument senses a falling hydrometeor by measuring the
52 magnitude and duration of attenuation of a temporally continuous 2-dimensional laser beam (780

53 nm) through which the hydrometeor passes. It, therefore, directly detects the presence of falling
54 hydrometeors without the time delay of typical tipping bucket gauges and with greater particle
55 size sensitivity than typical weighing gauges. Particle size is estimated from the magnitude of
56 beam attenuation. Particle fall speed is determined from the duration of beam attenuation while
57 overall precipitation rate and microphysical classification estimates are generated from a
58 combination of the size and fall speed measurements. The sensor detects liquid hydrometeor
59 particles ranging in size from 0.2 to 5 mm in diameter, solid hydrometeors ranging in size from
60 0.2 to 25 mm and provides estimates of particle velocities from 0.2 to 20 m/s. The stated
61 accuracy of liquid precipitation rate estimates is +/- 5%. Only the rainfall rate (mm/hr) is
62 discussed in this text.

63
64 Leaf wetness state was characterized using a dielectric Leaf Wetness Sensor (LWS; Decagon
65 Devices, Inc.). The sensor detects and provides a relative measure of the water or ice content on
66 or near the sensor surface (within ~1 cm) by measuring the dielectric constant of the surface. The
67 sensor outputs a voltage (measured in millivolts, mV) which is directly proportional to the
68 amount of water or ice in or near the sensor upper surface. Attribution of the cause of wetness
69 due to rain or dew formation (i.e. local condensation) is determined by comparing LWS voltage
70 with optical precipitation measurements and by the pattern of voltage readings from the sensor.
71 Sharp increases in the mV signal that are concurrent with precipitation events are characterized
72 as 'rainfall wetness' while slowly increasing mV values that are unaccompanied with
73 precipitation events are characterized as dew formation.

74

75 Other meteorological parameters such as barometric pressure, air temperature, humidity and
76 wind speed and direction were measured using a WXT520 Weather Transmitter (Vaisala, Inc.,
77 Helsinki, Finland). The weather transmitter, the disdrometer and the LWS were all located at the
78 Manitou Experimental Forest observatory within 100 m of the rest of the particle measurements
79 described below.

80

81 ***SI.3 UV-APS***

82 An ultraviolet aerodynamic particle sizer (UV-APS; TSI Inc. Model 3314, St. Paul, MN) was
83 utilized for this study following the procedure described by Huffman et al. (Huffman et al.,
84 2010). Aerodynamic particle diameter (D_a) is provided in the range of 0.54 – 19.81 μm by
85 measuring the time of flight between two continuous-wave red (633 nm) He-Ne lasers. Total
86 fluorescence of aerosol particles (non-wavelength-dispersed) in the wavelength range of 420 –
87 575 nm is detected after pulsed excitation by an Nd:YAG laser ($\lambda_{\text{ex}} = 355 \text{ nm}$). UV-APS number
88 concentrations are reported here as integrated between 1 – 20 μm . Smaller particles are
89 transmitted within the instrument less efficiently and thus should be considered as lower limit
90 values. Aerosol sampling was performed with a volumetric flow of 5 $\text{L}\cdot\text{min}^{-1}$ (LPM) at ambient
91 pressure and temperature, split within the instrument into a sample flow of 1.0 ± 0.1 LPM and a
92 sheath flow of 4.0 ± 0.1 LPM (pressure difference feedback control). The instrument was
93 controlled and the measurement data were recorded with an external computer connected via
94 serial port using the manufacturer's Aerosol Instrument Manager software (TSI AIM).
95 Measurements were initiated every 5 minutes and integrated over a sample length of 285 s. Five-
96 minute sample measurements were continuously repeated over a period of five weeks from 20
97 July to 23 August, 2011 (35 days) and only briefly interrupted for maintenance procedures

98 (usually less than 30 minutes per week). The local time (LT) used for data analysis and plotting
99 refers to Mountain Daylight Time (MDT). All times reported here are listed as LT.

100

101 Fluorescent particles (N_f) detected by the UV-APS can be regarded as a lower limit for the
102 abundance of primary biological aerosol particles (Huffman et al., 2010; Pöhlker et al., 2012),
103 utilizing nicotinamide adenine dinucleotide (NADH) and riboflavin as dominant biological
104 fluorophores. The UV-APS instrument sampled air from a 0.75 inch laminar flow inlet from ~4
105 m above ground into a climate-controlled trailer at ground level.

106

107 ***SI.4 WIBS***

108 The waveband integrated bioaerosol sensor – model 4 (WIBS4; University of Hertfordshire) is a
109 dual channel single particle fluorescence spectrometer (Kaye et al., 2005; Foot et al., 2008;
110 Gabey et al., 2010). Upon detection of a particle, xenon lamps provide two consecutive pulses of
111 light at 280 nm and 370 nm, in order to stimulate fluorescence of the tryptophan and NADH
112 biofluorophores respectively. The fluorescence of a particle is measured between 310-400 nm
113 (the FL1 channel) and 400-600 nm (the FL2 channel), capturing tryptophan fluorescence, and
114 400-600 nm, capturing NADH fluorescence. This leads to three separate fluorescence channels:
115 FL1_280, FL2_280 and FL2_370. The forward scattering signal of the particle is also measured
116 at four angular offsets using a quadrant photo-multiplier tube. This allows for measurements of
117 size and asymmetry. The WIBS4 model is essentially the same as the WIBS3 model employed
118 by Gabey et al. (2010), but with improved optics and electronics providing a more precise signal.
119 Baseline fluorescence is recorded by regularly measuring the internal fluorescence of the
120 instrument when no particles are present. The increased precision of the model 4 WIBS allows

121 for the detection of more marginally fluorescent particles than was possible using previous
122 WIBS models.

123
124 The WIBS4 was located on an automated profiling system running up the main measurement
125 tower, which allowed profile measurements to be made between 3 m and 22 m. Profiles
126 consisted of an eight stage profile up, lasting around 45 minutes, and a corresponding continuous
127 profile down, lasting about 3 minutes. The WIBS4 total particle size distribution compared well
128 with a co-sampling Grimm OPC, particularly in the super-micron regime. A subset of the WIBS4
129 single particle data (8000 particles) was analyzed using hierarchical agglomerative cluster
130 analysis using a group average distance metric. This clustering was analyzed in five dimensions
131 which were z-score normalized before analysis: the three fluorescence channels, size, and
132 asymmetry. A suitable solution was assessed by inspecting the coefficient of determination and
133 the root mean squared distance between clusters for each (e.g. Robinson et al., 2011).
134 Concentration time series for each cluster were established by comparing each of the remaining
135 particles to the centroid of each cluster. Each time series was apportioned a fraction of the
136 particles' count which was inversely proportional to the distance of the particle from each cluster
137 centroid (expressed in number of standard deviations of the centroid).

138
139 Bioaerosol fluxes were estimated for each cluster by combining the concentration gradient with
140 vertical wind speed data using:

141

142 Equation S1
$$F = -0.16 \left(\frac{\Delta z_u \Delta u}{\left(\ln \frac{z_{u2}}{z_{u1}} \right)^2} \right) \left(\frac{\Delta c}{\Delta z_c} \right)$$

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Where Δu is the difference between vertical wind speed measurements measured at heights z_{u1} and z_{u2} which are Δz_u apart, and Δc is the difference in concentrations between two heights which are Δz_c apart (Lindemann et al., 1982).

S1.5 Filter and Impactor Aerosol Samples

S1.5.1 Sample Collection

S1.5.1.1 Cascade Aerosol Impactor (MOUDI)

Size-resolved particle samples were collected using a micro-orifice uniform deposition impactor (MOUDI; MSP model 110-R) at a flow-rate of 30 LPM via a dedicated inlet. Samples used for offline ice nucleation analysis were collected onto hydrophobic, siliconized glass slides (Hampton Research, HR3-2125). The MOUDI sampler provided aerosol fractionation according to the following aerodynamic diameter size cuts (D_{50} , μm) (Marple et al., 1991):

Stage 1	18.0
Stage 2	10.0
Stage 3	5.6
Stage 4	3.2
Stage 5	1.8
Stage 6	1.0
Stage 7	0.56
Stage 8	0.32
Stage 9	0.18
Stage 10	0.10

167 Stage 1 is typically referred to as the pre-impactor, and stages 2-11 refer to stages in the MOUDI
 168 impactor. Because we are interested in large particles we refer to the pre-impactor as Stage 1 and
 169 list all stages as 1-11. Thus, the numbering scheme utilized here is shifted lower by one with
 170 respect to the common usage for MOUDI samplers.

171
 172 MOUDI samples collected at the following times were analyzed by fluorescence microscopy and
 173 used for microscopic ice nucleation activation experiments as discussed in the manuscript:

174	M01 (dry period)	7/22 14:29 – 7/23 09:41	(1152 min.)
175	M10 (rain period)	8/2 05:55 – 8/3 05:55	(1440 min.)
176	M26 (rain period)	8/16 20:26 – 8/17 06:32	(606 min.)
177	M27 (dry period)	8/17 06:35 – 8/17 19:46	(791 min.)

178
 179 Size distribution of ice nuclei shown in Figure 2C for dry periods are average of samples M1 and
 180 M27; Figure 2D for rain periods are average of samples M10 and M26. Corresponding time
 181 periods for UV-APS are identical to MOUDI sample periods.

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183 ***S1.5.1.2 High-volume Sampler***

184 Total aerosol samples for DNA analysis were collected onto 150 mm glass fiber filters
 185 (Machery-Nagel, Type MN 85/90, 406015) using a self-standing high-volume sampler (Digital
 186 DHA-80) operated at 1000 LPM and located approximately 50 m from the sampling trailer.
 187 Filters were pre-baked at 500 °C (12 h) to remove any contaminant DNA and stored in pre-baked
 188 aluminum bags before and after sampling.

189

190 ***S1.5.1.3 Glass Slide Impactor Samples***

191 Total aerosol samples were collected onto glass cover slides (13 x 13 mm) using a home-built,
192 single-stage impactor (Flow-rate 1.2 LPM, D₅₀ cut 0.5 μm). The impactor glass substrates were
193 coated with a thin layer of high viscosity grease (Baysilone grease, Bayer, Germany) administered
194 via hexane solution to reduce particle bounce. Single-stage impactor and housing for Nuclepore®
195 filters (below) sub-sampled from a separate inlet immediately next to MOUDI and UV-APS
196 inlets.

197

198 Glass slide impactor samples collected at the following times are shown in Figures 2A and 2B
199 and discussed in the manuscript:

200	G09 (dry period)	7/31 12:17 – 12:49	(32 min.)
201	G21 (rain period)	8/3 23:56 – 8/4 0:27	(31 min.)

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203 ***S1.5.1.4 Nuclepore® Filters***

204 Aerosol samples for electron microscopy analysis were collected with a stacked filter housing
205 using 12 mm diameter gold-coated Nuclepore® polycarbonate filters with pore sizes of 2 μm for
206 coarse particles and 0.2 μm for fine particles, respectively. The volume flow through the stacked
207 filter unit was nominally 2.0 LPM.

208

209 Stacked filter samples collected at the following times are discussed in the manuscript:

210	S10 (dry period)	7/31 11:57 – 15:58	(241 min.)
211	S12 (dry period)	7/31 19:58 – 23:55	(237 min.)

212	S20 (rain period)	8/4 3:52 – 8:04	(252 min.)
213	S23 (rain period)	8/4 16:23 – 20:24	(261 min.)

214

215 ***S1.5.1.5 Bio-Sampler Impactors***

216 Size-resolved viable bioparticles were collected via two types of impactors directly into growth
217 media (flow-rate 28 LPM). Andersen cascade impactors (Graseby Andersen; Atlanta, GA)
218 collect particles onto one of six sequential sample plates designed to collect large particles on
219 upper plates and smaller particles on lower plates. Slit samplers (New Brunswick Scientific Co.;
220 Edison, NJ) collect particles without selection due to sizing, but the collection stage rotates such
221 that particles are deposited in a circular arc to give time resolution of ~2 minutes over the course
222 of a 60 minute sample time. Samplers were placed ~2 m above ground on a piece of wooden
223 fencing that allowed air to pass through the support surface above ground and operated at 28
224 LPM. The surfaces of samplers were sterilized with isopropyl alcohol before each period of
225 collection to remove contaminant organisms. Samplers were operated separately for optimized
226 collection of fungi and bacteria. Fungal growth medium (malt extract medium) was prepared by
227 according to Medelin et al. (Madelin, 1994) with streptomycin (40 units, Sigma Aldrich) and
228 ampicillin (20 units, Fisher Scientific). Bacterial growth medium (Luria Bertani medium; LB)
229 was prepared according to Lighthart and Shaffer (Lighthart and Shaffer, 1995) with
230 cycloheximide (200 µg/mL, Sigma Aldrich). Samples for bacterial analysis were collected for 60
231 minutes, and samples for fungal analysis were collected for 20 minutes. Collection dishes were
232 immediately removed from samplers after each use and placed in an incubator (IncuMax,
233 IC150R) temperature-controlled at 25 °C. Fungal colonies were incubated for ~3 days before
234 counting and picking into 20 µl of sterile water. Bacterial colonies were incubated for ~7 days

235 before counting and picking into sterile water. The picked colonies were lysed at 95 °C for 10
236 min.

237

238 ***S1.5.2 Fluorescence Microscopy***

239 Fluorescence microscopy images were taken on a BZ-9000 Fluorescence Microscope (Keyence,
240 Inc., Osaka, Japan). The instrument was equipped with a super high-compression mercury lamp
241 (120 W) and a 2/3-inch, 1.5 mega pixel monochrome CCD. The following fluorescence filters
242 were used to take images in different spectral ranges: OP-66834 DAPI-BP ($\lambda_{\text{ex}}=360/20$ nm,
243 $\lambda_{\text{Dichroic}}=400$ nm, $\lambda_{\text{Absorp}}=460/25$ nm), OP-66836 GFP-BP ($\lambda_{\text{ex}}=470/20$ nm, $\lambda_{\text{Dichroic}}=495$ nm,
244 $\lambda_{\text{Absorp}}=535/25$ nm), OP-66838 TexasRed ($\lambda_{\text{ex}}=560/20$ nm, $\lambda_{\text{Dichroic}}=595$ nm, $\lambda_{\text{Absorp}}=630/30$
245 nm). Filter specifications are represented as wavelength and peak width (λ/FWHM).

246

247 In Fig. 2A and 2B an overlay of fluorescent emission from all three fluorescence microscope
248 channels (DAPI, GFP, TexasRed) onto a brightfield image of the same sample area is shown. For
249 comparability the exposure times of the individual fluorescence images in Fig. 2A and 2B were
250 set to the same values. The overlay image Fig. 2B is dominated by “blue-green” fluorescence
251 indicating strong emissions in the DAPI ($\lambda_{\text{ex}} = \sim 360$ nm, $\lambda_{\text{em}} = \sim 460$ nm) and GFP
252 ($\lambda_{\text{ex}} = \sim 470$ nm, $\lambda_{\text{em}} = \sim 535$ nm) channels. Blue-green fluorescence is characteristic for biological
253 material and mainly originating from protein and coenzyme fluorophores (Pöhlker et al., 2012).
254 In contrast “red-yellow” fluorescence is predominating in the overlay image in Fig. 2A
255 indicating strong emission in the TexasRed channel ($\lambda_{\text{ex}} = \sim 560$ nm, $\lambda_{\text{em}} = \sim 630$ nm). Red-yellow
256 fluorescence is regarded to be somewhat characteristic/typical for mineral dust (Bozlee et al.,
257 2005).

258

259 ***S1.5.3 SEM***

260 Scanning electron microscopy (SEM) images of aerosol particles were acquired using the
261 secondary electron in-lens detector of a high-performance field emission instrument (LEO 1530
262 FESEM, EHT 10 keV, WD 9 mm). The elemental composition of inorganic components was
263 characterized using the Oxford Instruments ultra-thin-window energy-dispersive X-ray (EDX)
264 detector.

265

266 The filter samples were scanned using a semi-automated spot counting technique (Sinha et al.,
267 2008; Pöschl et al., 2010) at a magnification of $6500 \times$ (pixel size 88.9 nm) for coarse and 19500
268 \times (pixel size 29.6 nm) for fine particle filters. Particles located on the predefined equidistant
269 spots of the counting grid were automatically counted, and the recorded data were used to
270 classify the particles according to size, composition, and mixing state. With spot counting, the
271 probability for particles of a certain size and type to be counted is directly proportional to the 2-
272 D surface area of the particles and the fraction of the filter surface covered by such particles.
273 This relationship is used to upscale the counting results from the investigated filter area to the
274 total filter area.

275

276 ***S1.5.4 DNA Analysis of Aerosol Samples***

277 To determine fungal diversity from the air filter samples (high-volume sampler, S1.5.1.2)
278 optimized methods of DNA extraction, amplification, and sequence analysis of the internal
279 transcribed spacer (ITS) regions as described in Fröhlich-Nowoisky et al. (2009; 2012) were
280 used. In addition to fungi, the primer pair ITS4Oo and ITS5 (Nikolcheva and Bärlocher, 2004)

281 was used for amplification of *Peronosporomycetes* (formerly *Oomycota*). Also specific for this
282 study, the internal transcribed spacer regions from fungal lysates, obtained from the cultivation
283 experiments of impactor samples (Anderson Cascade Bio-Sampler Impactors, S1.5.1.5), were
284 amplified with the primer pair ITS4 and ITS5 (White et al., 1990; Fröhlich-Nowoisky et al.,
285 2009; Fröhlich-Nowoisky et al., 2012). The obtained PCR products were sequenced using the
286 primer ITS5 and sequence analysis was performed as described in Fröhlich-Nowoisky et al.
287 (2009; 2012). The sequences from the obtained operational taxonomic units have been deposited
288 in the GenBank database under following accession numbers: JX135610 - JX136661 (Fungi) and
289 JQ976038 - JQ976273 (*Peronosporomycetes*).

290

291 For the determination of bacterial diversity from high-volume aerosol filter samples (see also
292 S.1.5.1.2) DNA was extracted as described by Després et al. (2007). The 16S ribosomal gene
293 was first amplified for taxonomic identification with primer pairs 9/27f and 1492r (Weisburg et
294 al., 1991) with PCRs conditions given by Després et al. (2007), and then cloned and sequenced.
295 The same primer pair was used for the bacterial lysates obtained from Andersen sampler culture
296 plates (Anderson Cascade Bio-Sampler Impactors, S1.5.1.5). Sequences are deposited in the
297 GenBank database under the following accession numbers: JX228219-JX228862.

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299 ***S1.5.5 Freezing Tests***

300 Fungal and bacterial colonies (Andersen Cascade Bio-Sampler Impactor, S1.5.1.5) were picked
301 and cultured in dextrose-peptone-yeast (DPY) medium (dextrose 10 g/L, peptone 3 g/L, yeast
302 extract 0.3g/L) in 96-well polypropylene plates and incubated at 16 °C. A 50 µl aliquot of the
303 inoculated DPY medium containing hyphal fragments and fungal spores was tested from each

304 well for its ice nucleation activity in a temperature range -12 °C to -2 °C. Aliquots were
305 transferred to a fresh, sterile, 96-well polypropylene PCR tray and these were cooled in a thermal
306 cycler (MJ Research, PTC-200). Temperature variation across the head was ± 0.2 °C of the true
307 temperature measured using a thermistor (Bio-Rad, VPT-0300). The cycler was programmed to
308 descend in 0.5 or 1 °C increments to -9.0 °C (the limit of the machine). After a 5 min dwell time
309 at each temperature, the number of frozen wells was counted and the temperature lowered to the
310 next level. Once at -9 °C, the tray was transferred to a 96-well aluminum incubation block
311 (VWR, 13259-260) which had been pre-cooled to \sim -12 °C inside a foam box in a freezer. The
312 thermistor was inserted into a side well and after 10 min the block temperature and number of
313 frozen wells was recorded. Aliquots of un-inoculated DPY medium were used as negative
314 controls. Ice active isolates were then cultured on DPY agar and incubated at RT for \sim 3 days.
315 Beside microscopic analysis as described under S1.5.2 hyphal fragments and spores were picked
316 into 20 μ l water, lysed at 95 °C for 10 min, and identified by DNA analysis as described above.

317

318 ***S1.6 Microscopic IN Activation Experiments***

319 Particles were collected on hydrophobic glass slides with a rotating MOUDI, as described above.
320 The freezing properties of particles collected on the slides were then determined with an optical
321 microscope and a flow cell with temperature and relative humidity control. The flow cell and
322 microscope set-up was very similar to the ones used by Iannone et al. (2011) and Dymarska et al.
323 (2006), to determine the ice nucleation properties of fungal spores and soot particles,
324 respectively.

325

326 In every IN activation experiment, a hydrophobic slide containing particles was located within
 327 the flow cell. The RH was first set to > 100% to condense water droplets on the particles. The
 328 droplets were grown to approximately 100 μm in diameter, and after droplet growth was
 329 completed each droplet contained between 30 and 100 particles. Next, the temperature was
 330 decreased at a rate of 10 K/min until a temperature of -40 °C was reached. During the
 331 experiment, between 11 and 66 droplets (average 36) were continuously monitored with an
 332 optical microscope coupled to a CCD camera. From the images recorded with the CCD camera,
 333 the freezing temperatures of the droplets were determined.

334
 335 The number of ice nuclei in a freezing experiment, $\#IN(T)$, was calculated from the freezing data
 336 using the following equation (Vali, 1971):

$$\text{Equation S2} \quad \#IN(T) = -\ln\left(\frac{N_{Total} - N_{Frozen}(T)}{N_{Total}}\right) \times N_{Total}$$

337
 338 where N_{Total} is the total number of droplets in a freezing experiment and $N_{Frozen}(T)$ is the number
 339 of frozen droplets as a function of temperature in a freezing experiment. Equation S2 accounts
 340 for the fact that multiple IN can exist in the same droplet (Vali, 1971). The number of ice nuclei
 341 per volume of air as a function of temperature, $[IN(T)]$, was calculated using the following
 342 equation:
 343

$$\text{Equation S3} \quad [IN(T)] = \frac{\#IN(T)}{VolumeAirSampled} \times \frac{Area_{MOUDIStage}}{Area_{Monitored}}$$

344
 345 where $VolumeAirSampled$ is the total volume of air sampled by the MOUDI, $Area_{MOUDIStage}$ is
 346 the total area covered by particles within a MOUDI stage and $Area_{Monitored}$ is the area
 347 monitored with the microscope.

348

349 In the freezing experiments, a majority of the droplets froze by immersion freezing while a
350 minority froze by contact freezing. Here immersion freezing refers to freezing of droplets by ice
351 nuclei immersed in the liquid droplets, and contact freezing refers to freezing of liquid droplets
352 by contact with neighboring frozen droplets (frozen droplets can grow by vapor transfer and
353 eventually can come in contact with their neighbors). Droplets that froze by contact freezing
354 were not considered when determining N_{Total} and $N_{Frozen}(T)$ from the freezing data. In addition to
355 immersion freezing and contact freezing, deposition freezing occasionally occurred in the
356 freezing experiments. Here deposition freezing refers to freezing on a particle not immersed in a
357 solution droplet. Deposition freezing was included in the calculations of $[IN(T)]$ by adding the
358 number of deposition freezing events to $\#IN(T)$ calculated with Equation S2 above.

359

360 Depending on the experimental conditions, the maximum concentration of ice nuclei, $[IN(T)]$,
361 that can be detected for any given slide (i.e. size interval sampled with the MOUDI) with the
362 microscope freezing technique is roughly 0.6-0.9 L⁻¹ depending on the number of droplets
363 condensed in an experiment and the total volume of air sampled by the MOUDI. As a result the
364 maximum concentration of IN determined by the microscope technique is small compared to the
365 maximum concentration determined with the CFDC method mentioned below.

366

367 ***S1.7 Real-Time Ice Nucleation Measurements with CFDC***

368 ***S1.7.1 IN Measurements***

369 A ground-based version of the Colorado State University continuous flow diffusion chamber
370 (CFDC) (Rogers et al., 2001) was employed for real-time measurements of IN concentrations.
371 The CFDC permits observation of ice formation on a continuous stream of particles at controlled

372 temperatures and humidities. In the CFDC, sampled air is directed vertically between two
373 concentric ice-coated cylinders held at different temperatures, creating a zone supersaturated
374 with respect to ice in the annular region. The sample air, ~15% of the total flow, is injected
375 between two particle-free sheath flows. As the particles in the sample flow are exposed to ice
376 supersaturations for several seconds, those particles active as IN under the sample temperature
377 and humidity conditions are nucleated and grown to ice crystals larger than a few μm in size.
378 These larger particles are distinguished from small non-IN aerosols by an optical particle counter
379 (OPC) at the outlet of the instrument. Physical impaction of larger aerosols ($>2.4 \mu\text{m}$) in advance
380 of the CFDC and reduction of humidity conditions to ice saturation in the lower third of the
381 chamber prevent false detection of large CN or cloud drops as ice nuclei. Temperatures ($\pm 1^\circ\text{C}$)
382 and humidities ($\pm 3\%$ RH with respect to water maximum uncertainty at -30°C) are well
383 controlled in the instrument. For data used in this study, measurements were made at -25°C at
384 relative humidity in the range of 103% to 106%. Under these conditions, the CFDC directly
385 measures IN activating by condensation/immersion freezing, and contributions are expected to
386 the IN population from both dust and biological particles (Prenni et al., 2009). For the data in
387 Figures 2E and 2F, particle concentrations were enhanced upstream using an MSP Corporation
388 (Model 4240) aerosol concentrator. Measurements made using the concentrator were corrected to
389 ambient concentrations based on the manufacturer's specifications for $1 \mu\text{m}$ particles, corrected
390 slightly for the sampling conditions at Manitou, as determined from direct measurements made
391 approximately every other day. IN number concentrations are reported at standard temperature
392 and pressure (STP; 1 atm and 0°C).

393

394 CFDC measurements were collected at the following times are shown in Figures 2E and 2F E-F
395 and discussed in the manuscript:

396	C01 (rain period)	8/2 10:27 – 17:57	(450 min.)
397	C02 (dry period)	8/17 16:27 – 23:47	(440 min.)

398
399 Periods C01 and C02 correspond to sub-periods during MOUDI samples M10 and M27,
400 respectively.

401

402 ***S1.7.2 DNA Analysis of IN Samples***

403 Ice crystals activated as IN in the CFDC were collected via impaction at the CFDC outlet (Prenni
404 et al., 2009; Garcia et al., 2012). Residual IN were impacted onto a glass slide, which was coated
405 with 5 mL of molecular grade mineral oil (Bio-Rad). DNA was then enzymatically extracted
406 using Proteinase K. The extracted DNA was PCR amplified using the universal 515F and 1391R
407 primers. The presence of biological IN was determined after PCR amplification via acrylamide
408 gel electrophoresis. PCR products were cloned into a plasmid vector using the TOPO TA
409 Cloning Kit® for sequencing (Invitrogen). Each clone was sequenced (Sanger method) and
410 identified via Blast search against the National Center for Biotechnology Information (NCBI)
411 genome database (2).

412 **References:**

- 413 Bozlee, B. J., Misra, A. K., Sharma, S. K. and Ingram, M.: Remote Raman and fluorescence
414 studies of mineral samples, *Spectrochimica Acta Part a-Molecular and Biomolecular*
415 *Spectroscopy*, 61, 2342-2348, 10.1016/j.saa.2005.02.033, 2005.
- 416 Després, V. R., Nowoisky, J. F., Klose, M., Conrad, R., Andreae, M. O. and Pöschl, U.:
417 Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by
418 DNA sequence and restriction fragment analysis of ribosomal RNA genes, *Biogeosciences*, 4,
419 1127-1141, 2007.
- 420 Dymarska, M., Murray, B. J., Sun, L. M., Eastwood, M. L., Knopf, D. A. and Bertram, A. K.:
421 Deposition ice nucleation on soot at temperatures relevant for the lower troposphere, *J. Geophys.*
422 *Res.-Atmos.*, 111, 10.1029/2005jd006627, 2006.
- 423 Foot, V. E., Kaye, P. H., Stanley, W. R., Barrington, S. J., Gallagher, M. and Gabey, A.: Low-
424 cost real-time multi-parameter bio-aerosol sensors, *Proceedings of the SPIE - The International*
425 *Society for Optical Engineering*, 711601 (711612 pp.), 10.1117/12.800226, 2008.
- 426 Fröhlich-Nowoisky, J., Pickersgill, D. A., Després, V. R. and Pöschl, U.: High diversity of fungi
427 in air particulate matter, *Proceedings of the National Academy of Sciences*, 106, 12814-12819,
428 10.1073/pnas.0811003106, 2009.
- 429 Fröhlich-Nowoisky, J., Burrows, S. M., Xie, Z., Engling, G., Solomon, P. A., Fraser, M. P.,
430 Mayol-Bracero, O. L., Artaxo, P., Begerow, D., Conrad, P. G., Andreae, M. O., Després, V. and
431 Pöschl, U.: Biogeography in the air: fungal diversity over land and oceans, *Biogeosciences*, 9,
432 1125-1136, 10.5194/bg-9-1125-2012, 2012.
- 433 Gabey, A. M., Gallagher, M. W., Whitehead, J., Dorsey, J. R., Kaye, P. H. and Stanley, W. R.:
434 Measurements and comparison of primary biological aerosol above and below a tropical forest
435 canopy using a dual channel fluorescence spectrometer, *Atmospheric Chemistry and Physics*, 10,
436 4453-4466, 10.5194/acp-10-4453-2010, 2010.
- 437 Garcia, E., Hill, T. C. J., Prenni, A. J., DeMott, P. J., Franc, G. D. and Kreidenweis, S. M.:
438 Biogenic ice nuclei in boundary layer air over two U.S. High Plains agricultural regions, *Journal*
439 *of Geophysical Research*, In Review, 2012.
- 440 Huffman, J. A., Treutlein, B. and Pöschl, U.: Fluorescent biological aerosol particle
441 concentrations and size distributions measured with an Ultraviolet Aerodynamic Particle Sizer
442 (UV-APS) in Central Europe, *Atmospheric Chemistry and Physics*, 10, 3215-3233, 2010.
- 443 Iannone, R., Chernoff, D. I., Pringle, A., Martin, S. T. and Bertram, A. K.: The ice nucleation
444 ability of one of the most abundant types of fungal spores found in the atmosphere, *Atmospheric*
445 *Chemistry and Physics*, 11, 1191-1201, 10.5194/acp-11-1191-2011, 2011.
- 446 Kaye, P. H., Stanley, W. R., Hirst, E., Foot, E. V., Baxter, K. L. and Barrington, S. J.: Single
447 particle multichannel bio-aerosol fluorescence sensor, *Optics Express*, 13, 3583-3593, 2005.
- 448 Lighthart, B. and Shaffer, B. T.: Airborne bacteria in the atmosphere surface layer: temporal
449 distribution above a grass seed field, *Applied and Environmental Microbiology*, 61, 1492-1496,
450 1995.

451 Lindemann, J., Constantinidou, H. A., Barchet, W. R. and Upper, C. D.: Plants as sources of
452 airborne bacteria, including ice nucleation-active bacteria, *Applied and Environmental*
453 *Microbiology*, 44, 1059-1063, 1982.

454 Madelin, T. M.: Fungal Aerosols - A Review, *Journal of Aerosol Science*, 25, 1405-1412,
455 10.1016/0021-8502(94)90216-x, 1994.

456 Marple, V. A., Rubow, K. L. and Behm, S. M.: A microorifice uniform deposit impactor
457 (MOUDI) - description, calibration, and use, *Aerosol Sci. Technol.*, 14, 434-446,
458 10.1080/02786829108959504, 1991.

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

461 Pöhlker, C., Huffman, J. A. and Pöschl, U.: Autofluorescence of atmospheric bioaerosols -
462 fluorescent biomolecules and potential interferences, *Atmospheric Measurement Techniques*, 5,
463 37-71, 10.5194/amt-5-37-2012, 2012.

464 Pöschl, U., Martin, S. T., Sinha, B., Chen, Q., Gunthe, S. S., Huffman, J. A., Borrmann, S.,
465 Farmer, D. K., Garland, R. M., Helas, G., Jimenez, J. L., King, S. M., Manzi, A., Mikhailov, E.,
466 Pauliquevis, T., Petters, M. D., Prenni, A. J., Roldin, P., Rose, D., Schneider, J., Su, H., Zorn, S.
467 R., Artaxo, P. and Andreae, M. O.: Rainforest Aerosols as Biogenic Nuclei of Clouds and
468 Precipitation in the Amazon, *Science*, 329, 1513-1516, 10.1126/science.1191056, 2010.

469 Prenni, A. J., Petters, M. D., Kreidenweis, S. M., Heald, C. L., Martin, S. T., Artaxo, P., Garland,
470 R. M., Wollny, A. G. and Pöschl, U.: Relative roles of biogenic emissions and Saharan dust as
471 ice nuclei in the Amazon basin, *Nature Geoscience*, 2, 402-405, 10.1038/ngeo517, 2009.

472 Robinson, N. H., Newton, H. M., Allan, J. D., Irwin, M., Hamilton, J. F., Flynn, M., Bower, K.
473 N., Williams, P. I., Mills, G., Reeves, C. E., McFiggans, G. and Coe, H.: Source attribution of
474 Bornean air masses by back trajectory analysis during the OP3 project, *Atmospheric Chemistry*
475 *and Physics*, 11, 9605-9630, 10.5194/acp-11-9605-2011, 2011.

476 Rogers, D. C., DeMott, P. J., Kreidenweis, S. M. and Chen, Y.: A continuous-flow diffusion
477 chamber for airborne measurements of ice nuclei, *J. Atmos. Ocean. Tech.*, 18, 725-741, 2001.

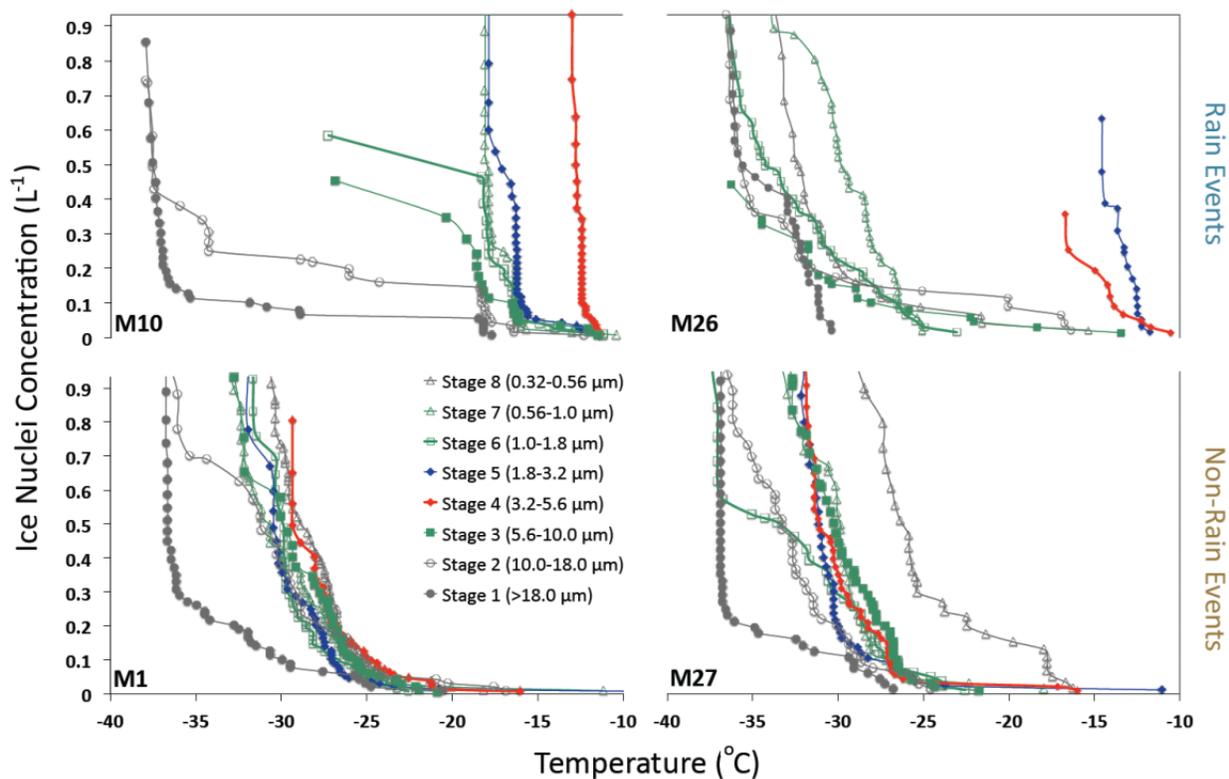
478 Sinha, B. W., Hoppe, P., Huth, J., Foley, S. and Andreae, M. O.: Sulphur isotope analysis of
479 individual aerosol particles in the urban aerosol at a Central European site (Mainz, Germany),
480 *Atmospheric Chemistry and Physics*, 8, 7217-7238, 2008.

481 Vali, G.: Quantitative evaluation of experimental results on heterogeneous freezing of
482 supercooled liquids, *Journal of the Atmospheric Sciences*, 28, 402-&, 10.1175/1520-
483 0469(1971)028<0402:qeoera>2.0.co;2, 1971.

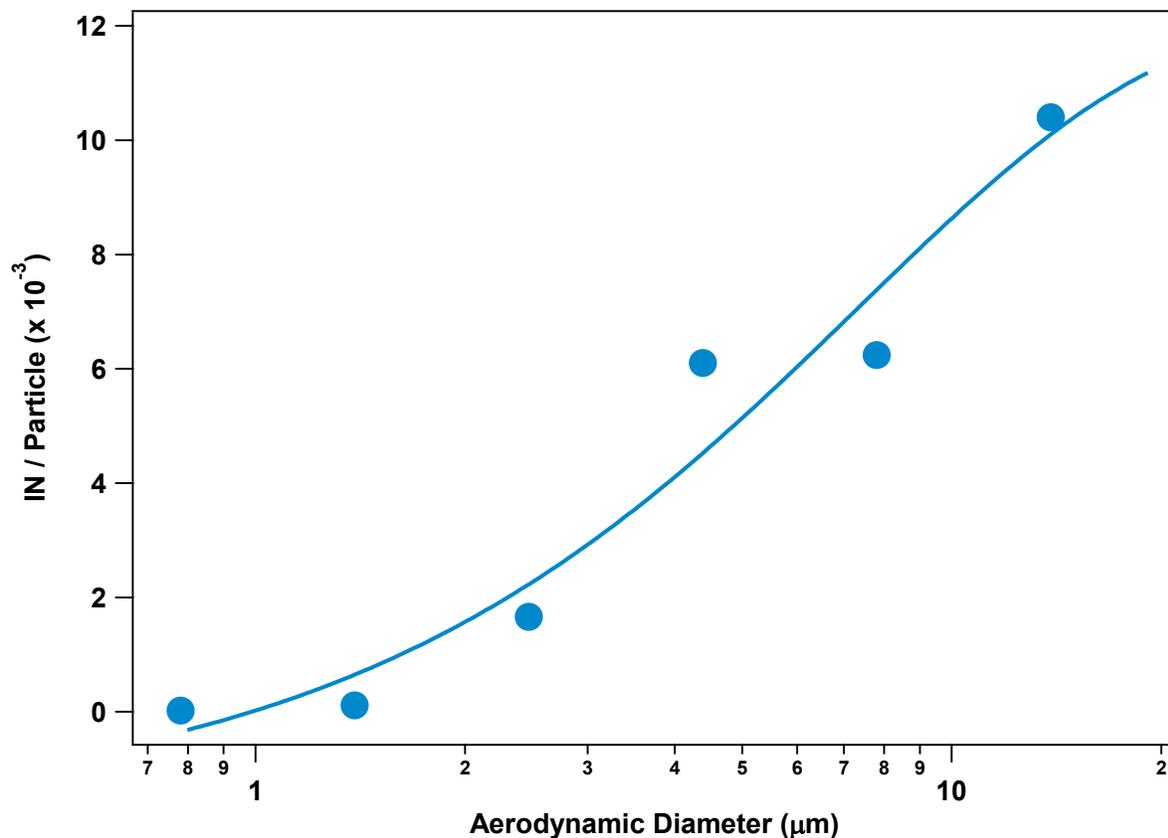
484 Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J.: 16S ribosomal DNA
485 amplification for phylogenetic study, *J. Bacteriol.*, 173, 697-703, 1991.

486 White, T. J., Bruns, T., Lee, S. and Taylor, J.: Amplification and Direct Sequencing of Fungal
487 Ribosomal RNA genes for Phylogenetics, in: *PCR Protocols: a Guide to Methods and*
488 *Applications*, edited by: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J., Academic
489 Press, Inc., New York, 315-322, 1990.

490



491
 492
 493 **Figure S1:** IN activation curves from microscopic IN activation experiments with size-resolved aerosol samples
 494 (MOUDI stages). Upper panels (A,B) for samples collected during rain events, and lower panels (C,D) for samples
 495 collected during dry periods. Red traces show Stage 4 (3.2 – 5.6 μm), blue traces show Stage 5 (1.8 – 3.2 μm), and
 496 light green traces show Stage 3, 6, 7 (5.6-10, 1.0-1.8, and 0.56-1.0 μm, respectively). See SOM section S1.5.1.1 for
 497 sampling dates.



498
 499
 500 **Figure S2:** An estimate of the fraction of particles collected during rain events (M10, M26) that can serve as IN at
 501 -15 °C. IN concentrations were calculated from microscopic IN activation experiments and particle concentrations
 502 were calculated from UV-APS measurements. See SOM section S1.5.1.1 for sampling dates. Note that the fraction
 503 of particles with IN activity is greater than 1 in 1000 for all particles >2 μm and exceeds 1 in 100 for particles >10
 504 μm. Exponential curve shown to guide the eye.