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Maurício C. Mantoani, Ana P.M. Emygdio, Cristiane Degobbi, Camila Ribeiro Sapucci, Lara C.C. Guerra, et al.. Rainfall effects on vertical profiles of airborne fungi over a mixed land-use context at the Brazilian Atlantic Forest biodiversity hotspot. *Agricultural and Forest Meteorology*, 2023, 331, pp.109352. 10.1016/j.agrformet.2023.109352 . hal-03974544

HAL Id: hal-03974544

<https://hal.inrae.fr/hal-03974544>

Submitted on 7 Feb 2024

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1 **Title:** Rainfall effects on vertical profiles of airborne fungi over a mixed land-use context at
2 the Brazilian Atlantic Forest biodiversity hotspot

3

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25 **Type of Submission:** Full Paper

26 **Author's Contribution:** MCM, CEM, and FLTG conceived and designed the research; MCM,
27 LCCG, GGA, SMB, and FLTG collected the data in the field; MCM, APME, CD, LCCG,
28 RHSZ, GGA, DMCS, and VBDF performed the laboratorial analyses; MCM, APME, CD,
29 CRS, LCCG, RHSZ, GGA, and FLTG analysed the data; MCM, APME, CD, CRS, LCCG,
30 MAFSD, PLSD, RHSZ, FR, GGA, DMCS, VBDF, SMB, JAM, FC, TŠ-T, CEM, and FLTG
31 wrote and edited the manuscript; MCM, CEM, and FLTG led the writing of the manuscript.
32 Critical contribution to drafts and final approval for publication was given by all authors.

33

34 **Acknowledgements:** The authors thank the support received from local landowners of
35 Arceburgo city and the COOXUPÉ-MG association, particularly Mr. Éder Ribeiro dos Santos.
36 We also thank Prof. Dr. Maria F. Andrade for lending fieldwork equipment, Mr. Jairo Fogaça
37 and his hot-air balloon team who gave exceptional help in the field, and Mrs. Solange Lima for
38 helping with fungal taxonomy and identification.

39

40 **Funding:** This work was supported by FAPESP (“*Fundação de Amparo à Pesquisa do Estado*
41 *de São Paulo*”); São Paulo Research Foundation; Grants: 2016/06160-8 to FLTG and
42 2020/14143-1 to MCM). T.Š.-T. was supported by The Danish National Research Foundation
43 (DNRF106, to the Stellar Astrophysics Centre, Aarhus University), the Novo Nordisk
44 Foundation (NNF19OC0056963) and the Villum Fonden (23175 and 37435).

45

46 **Conflict of Interests:** Authors declare no conflict of interest.

47

48 **Data Availability:** Data presented in this study are available on request from the corresponding
49 author. The data are not publicly available due to limitations of consent requested by the
50 participants of the study and landowners where the fieldwork experiment was done.

51 **Abstract:** Whilst fungi are a large fraction of primary biological aerosol particles (PBAPs) and
52 their impact on global climate has been widely recognised, few studies have empirically
53 assessed fungal vertical profiles and diversity relating those with rainfall. Here, we show the
54 results of fungal PBAPs before and after a rainfall event during a fieldwork campaign using a
55 hot-air balloon over a mixed land-use context at the Brazilian Atlantic Forest biodiversity
56 hotspot. Four flights of *c.* 1 hour each were performed in the early morning from 8th until 11th
57 of March 2022, and data were collected at three sampling heights (0, 150 and 300 m). Rainfall
58 estimation using IMERG data indicated the precipitation event was of 15-20 mm and
59 ERA5/ECMWF data highlighted that most of the airborne samples were taken above the
60 boundary layer height. After the rainfall, the concentration of fungal spores at the ground level
61 remained unchanged, whereas it was reduced to between 2- and 2.5-fold for the 150 and the
62 300 m heights, respectively. This was also accompanied by a reduction in the number of Pink-
63 CFU, indicating a major drop in fungal PBAPs at higher altitudes associated with the rain. In
64 addition, total spore concentration indicated *Cladosporium* sp. as dominant at all sampling
65 heights, accounting for more than 80% of all spores, whereas *Aspergillus/Penicillium*-like
66 represented less than 20%. Our results show the effects of rainfall and altitude on the
67 concentration of fungal PBAPs, indicating how wet removal impacts fungi vertical profiles
68 which has knock-on-effects on cloud and precipitation formation.

69

70 **Keywords:** *Aspergillus/Penicillium*-like; *Cladosporium*; cloud formation; ice nucleation
71 activity; PBAP.

72

73 **Introduction:**

74

75 Fungi are part of atmospheric aerosols (Heald & Spracklen, 2009; Janssen et al., 2021)
76 and may have a role in local, regional, and global climate through their ice nucleation activity
77 (Després et al., 2012) that impacts and cloud formation, optical properties, and lifetime (Bauer
78 et al., 2002; Sesartic et al., 2013; Kanji et al., 2017). Although the importance of primary
79 biological aerosol particles (hereafter, PBAPs) has been recognised to interplay with climate
80 (Després et al., 2012; Martinez-Bracero et al., 2022; Šantl-Temkiv et al., 2022), key processes,
81 such as emissions from surfaces and transfer of PBAPs to higher layers of the troposphere are
82 not well understood and only few studies have empirically assessed if PBAPs, in particular
83 fungi, show any patterns of stratification (Els et al., 2019a; 2019b; Emygdio et al., 2022).
84 Research focusing on fungi as a compound of climate regulation is still developing, and data
85 for the Brazilian Atlantic Forest, one of the global biodiversity hotspot areas, not only is scarce
86 (Emygdio et al., 2018; 2022; Mantoani et al., 2023), but it is also threatened by its high-level
87 degradation and loss of biological information (Lima et al., 2020).

88 Most true fungi (i.e., Eumycota) disperse via the atmosphere (Golan & Pringle, 2016),
89 which has implications for their presence in the planetary boundary layer of the atmosphere –
90 the lowest part of the atmosphere influenced by the planetary surface. The dispersion of some
91 fungi can also be triggered by precipitation (Löbs et al., 2020), which would be highlighted in
92 areas that have high pluviosity and water availability as is the case of the Brazilian Atlantic
93 Forest (Dalagnol et al., 2022). Meteorological events, therefore, may contribute to fungal
94 emissions (or liberation) to the atmosphere (Grinn-Grofoń et al., 2019; Fagodiya et al., 2022).
95 Since several fungal genera, for instance *Cladosporium*, *Fusarium*, and *Penicillium*, impact on
96 cloud, rain, snow, and hail formation by means of ice nucleation activity (Fröhlich-Nowoisky
97 et al., 2012; Kunert et al., 2019), understanding whether fungal PBAPs present any

98 stratification patterns that could (partially) explain these processes would enhance our
99 comprehension of climate regulation (Šantl-Temkiv et al., 2022). Nonetheless, sampling at
100 high altitudes can be difficult due to the low retrieval of microbial material (Šantl-Temkiv et
101 al., 2020; Tignat-Perrier et al., 2020). Additionally, given the stochasticity of rainfall events,
102 particularly in the light of climate change (Dalagnol et al., 2022), there is a need to investigate
103 the drivers behind fungal vertical profiles in the planetary boundary layer, such as rainfall
104 events. This would be important not only to verify the effects that fungal PBAPs have on cloud
105 formation and rain facilitation, but also to understand how these meteorological events
106 contribute to the dispersion of airborne fungi.

107 Under this context, while we were carrying out a fieldwork campaign using a hot-air
108 balloon to assess the fungal vertical profile in the atmosphere within the Brazilian Atlantic
109 Forest biome, rainfall occurred during the fieldwork. Taking advantage of it, here, we present
110 the results of fungal PBAPs before and after a rainfall event, elucidating how meteorology may
111 interfere with fungi present in the atmosphere, and relating this with sampling altitude (0, 150
112 and 300 m). Whilst we have assessed fungi diversity, the analysis is focused on *Cladosporium*,
113 *Aspergillus/Penicillium*-like (hereafter, *Asp/Pen*-like), and *Fusarium*. Not only these fungi are
114 particularly abundant in the studied region, serving as a proxy for other species (Emygdio et
115 al., 2022), but they also might interfere with climate by means of ice nucleation (Fröhlich-
116 Nowoisky et al., 2012; Kunert et al., 2019). For this, our hypotheses were: (1) we expected
117 fungal spore concentrations to be bigger at lower compared to higher altitudes (Emygdio et al.,
118 2022); (2) fungal PBAPs would have a stratification pattern, with smaller richness of species
119 occurring at higher altitudes or atmospheric layers (Els et al., 2019a; 2019b; Tignat-Perrier et
120 al., 2020); and (3) rainfall reduces the concentration of fungal spores at higher altitudes, by
121 means of wet removal, bringing fungal PBAPs present at 150 and 300 m to the ground level
122 (Yue et al., 2016; Rathnayake et al., 2017).

123 **Material and Methods**

124

125 **Area of Study and Experimental Design**

126

127 The area of study encompasses the region of Arceburgo city, located in the Southeast
128 region of Brazil, Minas Gerais state. The region features a mixed land-use pattern, including
129 coffee plantations, pasture for cattle, and sugar-cane crops interspersed with Atlantic Forest
130 fragments (Emygdio et al., 2022). The climate in the region is classified as Aw (Köppen-
131 Geiger), characterised as tropical weather with dry winters, with an annual rainfall of *c.* 1600
132 mm and daily average temperatures ranging from 21.1 to 23 °C (Reboita et al., 2015). Ground
133 sampling was done at the Fazenda Cachoeira (21°23'36.50" S; 46°55'15.87 W), and the hot-
134 air balloon flights departed from the same location.

135 We planned the campaign during the rainy season as we were interested in the effects
136 of precipitation on fungal spore concentration in the atmosphere. To characterise fungal PBAPs
137 vertical profiles, we performed four consecutive flights (between 8th and 11th of March 2022)
138 with a hot-air balloon. Each flight lasted *c.* 1 hour. Flights were performed in early mornings
139 (06:00 – 08:00 local time) since this was the only period in which the balloon could be safely
140 flown. Early morning corresponds to the period of the atmospheric boundary layer growth and
141 this phenomenon can influence the vertical distribution of PBAPs. This is a limitation of the
142 study that was taken into account and appropriately discussed in the following sections. Flights
143 reached the maximum height of *c.* 800 m from ground level, and since we were not able to
144 collect replicate-samples above 300 m, we show data for 0-300 m only. There was a rain event
145 on March 10th just before our third flight, so the analysis is divided into prior to and after the
146 rainfall, with two flights in each period. According to data collated by the meteorological
147 stations in the region (COOXUPÉ, 2022 – available at: cooxupe.com.br), on the 10th of March,

148 the total amount of cumulative rainfall for the region was 17 mm, with an average temperature
149 of 23.3 °C and an average air humidity of 73.7%.

150

151 **Rainfall Estimation using IMERG**

152

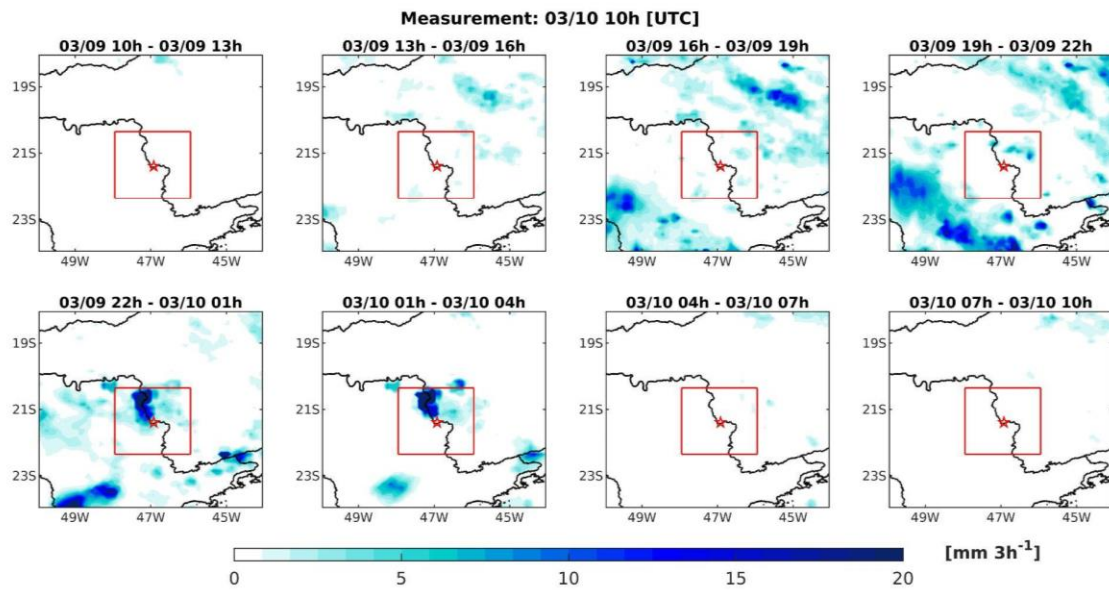
153 Half-hourly precipitation data from the Integrated Multi-satellitE Retrievals for the
154 Global Precipitation Measurement (GPM) (IMERG, Huffmann et al., 2019) were used to
155 estimate local precipitation during the fieldwork campaign. IMERG combines data from
156 passive microwave sensors comprising the GPM constellation to estimate the surface
157 precipitation with a 0.1° spatial resolution. We analysed the precipitation time series for the
158 campaign period for the closest grid point to the farm location in Arceburgo (21°23'36.50" S,
159 46°55'15.87" W). The IMERG rainfall spatial pattern in the 24 hours preceding the
160 measurements was also evaluated. The 3-hourly IMERG spatial precipitation pattern in the 24
161 hours preceding the measurement on the 10th of March at 10:00 UTC show an estimated
162 precipitation of 15-20 mm just before the third flight (Figure 1).

163

164 **ERA5/ECMWF Meteorological Data**

165

166 Meteorological data were obtained from the ERA5 reanalysis of the European Centre
167 for Medium-Range Weather Forecast (ECMWF). Specifically, height of the boundary layer (in
168 meters above ground) and the instantaneous surface heat flux (in W m⁻²) were extracted for the
169 period of the samplings from the “ERA5 hourly data on single levels from 1959 to present”
170 dataset (Hersbach et al., 2018). The reanalysis covers the entire globe with a horizontal
171 resolution of 0.25° x 0.25° and an hourly time resolution. For the present work, the closest
172 pixel to the experimental farm was chosen (-46.93° longitude, -21.40° latitude).



173

174 **Figure 1** – Three-hourly IMERG spatial precipitation pattern in the 24 hours preceding the
 175 measurement on the 10th of March at 10:00 UTC. The two bottom quadrants on the left,
 176 referring to 01:00-04:00 UTC, show an estimated precipitation of 15-20 mm just before the
 177 third flight. The red box represents an area of 200 km² centred on the point corresponding to
 178 the location of the farm in Arceburgo (red star, 21°23'36.50" S, 46°55'15.87" W).

179

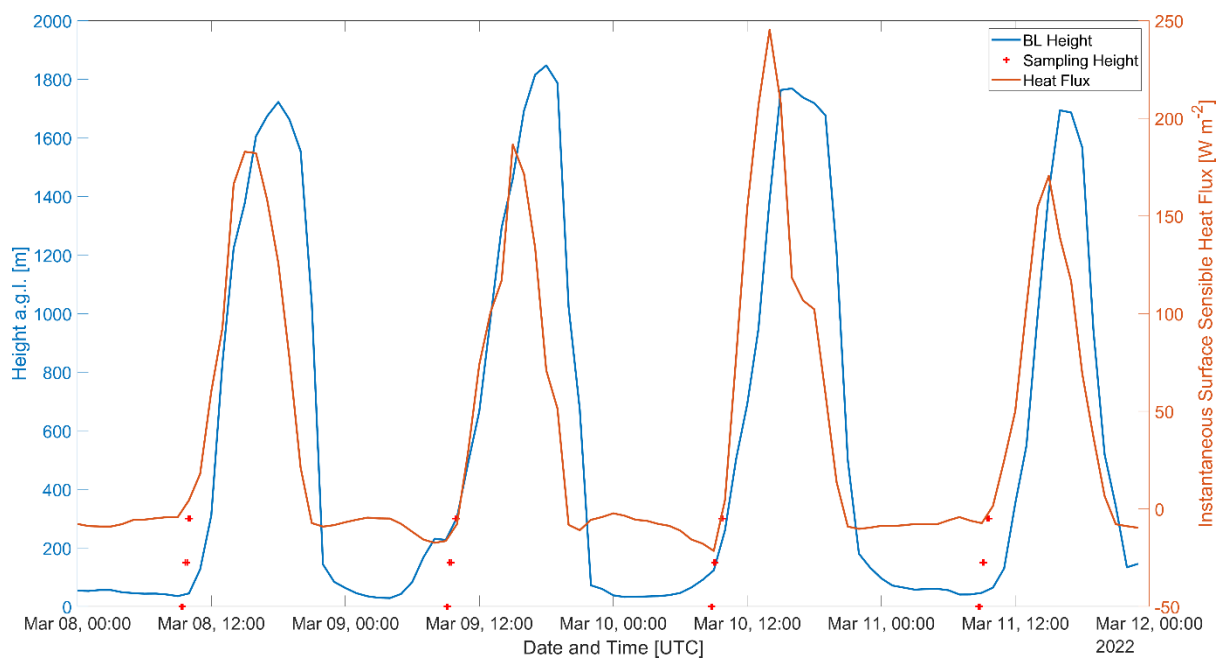
180 Given that the ECMWF convention specifies that negative fluxes are upward, the
 181 instantaneous heat flux was multiplied by -1 to have it in the standard micrometeorological
 182 convention for which upward fluxes are positive and negative fluxes are downward. Trends of
 183 meteorological parameters over the sampling campaign can be seen in Figure 2, which
 184 highlights how most of the airborne samples were taken above the boundary layer height. This
 185 suggests that airborne sampling happened in the residual layer derived from the decay of the
 186 mixed layer of the previous day (Stull, 1988). Furthermore, during the samplings, the
 187 instantaneous surface sensible heat flux showed negative or slightly positive values, indicating
 188 that thermal turbulence in the shallow morning boundary layer was still small.

189

190 We are aware of the limitations of models in simulating boundary layer height.
 Nevertheless, there was no available radiosonde close enough to the sampling region that could

191 be used in this study. ERA5 has, thus, been chosen to obtain boundary layer information
192 following a recent paper by Guo et al. (2021). The paper made a near-global comparison
193 between daytime boundary layer height from various reanalysis products and measurements
194 made by radiosonde. Even if the comparison was done only at synoptic times (00:00 and 12:00
195 UTC), ERA5 was shown to be the reanalysis having the smaller bias and the highest positive
196 correlations relative to radiosondes (Guo et al., 2021).

197



198

199 **Figure 2** – Trend of meteorological data from the ERA5 reanalysis during the sampling period.

200 On the left y-axis it is shown the height above ground of the boundary layer (BL, light blue
201 line) and the PBAP samplings (red crosses), while on the right y-axis it is shown the value of
202 the instantaneous surface sensible heat flux (orange line).

203

204 **Fungi Sampling using the Portable Burkard Air Sampler**

205

206 We collected *in situ* airborne fungal PBAPs using a portable Burkard air sampler
207 (Burkard Manufacturing Co., Hertfordshire, UK), as per Emygdio et al. (2022). Three sampling

208 altitudes were examined: 0 m or ground level, 150 m, and 300 m above ground level. At the
209 ground level, the instrument was placed at 0.3 m over a small table (Supplementary Figure 1A).
210 To sample at 150 and 300 m, the portable Burkard air sampler was mounted and stabilised in
211 the passenger basket of the hot-air balloon (Supplementary Figure 1B). Once the desired
212 sampling height was reached, the hot-air balloon floated steadily during sample collection. The
213 variation of flight height was calculated to be *c.* 10% of the desired one, so sampling heights
214 were 150 ± 15 m (i.e., 135-165 m) and 300 ± 30 m (i.e., 270-330 m).

215 Slides were prepared with a “Melinex” tape coated with an adhesive and the portable
216 Burkard air sampler sampled aerosols for 5 min, totalling 50 L of air on each slide (10 L/min;
217 see Aizenberg et al., 2000; Emygdio et al., 2022). Two slides per sampling height per day were
218 collected, totalling 6 slides per day and 24 slides analysed for the whole experiment (i.e., 2
219 slides x 3 heights x 4 flights = 24 slides). The Portable Burkard air sampler has a theoretical
220 cut-off size of 2.52 μm and a 2.3-2.4 μm experimental cut-off size (Aizenberg et al., 2000).
221 More information regarding the equipment can be found in Aizenberg et al. (2000). Following
222 the methodology presented by Rogers and Muilernberg (2001), sampled slides were fixed using
223 glycerine jelly that covered them entirely. *Cladosporium* sp. and *Asp/Pen*-like spores were
224 determined using a microscope at 1000x magnification (Emygdio et al., 2018; 2022). Fungal
225 spores were counted and identified as per Haines et al. (2000) and the whole slide was analysed.
226 Then, to infer the concentration of fungal spores per cubic meter, we divided the total number
227 of spores counted by the total volume sampled as per the Equation 1.

228 Equation 1:
$$\frac{\textit{Spores}}{m^{-3}} = \frac{\textit{number of spores counted}}{\textit{Flow rate (m}^{-3}\textit{)} \times \textit{sampled time (min)}}$$

229

230 **Fungi Sampling using the Microbial Air Monitoring System (MAS100)**

231

232 In addition to the data collected using the portable Burkard air sampler, a Microbial
233 Air Monitoring System (MAS100, Merck KGaA, Darmstadt, Germany; Supplementary Figure
234 1) was used to collect cultivable fungi. The instrument sampled a total volume of 250 L of air
235 into sterile Petri dishes, and plates contained a modified Dicloran Rosa Bengal culture medium
236 (Castro e Silva et al., 2015). After sampling, collected plates were immediately put into a
237 thermal box and, once the hot-air balloon flight was over, they were subsequently stored at
238 4 °C until analysis. Plates were incubated in a biological incubator at $30^{\circ} \pm 2^{\circ}$ C for up to 7
239 days for isolation and identification (Adolfo Lutz Institute Mycology Laboratory, São Paulo).
240 One plate for each sampling altitude (i.e., 0, 150 and 300 m) for each day prior (8th and 9th
241 March 2022) and after the rainfall (10th and 11th March 2022) were collected, totalling 12 plates
242 during the fieldwork campaign.

243 Fungal concentration was expressed as colony-forming units (hereafter, CFU) per
244 metre cubic of air (CFU m⁻³). Aside from estimating the total CFU number, we have classified
245 the colonies according to pigmentation (i.e., white, or pink) to determine concentrations of
246 fungi affiliating to *Fusarium* sp., which develops pink pigments after 7 days of growth (de
247 Hoog et al., 2020). Fungi were cultivated and molecular characterisation, as well as
248 classification at the genus level was performed by mass spectrometry using a MALDI Biotyper
249 (Bruker Daltonics, Billerica, Massachusetts, USA). The Matrix-Assisted Laser Desorption
250 Ionization Time-of-Flight Mass Spectrometry technique (or MALDI-TOF MS) assists protein
251 flight time, allowing the analysis of relatively large biomarkers, thus, identifying fungal genera
252 and species (Bizzini et al., 2010).

253

254 **Statistical Analysis**

255

256 As the assumptions of normality (Shapiro-Wilk's test) and homoscedasticity
257 (Levene's test) were not satisfied, data on the concentration of fungal spores and CFU, as well
258 as number of species were log-transformed. To compare differences in the concentration of
259 fungal spores collected at the different sampling heights prior to and after the rainfall, we used
260 Repeated Measures Analysis of Variance (i.e., rANOVA), followed by Tukey's HSD *post-hoc*
261 test. Analysis of Variance (ANOVA) was used to check differences on the data collected using
262 the MAS100 (i.e., total fungal CFU concentration, White- and Pink-CFU concentration, and
263 fungal richness) and also between fungal PBAP emission rates. Moreover, regression analysis
264 was used to evaluate the effects of sampling height on the concentration of *Cladosporium* sp.
265 and *Asp/Pen*-like spores. These analyses were performed with a significance level of $\alpha = 0.05$,
266 using Statistica v. 14.0.0.15 (Statistica, 2022).

267

268 **Results**

269

270 **Portable Burkard Air Sampler Results**

271

272 The total concentration of fungal spores varied with altitude, and it was much bigger
273 at the ground level (118,420 fungal spores m^{-3} or 76% of all spores counted), followed by the
274 150 m layer (19,700 spores m^{-3} or 17%), and the 300 m layer (8,280 spores m^{-3} or 7%) (Table
275 1). A significant interaction between the rainfall event and sampling heights was found ($F_{(2,18)}$
276 $= 14.26$; $P < 0.001$). We observed a pronounced reduction in the concentration of fungal spores
277 at 150 m (95% CI = 0.455, 1.066) and 300 m (95% CI = 0.324, 0.935) after the rainfall. This
278 resulted in a 4- to 5-fold decrease in fungal spore concentrations caused by rainfall (Table 1).
279 However, at the ground level, as the concentration of fungal spores showed only a minor
280 reduction (c. 15%), the total concentration of fungal spores after the rainfall was not

281 significantly different in comparison to before the rain ($F_{(2,18)} = 14.26$; $P = 0.799$; 95% CI = -
 282 0.191, 0.419). Thus, most spores (90%) after the rainfall event were found at the ground level.
 283
 284 **Table 1** – Concentration (spores m^{-3}) and percentage of *Cladosporium* sp. and
 285 *Aspergillus/Penicillium*-like fungal spores at the region of Arceburgo city, Minas Gerais state,
 286 Southeast Brazil. Note: “All Data” refers to all data collected in the whole experiment, whereas
 287 “Before Rainfall” or “After Rainfall” refers to data sampled prior or after the rain that occurred
 288 at the dawn of the third day of the fieldwork campaign (10th of March 2022) and before the
 289 third hot-air balloon flight.

All Data					
Sampling Height	Total	<i>Cladosporium</i>	<i>Asp/Pen</i>-like	% <i>Cladosporium</i>	% <i>Asp/Pen</i>-like
0 m	90,440	75,020	15,420	82.95	17.05
150 m	19,700	14,540	5,160	73.81	26.19
300 m	8,280	6,120	2,160	73.91	26.09
All Layers	118,420	95,680	22,740	80.80	19.20
Before Rainfall (8th and 9th of March 2022)					
0 m	48,440	39,440	9,000	81.42	18.58
150 m	16,760	12,600	4,160	75.18	24.82
300 m	6,700	4,980	1,720	74.33	25.67
All Layers	71,900	57,020	14,880	79.30	20.70
After Rainfall (10th and 11th of March 2022)					
0 m	42,000	35,580	6,420	84.71	15.29
150 m	2,940	1,940	1,000	65.99	34.01
300 m	1,580	1,140	440	72.15	27.85
All Layers	46,520	38,660	7,860	83.10	16.90

290
 291 Considering only the two main fungi groups in the studied region, *Cladosporium* sp.
 292 dominated all the three sampling heights examined, representing more than 80% of all spores
 293 counted, whilst *Asp/Pen*-like accounted for nearly 20%. Prior to the rain, *Cladosporium* sp.
 294 were 3- to 4-fold more abundant than *Asp/Pen*-like at all heights (ground level, $F_{(2,18)} = 5.168$;
 295 $P = 0.017$; 95% CI = 0.411, 0.882; 150 m, 95% CI = 0.251, 0.722; and 300 m, 95% CI = 0.228,
 296 0.699; Figure 3). After the rainfall, at the ground level, *Cladosporium* sp. were 5-fold more

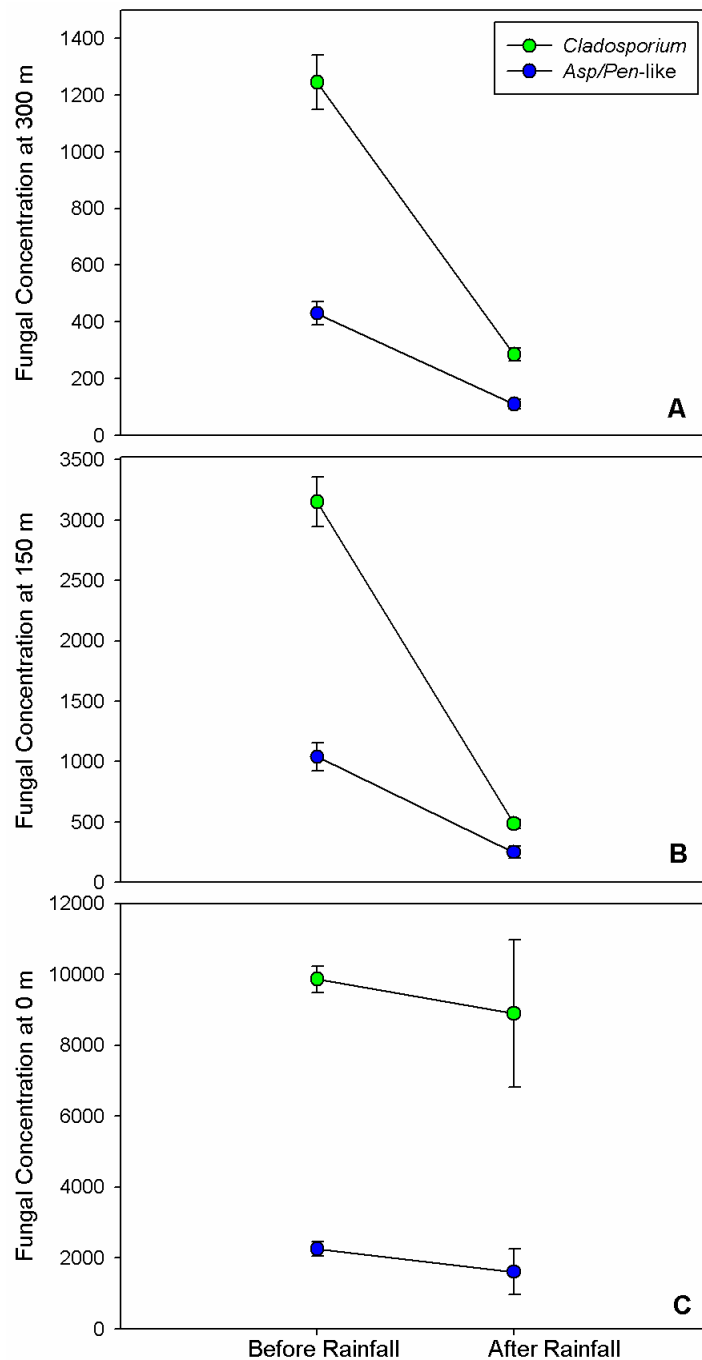
297 abundant ($F_{(2,18)} = 5.168$; $P = 0.017$; 95% CI = 0.606, 1.08) than *Asp/Pen*-like (Figure 3C).
298 Nevertheless, for the 150 m and 300 m, these ratios were reduced to 2-fold ($F_{(2,18)} = 5.168$; P
299 = 0.017; 95% CI = 0.076, 0.547; Figure 3B) and 2.5-fold ($F_{(2,18)} = 5.168$; $P = 0.017$; 95% CI =
300 0.188, 0.659; Figure 3A), respectively. These shifts in fungal concentration driven by the rain
301 were paralleled by the proportion of spores sampled at the different heights (Table 1).

302

303 **MAS100 Results**

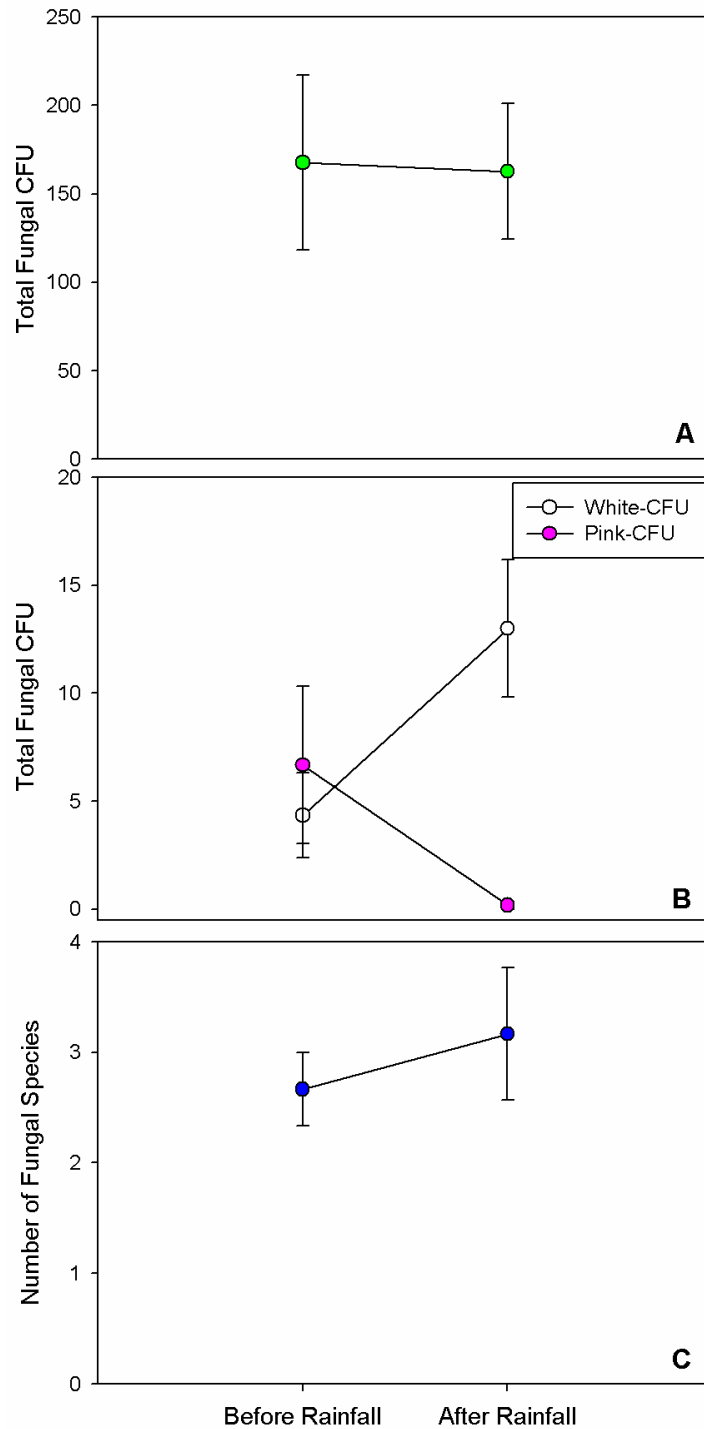
304

305 The MAS100 indicated that the number of CFU remained similar before (168 ± 49
306 CFU) and after the rain (163 ± 38 CFU) ($F_{(1,10)} = 0.006$; $P = 0.942$; Figure 4A). Nevertheless,
307 once the rain occurred, the abundance of White-CFU tripled (4.33 ± 1.96 CFU and $13.00 \pm$
308 3.17 CFU, before and after the rain, respectively; Figure 4B), although it was not significantly
309 different ($F_{(1,10)} = 2.889$; $P = 0.12$). In turn, the number of Pink-CFU shifted from an average
310 of 6.67 ± 3.65 CFU to less than one ($F_{(1,10)} = 5.086$; $P = 0.477$; 0.17 ± 0.17 ; Figure 4B). The
311 richness based on the number of fungal morphotype-species was not different between before
312 (2.67 ± 0.33) and after the rainfall event (3.17 ± 0.60 ; $F_{(1,10)} = 0.099$; $P = 0.759$; Figure 4C).
313 *Cladosporium* sp. was present in 11 out of 12 Petri dish samples and ranked the first in total
314 frequency (92%), followed by *Fusarium* sp. (50%) and *Penicillium* sp. (42%) (Supplementary
315 Table 1). *Alternaria* sp. was the only morphotype-species that appeared only after the rain and
316 *Curvularia* sp. was the morphotype-species with the lowest frequency amongst all fungi
317 analysed, with only two records (or a total of 17% of frequency; Supplementary Table 1).



318

319 **Figure 3** – Concentration (spores m⁻³) of *Cladosporium* sp. and *Aspergillus/Penicillium*-like
 320 spores at (A) 300 m, (B) 150 m, and (C) 0 m (i.e., ground level) sampled at the region of
 321 Arceburgo city, Minas Gerais state, Southeast Brazil (n = 4; mean ± SE; non-transformed data).
 322 Legend: “Before Rainfall” or “After Rainfall” refers to data sampled prior or after the rain that
 323 occurred at the dawn of the third day of the fieldwork campaign (10th of March 2022) and
 324 before the third hot-air balloon flight.



325

326 **Figure 4** – Total fungal CFU (A), White-CFU and Pink-CFU (B), and number of fungal species
 327 (C) sampled at the region of Arceburgo city, Minas Gerais state, Southeast Brazil (n = 6; mean
 328 ± SE; non-transformed data). Legend: “Before Rainfall” or “After Rainfall” refers to data
 329 sampled prior or after the rain that occurred at the dawn of the third day of the fieldwork
 330 campaign (10th of March 2022) and before the third hot-air balloon flight.

331 **Correlations between Fungal PBAPs and Sampling Heights**

332

333 We found a negative correlation between fungal spore concentration and sampling
334 height, meaning that there were smaller concentrations of fungal spores with increasing altitude
335 (Figure 5; Supplementary Table 2). We constructed linear regression models that described the
336 relationship between altitude and fungal spore concentrations, which fitted very well with our
337 observations prior to the rain (r^2 values > 0.9). Once the rain occurred, nevertheless, the
338 goodness of fit compared to prior the rainfall event decreased to a range of 0.73-0.84.

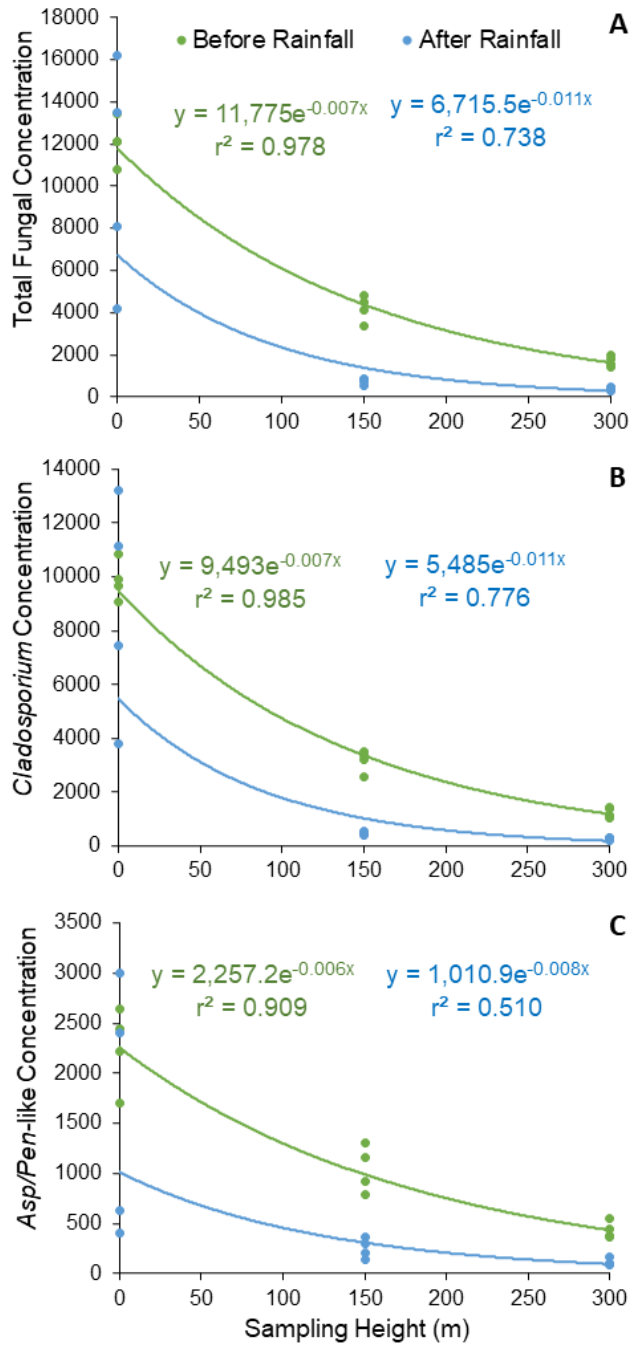
339

340 **Discussion**

341

342 Our hypothesis on a higher concentration of fungal spores at ground level was
343 confirmed as seen in other studies (Golan & Pringle, 2016; Emygdio et al., 2022), showing a
344 major drop on fungal PBAPs with increasing height above ground (Sesartic et al., 2013;
345 Emygdio et al., 2022). As per Emygdio et al. (2022) most airborne samples collected at 150
346 and 300 m so early in the morning are expected to be representative of the residual layer above
347 the growing convective boundary layer. Contrary to the findings of Els et al. (2019a, b) who
348 sampled microorganisms in the below and above the free troposphere, we did not find a
349 reduction on fungal species richness with increasing sampling height. It is not straightforward
350 to pinpoint the cause of this difference, which could be affected by location-associated factors,
351 such as available ground sources, meteorology, and climate, by different sampling strategies
352 and even by different techniques to evaluate richness. Furthermore, the residual layer tends to
353 preserve the characteristics of the previous day turbulent mixed layer and, therefore, may “trap”
354 remaining PBAPs from the day before, thus affecting species richness.

355



356

357 **Figure 5** – Regression analyses between (A) the total fungal concentration (spores m⁻³), (B)
 358 *Cladosporium* sp. concentration (spores m⁻³), and (C) *Aspergillus/**Penicillium*-like
 359 concentration (spores m⁻³) with sampling height (0-300 m) at the region of Arceburgo city,
 360 Minas Gerais state, Southeast Brazil (non-transformed data). Note: “Before Rainfall” or “After
 361 Rainfall” refers to data sampled prior or after the rain that occurred at the dawn of the third day
 362 of the fieldwork campaign (10th of March 2022) and before the third hot-air balloon flight.

363 This might also reflect the higher diversity of species found in the biodiversity hotspot
364 we researched (i.e., Brazilian Atlantic Forest; Lima et al., 2020), indicating that even under a
365 mixed land-use context as our study area, areas known to be biodiversity hotspots may have
366 high richness of fungal spores naturally. It is also important to note that regardless of the
367 altitude sampled (0, 150 or 300 m), *Cladosporium* sp. dominated all air layers investigated,
368 representing more than 80% of all spores in the study and was present in 92% of the samples
369 collected. Due to its observed concentration in the atmosphere both in our and previous studies,
370 *Cladosporium* sp. might be used as an indicator genus of fungal PBAPs (Grinn-Grofoń et al.,
371 2019; Emygdio et al., 2022). This genus is considered ubiquitous in the whole planet (Bensch
372 et al., 2012), so that studies aiming at investigating the links between atmospheric processes
373 and fungal PBAPs could focus on *Cladosporium* at the global scale to standardize protocols,
374 fostering insights and strengthening comparable research worldwide.

375 Whilst height was shown to be a factor structuring fungal communities in the
376 atmosphere both based on modelling and experimental studies (Sesartic et al., 2013; Els et al.,
377 2019a; 2019b; Tignat-Perrier et al., 2020; Emygdio et al., 2022), we demonstrate that rainfall
378 is an even stronger factor driving the removal of fungal PBAPs from the atmosphere (Yue et
379 al., 2016; Rathnayake et al., 2017). This confirms our third hypothesis that the concentration
380 of fungal spores present in the atmosphere would be reduced once rainfall occurs. After the
381 rain, the number of *Cladosporium* sp. and *Asp/Pen*-like spores were reduced from higher
382 layers. This indicates that rainfall drives the wet removal of fungal PBAPs from the atmosphere
383 to the ground, reducing their concentration at the studied sampling heights (Jensen et al., 2022).
384 Nevertheless, Huffman et al. (2013) have registered high concentrations of bioaerosol during
385 and after rain onset, which were linked to forest canopies that have triggered PBAPs emissions.
386 Since the region where the samples were collected features a mixed land-use pattern including
387 coffee plantations, pasture for cattle, and sugar-cane crops interspersed with Atlantic Forest

388 fragments (Eymygdio et al., 2022), differences in how rainfall events impact PBAPs fluxes may
389 have to consider the land-use context, warranting more research on this. Furthermore, the time
390 of day in which the sampling was done may influence the results obtained in this study, as well
391 as the meteorological conditions prior to and after the rain, so results should be taken with
392 caution. In fact, early morning flights might have happened above the atmospheric boundary
393 layer height, thus affecting the representativeness of fungi vertical gradients.

394 Although the concentration of spores, fungal species richness, total- and White-CFU
395 numbers at the ground level were not altered by the rain, the number of Pink-CFU was severely
396 reduced by the rainfall event. Although the reasons for these different outcomes remain unclear,
397 it might be related to dispersion mechanisms inherent to the different fungi species as suggested
398 before (Golan & Pringle, 2016; Löbs et al., 2020). Moreover, whilst fungal communities tend
399 to be less sensitive to precipitation in comparison to other microorganism such as bacteria
400 (Yang et al., 2021), at the same time rainfall reduces the concentration of some fungi, it could
401 boost the number of other PBAPs (Huffman et al., 2013). Environmental factors, for instance,
402 air temperature and vapour pressure, may play a major role in controlling the spore
403 concentration for some fungal species, such as *Cladosporium* sp. (Grinn-Grofoń et al., 2019).
404 Besides, rainfall might control the concentration of other fungal taxa, as for example *Alternaria*
405 sp. that we only observed after the rain has occurred, reinforcing the idea of rainfall dependence
406 to this fungi dispersion (Fagodiya et al., 2022).

407

408 **Conclusions**

409

410 Taking altogether, the results presented in our study demonstrate the differential
411 effects exerted by rainfall and altitude from ground level on fungal PBAPs, showing a use for
412 the fungal gradient at different sampling height to compare with a process induced by rain. In

413 general, an increase in height and rain occurrence lead to reductions in the concentration of
414 fungi present in the atmosphere in the study area within the Brazilian Atlantic Forest biome.
415 The higher prevalence of *Cladosporium* sp. at all sampling heights demonstrates the ubiquity
416 of this fungal species, which could be considered as a proxy for other fungal PBAPs in future
417 studies aiming to investigate cloud and precipitation formation by such microorganisms. This
418 has further implications for research on climate regulation, such as considering the land-use
419 context in where sampling is taken, as well as collating other types of data (e.g., PBAPs fluxes),
420 which warrants more investigation on the role of fungal PBAPs worldwide.

421

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564



565

566 **Supplementary Figure 1** – Fungal PBAPs sampling using the Microbial Air Monitoring
567 System (MAS100) and the portable Burkard air sampler at (A) ground level and (B) 300 m in
568 the hot-air balloon above the region of Arceburgo city, Minas Gerais state, Southeast Brazil,
569 during the fieldwork campaign between the 08th to the 11th of March 2022. Photos: MC
570 Mantoani and LCC Guerra.

571

572 **Supplementary Table 1** – Richness of species and frequency of fungi sampled using the
 573 Microbial Air Monitoring System (MAS100) at the region of Arceburgo city, Minas Gerais
 574 state, Southeast Brazil. Note: “Before Rainfall” or “After Rainfall” refers to data sampled prior
 575 or after the rain that occurred at the dawn of the third day of the fieldwork campaign (10th of
 576 March 2022) and before the third hot-air balloon flight.

Time	Height (m)	Fungi Genera/Species
Before Rainfall	0	<i>Cladosporium</i> sp.; <i>Fusarium oxysporum</i> ; Sterile Mycelium; Yeasts
	150	<i>Cladosporium</i> sp.; <i>Fusarium equisiti</i> ; <i>Penicillium</i> sp.; Yeasts
	300	<i>Cladosporium</i> sp.; <i>Curvularia</i> sp.; <i>Fusarium chlamydosporum</i> ; <i>Penicillium</i> sp.; Sterile Mycelium
After Rainfall	0	<i>Alternaria</i> sp.; <i>Cladosporium</i> sp.; <i>Curvularia</i> sp.; <i>Fusarium</i> sp.
	150	<i>Alternaria</i> sp.; <i>Cladosporium</i> sp.; <i>Fusarium equisiti</i> ; <i>Penicillium</i> sp.; Sterile Mycelium; Yeasts
	300	<i>Alternaria</i> sp.; <i>Cladosporium</i> sp.; <i>Fusarium equisiti</i> ; <i>Penicillium</i> sp.; Sterile Mycelium
Morphotype-Species	Presence in Samples	Frequency (%)
<i>Cladosporium</i>	11	92
<i>Fusarium</i>	6	50
<i>Penicillium</i>	5	42
<i>Alternaria</i>	4	33
Sterile Mycelium	4	33
Yeasts	3	25
<i>Curvularia</i>	2	17

577

578

579 **Supplementary Table 2** – Linear regression analyses results between the total concentration
 580 of spores (spores m⁻³), *Cladosporium* sp., and *Aspergillus/Penicillium*-like spores with
 581 sampling height (0-300 m) at the region of Arceburgo city, Minas Gerais state, Southeast
 582 Brazil. Note: “All Data” refers to all data collected in the whole experiment, whereas “Before
 583 Rainfall” or “After Rainfall” refers to data sampled prior or after the rain that occurred at the
 584 dawn of the third day of the fieldwork campaign (10th of March 2022) and before the third hot-
 585 air balloon flight.

	All Data	Before Rainfall	After Rainfall
Total Concentration of Spores (spores m⁻³)	y = -0.0037x + 3.949; r ² = 0.664; F _(1,24) = 43.37; P > 0.001; β = -0.81.	y = -0.0029x + 4.071; r ² = 0.976; F _(1,12) = 412.32; P > 0.001; β = -0.99.	y = -0.0046x + 3.827; r ² = 0.838; F _(1,12) = 51.69; P > 0.001; β = -0.92.
<i>Cladosporium</i> sp.	y = -0.0039x + 3.858; r ² = 0.666; F _(1,24) = 43.82; P > 0.001; β = -0.82.	y = -0.003x + 3.977; r ² = 0.979; F _(1,12) = 486.66; P > 0.001; β = -0.99.	y = -0.0048x + 3.739; r ² = 0.831; F _(1,12) = 49.13; P > 0.001; β = -0.91.
<i>Aspergillus/Penicillium</i>-like	y = -0.0029x + 3.179; r ² = 0.551; F _(1,24) = 27.02; P > 0.001; β = -0.74.	y = -0.0024x + 3.354; r ² = 0.936; F _(1,12) = 146.65; P > 0.001; β = -0.97.	y = -0.0035x + 3.005; r ² = 0.731; F _(1,12) = 27.23; P > 0.001; β = -0.86.

586