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Ana Paula Mendes Emygdio, Cristiane Degobbi, Federico Carotenuto, Dulcilena de Matos Castro E Silva, Thaysla Beluco Quintino, Rafael Henrique de Souza Zanetti, Mauricio Mantoani, Solana Meneghel Boschilia, Lara Chaves Carvalho Guerra, Pedro Leite da Silva Dias, et al.

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1 **Title: BIOAEROSOL VERTICAL FUNGAL SPORES PROFILE IN MINAS GERAIS**
2 **STATE, BRAZIL**

3
4 **Authors: Ana Paula Mendes Emygdio^a, Cristiane Degobbi^a, Federico Carotenuto^b,**
5 **Dulcilena de Matos Castro e Silva^c, Thaysla Beluco Quintino^a, Rafael Henrique de Souza**
6 **Zanetti^a, Mauricio C. Mantoani^a, Solana Meneghel Boschilia^d, Lara Chaves Carvalho**
7 **Guerra^e, Pedro Leite da Silva Dias^a, Cindy E. Morris^f, Fábio Luiz Teixeira Gonçalves^a**

8
9 **Affiliation:**

10 ^aInstitute of Astronomy, Geophysics and Atmospheric Science (IAG), University of São
11 Paulo (USP), Brazil

12 ^bNational Research Council of Italy (CNR), Institute of BioEconomy (IBE), Italy

13 ^cAdolfo Lutz Institute, Department of Micology, Brazil

14 ^dLaboratory of Research in Marine Environmental Monitoring (LAPMAR), Geosciences
15 Institute, Federal University of Pará, Belém, Brazil

16 ^eInterunities Graduate Program in Biotechnology, University of São Paulo, Brazil

17 ^fUnité de Pathologie Végétale UR407, INRA, Montfavet, France

18
19 *E-mail addresses:* ana.emygdio@alumni.usp.br, crisdegobbi@gmail.com,
20 federico.carotenuto@ibe.cnr.it, dulmatos.ial@gmail.com, thaysla@usp.br,
21 rafaelhszan@gmail.com, mcmantoani@gmail.com, solbos@gmail.com, laraguerra@usp.br,
22 pldsdias@gmail.com, cindy.morris@inrae.fr, fabio.goncalves@iag.usp.br

23
24 Corresponding author: Ana Paula Mendes Emygdio
25 *E-mail address:* ana.emygdio@alumni.usp.br
26 R. do Matão, 1226 - Butantã, São Paulo - SP, 05508-090

27
28
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43

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47 **manuscript; APME and FLTG led the manuscript writing; all authors contributed to all**
48 **versions and gave approval to the final version of the manuscript.**

49

50

51 **Abstract:** Primary biological aerosol particles (PBAPs) are involved in multiple phenomena
52 ranging from seasonal allergies to pandemic diseases. Furthermore, PBAPs that act as ice nuclei,
53 might interact with cloud physics affecting the formation of hail and, potentially, causing damage
54 to agriculture. These latter dynamics are still unclear, especially due to the lack of knowledge
55 about PBAPs concentration and emission rates. Here we characterized the fungal aerobiology of
56 Arceburgo, Minas Gerais State, Brazil, through ground level and airborne sampling of PBAPs via
57 a hot-air balloon. Total and cultivable fungal spores were collected using personal portable
58 Burkard and a MAS100 sampler respectively during the summer and winter of 2019. In the latter
59 season, daily dynamics were resolved by repeating flights and sampling in the morning and in the
60 afternoon. Both samplers identified a core fungal community (*Penicillium/Aspergillus* and
61 *Cladosporium* spp.) that are coupled with local meteorological dynamics and are able to undergo
62 atmospheric transport as indicated by their survival in the night-time residual boundary layer.
63 These results are invaluable in identifying a core set of aerobiological indicators that can be used
64 in future works to unravel PBAPs emission rates on the area of Arceburgo and form a basis to
65 close the gap in knowledge in the interplay between PBAPs and hail formation.

66

67 **Key-words:** Fungi, bioaerosol, hot-air balloon, Burkard, MAS100

68

69

70 1. Introduction

71 Aerosols can impact Earth's climate as well as human, animal and plant's health. Aerosol
72 particles can directly absorb or reflect solar radiation and can act as cloud condensation nuclei
73 (CCN) as well as ice nuclei (IN), which trigger cloud drops and ice/hail formation, respectively.
74 These abilities directly and indirectly affect the atmosphere's energy balance, being both of great
75 interest in Intergovernmental Panel on Climate Change (IPCC)'s studies (Murray et al., 2012;
76 Pösch et al., 2010; Seinfeld et al., 2016; Rosenfeld et al., 2008; Tao et al., 2011; Morris et al.,
77 2008; 2011; 2012).

78 A specific group of aerosols are the Primary Biological Aerosol Particles (PBAPs),
79 derived from living organisms (e.g., bacteria, fungi, and pollen) ranging from nanometers to more
80 than 100 μm in diameter with a very heterogeneous nature (Després et al., 2012). They cause a
81 large range of outcomes, from allergic reactions to pandemic diseases in humans, animals and
82 plants (Douwes et al., 2003). PBAPs may include viable or non-viable microorganisms
83 (e.g., bacteria, viruses and fungi), their fragments or a product released from them and other living
84 organisms such as essential oils, metabolites, pollen, cell debris and biofilms (Ariya and Amyot,
85 2004; Després et al., 2012).

86 At ground level and moderate altitudes (for example, below 10-15 m), there are hundreds
87 of thousands of particles of biological origin per cubic meter of air (Oke, 2002). On the other
88 hand, various types of bioaerosols have been found in the upper troposphere and even in the
89 stratosphere (Matthias-Maser et al., 1995; DeLeon-Rodriguez et al., 2013; Griffin, 2004;
90 Wainwright et al., 2003). Clearly, their concentrations are subject to significant spatial and
91 temporal variations depending on altitude, land use (such as rural, urban or forest) and
92 meteorological factors (temperature, radiation, relative humidity, precipitation, wind) (Morris et
93 al., 2008, Matthias-Maser, 2000a; Després et al., 2012; Fröhlich-Nowoisky et al., 2016; Gong et
94 al., 2020).

95 An interesting characteristic of biological particles is their ability to act as ice nuclei
96 (PBAP-IN) (Morris et al., 2004). Most of non-biological particles need temperatures colder than
97 -8°C to -10°C to act as ice nuclei, while PBAP-INs can initiate freezing at temperatures as warm
98 as -2°C (Murray et al., 2012, Morris et al., 2008; 2011; 2012). Some fungal spores and bacteria,
99 such as *Fusarium* and *Pseudomonas syringae*, play a catalytic role in the ice cloud formation
100 (Morris et al., 2004).

101 Over the last decades, PBAP-IN studies have been developed around the hypothesis of
102 bio-precipitation, which consists in a feedback cycle where land plants, even crops (and their
103 microbiota) generate airborne particles (aerosols) containing biological nuclei, which for their
104 turn contribute to the cloud ice formation and hail (Morris et al., 2014).

105 On the other hand, hailstorms are one of the most crop destructive meteorological
106 outcomes in many parts of the world, including in South and Southeast of Brazil (Martins et al.,

107 2017). The role of PBAP-IN on the impact of the hail formation and on the diseases caused by
108 fungi and bacteria, makes the characterization of the microbiota in the region of vital importance,
109 specially to support the policy maker's actions.

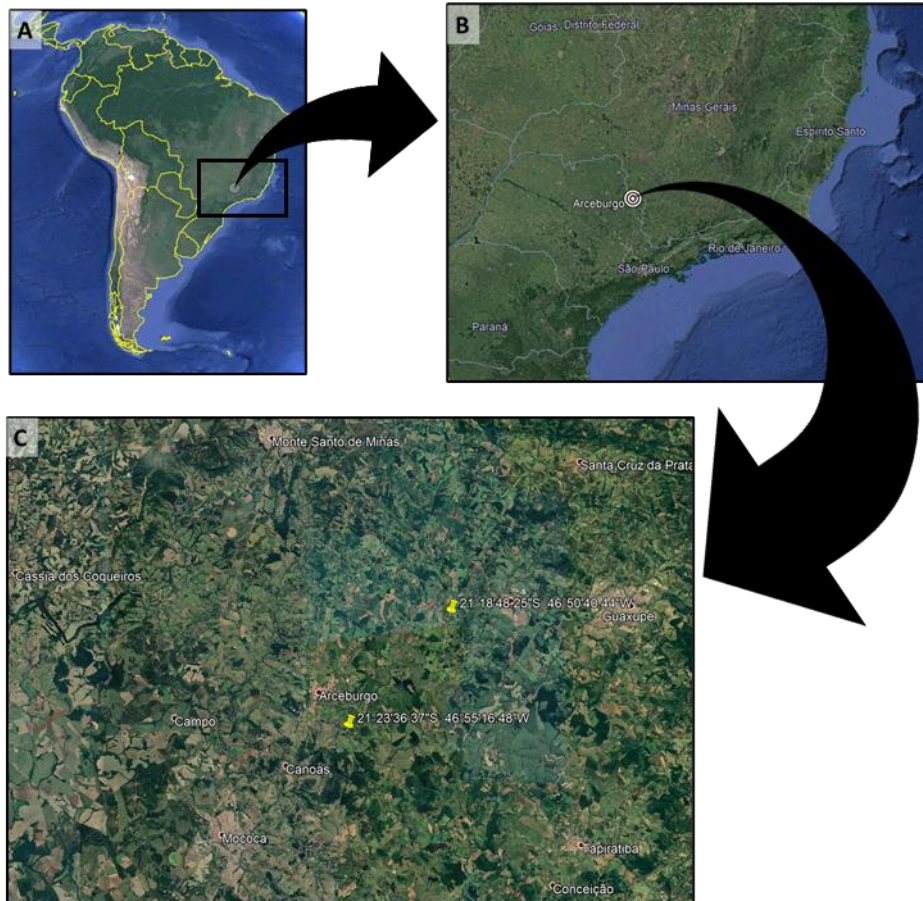
110 Based on the above described, our study aimed at analyzing the vertical variability of
111 fungal spores in the Minas Gerais State, Southeast Brazil. Data on fungal spore concentration,
112 particularly for three common genera (*Aspergillus*, *Cladosporium*, and *Penicillium*) were
113 evaluated concerning daily (i.e., morning and afternoon) and seasonal concentrations. Data was
114 collected at ground level, as well as at different altitudes using a hot-air balloon. We are aware of
115 the limitations that hot-air balloon sampling might have, for instance, the balloon does not exactly
116 follow the air parcels because for security reasons the vertical level is controlled. Nonetheless,
117 the absence of tall towers (i.e., > 100 m) in the studied region, which permit an Eulerian sampling,
118 served as an appeal to consider hot-air balloon flights as an alternative. In addition, when
119 compared to tethered balloons, in which it is not possible to sample bioaerosols easily since the
120 sampling device needs to be manipulated, hot-air balloons work well. This research aimed then
121 to calculate the fungal spore concentration related to different height levels.

122

123 **2. Methodology**

124 Sampling site

125 Both sampling sites are based on Arceburgo town, at Minas Gerais State of Brazil, as seen
126 in Fig. 1, showing the different land use such as coffee crops, sugar cane crops, pasture and natural
127 reservations. One of the sampling sites was located at southwest of the Arceburgo city, in a coffee
128 farm close to a pasture (21°23'36.37" S and 46°55'16.48" W) and the other was in the northwest
129 of the Arceburgo city, were only the sample from day 30.07.19 was collected (21°18'48.25" S
130 and 46°50'40.44" W). The ground samplings were collected immediately before the balloon
131 flight for bioaerosol measurements.



132
 133 **Fig. 1** Map with the sampling sites. A: South America; B: Southeast of Brazil; C: Region of the
 134 sampling site centered at the 21°23'36.37" S and 46°55' 16.48" W, and at 21°18'48.25" S
 135 46°50'40.44" W (yellow markers), nearby Arceburgo City (21° 20' 35" S and 46°56'21.56" W,
 136 ~716 m asl), where the balloon flights started (Source: Google Earth).
 137

138 Sampling methodology

139 The samples were collected at the ground level and at altitude with a hot-air balloon. Each
 140 flight took approximately 1 hour and, when possible, temperature, relative humidity, wind
 141 velocity, atmospheric pressure and altitude were recorded during the balloon sampling and at the
 142 ground level close to the balloon takeoff location. After the balloon takeoff, the first samples were
 143 collected when the altitude reached approximately 100 meters above the ground. The sampling
 144 consisted in several cycles involving the collection of fungi with a duration of 4 to 6 minutes each
 145 cycle.

146 Sampling devices

147 Samples were collected with the Burkard Personal Volumetric Air Sampler (portable,
 148 Burkard Manufacturing Co., Hertfordshire, U.K.) with a flux of 10 liters/minute and MAS100
 149 (Merck KGaA, Darmstadt, Germany) collecting at an air flow rate of 100 liters/minute. The
 150 Portable Burkard has a narrow inlet followed by a glass slide coated by a tape with adhesive where
 151 sampled particles are collected (Aizenberg et al., 2000). The Portable Burkard was used to obtain
 152 the concentration of fungal spores per cubic meter (more information about portable Burkard in

153 Aizenberg et al., 2000). After the sampling, the resulting slide with the tape was fixed with a
154 glycerin jelly and cover glass (Rogers & Muilenberg (2001) and the whole slide was analyzed
155 with a microscope at 1000x magnification (100x for the objective and 10x for the ocular). Fungal
156 spore identification was performed according with Haines et al. (2000) and others references in
157 the field. Burkard samplings were collected for 6 minutes during the Summer Campaign and for
158 4 minutes during the Winter Campaign. This change occurred because within 6 minutes (in
159 summer) the number of spores on the slide was too high, making it difficult to count and identify
160 the fungal spores. Portable Burkard has a 2.52 μm theoretical cut-off size (d_{50} , the diameter where
161 only 50% is collected) and an experimental cut-off size of 2.3 and 2.4 μm according to the
162 literature (Aizenberg et al., 2000).

163 The MAS100 impactor works by aspirating ambient air through a perforated plate (which
164 has 300 holes with a diameter of 0.6 mm each), and the particles are then deposited in a culture
165 medium (Meier and Zingre, 2000; Yao and Mainellis, 2006a). A statistical correction (positive
166 hole conversion) was performed using the values presented in the manual of the equipment and
167 considering the conversion formula devised by Feller (1950). The MAS100 impactor sampled
168 with a disposable plate containing 20 mL of culture medium with Dichloran Rosa Bengal
169 modified (DRBCm) for fungi (Castro and Silva et al., 2015). The sampled plates were incubated
170 at 30°C ($\pm 2^\circ\text{C}$) for up to 7 days for isolation and identification of genus and species. For each
171 sample, the MAS100 collected a total of 500 liters which took approximately 5 minutes, with
172 volume adjustable to avoid overlapping, and the concentration was expressed in CFU/m³. The
173 phenotypic identification of cultivated fungi was based on the taxonomy characterization (de
174 Hoog et al., 2014).

175 Sampling period

176 The samples were collected on February 3rd, 4th, 5th and 8th of 2019 (Summer) and on July
177 27th, 28th, 30th and 31st of 2019 (Winter), totalizing 8 sampled days. The Summer Campaign had
178 only morning flights (4 flights), due to security reasons, while the Winter Campaign had morning
179 (3 flights) and afternoon flights (3 flights). The samples were collected at the ground level and in
180 flight, with three to seven samples per flight, totaling 40 samples with portable Burkard (16 on
181 summer and 24 on winter) and 34 samples with MAS100 (13 on summer and 21 on winter) for
182 fungi. The Winter Campaign had 12 samples collected in the mornings and 12 samples in the
183 afternoons for portable Burkard and 13 samples collected in the mornings and 8 samples in the
184 afternoon for MAS100 for fungi.

185 Balloon flights flew in different directions, but mostly to S-SW, based on flight
186 trajectories, at heights from approximately 11-478 m above the ground level. The altitudes were
187 obtained from a GPS (GPSMAP 76CSx, with <10 m accuracy) that tracked all the flights. The
188 altitude of a sample was considered as the average GPS altitude over the sampling period.
189 Samples collected during winter were divided into categories. A first division considered the

190 samples taken at ground level (0 m, Burkard: number of samples (n)=3, MAS100: n=4) vs. those
191 taken above-ground (> 0 m, Burkard: n=21, MAS100: n=17). Secondly, samples collected at
192 different altitudes were categorized either as low altitude (up to 211 m – median of all altitude
193 values, Burkard: n= 10, MAS100: n= 7) or high altitude (>211 m, Burkard: n= 11, MAS100: n=
194 10).

195 Statistics

196 The data obtained were analyzed using Microsoft Excel and R (packages: *readxl*
197 (Wickham and Bryan, 2019), *dplyr* (Wickham et al., 2020), *openair* (Carslaw et al., 2012), *psych*
198 (Revelle, 2020), *pastecs* (Grosjean and Ibanez, 2018), *ggplot2* (Wickham, 2016), *pgirmess*
199 (Giraudoux, 2018)). The statistical analyzes were based on Field et al. (2012). Samples were
200 tested for their normality using the Shapiro test. Most of the subset had a non-normal distribution,
201 and due to that, all statistical analyzis was performed using non-parametric tests. Wilcoxon rank-
202 sum test was performed to compare the concentrations in both campaigns, in different periods of
203 the day, between the ground level and above-ground, and between the altitude categories. The
204 concentrations were compared with the Spearman's rank correlation (ρ) to better understand the
205 relationship between the two instrumental methodologies (Burkard and MAS100).

206 Auxiliary meteorological data were obtained at hourly resolution from the global ERA5
207 reanalyzes of the European Centre for Medium-Range Weather Forecasts (ECMWF).
208 Specifically, height of the boundary layer (BL) has been extracted from the ERA5 data on single-
209 levels (SL) dataset (Hersbach et al., 2018a), while relative humidity (RH) and geopotential height
210 were obtained from the ERA5 data on pressure-levels (PL) dataset (Hersbach et al., 2018b). Both
211 datasets report data from 1979 to the present at an hourly time resolution and with horizontal
212 resolution of $0.25^\circ \times 0.25^\circ$. The closest pixel to the Arceburgo sampling area was selected for both
213 datasets (-21.40° latitude, -46.93° longitude). The main difference between the two datasets is the
214 kind of variables found in them and their vertical distribution: geopotential and relative humidity
215 are reported at pressure levels in the atmosphere, specifically on 37 pressure levels from 1 to 1000
216 hPa. The actual height above ground of the relative humidity data was obtained in two steps: first
217 geopotential was converted to geopotential height by dividing it by Earth's gravitational
218 acceleration (obtaining the height above sea level of the corresponding pressure level) and finally
219 by subtracting the ground elevation from the geopotential height in order to obtain the height
220 above ground level of the pressure levels. Ground elevation for the ERA5 pixel's coordinates
221 (666 m) was obtained from the Terra Advanced Spaceborne Thermal Emission and Reflection
222 Radiometer (ASTER) Global Digital Elevation Model (GDEM) Version 3 (NASA et al., 2019).
223 After this transformation was made, only the levels with height above grounds compatible to the
224 balloon flights were selected which, for both measurement campaigns, where the levels between
225 925 and 700 hPa, corresponding to height above grounds ranging between roughly 100 and 2500

226 m (more specifically between 81.82 and 2531.97 m for the Summer Campaign and between
227 141.17 and 2549.83 m for the Winter campaign).

228 3. Results

229 3.1. Overall description of fungal analyzis

230 Samples collected with portable Burkard had a total of 47 fungal types identified (Table
231 1), while samples collected with MAS100 had a total of 12 fungal genera and type identified. As
232 so, the fungal samples identified by Burkard will be referred as “Fungal types”, while for MAS100
233 will be referred as “Fungal genera”. Due to the high number of colonies found in each plate from
234 the MAS100, it was not possible to count the number of colonies for each genus, therefore, the
235 only concentration obtained was CFU/m³ of the total fungi.

236 The average fungal concentration obtained by portable Burkard and by MAS100 were
237 67,072 ($\pm 57,455$) spores/m³ (max of 324,520 spores/m³) and 210 (± 124) CFU/m³ considering all
238 sampled periods, respectively. A higher concentration of total fungal spore was found in the
239 winter campaign in the afternoon for Burkard (Figure SI 1 and Table SI 1). The total spore
240 concentration from portable Burkard showed a weak positive and non-significative Spearman's
241 rank correlation with the CFU from MAS100 ($\rho=0.29$).

242 The fungal types/genera that were identified by both equipment are *Cladosporium*,
243 *Alternaria*, *Penicillium*, *Aspergillus*, *Curvularia*. The average total MAS100 concentration
244 (CFU/m³) represented approximately 16% of the average concentration of five fungal types
245 (*Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Curvularia*) obtained with Burkard
246 (spores/m³), considering both campaigns. If all the fungal types collected by Burkard are
247 considered, this percentage decreased to less than 1% (0.4%).

248 The fungal type identified by Burkard methodology with higher concentration were the
249 Hyaline Basidiospores, followed by *Penicillium/Aspergillus*-like and *Cladosporium* spp (Table
250 S1). Nevertheless, the Hyaline Basidiospores in average represents ~78% of the total spore
251 concentration in our study.

252 The fungal genera found by the MAS100 were *Alternaria* spp. (alt), *Fusarium* spp. (fus),
253 *Curvularia* spp. (cur), *Aspergillum* spp. (asp), *Penicilium* spp. (pen), *Rhizopus* spp. (rhi),
254 *Trichoderma* spp. (tri), *Cladosporium* spp. (cla), *Paecilomyces* spp. (pae), *Mucor* spp. (muc),
255 *Exerohilium* spp. (exe), Sterile mycelium (mic). *Fusarium* spp. followed by *Cladosporium* spp.
256 and *Penicillium* spp. had the highest frequency, indicating their relevance throughout the
257 sampling period.

258 Some of the most frequently found fungal types/genera by Burkard and MAS100 were
259 similar (*Cladosporium* and *Penicillium*), although this was not the case for *Fusarium* spp. and for
260 Hyaline Basidiospores. *Fusarium* spp. is not easily identified by the Burkard methodology, and
261 Hyaline Basidiospores usually does not show a reproductive form in culture media, and
262 consequently are not identified in the samples from MAS100. *Cladosporium* sp. and *Pen/Asp-*

263 like from Burkard, had an average concentration of 3,368 ($\pm 8,124$) spores/m³ and 3,593 ($\pm 11,625$)
264 spores/m³, respectively and were found with higher concentrations on the winter campaign during
265 the afternoon. Overall, *Pen/Asp*-like had a higher standard deviation indicating their higher
266 variability through the sampled period.

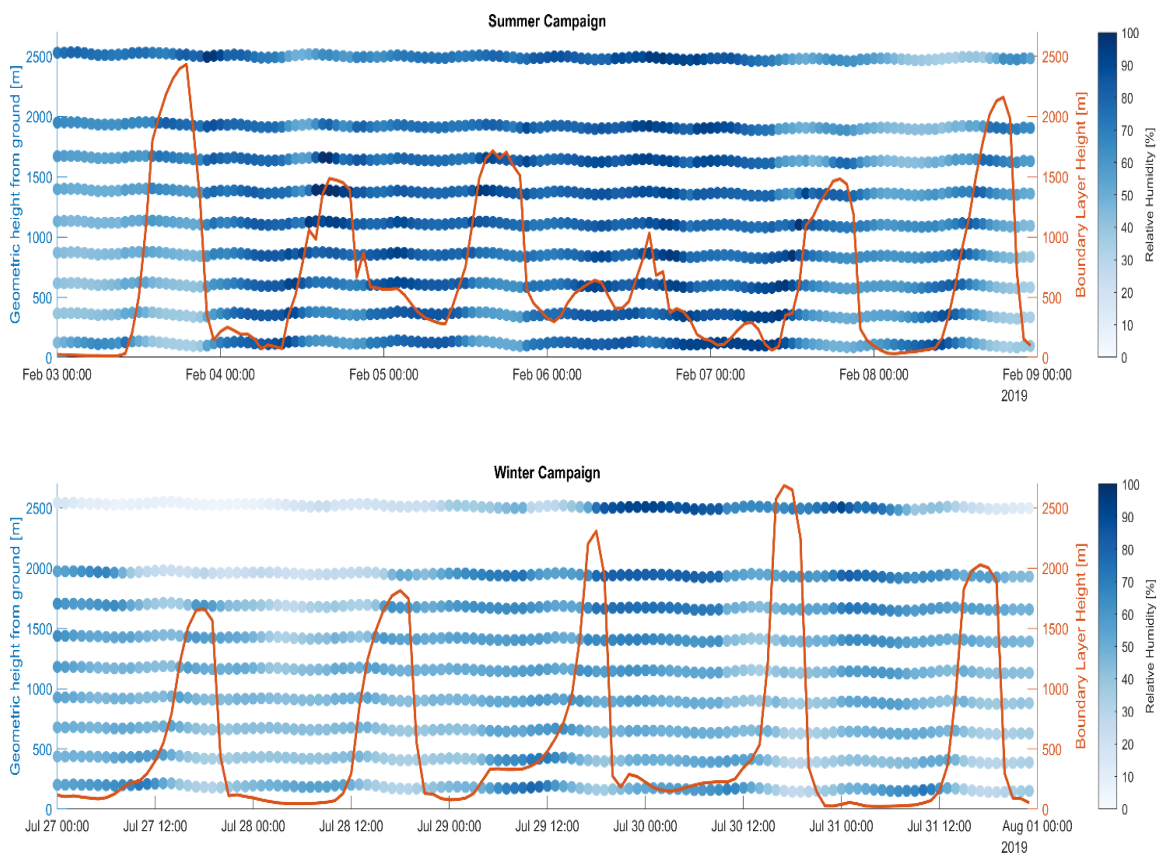
267 Thus, it is understood that *Cladosporium* sp. and *Penicillium/Aspergillus*-like were found
268 in both methodologies and are the two of the spores found most often during the campaign (each
269 representing ~3% of the total fungal concentration from portable Burkard). Moreover, they were
270 found in both campaigns and have a consistent concentration through the sampled period. Hence,
271 both spore types, mainly *Cladosporium* sp., can be used in future research efforts as indicators of
272 upward PBAP flux.

273

274 3.2. Meteorological data

275 Relevant meteorological data for the summer and the winter campaigns are shown in Fig.
276 2. The figure shows the interplay between the development of the diurnal convective boundary
277 layer and RH. As the day moves forward from the night-time, the boundary layer (BL) height
278 increases with the increased solar radiation and RH decreases following a similar, albeit delayed,
279 pattern (RH decreases during the day due to entrainment of dry air at the top of the BL and to the
280 decrease, or eventually, shutdown of evapotranspiration due to the solar heating and soil moisture
281 limitation).

282



283

284 **Fig 2** Relative humidity and boundary layer (BL) heights for the two sampling Campaigns.
 285 Position of the colored dots indicates height above ground (left y-axis), while the intensity of the
 286 blue color of the dots indicates RH percentage (colorbar). The orange line indicates the height of
 287 the BL (right y-axis). The top plot is hourly data for the Summer Campaign period (3-8 February
 288 2019), while the bottom one is for the winter campaign period (27-31 July 2019).
 289

3.3. Overall Fungal concentration in different altitudes

290 Samples were collected in different altitudes in both campaigns (winter and summer),
 291 with MAS100 and portable Burkard, however, only winter campaign concentrations were
 292 considered in the comparison between altitudes. Results for the differences of fungal type
 293 concentrations for each altitude may be found on table 1.
 294

295 **Table 1.** Differences of fungal type concentrations from Burkard and total fungal colonies
 296 obtained by MAS100 for each altitude group during the Winter Campaign.
 297

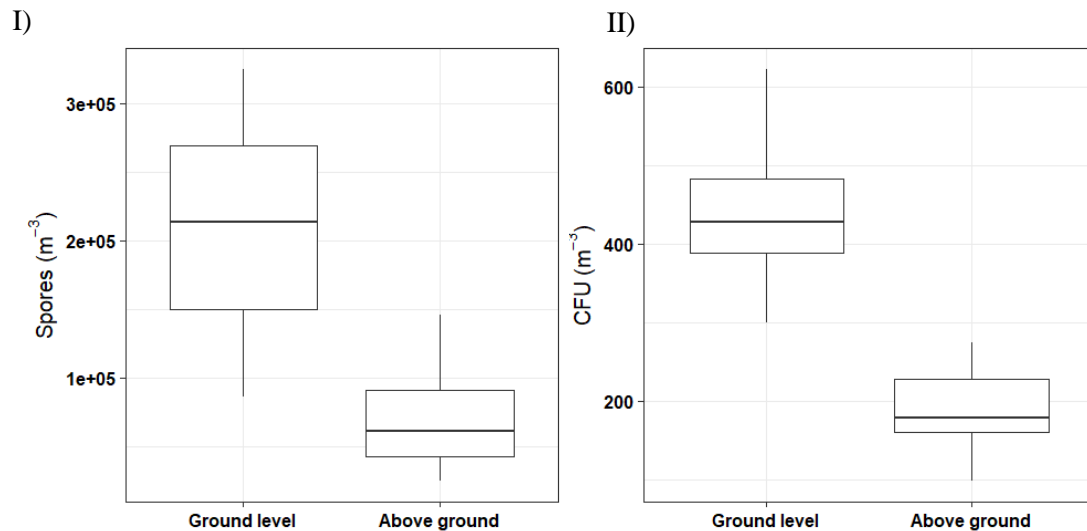
Fungal types	GL	AG	GL (M)	AG (M)	GL (A)	AG (A)	LA	HA
<i>Alternaria</i> sp.	+	-	+	-	+	-	+	-
<i>Pen/Asp.-like</i>	+	-	+	-	+	-	-	+
<i>Cercospora</i> sp.	0	+	0	+	0	+	+	-
<i>Cladosporium</i> sp.	+	-	-	+	+	-	+	-
<i>Curvularia</i> sp.	+	-	+	-	+	-	+	-
<i>Drechslera-like</i>	+	-	+	-	+	-	+	-
<i>Helminthosporium</i> sp.	0	+	0	0	0	+	+	0
<i>Periconia</i> sp.	+	-	0	+	+	-	+	-
<i>Epicoccum</i> sp.	+	-	+	-	+	-	+	-
<i>Botrytis</i> sp.	0	0	0	0	0	0	0	0
<i>Nigrospora</i> sp.	+	-	0	+	+	-	+	-
<i>Pithomyces</i> sp.	+	-	0	0	+	-	0	+
<i>Spegazzinia</i> sp.	+	-	0	0	+	-	+	-
<i>Stemphylium</i> sp.	0	+	0	0	0	+	+	0
<i>Tetraploa</i> sp.	+	-	0	+	+	-	+	-
<i>Torula</i> sp.	+	-	0	+	+	-	+	-
Unknown MS	+	-	+	-	+	-	+	-
Smuts	+	-	0	+	+	-	+	-
<i>Coprinus-like</i>	+	-	+	-	+	-	+	-
<i>Agrocybe/Conocybe-like</i>	0	0	0	0	0	0	0	0
<i>Calvatia lycoperdon</i>	0	0	0	0	0	0	0	0
Boletaceae	0	0	0	0	0	0	0	0
<i>Ganoderma</i> sp.	+	-	0	+	=	=	+	-
Hyaline Basidiospores	+	-	+	-	+	-	+	-
<i>Panoellus/Psathyrella</i>	0	+	0	+	0	+	+	0
Colored Basidiospores	-	+	0	+	-	+	+	-
Unknown Basidiospore	0	+	0	0	0	+	+	0
Puffball	0	+	0	+	0	+	-	+
<i>Saccobolus</i>	0	+	0	0	0	+	+	0
<i>Chaetomium</i> sp.	0	+	0	0	0	+	+	0
Diatrypaceae	0	+	0	0	0	+	-	+
<i>Phaeosphaeriaceae</i>	0	+	0	0	0	+	0	+
<i>Leptosphaeria-like</i>	+	-	0	+	+	-	+	-
<i>Ophiobolus</i> sp.	+	-	0	0	+	-	0	0

<i>Paraphaeosphaeria michotii</i>	0	+	0	0	0	+	+	0
<i>Pleospora</i> -like	+*	-*	0	0	+*	-*	+	-
Xylariaceae	-	+	-	+	+	-	-	+
<i>Sporormiella</i> sp.	0	0	0	0	0	0	0	0
2 cell colorless Ascospores	-	+	0	+	+	-	+	-
4 cell colorless Ascospores	+	-	0	+	+*	-*	+	-
Colorless Ascospores	+*	-*	+*	-*	+	-	-	+
2 cell colored Ascospores	+	-	0	0	+	-	+	0
Unknown Ascospores	+*	-*	0	+	+*	-*	=	+
<i>Venturia</i> -like	0	+	0	+	0	+	+	0
<i>Sordaria</i> sp.	0	0	0	0	0	0	0	0
<i>Herbothidia</i> sp.	0	0	0	0	0	0	0	0
Myxomycete	+*	-*	0	+	+*	-*	-	+
Rusts	0	+	0	+	0	0	0	+
Unknown Spores	+	-	-	+	+*	-*	-	+
Total	+*	-*	+	-	+*	-*	+	-
Fungi colonies	+*	-*	+*	-*	+	-	+	-

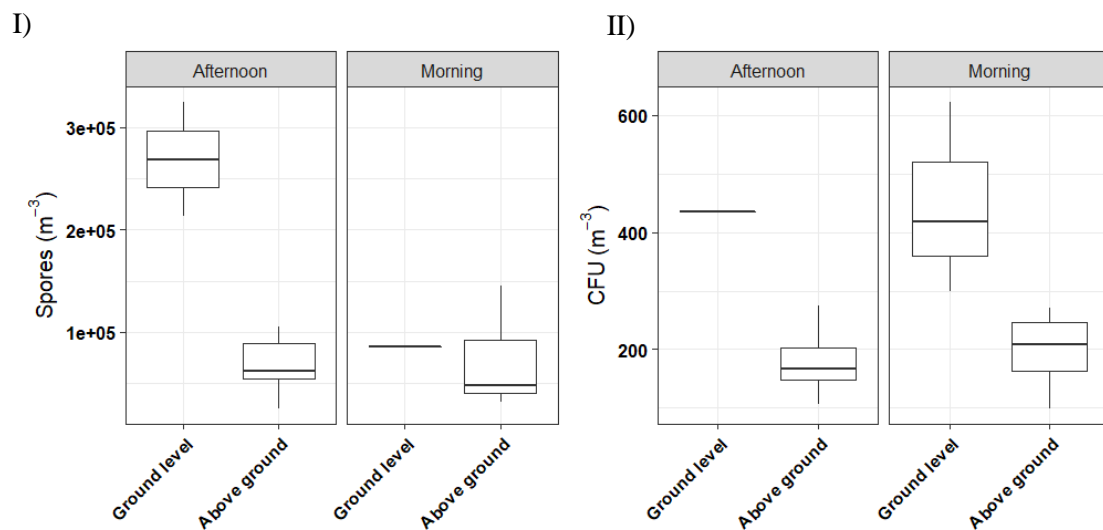
298 Legend: ground level (GL, 0 m), above ground (AG, > 0 m), low altitude (LA, up to 211 m), high
299 altitude (HA, >211 m), Afternoon (A) and morning (M). “+”: higher concentration compared to
300 its equivalent (i.e., GL with AG; GL (M) with AG (M); GL (A) with AG (A); LA with HA); “-”:
301 lower concentration compared to its equivalent; “0”: null -nothing occurred. *p<0.05
302

303 The total spore average concentrations collected with portable Burkard was 3 times higher
304 at the ground level (n=3) compared to the above ground level (n=21, p<0.05) for the entire Winter
305 Campaign (Fig. 3). Considering only the afternoon period of the Winter Campaign, the difference
306 between ground level (n=2) and above ground (n=10) average concentration was 3.9 times
307 (p<0.05 – Fig. 4). No significant difference between ground and above ground was observed
308 considering only the morning period (Figure SI 1 and Table SI 1).

309 The average CFU/m³ sampled with MAS100 showed the same tendency, with the ground
310 level (n=4) concentration significantly higher (p<0.05) by 2.3 times than above ground levels
311 (n=17) considering the Winter Campaign (Fig. 3). The afternoon period and the morning period
312 of the Winter Campaign also present a higher concentration in the ground level (2.4 times and 2.2
313 times higher, respectively) compared to the above ground (Fig. 4) for the CFU/m³ sampled with
314 MAS100, however only the morning period was significant (p<0.05).



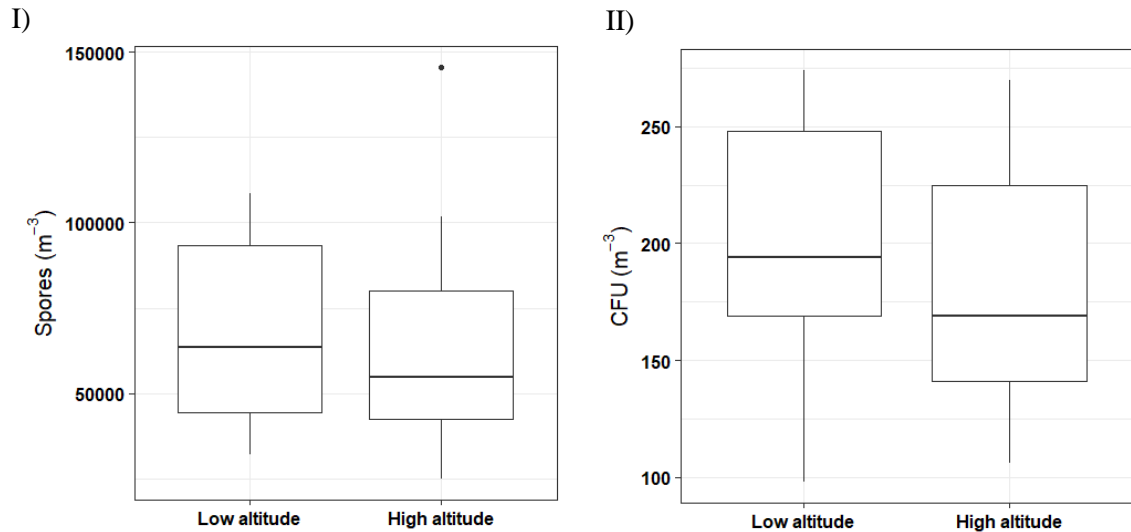
315
 316 **Fig. 3** Boxplot of the concentration at ground level and above ground considering the Winter
 317 Campaign. I) average fungal spore concentration (spores/m³) from portable Burkard (W = 56, p
 318 <0.05); II) average fungi colony forming unit (CFU/m³) from MAS100 (W = 68, p <0.05). For
 319 Burkard, the ground level had a n=3, and for above ground a n=21. For MAS100 the ground level
 320 had a n=4, and for above ground a n=17.



321
 322 **Fig. 4** Boxplot of the concentration at ground level and above ground considering the Winter
 323 Campaign and different periods of day (morning and afternoon). I) average fungal spore
 324 concentration (spores/m³) from portable Burkard (Afternoon: W=20, p<0.05); II) average fungi
 325 colony forming unit (CFU/m³) from MAS100 (Morning: W=30, p<0.05). For Burkard in the
 326 afternoon the ground level had a n=2, and for above ground a n=10, while in the morning the
 327 ground level had a n=1, and for above ground a n=11. For MAS100 in the afternoon the ground
 328 level had a n=1, and for above ground a n=7, while in the morning the ground level had a n=3,
 329 and for above ground a n=10.
 330

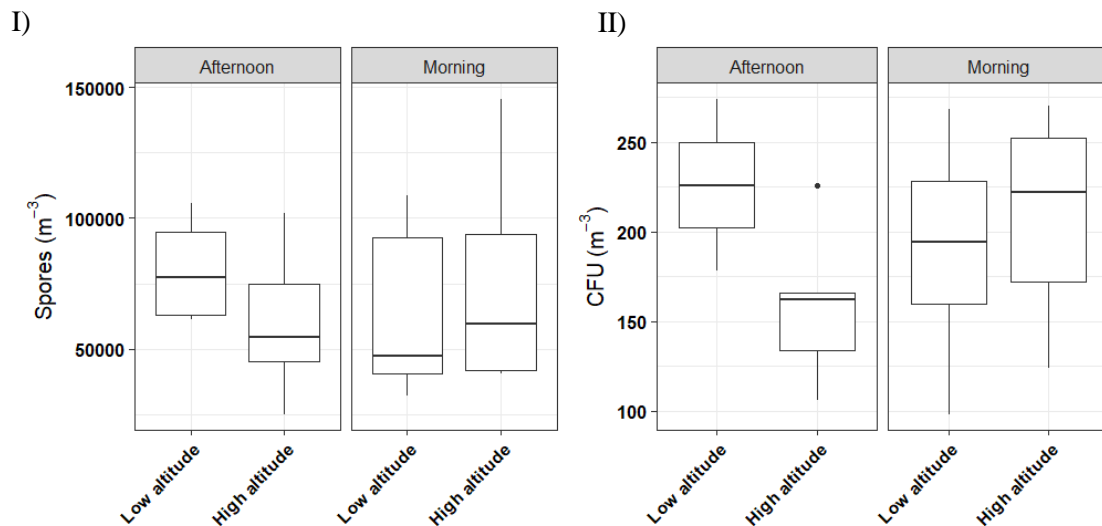
331 The average concentrations of fungal spores from Burkard and the CFU from MAS100
 332 in the low altitude (up to 211m) and high altitude (>211m) of the Winter campaign did not differ
 333 significantly (Figure SI 1 and Table SI 1). The same tendency is observed when analyzing the

334 afternoon and morning period of the Winter Campaign. Although not significant, the CFU from
 335 MAS100 and the spores, from Burkard, show an overall tendency of higher average concentration
 336 in the low altitude (Fig. 5). Both also show a tendency of higher average concentration in the
 337 higher altitude (>211) in the morning period, and higher concentration in the low altitude (up to
 338 211) in the afternoon period (Fig. 6).



339 **Fig. 5** Boxplot of the concentration at low altitude (up to 211 m) and high altitude (>211 m)
 340 considering the Winter Campaign. I) average fungal spore concentration (spores/m³) from
 341 portable Burkard; II) average fungi colony forming unit (CFU/m³) from MAS100. For Burkard,
 342 the low altitude had a n=11, and for high altitude a n=10. For MAS100 the low altitude had a n=7,
 343 and for high altitude a n=10.

345



346 **Fig. 6** Boxplot of the concentration at low altitude (up to 211 m), and high altitude (>211m)
 347 considering the Winter Campaign and the morning and afternoon period. I) average fungal spore
 348 concentration (spores/m³) from portable Burkard; II) average fungi colony forming unit (CFU/m³)
 349 from MAS100. For Burkard in the afternoon the low altitude had a n=4, and for high altitude a
 350 n=6, while in the morning the low altitude had a n=7, and for high altitude a n=4. For MAS100
 351 in the afternoon the low altitude had a n=2, and for high altitude a n=5, while in the morning
 352 the low altitude had a n=5, and for high altitude a n=5.

353

3.4. *Cladosporium*, *Penicillium* and *Aspergillus*

The *Cladosporium*, *Penicillium* and *Aspergillus* were among the main fungal types observed in both methodologies, as such a more detailed analysis was performed considering the Burkard concentrations. Average concentration of *Cladosporium* sp. and *Pen/Asp*-like at ground level was 24,672 ($\pm 22,567$) spores/m³ and 26,858 ($\pm 41,353$) spores/m³ respectively, and above ground was 2,021 ($\pm 1,700$) spores/m³ and 2,321 ($\pm 1,708$) spores/m³, respectively.

The *Cladosporium* sp. and *Pen/Asp*-like average concentrations collected with portable Burkard at the ground level and above ground level of the Winter campaign did not differ significantly (Fig. 7). However, although not significant, both showed a tendency of higher concentration at ground level. Even though both have an average concentration much higher at the ground level, the standard deviation is exceedingly high, sometimes even higher than the average concentration itself. This indicates that the variability of the data is elevated. *Cladosporium* sp. did show a significant difference between ground level (n=2) and above ground (n=10) when considering only the afternoon period of the Winter Campaign with an average concentration 18 times higher at ground level (Fig. 8). No significant difference was observed for the morning period or for the *Pen/Asp*-like.

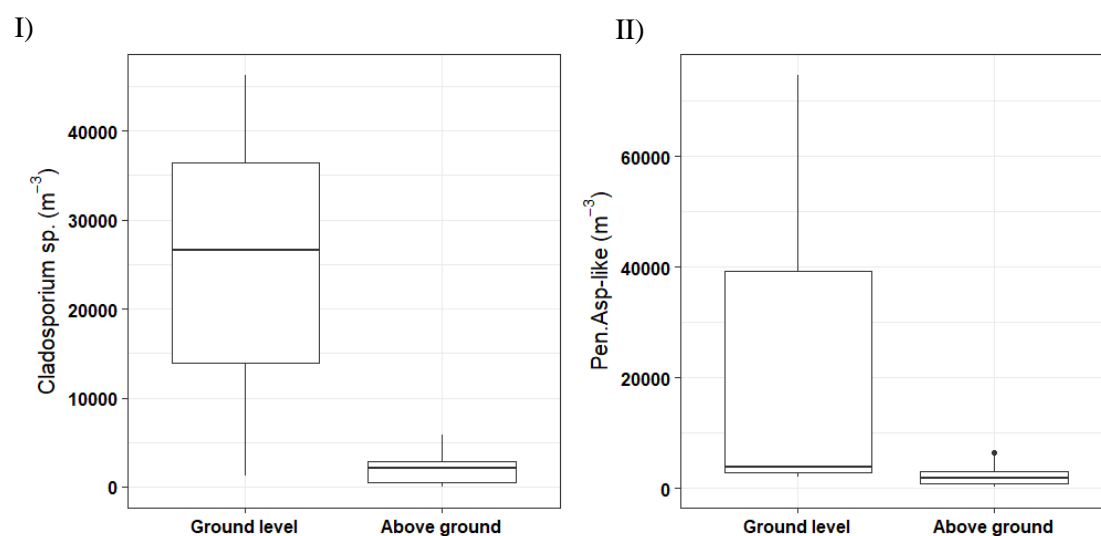
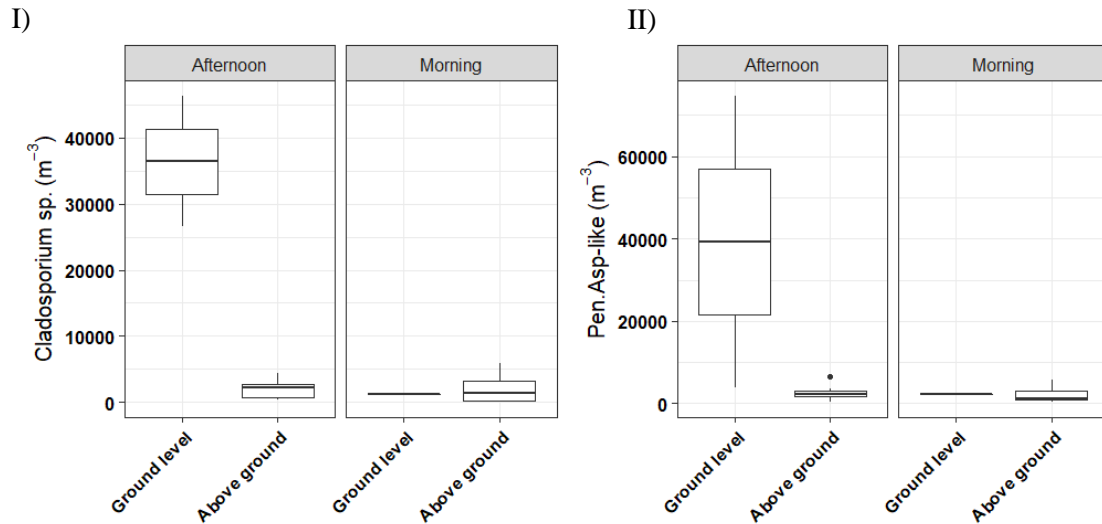


Fig. 7 Boxplot of the concentration at ground level and above ground from portable Burkard and considering the Winter Campaign. I) average *Cladosporium* sp. concentration (spores/m³); II) average *Pen/Asp*-like concentration (spores/m³); For *Cladosporium* sp. and *Pen/Asp*-like the ground level had a n=3, and for above ground a n=21.

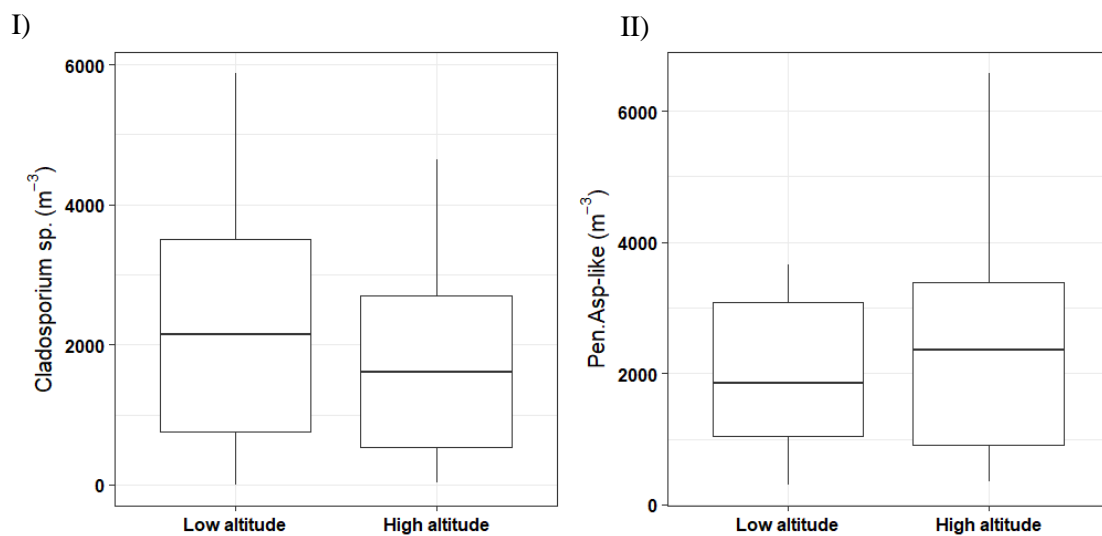


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Fig. 8 Boxplot of the concentration at ground level and above ground from portable Burkard considering the Winter Campaign and different periods of day (morning and afternoon). I) average *Cladosporium* sp. concentration (spores/m³) (Afternoon: W=20, p<0.05); II) average *Pen/Asp*-like concentration (spores/m³). For *Cladosporium* sp. and *Pen/Asp*-like in the afternoon the ground level had a n=2, and for above ground a n=10, while in the morning the ground level had a n=1, and for above ground a n=11.

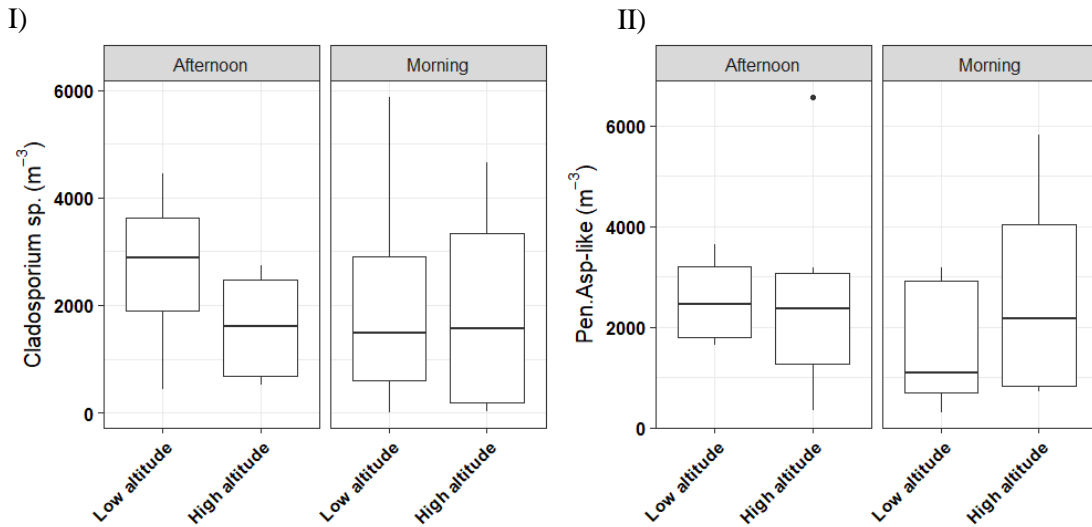
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The average concentrations of *Cladosporium* sp. and *Pen/Asp*-like from Burkard in the low altitude (up to 211m) and high altitude (>211m) of the Winter campaign did not differ significantly (Fig. 9). This also is true when considering the afternoon and morning period (Fig. 10). Different from the total concentration of spores from Burkard, *Pen/Asp*-like shows a tendency of higher concentration at high altitude when considering the whole Winter campaign, and also when considering only the morning period, although not significant.



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399

400 **Fig. 9** Boxplot of the concentration at low altitude (up to 211 m) and high altitude (>211 m)
 401 considering the Winter Campaign. I) average *Cladosporium* sp. concentration (spores/m³) from
 402 portable Burkard; II) average *Pen/Asp*-like concentration (spores/m³) from portable Burkard. For
 403 *Cladosporium* sp. and *Pen/Asp*-like the low altitude had a n=11, and for high altitude a n=10.
 404



405
 406 **Fig. 10** Boxplot of the concentration at low altitude (up to 211 m), and high altitude (>211 m)
 407 considering the Winter Campaign and the morning and afternoon period. I) average
 408 *Cladosporium* sp. (spores/m³) from portable Burkard; II) average *Pen/Asp*-like (spores/m³) from
 409 portable Burkard. For *Cladosporium* sp. and *Pen/Asp*-like in the afternoon the low altitude had a
 410 n=4, and for high altitude a n=6, while in the morning the low altitude had a n=7, and for high
 411 altitude a n=4.
 412
 413

414 There was no significant difference between low and high altitudes, which may be due to
 415 a small number of samples or that the difference in altitude was not high enough to significantly
 416 influence the concentration of fungal spores. Nevertheless, some spore types, such as,
 417 Phaeosphaeriaceae and rusts, were only found at high altitudes (> 211 m), while other as puffballs
 418 and *Chaetomium* sp. were only found in high altitude or in the transition altitude between low
 419 altitude and high altitude (192 m). However, all these fungal types were found in low
 420 concentrations (Phaeosphaeriaceae: av: 1.04 spores/m³, max: 25 spores/m³, min: 0 spores/m³;
 421 Rust: av: 1.04 spores/m³, max: 25 spores/m³, min: 0 spores/m³; Puffballs: av: 4.17 spores/m³,
 422 max: 50 spores/m³, min: 0 spores/m³; *Chaetomium* sp.: av: 20.83 spores/m³, max: 500 spores/m³,
 423 min: 0 spores/m³).
 424

425 4. Discussion

426 Findings from the present work are consistent with previous literature. The main genera
 427 found with Burkard (Hyaline Basidiospores, *Cladosporium* spp. and *Penicillium/Aspergillus*-
 428 like) were also found by Degobbi et al. (2011) and Emygdio et al. (2018) analyzing samples in
 429 São Paulo city (Brazil) with similar methodology. Moreover, the fungal genera found with
 430 MAS100 (*Fusarium* sp., *Cladosporium* spp. and *Penicillium* spp.) are commonly found in the

431 atmosphere of São Paulo city and other regions of Brazil using similar methodology (Brickus et
432 al., 1998; Gonçalves et al., 2010; Bezerra, 2014).

433 *Cladosporium* is one of the most frequently found genus in the atmosphere in the majority
434 of the countries (Burch & Levetin, 2002; Després et al., 2012; Ščevková and Kováč, 2019; Wu et
435 al., 2007; Ataygul et al., 2007), as well as *Penicillium* and *Aspergillus* (Després et al., 2012).
436 *Cladosporium*, *Fusarium*, *Aspergillus* and *Penicillium* are important genera of fungi, with species
437 distributed worldwide and found, in the air, soil, food, organic materials and plants (Bensch et al.,
438 2012; Crous et al., 2007; Lucca et al., 2007). These genera include species that can be pathogenic
439 to humans and to plants (phytopathogenic), saprobic, endophytic and fungicolous (Crous et al.,
440 2007; Lucca et al., 2007). They can cause several types of injuries in the alive or senescing leaves
441 (Bensch et al., 2012, Lucca et al., 2007), and can also results in damage and decay in grains,
442 legumes, fruits, and vegetables after harvesting (Agrios et al., 2005; Lucca et al., 2007).
443 Remarkably, species from these genera can cause several damages to agriculture and
444 consequently economic losses (Agrios et al., 2005; Lucca et al., 2007).

445 Comparing the total concentration of fungal genera found in MAS100 with some
446 equivalent (mitospores) found with Burkard, the MAS100 represents only ~16% of the Burkard
447 total concentration. However, when comparing all fungal types found with Burkard with MAS100
448 concentration, this represents only 0.4%. These percentages are related to the viability and
449 culturability of PBAPs and indicate a reduced number of viable/culturable spores versus the total
450 number of spores, as expected from existing literature (Després et al., 2012). Moreover, Burkard
451 had about 4 times more fungal genera/type identification when compared to MAS100. According
452 with Hawksworth (1991) only 17% of known fungal species are found in culture collection, and
453 as cited by Bridge and Spooner (2001) can be readily grown in culture. Moreover, if we consider
454 the currently known accepted species of 120 thousand (Hawksworth and Lücking, 2017) and the
455 amount of currently species in the culture collection of 25,611 (WFCC, 2020), the percentage of
456 known fungi species existed in culture collection, and as so, the percentage of fungi that can grow
457 in culture, increase to ~21%.

458 Concerning altitude variability, the overall higher concentration of fungal spores at
459 ground level is expected, because the primary source of fungal spores is in the ground and some
460 larger spores cannot reach high altitudes and deposit faster (Lighthart and Stetzenbach, 1994).
461 Pace et al. (2019) found around half of the fungal spore concentration in a high-altitude site
462 compared to low-altitude site in Italy, explained by difference in temperature, relative humidity,
463 and wind speed. However, *Cladosporium* and *Alternaria* remained the most abundant genera for
464 both cases. In our work, the fungal types did vary considering the altitudes, with some fungal
465 spores type only present in high altitude or low altitude, for example. However, the main fungal
466 types, were present in all levels (ground, above ground, low and high altitude)

467 Spore types such as Phaeosphaeriaceae and rusts, were only found at high altitudes (>
468 211 m), while others were found only at high altitude or at the transition altitude between low
469 altitude and high altitude (192 m) such as puffballs and *Chaetomium* sp. Concentrations were,
470 however, low and below the ranges described in the literature (Degobbi, 2010).

471 Although puffballs belong to an extensive group, the average spore size across the group
472 is 5 μm , and the cloud of spores released from a fruiting body occurs in response to raindrops
473 (Amador et al., 2012) or other types of impacts. Some puffball species have shown to maintain
474 structural characteristics for long periods under controlled dry conditions (up to 2 years) (Zhirnov
475 et al., 2019). Since each fruiting body may release trillions of spores as an explosive discharge
476 (Zhirnov et al., 2019), this characteristic reinforces the explanation of the results in the whole
477 boundary layer, including altitudes as high as 350 m. In a similar way, the ascospore *Chaetomium*
478 sp. (average spore size c.10 μm ; Wang et al., 2016) has its spores released from the perithecium
479 after being disturbed with a raindrop. The entire perithecium may become airborne and release
480 spores afterwards, when exposed to wind (Dixon, 1961), aiding the dispersal at higher altitudes.

481 Phaeosphaeriaceae is a family of spores found in Brazil (spore sizes > 11 μm ; Shoemaker
482 et al., 1989) and the spore type found in this study resembles *Phaeosphaeria annulata*, which is
483 very distinctive. Rusts are another category that causes diseases in crops and have a relatively
484 sizable spore aerodynamic diameter of 20 μm or larger (Smith, 1984). Although these spores are
485 much bigger than most of all fungal species, size is just one of the factors affecting airborne
486 transportation. Spores' characteristics (particle density and hygroscopicity) and atmospheric
487 characteristics (scavenging in-cloud as well as below cloud, wind speed, air temperature and air
488 humidity) are the others. In fact, predictive models have shown that airborne transfer among
489 different countries is a complex phenomenon highly dependent on meteorological conditions such
490 as wind, temperature, humidity of local area as well as overall synoptical conditions (Nagarajan
491 et al., 1990). Consequently, concentration measurements alone are insufficient to evaluate spore
492 transportation. More comprehensive studies including particles characteristics as well as their
493 actual emission rate (i.e.: flux) need to be carried out in order to being able to evaluate spore
494 transportation with sufficient accuracy. Flux measurements in particular will be the focus of the
495 next studies and they will take in account the more prominent fungal genera/types found in the
496 present study.

497 Spore discharge mechanism is an important factor when considering the spore
498 concentration in the atmosphere. According to the literature, in general, it can have different types
499 of spore release. One type is the so-called "actively wet discharge", which involves the release of
500 spores with liquid jets or droplets and is usually related with humid conditions (e.g., night and
501 early morning) (Elbert et al., 2007; Després et al., 2012). The other case is called "dry discharged
502 spora", which does not accompany liquid and is usually related with weather conditions with low
503 relative humidity, high wind speed and temperature. Some fungal species belonging to

504 *Aspergillus*, *Penicillium* and *Cladosporium*, have this so-called “dry discharge spores” (Elbert et
505 al., 2007; Grinn-Gofroń & Rapiejko, 2009; Després et al., 2012). This tendency could explain the
506 higher concentrations of these fungal types/genera during the afternoon period.

507 The meteorological trends presented in topic 3.2 also bring some consideration for PBAPs
508 samplings. Early morning is characterized by a moist shallow boundary layer (BL) which is not
509 very conducive to spore release and transport: the reduced height of the early morning BL is due
510 to reduced solar radiation which translates in weak or absent thermal eddies that favor the mixing
511 of spores into the air (especially considering the spores’ size and hygroscopicity, Reponen et al.,
512 2001). This trend would progressively change as the day goes on: the increased solar radiation
513 deepens the BL due to increased thermal eddies and turbulence thus favoring the uplifting of the
514 spores. The increase in solar radiation and thus air temperature would also explain the progressive
515 reduction in RH that, in turn, could favor the release of spores of some fungal species within
516 *Cladosporium* and the *Pen/Asp.*-like fungi that can have the “dry discharges spores” mechanism.
517 This interplay between solar radiation, turbulence and relative humidity would explain the
518 difference in ground level concentrations between mornings and afternoons during the winter
519 campaign (Stull, 1988; Oke, 1987). Above ground concentrations are expected to be low due to
520 vertical dilution and distance from the source, but at least for early morning flights there’s a
521 further interesting consideration.

522 The average BL height between 05:00 and 07:00 (local time) was of roughly 198 m during
523 the summer campaign and of 174 m during the Winter Campaign, a difference of 12%, higher in
524 summertime as expected. While this height is not without uncertainties due to the assumptions
525 inherent in its calculation (see e.g., Siebert et al., 2000), it is possible that most balloon flights
526 performed in the morning were cruising in the residual mixed layer from the previous day. The
527 latter was formed during the previous day and survives during the night, getting progressively
528 eroded during the first hours of the new day by the formation and growth of the boundary layer
529 underneath (with which a dynamical coupling can be developed as suggested by Fochesatto et al.,
530 2001). Nevertheless, the characteristics of the nocturnal residual mixed layer are, at least initially,
531 the same as the decayed mixed layer of the day before (Stull, 1988). This could imply that
532 whatever was sampled on the balloon during morning flights were actually remaining bioaerosols
533 from the day before, potentially diluted by the passage of time. Genera that resulted viable in early
534 morning flights are therefore of potential interest for long-range transport and dissemination given
535 their persistence in the unfavorable airborne environment.

536

537 **5. Conclusion**

538 Bioaerosols are increasing their relevance in many studies around the world, but they are
539 not yet fully understood. This work aimed to analyze fungal samples at different altitudes as well
540 as through two different sample devices to serve as a basis for further investigation on fungal

541 spore fluxes in Brazil. Samples were collected using a portable Burkard and MAS100 at ground
542 level near the studied crops and above ground with a hot-air balloon. The main fungal types in
543 the studied region were Hyaline Basidiospores, *Penicillium/Aspergillus*-like and *Cladosporium*
544 spp. In comparison to the classic Burkard sampler, the MAS100 reported smaller concentrations
545 and lower fungal diversity, as expected. The average CFU (from MAS100) and fungal spore (from
546 Burkard) concentration at ground level was ~2 and ~3 times higher than above ground levels
547 . *Cladosporium* sp. and *Penicillium/Aspergillus*-like were consistently found during the
548 sampled period and are frequently in both equipment. Therefore, they can be used in future works
549 as main indicators to calculate vertical flux of fungal spores in the studied area, especially because
550 daily cycles of boundary layer growth and relative humidity well explain the differences in ground
551 concentrations between morning and afternoon samplings. It is interesting to point out that some
552 fungal types were found in concentrations that were dependent on the altitude, being some types
553 found only in high altitudes. Further research is needed in order to confirm these results. To
554 conclude, these findings will help to better understand the bioaerosol diversity in the atmosphere
555 in the studied region and provide valuable information on how to calculate the bioaerosol flux to
556 the higher levels, and its interaction with many meteorological variables such as precipitation,
557 including hail formation.

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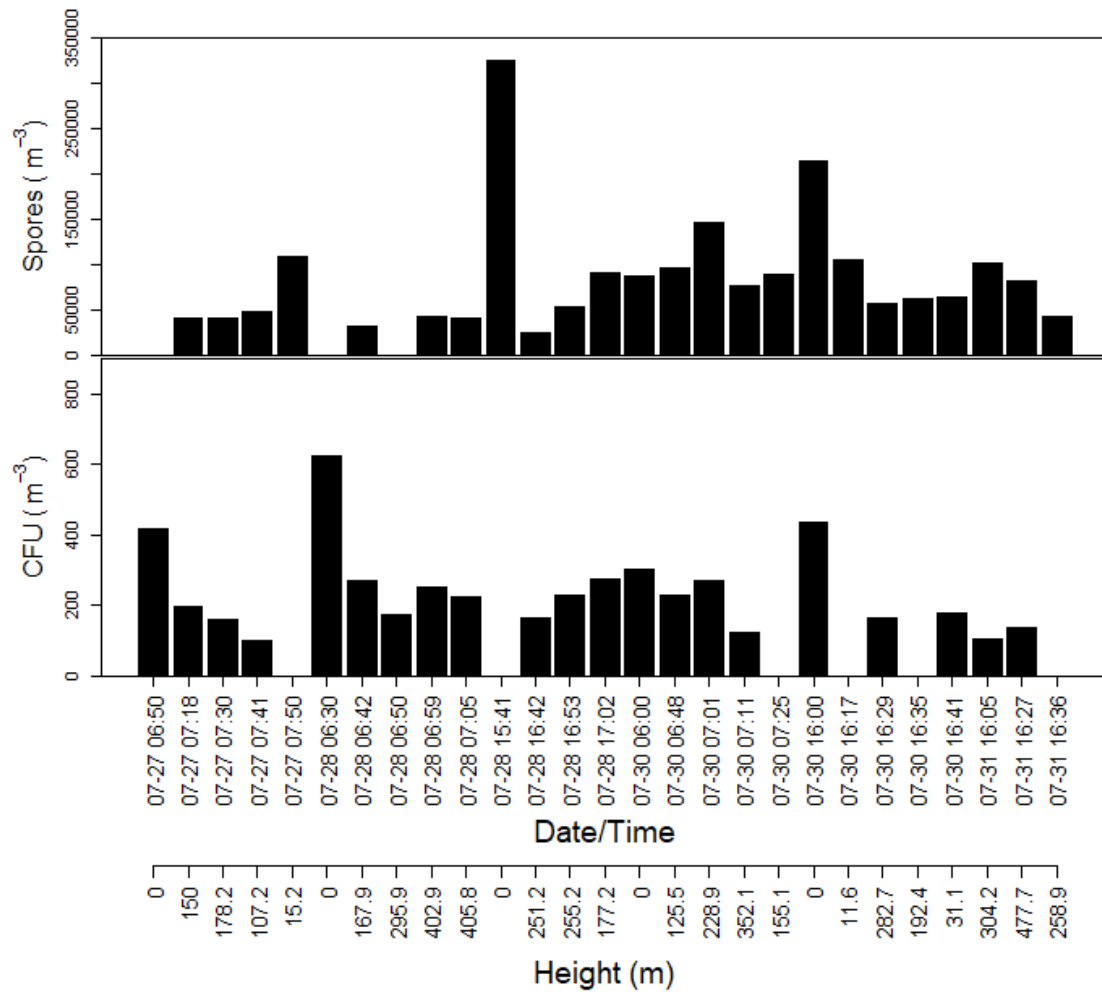
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722

7. Supplementary information



724

725 **Fig. SI 1** Temporal variation and respective sampling heights considering the Winter Campaign.
 726 I) Fungal spore concentration (spores/m³) from portable Burkard; II) Fungi colony forming unit
 727 (CFU/m³) from MAS100.

728

729 **Table SI 1** Fungal spore (spores/m³) concentration from portable Burkard and Fungi colony
 730 forming unit (CFU/m³) concentration from MAS100 considering the date/time and respective
 731 sampling heights during the Winter Campaign.

Date	Height (m)	Spores/m ³	CFU/m ³
27/07/2019 06:50	0.0 (GL)	NA	418
27/07/2019 07:18	150.0 (AG and LA)	41225	194
27/07/2019 07:30	178.2 (AG and LA)	40375	160
27/07/2019 07:41	107.2 (AG and LA)	47575	98
27/07/2019 07:50	15.2 (AG and LA)	108575	NA
28/07/2019 06:30	0.0 (GL)	NA	622
28/07/2019 06:42	167.9 (AG and LA)	31950	268
28/07/2019 06:50	295.9 (AG and HA)	NA	172
28/07/2019 06:59	402.9 (AG and HA)	42500	252
28/07/2019 07:05	405.8 (AG and HA)	40525	222
28/07/2019 15:41	0.0 (GL)	324520.5	NA
28/07/2019 16:42	251.2 (AG and HA)	24925	162
28/07/2019 16:53	255.2 (AG and HA)	53475	226

28/07/2019 17:02	177.2 (AG and LA)	91200	274
30/07/2019 06:00	0.0 (GL)	86175	300
30/07/2019 06:48	125.5 (AG and LA)	95425	228
30/07/2019 07:01	228.9 (AG and HA)	145500	270
30/07/2019 07:11	352.1 (AG and HA)	76525	124
30/07/2019 07:25	155.1 (AG and LA)	89500	NA
30/07/2019 16:00	0.0 (GL)	213568.5	436
30/07/2019 16:17	11.6 (AG and LA)	105500	NA
30/07/2019 16:29	282.7 (AG and HA)	55900	166
30/07/2019 16:35	192.4 (AG and LA)	61275	NA
30/07/2019 16:41	31.1 (AG and LA)	63400	178
31/07/2019 16:05	304.2 (AG and HA)	101850	106
31/07/2019 16:27	477.7 (AG and HA)	81300	134
31/07/2019 16:36	258.9 (AG and HA)	42400	NA

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